

Toxoplasma gondii seroprevalence in women with bad obstetric history

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ABSTRACT

Background: Toxoplasmosis is a well documented cause of bad obstetric history (BOH).

Objective: To determine the seropositivity of toxoplasmosis in women with bad obstetric history and factors that influence seroprevalence.

Study design: Case control descriptive study.

Patients and Methods: The study included 293 women with BOH and 245 women with normal pregnancy outcome. Serological study carried out to determine T.gondii IgG and IgM using ELISA kits.

Results: The overall seroprevalence rate of T. gondii IgG was 29%, with no significant difference between women with BOH (27%) and women with normal pregnancy (31.4%). However, there was a significant difference between pregnant (20.3%) and non pregnant (31.4%) women. The current T. gondii infection overall rate was 0.9%, with significantly higher rate in women with BOH (1.7%). Both T. gondii IgG and IgM were significantly varied with women's age. Odd ratio confirmed the association of T. gondii IgG and women's age, residence, and education.

Conclusion: The seropositivity of Toxoplasma was significantly influenced by age, residence, and education levels.

Key words: TORCH, BOH, Toxoplasma, Pregnancy, Iraq

Introduction

Primary infection with any of the TORCH agents during pregnancy can lead to adverse outcomes, which are initially inapparent or asymptomatic and thus difficult to diagnose on clinical grounds (1). The ultimate consequences of intrauterine infection can be any one of the following (2): no significant problem, asymptomatic chronic infection, spontaneous abortion, intrauterine fetal death, stillbirth or neonatal death, fetal malformation or neonatal infection.

Spontaneous abortion is a new issue in terms of its social and economic impact. Pregnancy loss has been attributed to several factors involved in human reproduction; genetic and uterine abnormalities, endocrine and immunological dysfunctions, environmental pollutants, psycho-genetic factors and endometriosis, infectious agents, are most important causes of spontaneous abortion (3). The rate of spontaneous abortion from foetal infection by the infectious agents like TORCH is believed to range from 10-15% (4).

Toxoplasma gondii is considered one of the most widespread parasites in the world causing abortion. It is an intracellular protozoan that infects humans and other warm-blooded animals (5). The organism is transmitted to humans by accidental ingestion of water, food, or soil contaminated with *T. gondii* oocysts or consumption of meat containing *T. gondii* cysts that is eaten raw or undercooked (6). This disease is clinically insignificant in immunocompetent adults. The immunologic response to primary infection is followed by encystment of the parasite (latent toxoplasmosis), providing life-long immunity. Possible reactivation of latent infection in an increasingly immunosuppressed population, however, makes toxoplasmosis an important opportunistic infection. In addition, toxoplasmosis has long been known as a major cause of perinatal morbidity. *T. gondii* infection in pregnant women can be transmitted to the fetus and cause mental retardation, blindness, epilepsy and death (7).

In Iraq [Baghdad], Abdulmohymen (8) reported that there was a significant difference ($p < 0.05$), in the serum level of *Toxoplasma gondii* specific IgM among the three investigated patients groups (Recurrent spontaneous abortion, non-recurrent spontaneous abortion, and successful pregnancy). A similar result was obtained by Abbas (9), who showed that 21.5% of women with first abortion have positive only IgM. Al-Fertosi (1-) and Salman (11) showed 19.17% of women with single or repeated abortion. In addition, there is more than one *T. gondii* strain with difference in virulence among isolates in the nature (12). This strains' difference could be a potential explanation regarding the high prevalence of toxoplasmosis.

The relatively high frequency of toxoplasmosis in women with abortion as Abdulmohymen (8) reported could be due to the sample selection. Their samples were collected from Al-Kadhimiya Teaching Hospital which is a reference hospital for the surrounding rural areas where they have habits in favor of acquiring toxoplasmosis by eating unwashed raw vegetables or unpeeled fruits. In addition, in the rural cities there is close contact with cats and consequent exposure to sporulated

oocysts by ingestion of these oocysts that contaminate soil during gardening, or eating undercooked meat contaminated with cysts (8). Moreover, the low level of education in the women about the risk factors for toxoplasmosis may play an important role in the high rate of infection (13).

Furthermore, Abdulmohymen's (8) study showed a highly significant difference between the women group with recurrent spontaneous abortion and the women group with successful pregnancy in acute infection of *T. gondii*, but no significant difference in the mean value between recurrent spontaneous abortion and non recurrent spontaneous abortion (non-RSA) and non-RSA and those with successful pregnancy. It has been proposed that during pregnancy, systemic maternal immune response is biased in favor of Th2 cytokine (14,15). Moreover, Th2 cytokines pattern of pregnancy induces the susceptibility to toxoplasmosis infection, together with risk of placental infection and congenital transmission (16). Evidence from murine and human pregnancy showed that since Th1 type cytokine mediated pregnancy loss, a shift towards Th1-type immunity during *T. gondii* infection may help to explain pregnancy failure (17,18). Thus, a considerable amount of evidence suggests that Th1 cytokine might well be implicated in adversely affecting pregnancy, directly by interfering with trophoblast survival and function, and indirectly by activating cell-mediated immune effectors (19).

The aim of the present study is to provide a picture about frequency, distribution, risk and determinants of the seroprevalence of *T. gondii* infections during pregnancy and their association with high delivery risk factors.

Patients and Methods

Settings:

Kirkuk General Hospital, Primary Health Care Centers in Kirkuk Governorate.

Study Design:

The study design is a Descriptive Case Control Study.

Study area:

This descriptive case-control study was conducted at the antenatal clinic of Kirkuk General Hospital and Primary Health Care Centre in Tesean. Women (pregnant or non pregnant) with bad obstetric history were recruited from those attending outpatient Gynaecology Clinic Kirkuk General Hospital or the outpatient Clinic at Tesean PHC.

Study Population:

The study population is women of childbearing age. Study population was recruited from Primary Health Care Centers located in urban and rural areas in Kirkuk Governorates. In addition, one of the study population group was recruited from pregnant women who were in labor to select the group of pregnant women with risky outcomes.

Group 1: Pregnant women with age range of 17-45 years, and with normal pregnancy.

Group 2: Non pregnant women with age range of 17 - 45 years, and

with normal pregnancy.

Group 3: Pregnant women with Risk factor (BOH) depending on their previous pregnancy and /or delivery outcome which included pregnancy loss, intrauterine deaths, preterm deliveries and intrauterine growth retardation. Their age ranged from 17 to 45 years.

Group 4: Non-pregnant women with Risk factor depending on their previous pregnancy and /or delivery outcome which included pregnancy loss, intrauterine deaths, preterm deliveries and intrauterine growth retardation. Their age ranged from 17 to 45 years.

The demographic information of these groups is shown in Table 1. The target number recruited for each group was 150 women. However, the total number of women included in the study was 538, of these 293 (54.5%) had BOH, and 245 (45.5%) had normal pregnancy history. In the BOH group, 144 (49.1%) women were pregnant, while in the normal pregnancy group, 117 (47.7%) were pregnant.

Collection of data

The designated investigators visited the outpatient department daily, selected the study subjects, and screened them using a predesigned pretested schedule considering the inclusion and exclusion criteria till the study subjects recruitment could be identified. The next available age-matched multiparous antenatal woman without BOH was included in the control group subjects.

Clinical examination and laboratory investigations were carried out for the study subjects to exclude other causes of foetal wastage, such as hypertension, diabetes mellitus, syphilis, Rh (rhesus) incompatibility, physical causes of abortion, and consanguinity. Subjects with known causes of foetal wastage were excluded from the study. All of them were interviewed to ascertain age, medical and obstetric information.

Sample Collection:

For serological analysis, 5-10 mL of venous blood was collected in a sterile container with strict aseptic precautions from each study subject. The serum was separated and stored in

numbered aliquots at -20 °C till assayed. All the serum samples collected from the study and control groups were tested for HSV 2 IgM and IgG antibodies by commercially available (ELISA) kits. The results were read by a Microwell reader and compared in a parallel manner with controls; optical density was read at 450 nm on an ELISA reader.

Ethical approval

The ethical committee of the concerned institute approved the research protocol. The purpose and procedures of the study were explained to all the study subjects, and informed consent was obtained from them. The study design was approved by the ethical committee of TUCOM that is now registered in the USA [U.S. Department of Health and Human Services (HHS)& Registration of an Institutional Review Board (IRB)]. IORG #: IORG0006885. Institution: Tikrit University College of Medicine [TUCOM] OMB No. 0990-0279

Methods:

ELISA was used for determination of IgM and IgG for HSV-2 and the test was performed according to manufacturer instructions. The kit was purchased from BioCheck, Inc, 323 Vintage Park Dr, Foster City, CA 94404.

Analysis of data

Collected data were compiled in Microsoft Excel spreadsheet. The proportion and the mean value were computed in appropriate situations. To find out any association between categorical data, Chi square test was employed using the SPSS (Version 16). If the sample size in BOH group did not reach the targeted number, Power Analysis was performed to determine the accuracy of findings.

The study finding data are presented as frequency \pm SD and 95% Confidence Interval. Bivariate Regression Line Analysis calculated Odd Ratio for determination of association between the two variables. The determinants for T.gondii infection was determined by calculation of Odd Ratio using Logistic Regression Line Analysis. Confounding factors such as age, socio-economic status, etc. were standardized when serological determinants were calculated.

Table 1: Study population

Group		Number	Mean age \pm SD in years
Women with bad obstetric history	Pregnant	144	27.38 \pm 7.5
	Non pregnant	149	28.56 \pm 6.7
	Total	293	27.97 \pm 7.1
Women with normal pregnancy	Pregnant	117	26.00 \pm 6.2
	Non pregnant	128	30.16 \pm 10.9
	Total	245	28.16 \pm 9.2
Grand total		538	28.06 \pm 8.1
P value	ANOVA	NS	

Results

The overall seroprevalence rate of *T. gondii* IgG was 29%, with no significant difference ($X^2=1.293$) between women with BOH (27%) and women with normal pregnancy (31.4%) (Table 2). However, there was a significant difference ($X^2=18.59, P=0.000$) between pregnant (20.3%) and non pregnant (31.4%) women (Table 3). The current *T. gondii* infection overall rate was 0.9%, with significantly ($X^2 =4.22, P=0.04$) higher rate in women with BOH (1.7%).

Table 2: Toxoplasma seroprevalence in women with bad obstetric history

Group [Number]		Number positive [Percent]	
		IgM	IgG
Bad obstetric history	Pregnant [144]	4 [2.8]	40 [27.8]
	Non- pregnant [149]	1 [0.7]	39 [26.2]
	X^2	1.937	0.096
	P value	NS	NS
	Total [293]	5 [1.7]	79 [27]
Normal pregnancy	Pregnant [117]	0 [0]	13 [11.1]
	Non- pregnant [128]	0 [0]	64 [50]
	X^2	-	42.89
	P value	-	0.000
	Total [245]	0 [0]	77 [31.4]
Grand total [538]		5 [0.9]	156 [29]
X^2 BOH versus Normal Pregnancy		4.22	1.293
P value BOH versus Normal Pregnancy		0.04	NS

Table 3: Toxoplasma seroprevalence in pregnant compared to non-pregnant women

Group [Number]	Number positive [Percent]	
	IgM	IgG
Pregnant [261]	4 [1.5]	53 [20.3]
Non- pregnant [277]	1 [0.4]	103 [37.2]
X^2	2.003	18.59
P value	NS	0.000

Both *T. gondii* IgG and IgM were significantly varied with women's age (IgG, $X^2 =54.4, P=0.000$; IgM, $X^2=6.36, P=0.01$). Current *T. gondii* infection was detected in the age group of 20 to 29 (2.1%) years only. *T. gondii* IgG seroprevalence was 31.1% in women with age of 15-19 years, then declined to reach 17.2% in women with age of 20-29 years. However, IgG seroprevalence increased in women aged 30 and above to the plateau of 66.7% in age group of 40 - 48 years (Table 4). Odd ratio confirmed the association of *T. gondii* IgG and women age of lower or upper than 30 years ($OR=2.66, P<0.0001$). Although, *T. gondii* IgM demonstrated high OR, it did not give significant confirmation of the association and this was due to the small number included in the analysis (Table 5).

Table 4: Frequency of *T. gondii* in regard to age

Age group in years	Number	T. gondii Number [%]	
		IgM	IgG
15 – 19	74	0 [0]	23 [31.1]
20-29	238	5 [2.1]	41 [17.2]
30-39	172	0 [0]	56 [32.6]
40 -48	54	0 [0]	36 [66.7]
Chi Square		6.36	54.4
P value		0.01	0.000

Table 5: Odd ratio of T. gondii in regards to age of women lower than 30 years

Variable	Odd ratio [95% Confidence interval]	P value
T. gondii IgM	8.1024 [0.4457-147.2835]	NS
T. gondii IgG	2.66 [1.8157 – 3.8982]	<0.0001

T.gondii IgG seroprevalence was significantly higher ($X^2=7.439$, $P=0.006$) in women living in urban areas (32.2%) as compared to women living in rural (20%) areas. However, current infection was significantly ($X^2=7.638$, $P=0.006$) predominant (about 10 times) in rural areas (2.9%) than in urban (0.3%) areas (Table 6). OR confirmed the association between residence and both T.gondii IgM ($OR=11.6765$, $P=0.02$) and IgG ($OR=1.896$, $P=0.006$) (Table 7 - next page).

Table 6: Frequency of HSV 2 IgG and IgM in regard to sociodemographic characteristics

Variable	[Number]	Number positive [Percent]	
		IgM	IgG
Residence	Rural [140]	4 [2.9]	28 [20]
	Urban [398]	1 [0.3]	128 [32.2]
	X^2	7.638	7.439
	P value	0.006	0.006
Occupation	House wife [497]	5 [1]	149 [30]
	Working [41]	0 [0]	7 [17.1]
	X^2	0.416	3.065
	P value	NS	NS
Education	Uneducated [34]	0 [0]	11 [32.4]
	Primary [331]	1 [0.3]	109 [32.9]
	Secondary [105]	4 [3.8]	26 [24.8]
	College & above [68]	0 [0]	10 [14.7]
	X^2	11.83	12.53
	P value	0.019	0.014
Crowding Index	≤ 3 [478]	5 [1.04]	141 [29.5]
	3.1 – 8 [60]	0 [0]	15 [25]
	X^2	0.634	0.524
	P value	NS	NS

T.gondii IgG seroprevalence was higher (about double) in housewives (30%) as compared to working women (17.1%), but the difference did not reach a significant level. In addition, all the current T. gondii infections were in housewives. Furthermore, OR did not confirm the association between occupation and both IgG and IgM seropositivity (Tables 6 & 7).

About 96% of women who participated in this study were educated and only 4.1% were uneducated. Relationship between infection with T.gondii and education was significant ($P=0.014$), also current infection had a significant association with education ($P=0.019$). OR confirmed T.gondii seropositivity and education for remote (IgG), but not current infection (IgM) (Tables 6 & 7).

T.gondii IgG and IgM seroprevalence was higher in small size families (<3 crowding index). However, OR did not confirm an association between T.gondii infection and family size in women included in our study population (Tables 6 and 7).

Table 7: Association of HSV 2 seropositivity with sociodemographic characteristics using Bivariate analysis.

Variable		Odd ratio [95% Confidence interval]	P value	
Occupation [Housewife versus Employed]	IgM	0.9296 [0.0504-17.0558]	NS	
	IgG	2.2020 [0.9582-5.0603]	0.06	
Crowding Index [<3 versus >3]	IgM	1.4055 [0.0768-25.7347]	NS	
	IgG	1.2552 [0.6776-2.3253]	NS	
Education	Uneducated	IgG	2.7740 [1.038-7.415]	0.04
	Primary		2.8477 [1.401-5.787]	0.003
	Secondary		1.9089 [0.8541-4.2662]	NS
	College & above		2.7739 [1.0378-7.4146]	0.04
	Uneducated vs. Educated	IgM	2.9353 [0.3433-26.641]	NS
Residence [Rural versus Urban]	IgM	11.6765 [1.2938 – 105.3785]	0.02	
	IgG	1.896 [1.1818-3.0174]	0.006	

Discussion

The result of TORCH testing suggests that 29% of women of 15-48 years in age in Kirkuk , Iraq were seropositive for T. gondii. Thus 71% of studied population were susceptible to infection with Toxoplasmosis. Pregnant women should take appropriate precautions to protect themselves against infections. Such precautions include cooking meat, handling raw meat or working in soil ; and avoiding contact with cat faeces (20).

The result of this study and that reported by others (21,22), based on serological tests, confirmed expectation that Toxoplasmosis is indeed endemic in Kirkuk and that a substantial proportion of the examined patients' sera showed evidence of earlier infection (IgG), whilst fewer had evidence of current infection (IgM). The prevalence and risk factor for transmission of T. gondii varies substantially between countries and geographical regions (23). In this study Toxoplasma gondii IgG antibodies were found to be 32 times more common than IgM antibodies (29% versus 0.9% in the study group). Clearly the overall prevalence of IgG antibodies, reflecting earlier infection with Toxoplasma gondii among the tested samples from the study population indicated that between 1 in 3 and 1 in 4 had experienced earlier infection and these values are broadening in line with reports from other countries (23,24). However, changes of prevalence of IgG seropositivity across the age groups revealed a more complex story. In 82.8% of woman with age group of 20- 29 years (44.2% of study

population) were negative for Toxoplasma gondii IgG and these 4/5ths were susceptible for Toxoplasma gondii infection during their pregnancy.

However, the Toxoplasma gondii IgG seropositivity was higher in the 15-19 years age group. This could be attributed to less meat consumption and catabolism of passively transferred antibodies; prevalence then increased markedly with each successive age class as reported previously in other studies (25-31), peaking in the older subjects at over 66%. This rising trend with age, reflects the constituting risk of infection throughout adult life and arises from cumulative infection . However studies reported for Iraq indicated that Toxoplasma gondii IgG seroprevalence reduced with age (32-36). Risk of exposure and infection with age in an environment where transmission is encouraged by the high density of feral cats (23).

Interestingly the age related trend in prevalence of IgG antibodies in the combined study group (A=all tested samples), was also seen in each of the groups (BOH pregnant, BOH not pregnant, normal pregnancy and normal non pregnancy) representing their subdivision in regard to bad obstetric history). The overall Toxoplasma gondii IgG seroprevalence (29%) in woman from Kirkuk governorate was similar to reported studies from Egypt (37-42), Morocco (43,44), Saudi Arabia (45,46), Iraq (29,21,22,36,38,47-54), Libya (55,56), Tunisia

(57). Although our finding for IgG seropositivity was higher than that reported for Iraq (8,28,58,59,60), Bahrain (61) and Palestine (62). These studies performed in Arab countries indicated that seroprevalence of *T. gondii* varies substantially between geographical regions, and the variation demonstrated between the studies performed in Iraq.

In Kirkuk, 3 studies were reported. Ali (21) reported seroprevalence of 61 % in 100 pregnant woman where Kader et al (22) reported a seroprevalence of 36.6% in 319 pregnant woman and 52% in 121 aborted woman, giving an overall seroprevalence of 44.3%. In addition, Salman (60) reported a very low (4.84 %) seroprevalence of IgG in 184 woman with bad obstetric history. The present study finding indicated that overall seroprevalence was lower than that reported by Ali (21) and Kadir et al (22), but higher than that reported by Salman (60). Furthermore the global *T. gondii* IgG seroprevalence in pregnant woman was from 0.48 in Brazil (90 %) to (83 .5%)

in Madagascar (63), while the range in woman with BOH was from 19.44 % reported by Natu et al (64) to 55.2% in Nepal (65). The above information collected together indicated that there was a worldwide variability in the prevalence rate of *Toxoplasma gondii* seropositivity among pregnant and non pregnant woman. The relationship between education and *Toxoplasma* infection was recorded in this study, thus women who cannot read and write and with primary school level were more infected than educated woman (32.4 % and 32.9 % respectively), with a highly significant difference between the education level groups. In the United States the risk of *Toxoplasmosis* is associated with low level of education (66). The *T. gondii* IgG seroprevalence significantly varies ($P=0.014$) with the education level. In addition, the OR confirm that uneducated women and those with primary education have a chance to be detected three times more than women with secondary education and above.

Although , 94 % of educated but uneducated woman still are more prone to infection by *Toxoplasma gondii* . This might be due to fact that uneducated women were of ideal health condition or may not be caring about that. However, uneducated woman may be less likely to wash boards with soap or bleach after cutting raw meat or using the same knife for salad preparation. The present study finding was in agreement with that reported for Basrah (28), Tikrit (36) and Sudan (67), while none of the 9 studies performed in Iraq reported any information on such variables (26,29,30,32,33,34,35,43,68). From the present study, it was speculated that high education linked with good hygiene sanitary practices reduce the transmission of the parasite. This finding confirmed using both Logistic regression and Bivariate analysis, as it indicated that higher education was protective against infection ($X^2=0.614$, $P=0.000$).

The present study indicated that *T. gondii* infection was more predominant in housewives (30 %) compared to employed (17.1 %). Thus occupation plays a key role in the transmission of the disease when occupation is related to direct contact with meat, soil and animals. The majority of women (92.2%) in this study were housewives and few of them are working

in jobs not related to soil or meat but (?) mostly they are house wives. The predominance of infection in housewives as this study indicated was in agreement to that reported for Kirkuk (22,60), Almothana (69), Al-Najaf (30), and Tikrit (36). However, prevalence rate was much higher than that reported by Salman (3.49 %)(60) for Kirkuk in 2007, while it was about similar to that reported by Kadir (22) for Kirkuk in 2011.

Another two studies performed in Iraq , for Najaf (68) and Basrah (34) reported lower predominance rate of *Toxoplasma gondii* infection. One study reported for Basrah, Iraq (28), did not find a relationship between *Toxoplasma gondii* infection and occupation. Although the prevalence rate for *Toxoplasma gondii* infection was higher in housewives but the OR did not confirm the responsibility of such occupation as bivariate as logistic regression analysis demonstrated.

The present study has revealed that the prevalence rate of *Toxoplasmosis* varies remarkably in relation to residence and it was significantly more predominant in urban areas (32.2 %) than that for rural (20 %) areas. The OR confirmed that women living in urban areas have more of a chance to be infected than those living in rural areas. This finding was in agreement to that reported in Iraq, for Najaf (30), Karbala (33), and Tikrit (36). However, other studies in Iraq reported that prevalence rate was more in rural areas, in AL-Anbar (29), Diyala (32), Tikrit (35) and Kirkuk (22). All the studies reported that high prevalence in rural areas presented their data as frequency and did not perform any statistical test to determine the association. In addition, the study population size of most of the studies (except 22) was smaller than ours, which may influence their findings. Globally, seroprevalence estimates from human population vary greatly among different countries, among different geographical areas within one country, and among different ethnic groups living in the same area (70).

Toxoplasma gondii IgG seroprevalence was higher in families with crowding index of less than 3 (29.5 %). However, OR did not confirm a significant association between family size and *Toxoplasma* IgG seroprevalence. The presence of IgM antibody in 5 women suggests that 0.9% active infection may be present in the studied population. Four of them are pregnant and thus IgG avidity testing is the preferred method to confirm the recent infection (71), because IgM antibodies can persist for months after initial infection in some individuals (72,73). Alternate testing algorithms use higher IgM serologic titers as good indicators of acute infection; economic considerations prompt additional tests (74,75).

T. gondii IgM seroprevalence in high risk women declined from 1.05 % in the previous 3 years (Retrospective study) to 0.9 % in our prospective study (15 % decline), but this was not the case for IgG seroprevalence. The latter is a measure of individuals infected months or years earlier; therefore, it is unlikely we would detect a decline until incidence decreases significantly for 5 to 10 years. Declining of IgM seroprevalence suggests that knowledge about how to prevent *T. gondii* infection may have improved, the prevalence of *T. gondii* cysts in food animals in the region are declining , or the feral cat control is reducing the quality of oocysts entering the environment. The association of *T. gondii* with abortion, does

act as an impact on society to increase the awareness towards prevention of infection. There is one problem that emerged recently which may affect *T. gondii* in Iraq, which is the prevalence of *T. gondii* in meat, most likely sheep and goats, imported into Iraq for human consumption is unknown.

Reports from other countries in the region since 2001 found *T. gondii* prevalence of 23 % to 25 % in sheep (76-82), suggesting that lamb, if it is under cooked, could be a source of *T. gondii* infection. Since there is no well designed feral cat control program, thus infected cats may be responsible for parasite spread. *T. gondii* seroprevalence was significantly higher in non-pregnant (37 %) women as compared to pregnant women (20.3 %). This finding is in agreement with that reported by others (21) for Kirkuk.

In conclusion, the seropositivity of *Toxoplasma* was significantly influenced by age, residence, and education levels.

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