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ORIGINAL ARTICLE

Diagnostic Value of Serum ADA in Smear-Negative Pulmonary Tuberculosis

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Abstract:

Background & Objective: *Diagnosis of tuberculosis (TB) is not always easy, particularly if it is a case of smear-negative pulmonary tuberculosis (SNPTB). Patients with respiratory symptoms resembling SNPTB is difficult to differentiate on the basis of clinical features, X-ray chest and Xpert MTB/RIF negativity. So additional diagnostic tests with high sensitivity and specificity is needed to increase the yield of the ongoing diagnostic strategy for SNPTB. That purpose the present study tested the value of serum adenosine deaminase (ADA) as an adjunct to the existing diagnostic aids.*

Patients & Methods: *The present cross-sectional analytical study was carried out in the Department of Respiratory Medicine, National Institute of Diseases of the Chest & Hospital (NIDCH), Mohakhali, Dhaka over a period of one year from April 2018 to March 2019. Patients attending in the above-mentioned hospital with respiratory ailments and were suspected of having pulmonary tuberculosis from their clinical presentation, chest radiography, sputum smear and Xpert MTB/RIF negativity were the study population. A total of 60 such patients (suspected SNPTB), 30 smear-positive pulmonary tuberculosis (SPPTB) cases and 30 healthy controls were included in the study. According to National Guidelines for Management of Tuberculosis, if a patient with symptoms suggestive of TB with two consecutive sputum specimens being negative for AFB, Xpert MTB/RIF negative, chest X-ray abnormalities consistent with active TB and the diagnosis was made by a qualified physician, the case was considered as having SNPTB.*

Result: *The SNPTB patients had a moderate rise of serum ADA (35.4 U/L) compared to the SPPTB patients who had the highest serum ADA and the healthy controls who had the lowest serum ADA (41.1 ± 11.8 vs. 22.7 ± 5.5 U/L respectively). In order to find a cut-off value for serum ADA at which it is fairly sensitive and specific to diagnose SNPTB, a receiver-operating characteristic (ROC) curve was constructed with an area under the curve being 0.851(95% CI = 0.745-0.957, p < 0.001). The ROC curve gave a cut-off value 27.5 U/L at which the serum ADA had a sensitivity, specificity, PPV, NPV and the diagnostic accuracy of 80, 80, 88.9, 66.7 and 80% respectively. The LR+ and LR- were 4.0 and 0.25 respectively.*

Conclusion: *From the findings of the study, it can be concluded that the serum ADA has a modest sensitivity and specificity in the diagnosis of SNPTB. However, the results of ADA assays should be interpreted in conjunction with clinical presentations and other laboratory test findings. As the LR+ is only 4, the test is of little clinically useful in the diagnosis of SNPTB. Therefore, estimating ADA levels should not be a valuable additional test, in the rapid diagnosis of SNPTB patients provided a large-scale study on a cross-section of diverse SNPTB population to confirm its limited usefulness.*

Key words: *Serum ADA, Smear-negative Pulmonary Tuberculosis (SNPTB), sensitivity and specificity etc.*

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Introduction:

Tuberculosis (TB) is one of the leading causes of morbidity and mortality, amongst infectious diseases.[1] It has been estimated that about one-third of world's population was affected with TB and more than 95% patients died in developing countries.[2] According to European Centre for Disease Prevention and Control, TB remains responsible for the deaths of nearly 1.7 million people each year and representing the ninth leading cause of death globally.[3] Bangladesh ranks sixth among the world's 22 high-burden TB countries with estimated 350,000 new cases and 70,000 deaths each year.[4]

Although TB is a known infectious disease with a definite epidemiological pattern and known principles of treatment since last 60 years, there are still a considerable number of TB cases in many parts of the world who are not timely diagnosed and properly treated.[5] Tuberculosis, can present as pulmonary tuberculosis (PTB) or extra-pulmonary tuberculosis (EPTB). Sputum smear microscopy is routinely used for diagnosis of PTB. The diagnosis of smear-positive pulmonary tuberculosis (SPPTB) does not pose any problem. However, definitive laboratory diagnosis and confirmation of sputum smear-negative pulmonary tuberculosis (SNPTB) still remains elusive and poses a major challenge in the management and control of active pulmonary tuberculosis. Cliniciansoften have to face difficulties in smear negative patients, and sometimes, it becomes almost impossible to diagnose this entity.[6]

The symptoms of active pulmonary TB are coughing, sometimes with sputum or blood, chest pain, weakness, weight loss, fever, and night sweats and it is treatable with a 6-months course of antetubercular chemotherapy.[7] Chest radiograph provides only a probable diagnosis of tuberculosis; they are sometimes difficult to differentiate from other causes of lung shadows, such as, pneumonia and malignancies.[8]In resource-poor settings, SNPTB is difficult to diagnose and also difficult to exclude, especially in HIV infected patients.[9] Although the standard method for TB diagnosis is direct observation of acid-fast bacilli (AFB) in sputum smear or *M. tuberculosis* isolation in specific culture media,[5] this method is not always easy to perform. The sensitivity of acid-fast bacilli (AFB) staining result is known to be poor varying between 30-70% depending on a number of factors relating to how the test is implemented.[10] Thus, nearly half of all cases of pulmonary TB are smear-negative, meaning that the overall disease burden is substantial and is associated with treatment delay and hospitalization.[11]Moreover, the presence of comorbidities like diabetes mellitus, HIV and other immune- compromised conditions further complicate the picture as they lead to atypical clinical and radiological presentations.[12] This delay in diagnosis and subsequent treatment leads to increased disease transmission and chances of drug resistance.[13]Therefore, finding a laboratory test for SNPTB cases, that is simple, easy-to-perform, rapid, reliable and inexpensive is an urgency and efforts to improve the quality of existing diagnostic methods are necessary.[14]

Adenosine deaminase (ADA) is one such biomarker which is now a days being studied as a diagnostic tool in tuberculosis.[15] Studies are available on its role in effusion fluids.[16] However, limited literature is available regarding the use of serum ADA in active disease, and whether the levels fall with the recovery of the patients from infection.[17-19] Human adenosine deaminase (an enzyme of purine catabolism) activity has been found to increase in various diseases such as tuberculosis,[20] HIV, typhoid, infectious mononucleosis and certain malignancies especially those of hemopoietic origin.[21] ADA assay in various body fluids had established its usefulness in the laboratory diagnosis of extrapulmonary TB,[22] smear-positive TB and SNPTB.[23]Now there is sufficient data suggestingthat ADA assays can be performed in many health care centres with limited diagnostic facilities other than mycobacterial culture, PCR etc. In addition, it is cheap and has good sensitivity. ADA may be used for early diagnosis of TB, especially in case of negative AFB smear from the body specimens.[24] Considering the issues and constraints in the diagnosis of TB, this study was designed to determine the diagnostic accuracy of serum ADA in the diagnosis of SNPTB.

Materials and Methods:

This cross-sectional study was carried out in the Department of Respiratory Medicine, National

Institute of Diseases of the Chest & Hospital (NIDCH), Mohakhali, Dhakaover a period of one year between April 2018 to March 2019.Patients (whose age ranged from 18-65) attending in the study hospital with respiratory ailments and were suspected of having pulmonary tuberculosis from their clinical presentation, chest radiography, sputum smear and Xpert MTB/RIF negativity were the study population.Patients with characteristics of extra pulmonary tuberculosis, old treated cases of pulmonary tuberculosis and patients diagnosed with other respiratory diseaseswere excluded from the study. A total of 60 SNPTB cases, 30 SPPTB cases and 30 healthy controls were taken in the study.

Having obtained ethical clearance from the Ethical Committee and verbal consent from the patients, the data collection was commenced. Statistical analyses were carried out using Statistical Package for Social Sciences, version 25.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Categorical data were presented as frequency and percentage and continuous data were expressed as mean ± SD (standard deviation). While categorical data were compared between groups using Chi-square ($Ç^2$) Test, continuous data were compared between groups using Independent sample t-Test. The cut-off value of serum ADA at which it had optimum sensitivity and specificity was found out using receiver-operating characteristic (ROC) curve with 95% confidence interval. The area under the curve (AUC) with 95% confidence interval (CI) was statistically determined to find the accuracy of serum ADA in diagnosing smear negative pulmonary tuberculosis (SNPTB). For all analytical tests, the level of significance was set 5% and p-value < 0.05 was considered significant. The findings obtained from data analyses are presented below:

Results:

Age distribution shows that the subjects of SPPTB were older compared those of SNPTB, who were again older than healthy control subjects. ANOVA test revealed that the groups were significantly heterogeneous in terms of age ($p < 0.001$). Sex distribution among the three study groups was also significantly different with a male predominance in SPPTB and in healthy controls ($p < 0.001$) (Table I). The SPPTB, SNPTB patients were predominantly rural residents, while the healthy subjects were invariably urban residents ($p < 0.001$). Around two-thirds of the study subjects in the SPPTB and SNPTB were married, whereas 96.7% of the healthy subjects were married ($p = 0.004$). In terms of occupation, farmers, labor and business together comprised 63% of the SPPTB cases, while 66.6% of the SNPTB cases were students and other occupants. Healthy controls were all service-holder ($p < 0.001$). Around three-quarters of the SPPTB and SNPTB cases belonged to poor and lower middle class. SPPTB cases had the lowest monthly income compared to other two groups ($p < 0.001$). Average weights of SPPTB and SNPTB patients were also lower than that of healthy controls ($p < 0.001$) (Table II).

The symptoms like chest pain, dyspnoea, weight loss, haemoptysis and fever were considerably higher in the SPPTB subjects than those in the SNPTB subjects(Fig.1). There was no significant difference between the groups with respect to haematological parameters, except the percentage of neutrophil, which was significantly higher in SNPTB than that in the SPPTB ($p = 0.004$). The lymphocyte waspredominentin the SPPTB subjects than that in the SNPTB subjects, although the difference was not statistically significant ($p= 0.064$). Cavitation and patchy opacity were more readily found in SPPTB cases, whereas consolidation was more frequent in SNPTB cases ($p = 0.023$) (Table IV). Analysis of the distribution of serum ADA level among the three study groups revealed that SPPTB group had the highest mean serum ADA (41.1 ± 11.8 U/L) followed by SNPTB (35.4 ± 11.7 U/L) and healthy controls (22.7 ± 5.5 U/L) ($p < 0.001$) (Table V). Before determining the accuracy of serum ADA in diagnosing SNPTB, an optimum cut-off value for serum ADA was determined using Receiver Operating Characteristic (ROC) curve (Fig.2):

The sensitivity of serum ADA, at a cut-off value of 27.5 U/L, in diagnosing SNPTB was, therefore, 48/60 ´ 100 = 80.0% (95% CI = 0.682 – 0.882) and the specificity of the test in correctly excluding those who did not have PTB was 24/30 ´ 100 = 80.0% (95% CI = 0.627 – 0.905). The positive and negative predictive values of the test were 48/54 ´ 100 = 88.9% (95% CI = 0.778 – 0.948) and 24/36 × 100 = 66.7% (95% CI = 0.503 – 0.798) respectively. The

percentages of false positive and false negatives are 6/54 ´ 100 = 11.1 and 12/36 ´ 100 = 33.3% respectively. The positive likelihood ratio (LR+) = sensitivity/(1-specificity) = 4.0 (95% CI = 1.93 – 8.27) and negative likelihood ratio (LR-) = 1- sensitivity / specificity = 0.25 (95% CI = 0.15 - 0.43) The overall diagnostic accuracy of the test was (48 + 24)/ (48 + 6 + 12 + 24) × 100 = 80.0% (table VII, Table III).

The best cut-off value for optimum sensitivity without much compromise with specificity obtained from the table below was 27.5 with an area under the curve being 0.851(95% CI = 0.745-0.957), p < 0.001 (Table VI & VII). The area under the curve indicates that 85.1% of the SNPTB could be correctly diagnosed with serum ADA level 27.5 and more in patients with SNPTB.

Table-I

Comparison of patients' demographic characteristics between groups

Demographic characteristics	Group			P-value
	SPPTB (n = 30)	SNPTB (n = 60)	Healthy control (n = 30)	
Age (years)#	43.8 ± 18.8	35.9 ± 19.1	31.6 ± 3.9	< 0.001
Sex*				
Male	22(73.3)	28(46.7)	29(96.7)	< 0.001
Female	8(26.7)	32(53.3)	1(3.3)	

Figures in the parentheses indicate corresponding %;
*Chi-squared Test (c^2) was done to analyze the data.
#Data were analyzed using ANOVA statistics and were presented as mean ± SD.

Table-II

Patients' demographic characteristics among groups (Contd.)

Demographic characteristics	Group			P-value
	SPPTB (n = 30)	SNPTB (n = 60)	Healthy control (n = 30)	
Residence*				
Urban	12(40.0)	23(38.3)	30(100.0)	< 0.001
Rural	18(60.0)	37(61.7)	0(0.0)	
Marital status*				
Married	20(66.7)	39(65.0)	29(96.7)	0.004
Unmarried	10(33.3)	21(35.0)	1(3.3)	
Occupation*				
Farming	6(20.0)	4(6.7)	0(0.0)	
Labor	7(23.3)	12(20.0)	0(0.0)	
Business	6(20.0)	4(6.7)	0(0.0)	< 0.001
Service	4(13.3)	0(0.0)	30(100.0)	
Student	2(6.7)	24(40.0)	0(0.0)	
Others	5(16.7)	16(26.6)	0(0.0)	
Socioeconomic status*				
Poor	17(56.7)	24(40.0)	2(6.7)	
Lower middle class	5(16.7)	22(36.7)	8(26.7)	
Middle class	7(23.3)	14(23.3)	8(26.7)	< 0.001
Upper middle class	1(3.3)	0(0.0)	8(26.7)	
Rich	0(0.0)	0(0.0)	4(13.3)	
Income#	19866 ± 12340	23733 ± 12423	28216 ± 21640	< 0.001
Weight#	50.6 ± 5.1	47.4 ± 5.8	64.9 ± 6.0	< 0.001

Figures in the parentheses indicate corresponding %;
*Chi-squared Test (c^2) was done to analyze the data.
#Data were analyzed using ANOVA and were presented as mean ± SD.

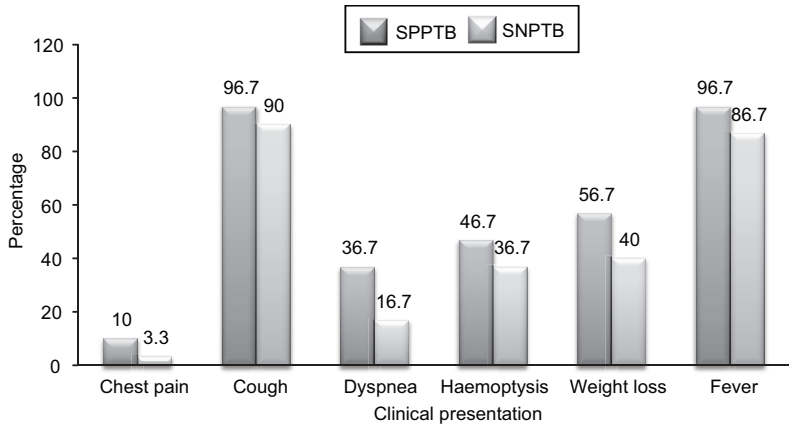

Fig. 1: Comparative clinical presentations of SPPTB and SNPTB

Table-IV

Comparison of patient's Investigation between groups

Investigations	Group		P-value
	SPPTB(n = 30)	SNPTB(n = 60)	
Total count of WBC (cu-mm of blood)#	10094 ± 2602	13712 ± 3440	0.146
Neutrophil (%)#	61.8 ± 11.1	68.4 ± 6.1	0.004
Lymphocyte (%)#	32.4 ± 11.5	28.2 ± 5.9	0.064
Level of Hb (gm/dl)#	12.1 ± 0.8	11.7 ± 0.9	0.134
ESR (mm at the 1st hr)#	83.3 ± 12.3	81.1 ± 11.4	0.420
X-ray chest findings*			
Consolidation	5(16.7)	14(23.3)	
Cavitation	3(10.0)	2(3.3)	0.023
Patchy opacity	20(66.7)	32(53.4)	
Fibrosis	2(6.6)	4(6.7)	
Others	0(0.0)	8(13.3)	

Figures in the parentheses indicate corresponding %;
*Chi-squared Test (c^2) was done to analyze the data.
#Data were analyzed using Unpaired t-Test and were presented as mean ± SD.

Table-V

Comparison of patient's Investigation between groups

Investigations	Group			P-value
	SPPTB (n = 60)	SNPTB (n = 30)	Healthy control (n = 30)	
Serum ADA level (U/L)#	41.1 ± 11.8	35.4 ± 11.7	22.7 ± 5.5	< 0.001

Figures in the parentheses indicate corresponding %;
#Data were analyzed using ANOVA and were presented as mean ± SD.

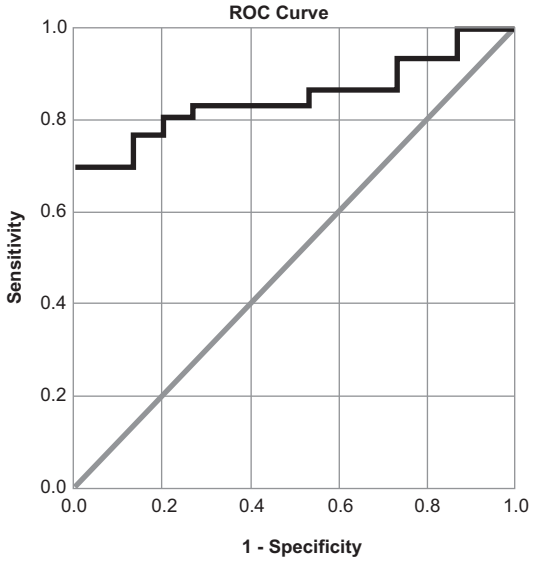

Fig. 2 *showing area under the ROC curve*

Table-VI

Area Under the Curve

Test Result Variable(s): Serum ADA

Area	Std. Error[a]	p-value[b]	95% Confidence Interval of Area Under the Curve	
			Lower Bound	Upper Bound
0.851	0.054	< 0.001	0.745	0.957

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

Table-VII

Accuracy of serum ADA in predicting SNPTB with clinical pictures and X-ray chest suggestive of PTB

Serum ADA (U/L)	SNPTB		Total
	Present	Absent	
≥27.5	48	06	54
< 27.5	12	24	36
Total	60	30	90

Discussion:

The present study intended to evaluate the usefulness of serum ADA in the diagnosis of SNPTB demonstrated that SNPTB patients had a mean serum ADA of 35.4 U/L, while the SPPTB patients had the highest and the healthy controls had the lowest serum ADA (41.1 ± 11.8 vs. 22.7 ± 5.5 U/L respectively). The study indicates that there is moderate rise of ADA in the SNPTB – lower than the SPPTB but higher than the normal individuals. Previous studies had also shown elevated levels of serum ADA in SNPTB patients.[23,25] Chander and associates[17] showed significantly increased serum ADA levels in SNPTB patients compared to that in healthy controls (42.26 ± 21.22 U/L vs. 18.88±6.67 U/L, $p < 0.001$), which is fairly comparable to the findings of the presents study. They, however, did not include SPPTB cases rather they included the non-tubercular chest disease – COPD cases which exhibited a moderate rise of ADA (23.35±8.22 U/L, $p < 0.001$). Gupta and associates[26] also demonstrated mean serum ADA level of their

smear negative pulmonary tuberculosis patients to be clearly elevated (43.5±6.10 U/L).

In order to find a cut-off value for serum ADA at which it is fairly sensitive and specific to diagnose SNPTB, we constructed receiver-operating characteristic (ROC) curve. The ROC curve gave a cut-off value 27.5 U/L at which the serum ADA was 80% sensitive and 80% specific. From ROC curve it appears that increasing the cut-off value, increases the specificity of the of serum ADA to exclude SNPTB but at the cost of sensitivity, while decreasing the cut-of value increases its sensitivity but with compromise of specificity. So for the present study the serum ADA 27.5 U/L seems to be an optimum cut-off value. At this cut-off value, the sensitivity, specificity, PPV, NPV and the diagnostic accuracy of serum ADA were 80, 80, 88.9, 66.7 and 80% respectively. The LR+ and LR- were 4.0 and 0.25 respectively. As the LR+ is 4 (much greater than 1), the test is of greater value in the diagnosis of SNPTB, because the odds of having the condition have changed significantly after the test.Chander and associates[17]using a cut-value of 30 U/L, demonstrated a high sensitivity (91.2%) and specificity (83.1%) of serum ADA in the diagnosis of SNPTB. Their reason for choosing the cut-off value at 30 U/L was that a previous study, at this cut-off value, had shown the specificity and sensitivity of ADA to be nearly 100%. A high positive predictive value (88.9%) in the present study as well as in Chandler's study (94%) indicate that ADA activity measurements could be a promising diagnostic marker in the differentiation of SNPTB from COPDs.

Increased serum ADA levels in pulmonary TB may be due to a stimulation of cell mediated immunity. A fully functioning cell mediated immune response is dependent on normal lymphocyte metabolism which is, in part regulated by the purine salvage enzyme, adenosine deaminase. ADA catalyzes the deamination reaction from adenosine to inosine that increases in TB because of the stimulation of T-cell lymphocytes by mycobacterial antigens[27]Increased serum ADA activity is also found in other diseases involving stimulation of cell-mediated immunity such as typhoid fever, infectious mononucleosis and bronchogenic carcinoma.[25]These non-tubercular infections can be ruled out on the basis of clinical presentations and other laboratory investigations. But patients with respiratory symptoms mimicking SNPTB is difficult to diagnose on the basis of clinical signs and symptoms, X-ray chest and Xpert MTB/RIF negativity. So additional diagnostic tests with high sensitivity and specificity may act as an adjunct to the existing diagnostic aids for SNPTB. Considered in this context, the serum ADA as a screening test for differentiating SNPTB from other cases of COPD has immense value and may add an impetus to the diagnostic yield of SNPTB.

Despite availability of culture facilities for the tubercle bacilli at our hospital, culture confirmation of the SNPTB cases was not done, for our diagnosis of SNPTB was based on National Tuberculosis Management Guidelines. Patients with active TB are also capable of transmitting the infection. Existing diagnostic approaches have largely failed to interrupt TB transmission in populations with a high prevalence of HIV and drug-resistant TB.[28]Although, persons with SNPTB are less infectious than the smear-positive patients, their overall contribution to disease transmission is considerable because half of all patients with TB can present with negative sputum smear findings. Thus, accurate diagnosis of SNPTB patients is of utmost significance. Though newer rapid diagnostic tests for TB are being developed, but these are either not available in developing countries or are technology-intensive and expensive, have poor sensitivity and specificity for smear-negative sputum samples and are not yet considered as diagnostic of the cases. In the developing countries where TB is endemic, an ideal test for tuberculosis should be economic, minimally invasive, of high accuracy,easy and quick to perform.[15]

The present study revealed that measuring ADA level is a rapid, sensitive, inexpensive diagnostic marker for diagnosing SNPTB patients, in whom otherwise the diagnosis is missed by sputum smear findings. However, the results of ADA assays should be interpreted with clinical presentations and with other laboratory examination findings. Now before drawing conclusion from the findings of the study, it would be worthwhile to discuss the strengths and limitations of the study. The following strengths and limitations deserve mention.

Strengths and limitations of the study:

The strength of the study lies in its sample size, which in the present study was more than the required size. Another strength of the study was that we constructed a ROC curve to find a cut-off value of serum ADA which is optimally sensitive and specific. The limitations of the study were that there was no 'Gold standard' for the diagnosis of SNPTB and the diagnosis was not confirmed by culture of tubercle bacilli. Another limitation is that we included SPPTB patients. Had we included chronic lung disease (like bronchial asthma or COPD) cases instead of SPPTB, the findings could have better explained the role of ADA in the diagnosis of SNPTB.

Conclusion:

From the findings of the study, it can be concluded that the serum ADA has a modest sensitivity and specificity in the diagnosis of SNPTB. However, the results of ADA assays should be interpreted in conjunction with clinical presentations and other laboratory test findings. As the LR+ is only 4, the test is of little clinically useful in the diagnosis of SNPTB. Therefore, estimating ADA levels should not be a valuable additional test, in the rapid diagnosis of SNPTB patients provided a large-scale study on a cross-section of diverse SNPTB population to confirm its limited usefulness.

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