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Prevalence of HIV Sero-conversion and associated risk factors among antenatal clinic attendees in Enugu state university of science and technology teaching hospital, Enugu Nigeria

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Abstract

Background: Human immunodeficiency virus (HIV) infection is a leading cause of morbidity and mortality among women of reproductive age and it is a major contributor to maternal, infant and child morbidity and mortality. Globally, in 2015, an estimated, 78 million people had become infected with HIV while 35 million people had died from Acquired Immune Deficiency Syndrome (AIDS)-related illness since the start of the epidemic. In Nigeria, a national HIV prevalence rate of 3.4% was reported in 2012 with 1.3% in Enugu State.

HIV retesting is a WHO recommendation that was adopted by Nigerian her national guideline. The essence of antenatal HIV retesting includes the identification of pregnant women who were first tested during their window periods and subsequently sero-converted, and those who acquired new HIV infection after their initial screening test given the fact that a single HIV testing may not be enough to diagnose HIV with findings of high rates of incident HIV infection during pregnancy and at the post-partum periods. It has been shown that the rate of MTCT was higher from sero-converting women compared with women with already established HIV infection. Hence, this study which gave a clue to the rate of HIV sero-conversion among our antenatal women which may possibly popularize the concept of retesting especially among these women.

Aim: To determine the prevalence of HIV sero-conversion and the associated risk factors among previously sero-negative antenatal clinic attendees in Enugu State University of Science and Technology Teaching Hospital, (ESUTTH).

Methods: This was a prospective, cohort study involving 415 sero-negative HIV women recruited from antenatal clinic in ESUTH from January to April, 2019 and followed up for 12 weeks. Information on their socio-demographic characteristics, pregnancy history, sexual lifestyle, and clinical profile were obtained using an interviewer-administered questionnaire. HIV tests were done at booking and repeated after 12 weeks using the Rapid Diagnostic Test kits by the parallel algorithm format. Data was analyzed with the IBM SPSS version 23.0. Measures of associations were determined with the Chi-Square test and binary logistic regression. P-value < 0.05 was assessed as statistically significant.

Results: The mean age of the participants was 29.44±4.91SD years with a range of 16-43 years. Majority of them were married (397; 95.7%) and had post-secondary education (321; 77.3%).

The incidence of HIV sero-conversion was 1.0%.

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Less than 35 years of age, being single, tobacco use, multiple sexual partners in pregnancy and knowledge of partners' HIV status were significantly associated with HIV sero-conversion ($p < 0.05$). However, none of these factors was a significant predictor following logistic regression.

Conclusion: The rate of HIV sero-conversion in pregnancy is of public health importance in Enugu and obligatory repeat testing, especially among women with these identified associated risk factors is therefore advised to forestall the menace of HIV to the child and the society at large.

Key words: HIV; Sero-conversion; Pregnancy; Enugu

1. Introduction

Human immunodeficiency virus (HIV) infection is a leading cause of mortality and morbidity among women of reproductive age and it is a major contributor to maternal, infant and child morbidity and mortality [1]. Globally, in 2015, an estimated78 million people had become infected with HIV while 35 million people had died from Acquired Immune Deficiency Syndrome (AIDS)-related illness since the start of the epidemic [2]. Regional reports showed that in 2016, in Central and Western Africa, 6.1 million people were living with HIV and women accounted for 56% of this population [3]. In Nigeria, a national HIV prevalence rate of 3.4% was reported in 2012 with 1.3% in Enugu State, [4]

HIV retesting is a WHO recommendation that was adopted by Nigerian in her national guideline [5,6]. The essence of antenatal HIV retesting includes the identification of pregnant women who were first tested during their window periods and subsequently sero-converted, and those who acquired new HIV infection after their initial screening test [7], given the fact that a single HIV testing may not be enough to diagnose HIV infection [8]. Studies have shown that there are high rates of incident HIV infection during pregnancy and at the post-partum period [9,10]. This increased HIV infectivity during pregnancy has been shown to be related to the woman's or her partner's sexual behaviour, genital mucosal changes, and/or hormonal changes [10]. Unprotected sexual intercourse, multiple sexual partners, inconsistent/non-use of condoms, use of recreational drugs, and poor knowledge of HIV/PMTCT and partners' HIV status are some of the documented risk factors for HIV acquisition during pregnancy [9,11-14]. Perinatal transmission of HIV has also been shown to be higher from acutely infected women compared with their chronic counterparts [10].

HIV sero-conversion rates of 3.0 to 14.7% [9,10,11,15] have been reported following HIV retesting in various settings across the globe. A systematic meta-analysis [16] also showed that the aggregate HIV sero-conversion rate among African countries was 3.6%.Despite being a national policy in many African countries including Nigeria; HIV retesting is rarely carried out [11]. Reports are varied on the frequency of retesting, characteristics of those re-tested, and the rate of HIV diagnosis among retesting clients [17]. It has been shown that the rate of MTCT was higher from sero-converting women compared with women with established HIV infection [18]. A study from Zambia [19] reported that there is paucity of data on the implementation of repeat HIV testing in Sub-Saharan Africa when it found that despite over 90% ANC coverage, opportunities for repeat HIV testing were missed in almost half of all pregnant women who delivered in a rural hospital in Zambia as only 24.5% underwent HIV retesting. No data was found on the current rate of HIV retesting among pregnant women in Nigeria.

Despite the institutional and patient-related challenges and barriers to repeat HIV testing in pregnancy [20], studies have shown that repeat HIV testing in pregnancy is both cost effective and achievable [21,22]. These studies clearly showed that the cost of repeating HIV test later in pregnancy is offset by the cost of treating an HIV positive child for life. In a high risk resource-poor setting where polymerase chain reaction (PCR) that detects viral antigens is not readily available, repeat HIV testing becomes a precautionary and life-saving measure. The ten year old South-Eastern Nigerian study in Nnewi [9] which reported a 3.9% sero-conversion rate did not investigate the associated risk factors. Given this background, this study therefore sought to explore the incidence of HIV sero-conversion in pregnancy with the associated risk factors in Enugu, not only to inform obstetric practices and policies but also with the intention to treat discovered cases in order to avert the threat of HIV to the child and the society at large.

Aims

To determine the prevalence of HIV sero-conversion and the associated risk factors among previously sero-negative antenatal clinic attendees in Enugu State University of Science and Technology Teaching Hospital, (ESUTTH).

Objectives

The objectives of this study were to

- Determine the prevalence HIV among antenatal attendees in ESUTTH
- Determine the prevalence of HIV sero-conversion among antenatal women in ESUTTH
- Determine the relationship between the socio-demographic variables and HIV sero-conversion in pregnancy
- Determine the risk factors to HIV sero-conversion among the antenatal attendees in ESUTTH

2. Material and methods

2.1. Study Area

The study was conducted at the department of Obstetrics and Gynaecology, ESUTTH. Its location at the centre of Enugu metropolis makes it accessible to the people of Enugu. It serves as a major referral center for primary and secondary health centres within the state and other South-Eastern States, as well as South-South Nigeria and beyond.

2.2. Methodology

This was a prospective, cohort study involving 415 sero-negative HIV women recruited from antenatal clinic in ESUTH from January to April, 2019 and followed up for 12 weeks. Information on their socio-demographic characteristics, pregnancy history, sexual lifestyle, and clinical profile were obtained using an interviewer-administered questionnaire. HIV tests were done at booking and repeated after 12 weeks using the Rapid Diagnostic Test kits by the parallel algorithm format.

2.3. Study population

This comprised a total of 430 sero-negative HIV pregnant women recruited from antenatal clinic in ESUTH from January to April, 2019. However, 15 were lost to follow up.

2.4. Inclusion criteria

Participants who booked at a gestational age not greater than 24 weeks using ultrasound estimation with a HIV negative test result at the time of booking for antenatal care in ESUTTH, who also consented to participation.

2.5. Exclusion criteria

Pregnant women who did not give consent, who were more than 24 weeks gestational age at the time of booking, and with known HIV- positive status.

2.6. Ethical considerations

Approval for the study was obtained from the Health Research and Ethics Committee (Institutional Review Board) of the Enugu State University of Science and Technology Teaching Hospital Enugu according to the tenets of the Helsinki Declaration. Written informed consent was obtained from the subjects. Confidentiality was strictly maintained through the use of coded number tags on the questionnaires, folders and all specimen containers. Participation in the study was voluntary and at no cost to the subjects. Those who were diagnosed with HIV were referred to the appropriate unit for expert care.

2.7. Minimum Sample Size Determination

Cochrane's formula for prevalence studies was applied[23]

$$\text{Sample Size N} = \frac{z_{1-\times/2^2} - \mathrm{x}/2^2\ \mathrm{p}(1-\mathrm{p})}{d^2}$$

Where;
N = Sample Size
$Z_{1-x/2}{}^2$= standard normal variate corresponding to 95% confidence interval.
P = Expected Proportion based on previous study or pilot study
d = Absolute error or precision.

Using a HIV sero-conversion rate (p) of 3.91% among pregnant women in Nnewi South-East Nigeria (14), at a confidence limit of 95%, and 5% type 1 error, the sample size was

Therefore,
d = 5%=0.05; Z =1.96; p= 3.91% =0.391

$$`N = \frac{1.96^2 \text{ X } 0.0391(1-0.0391)}{0.05^2} = 365.9 = 366$$

Assuming a non-response rate of 10% = 37, then the minimum sample size = 403 mothers. However, a total of 430 participants were recruited for the study.

2.8. Sampling technique

By simple balloting of the weekdays, Monday, Wednesday and Thursday were the selected antenatal clinic days. To obtain the required sample size, a consecutive enrolment was used. All consecutive, consenting subjects who met the inclusion criteria on the selected days were enrolled into the study until the estimated sample size was reached.

2.9. Study procedure

On each of the selected clinic days which run from 8:00am to 4:00pm, each attendee for booking was given a serially numbered tally after a brief explanation of the study protocol. The importance of repeating the test after 12 weeks was emphasized and their cooperation solicited.HIV test counseling was done on individual basis. The questionnaires were administered and eligible attendees selected after ensuring that they met the initial inclusion criteria, and the study GA confirmed using ultrasound estimation. Thereafter, each questionnaire was marked using a coded number written with a permanent green marker. This same number code was issued to the respondent with which she presented to the side laboratory. The HIV testing was conducted using the parallel algorithm. A tourniquet was applied about 4cm above the elbow joint after cleaning the cubital fossa with a spirit swab. A 10ml syringe and needle was then used to draw 10mls of venous blood from the brachial vein and 2mls was transferred into an ethylene diaminetetraacetic acid (EDTA) specimen bottle. This specimen container was labelled according to patient's coded number tag. The remaining 8mls was transferred into other specimen containers for the other booking investigations.Before running the tests it was established that the test kits were not expired, and that they were kept at the designated room temperature. The solvent was also checked to ensure it was not leaking. All samples were tested simultaneously with the STAT-Pak and the Determine Kits according to the instructions on the kit manuals. The test strips were displayed on the bench and the covering foil removed. Each strip was marked with the corresponding attendees' code number to avoid mix-up while a pipette was used to aspirate blood from the specimen bottle and 2 drops were left on the appropriate region of each test strip which was then followed by 2 drops of the appropriate solvent buffer solution. These were allowed to stand for a minimum of 15 minutes before the results were read following the company's specifications. The appearance of pink/red lines at both the control and test regions of both kits confirmed a positive HIV result, while its absence was a negative HIV result.Indeterminate or discordant results occasioned by a positive result from one kit and a negative result from the second kit would have led to a repeat test using the Uni-Gold Kit as a tie breaker but there was no record of indeterminate or discordant result throughout the testing period. All attendees had same-day tests and same-day results. All sharps were appropriately disposed into the biohazard boxes. The results of this baseline testing were then documented in the research register against the attendees' coded number, labelled Test - 1. The results were then announced and given to the attendees individually during post-test counselling. The counselling and testing procedures were carried out at every selected booking clinic day until a total of 430 HIV negative attendees were recruited and followed up. After this initial testing at booking, 3 new HIV positive attendees were diagnosed and were referred to the PMTCT unit after appropriate counselling for initial evaluation and possible commencement of ART. After each recruitment day, all the questionnaires were collated along with the records of the test results for safe-keeping.

2.10. Follow-up and repeat HIV testing

Participants were tracked with their phone numbers and those that missed their antenatal clinic visit were called on phone to ascertain if they had any problem and then reminded not to miss their repeat test appointment. Notices were pasted across the antenatal clinics and in each of the consulting rooms and at the labour ward so that every unit would help to track the participants.

At 12 completed weeks post-test-1, a second questionnaire on the predisposing risk factors was administered, thereafter, 2mls of venous blood was collected for the repeat HIV testing as described above. The results labelled **Test -2** were documented against the coded number tags on the patients' case file. Post-test counselling was given to all the participants individually irrespective of the outcome of their test results. In addition, those that tested positive were referred to the PMTCT unit for further evaluation and care.

2.11. Data Analysis

Data was cleaned, coded, and entered electronically into the computer and analyzed using Statistical Package for Social Sciences [SPSS] software version 23.0 (IBM Armonk, NY. 2015).A scoring system was used to assess respondents' level of knowledge of HIV and PMTCT. Statistical relationships were assessed with Chi-square. In other to identify predictors of HIV sero-conversion, all variables which demonstrated statistically significant relationship with sero-conversion in Chi square analysis were introduced into the logistic regression model. P-value <0.05 was regarded as statistically significant

3. Results

The result showed that the mean age of the participants was 29.44 ±4.91SD years with a range of 16-43 years. Majority of them were married 397(95.7%) and had post-secondary education 321(77.3%). Other characteristics were as shown in table 1 below.

Table 1 Socio-demographic characteristics of participants

Variable	Frequency	Percentage(%)	Mean
Age group (years)			
<19	5	1.2	29.44 ±4.91SD
20 – 24	60	14.5	
25 – 29	145	34.9	
30 – 34	142	34.2	
35 – 39	56	13.5	
>39	7	1.7	
Educational level			
None	2	0.5	
Primary	8	1.9	
Secondary	84	20.2	
Tertiary	321	77.3	
Marital status			
Married	397	95.7	
Single	14	3.4	
Divorced/Separated	1	0.2	
Widowed	3	0.7	
Occupation			
Civil servant	144	34.7	
Farming	7	1.7	
Student	72	17.3	
Unemployed	75	18.1	
Others	117	28.2	
Parity			
Nulliparous	168	40.4	
Primiparous	121	29.2	
Multiparous	119	28.7	
Grand multiparous	7	1.7	

It was also found that out of a total of 701 attendees who participated in the study, 680 tested negative to HIV at booking whereas 21 tested positive. Out of this figure 18 were known HIV positive women on HAART while only 3 were newly diagnosed given a prevalence of about 3% as shown in table 2 below.

Table 2 Pattern and prevalence of HIV among antenatal attendees at booking

Month(2018)	Total monthly attendance	HIV status at booking		Known HIV positive on HAART
		Negative	Positive	
July	62	59	0	3
August	238	231	2	5
September	220	213	1	6
October	181	177	0	4
Total	701	680	3	18
Total positive cases = 21; Prevalence = 3%				

A total of 415 sero-negative women were retested at 36 weeks and 4 out of the number were found to be sero-positive giving a sero-conversion rate of about 1% as shown in table 3 below.

Table 3 Result of HIV retesting at 36 weeks

Test result	Frequency	Percentage
Positive (sero-conversion)	4	1
Negative	411	99
Indeterminate	0	0

Considering the relationship between sero-conversion and various socio-demographic variables, age and marital status were found to have significant influence on the rate of sero-conversion having a p-value of less than 0.005 as shown in table 4 below. Subsequently, when compared with other lifestyle and behavioural activities, tobacco use and knowledge of partner's HIV status showed a significant influence on the rate of sero-conversion with p-values of less than 0.005 as shown in table 5 below. However, using multiple binary logistic regression analysis on the variables none was found to have any statistical significant influence on the rate of sero-conversion among the women as also shown in table 6.

Table 4 The sero-conversion rates and their associated socio-demographic variables

Variables	Yes (%)	No (%)	Total (%)	(X2) p value
Age (years)				
<19	1(0.25)	4(1)	5(1.2)	20.58(0.001)
20 – 24	0(0)	60(14.5)	60(14.5)	
25 – 29	1(0.25)	144(34.7)	145(34.9)	
30 – 34	2(0.5)	140(33.7)	142(34.2)	
35 – 39	0(0)	56(13.5)	56(13.5)	
>39	0(0)	7(1.7)	7(1.7)	
Marital status				
Married	1(0.25)	396(95.4)	397(95.7)	61.07(0.001)
Single	2(0.5)	12(2.9)	14(3.4)	

Separated	0(0)	1(0.2)	1(0.2)	
Widowed	1(0.25)	2(0.5)	3(0.7)	
Occupation				
Civil servant	2 (0.5)	142 (34.2)	144(34.7)	4.46(0.35)
Farming	0 (0.0)	7 (1.7)	7(1.7)	
Student	0 (0.0)	72 (17.3)	72(17.3)	
Unemployed	0 (0.0)	117 (28.2)	117(28.2)	
Others	2 (0.5)	73 (17.6)	75(18.1)	
Education level				
None	0 (0.0)	2 (0.5)	2(0.5)	11.61(0.009)
Primary	1 (0.25)	7 (1.7)	8(1.9)	
Secondary	1 (0.25)	83 (20)	84(20.2)	
Tertiary	2 (0.5)	319 (76.9)	321(77.3)	
Parity				
Nulliparous	2 (0.5)	166 (40)	168(40.5)	1.98(0.58)
Primiparous	0 (0.0)	121(29.2)	121(29.2)	
Multiparous	2 (0.5)	117 (28.2)	119(28.7)	
Grand multiparous	0 (0.0)	7 (1.7)	7(1.7)	
Religion				
Christianity	4(1)	406(98.1)	410(99)	0.04(0.98)
Islam	0(0)	3(0.7)	3(0.7)	
ATR	0(0)	1(0.2)	1(0.2)	

Table 5 Relationship between other variables with HIV Sero-conversion

Variables	**Sero- conversion**				
	Yes (%)	**No (%)**	**Total (%)**	**(X^2) p value**	**RR**
How often do you take alcohol in this pregnancy?					
Always/Occasionally	3(0.7)	117(28.2)	120(28.9)	2.21(0.137)	0.98:7.37(7.5)
Never	1(0.2)	294(70.8)	295(71.1)		
How often do you use tobacco?					
Always/Occasionally	3(0.7)	2(0.5)	5(1.2)	127.48**(0.001)***	0.4:6.1(15.2)
Never	1(0.2)	409(98.6)	410(98.8)		
HIV Knowledge category					
Good	0 (0.0)	21 (5.06)	21(5.06)	0.001(0.99)	---
Poor	4 (1.0)	390 (93.98)	394(94.94)		

Aware that MTCT is preventable					
Yes	2 (0.5)	331 (79.76)	333(80.24)	0.802(0.371)	0.9:4.06(4.5)
No	2 (0.5)	80 (19.28)	82(19.76)		
Knowledge of partners HIV status					
Yes	3 (0.75)	9 (2.2)	12(2.9)	51.11**(0.001)***	0.75:10.75(14.3)
No	1 (0.25)	402 (96.9)	403(97.1)		
History of blood transfusion					
Yes	0(0)	9(2.2)	9(2.2)	0.001(0.99)	
No	4(1)	402(96.9)	406(97.8)		
History of unprotected sex in past the 3 months pregnancy?					
Yes	4 (1.0)	392(94.5)	396(95.4)	0.01(0.99)	
No	0	19 (4.6)	19(4.6)		

Table 6 Multiple binary logistic regression analysis to identify predictors of HIV sero-conversion among participants

Subject Characteristic	**Adjusted Odds ratio**	**p value**	**95% Confidence Interval**	
			Lower limit	**Upper limit**
Age	0.31	0.58	0.11	0.42
Marital status	0.12	0.76	0.23	0.46
Educational level	0.41	0.52	0.19	0.23
Alcohol intake	0.004	0.95	0.02	0.14
Tobacco use	0.19	0.66	0.33	0.98
Sex partner in pregnancy	0.02	0.97	0.02	0.11
Knowledge of partners HIV	1.53	0.22	0.99	1.19

4. Discussion

The aim of this study was todetermine the prevalence of HIV sero-conversion and the associated risk factors among previously sero-negative antenatal clinic attendees in ESUTTH. The result from the study showed a prevalence of HIV sero-conversion of 1.0%. This means that for every 100 HIV negative women that booked for antenatal care, one would turn out to be HIV positive after 12 weeks of her initial screening test. This finding was less than the value found in a similar study done in Nnewi [9] where the prevalence of sero-conversion was found to be 3.91%. The difference could have been due to difference in the number of participants in that study which was 230 as against 415 participants in our study. The overall prevalence of HIV in that centre was quite high at the time of the study, 20.64%, against the overall HIV prevalence of 3% among the antenatal attendees in our centre during the study period. The result also differed a little from the findings of Nyoyoko NP and Umoh AV in Uyo, Akwaibom State [11] where the prevalence of HIV sero-conversion in pregnancy was found to be 3%. The difference could be accounted for by the difference in the participant demographics and lifestyle. The study was done in the South-South region of Nigeria as against our study done in the South-East region with different population demographics. This also differed from the findings in Jos, North-Central, Nigeria where a sero-conversion prevalence rate of 2.1% was found among women in labour who were previously found to be sero-negative earlier in the index pregnancy [24]. The difference in methodology, the different population and the period of the study may account for the discrepancies in the results. Our result maintained a steady lower value when compared with results from other African countries [10,15,16] and even beyond Africa [25]**.** The differences could be due to the different populations studied and the difference in HIV prevalence, knowledge and

awareness of HIV in those countries. However, our prevalence was found to be higher than what was obtained in Russia by Kissinet et al [26]. They found a sero-conversion prevalence of 0.4%. Russia is a developed country and has a better developed educational and health systems which may influence their health positively accounting for the low sero-conversion rate. The dissimilarities in the study settings, study definitions, socio-demographic characteristics and the time interval of repeat test in these studies may explain the varying results. While the maximum repeat test time in the present study was at 36 weeks gestational age, others were at 40 weeks [10], during labour [9,10] and 6 weeks post-partum [27].

This extra time gained might have contributed to their higher sero-positive rates compared with the result of this study.It is possible that if the researchers had extended the retesting period, a few more sero-positive cases might have been found given the on-going risk of HIV acquisition in pregnancy. However, these results confirmed the existence of HIV sero-negative window period [28] and showed that HIV negative attendees might actually be carrying the virus and were potentially infectious.

The sero-conversion rate was significantly higher in women less than 35years of age in this study. This agrees with the report made by the National Agency for the Control of AIDS [29]. The numerous implications of a young woman becoming HIV positive during the most sexually and economically productive stage of her life cannot be over-emphasized. It places a high risk of transmission on all of her unborn children and financial, social, emotional and physical stress on her at a critical period when she should thrive.

Sero-conversion was higher among single women than married women in this study. This was similar to the findings in Tanzania [14], South Africa [10] and Brazil [12]; but differs from the result by Kinuthia et al [27] in which HIV sero-conversion was more common among married women. The fact that single women were likely to indulge in risky sexual behaviours compared to married women may explain this finding [10].

In this study, knowledge of partners' HIV status was significantly associated with sero-conversion. Previous report made by Okaforet al [30] in the same facility showed that there was high rate of HIV sero-discordance among couples and that 68.1% of the discordant female partners tested positive. Couples should therefore be encouraged to declare their real status to each other and adopt possible means of protecting their unborn children. The fact that none of these identified risk factors was truly an independent predictor when subjected to logistic regression might be related to the sample size, the prevalence rate and the possible influence of other confounding variables in this study

Recommendation

- We recommend that all sero-negative pregnant women be re-tested after 12 weeks to the first screening
- Also, that husbands should be part of the antenatal booking protocol and their status promptly evaluated and possibly repeated after 12 weeks if found to be sero-negative

5. Conclusion

There is a significant HIV sero-conversion among antenatal attendees in ESUTTH and a statutory repeat test after the initial screening may help detect this condition with aim of preventing mother-to-child-transmission of the virus and its attendant health and economic consequences.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest

Statement of ethical approval

Ethical approval was obtained from the Ethics Committee of ESUT Teaching Hospital before the commencement of the study

Statement of informed consent

Informed consent was obtained from all individual participants included in this study.

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