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Toxoplasmosis Prevalence in Pregnant Women of Plain Gangetic Region, Allahabad

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Toxoplasmosis is caused by the intracellular parasite *Toxoplasma gondii* and may be contracted by consuming contaminated meat or by contact with cat feces containing oocysts. In adolescence and adulthood, most infections are subclinical. However, if a pregnant woman contracts toxoplasmosis, it may be passed through the placenta to the fetus, resulting in congenital toxoplasmosis, which is a cause of mortality and malformation. Asymptomatic infants may develop anomalies later in life.The objective of this study was to determine the seroprevalence of *Toxoplasma gondii* infections. Samples were collected about 103 samples from Arogya Niketan obstetric clinic, Lukerganj in Allahabad over period of one year, from November 2012 to November 2013, to estimate seroprevalence of IgM and IgG antibodies to *Toxoplasma gondii*. Serum samples were collected and assayed quantitatively by using automated ELISA technique. Out of 103 serum sample, 26(25.2%) and 02(1.9%) were positive for Toxoplasma IgG and *Toxoplasma* IgM respectively. Toxoplasma IgM was in first trimester 01(5.3%) and second trimester 01(1.7%) while *Toxoplasma* IgG was seen 03(15.8%), 15(25.4%), and 08(32%) for first trimester, second trimester and third trimester respectively. Seroprevalence *T. Gondii* IgM and IgG were seen statistically insignificant (P > 0.05) association to gestation age (1st, 2nd and 3rd trimesters). IgG and IgM were statistically insignificant (P > 0.05) in relation to age group of pregnant women. Seroprevalence of Toxoplasma IgG^+ IgM^- in pregnant women were 03(15.8%), 15(25.4%), and 08(32%) for first trimester, second trimester and third trimester respectively, these results indicate to past infection toxoplasmosis. Toxo IgG^+ IgM^+ was 02(3.4%), for second trimester, these results indicate to recent infection. Toxo IgG^- IgM^- was 16 (82.2%), 44(74.6%), and 17(68%) for first trimester, second trimester and third trimester respectively, these negative results indicate no prior exposure to *Toxoplasma gondi i*nfection, these individuals are presumed to be susceptible to a primary infection.

Key words: Toxoplasmosis, Seroprevalence IgG & IgM, quantitative ELISA technique.

Toxoplasmosis is a zoonotic infection of humans and animals, caused by the opportunistic obligate intercellular protozoan *Toxoplasma gondii*, a parasite belonging to the phylum Apicomplexa (Zhou *et al.*, 2011). Humans can acquire infection by ingestion of raw or poorly cooked meat containing the *T. gondii* cysts or by ingestion of food or water contaminated with oocysts shed by cats (Richard *et al*., 2006),and also *via* contact with cats faeces (directly or indirectly through the soil) and via transplacental transfer, notably when the mother becomes infected for the first time while pregnant (Tenter*et al.*, 2000; Kravetz & Federman, 2005*;* Sroka *et al.*, 2006*;* Qublan *et al.*, 2002*;* Dubey, 2004 & Ahmed *et al.*, 2008). Vertical transmission only occurs if the

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mother becomes infected for the first time during her pregnancy. The highest risk of giving birth to a child with symptomatic congenital toxoplasmosis (about 10%) is when seroconversion occurs at 24–30 weeks' gestation (Foulon *et al.,* 1999; Dunn *et al.,* 1999 & Villena *et al.,* 2010).

In most adults it does not cause serious illness. However, blindness and mental retardation can be caused in congenitally infected children and severe diseases in those with compromised immunity. A recent study indicated that infection with *T. gondii* is associated with abdominal hernia (Alvarado and Estrada, 2011).

Possible reactivation of latent infection in an increasingly immunosuppressed population, however, makes toxoplasmosis an important opportunistic infection (Jumaian, 2005).

Congenital toxoplasmosis occurs almost exclusively as a result of primary maternal infection during pregnancy (John *et al.,* 2008). Congenital disease is more severe when infection is acquired in the first trimester (Remington *et al.,* 2001).

The risk of foetal infection is greater in the case of Toxoplasma recent infections in the first trimester due to the formative stage of the foetus, hence the likelihood of deformations and even death. The frequency and severity of foetal infection (*via* transplacental transmission) varies depending on factors such as the date of onset of the maternal infection, the virulence of the parasite strain, the size of the inoculums and the maternal immunity (Pelloux*et al.,* 1998).

Congenital infection caused by trans placental transmission can lead to a wide variety of manifestations in the foetus including spontaneous abortion, still-birth and preterm deliveries (Goldenberg & Thompson, 2003; Gibbs, 2002). Spontaneous abortion is defined as the termination of pregnancy at less than 20 weeks' gestation in the absence of elective medical or surgical measures. After 20 weeks the pregnancy losses are called preterm deliveries (Scroggins, 2000).

A newborn with classic signs of congenital toxoplasmosis such as hydrocephalus or microcephalus, cerebral calcifications, retinochoroiditis, hepatosplenomegaly, ectogenic bowel and hepatic calcifications or (intrauterine growth retardation (Goldenberg & Thompson, 2003; Gibbs, 2002). IgM antibodies were demonstrated in 142 (86.6%) of the 164 infants, and its detection decreased with increasing age (neonates vs. those aged 91–180 days; $P = 0.036$). IgA antibodies were demonstrated in 127 (77.4%) of the 164 infants and in 11 (6.7%) infants, they were noted in the absence of demonstrable IgM antibodies. Positive IgA test results were more commonly detected in neonates, and their rate of detection slightly decreased with increasing age of the infants (Tudor *et al.,* 2011). Most infected newborns have no apparent physical abnormalities at birth, but without treatment, the infection will progress resulting in serious sequels such as chorioretinitis, neurologic damage or growth and hearing impairment can be developmental later in the life. *T. gondii* infection in disabled children with symptoms of hypophrenia, epilepsy, retinochoroiditis, cardiovascular defects and respiratory system defects were 21.7%, 20%, 26.1%, 25% and 14.3%, respectively (Zhou *et al.,* 2002) ,and more than 75% infected newborn by toxoplasmosis are asymptomatic and free of symptoms at birth(Freeman *et al*., 2005; Boyer *et al*., 2005). On average, one third of people are chronically infected worldwide with toxoplasmosis (Zhou *et al*., 2011). Thus, to identify these 27 cases, only 20% of the women would need repeated screening if the seroconversion rate was 7.5% per year vs. 80% of the women in the other scenario. Nonetheless cost of analysis suggests that even in areas with low incidence of *T. gondii* infections screening for primary maternal infections during pregnancy is economically worth while (Lappalaine *et al.,*1995).

Laboratory documentation of past exposure or to recent infection by *Toxoplasma gondii* in a pregnant women is best found by serology tests such as capture IgM ELISA or IgM immunosorbent agglutination assay (ISAGA) (Lester,1983). ELISA technique is usefulness as a toxoplasmosis diagnostic test and it is a valuable technique for diagnosis of congenital toxoplasmosis (Paul *et al*., 2001).

ELISA is more sensitive than IFA test (Lone *et al*., 2004). IgM-negative, IgG-positive results on serum generally exclude a recent infection(Remington *et al*., 1995; Thulliez *et al.,* 1992).The detection of a positive *T. Gondii* IgG titre and a positive IgM indirect fluorescent antibody(IFA) or ELISA titre must be presumed to

indicate recently acquired infection with *T. gondii* (Armstrong *et al*., 2004).

Prenatal diagnosis of foetal infection may be also suggested by findings of nonspecific tests such as ultrasound examination with hydrops, hydrocephalus, microcephaly, intracranial calcifications, hepatosplenomegaly, ectogenic bowel, hepatic calcifications or intrauterine growth retardation (Pujol*et al*., 1992). Definitive diagnosis requires specific testing as mentioned above.

Maternal treatment with spiramycin (Rovamycine) is recommended to prevent transplacental transmission of acute toxoplasmosis to the foetus (Couvreur *et al*., 1988). While spiramycin is not teratogenic, it does not cross the placenta. When fetal infection is confirmed through amniocentesis, the woman may be switched to the combination of pyrimethamine (Daraprim), sulphadiazine and folinic acid (leucovorin) is used (Hohlfeld *et al*., 1989). Generally after the first trimester or, after 20 weeks' gestation, as Folinic acid (leucovorin) is given with combination of pyrimethamine and sulfadiazine to protect bone marrow from the suppressive effects of pyrimethamine (Daffos*et al*., 1988).

MATERIAL AND METHODS

Sample Collection

Blood samples were collected from pregnant women by venepuncture in containers under strict aseptic precaution. Samples were then centrifuged at 1500 rpm for 10 minutes to separate serum. Serum samples were then kept at – 20CÚ until assayed. Samples were collected about 103 samples from Arogya Niketan obstetric clinic, Lukerganj in Allahabad during November 2012 to November 2013.

Specimen Analysis

Sera samples were screened for the presence of Toxoplasma IgM, and Toxoplasma IgG antibodies by ELISA: Enzyme Linked Immunosorbent Assay Immunoassay method using principle: solid phase Enzyme Immunoassay for Toxoplasma IgG and using principle: solid phase capture Enzyme Immunoassay for toxoplasma IgM.

Interpretation of results

A negative result indicates that there was no prior exposure to *Toxoplasma gondii*, these individuals are presumed to be susceptible to a primary infection. A positive result indicates that there was a prior exposure at some undetermined time to *Toxoplasma gondii*. A highly positive result may indicate acute or recent disease.

Statistical Analysis

Data obtained in this study were analyzed using CHI-Square. A statistically significant difference was considered if P-value less than 0.05 ($P < 0.05$) was obtained.

RESULTS

Out of the 103 pregnant women examined for seroprevalence of *Toxoplasma*, 26(25.2%) and 02(1.9%) were positive for Toxoplasma IgG and *Toxoplasma*IgM respectively (Table 1).

Refer to gestation age of Pregnant women examined were 19(18.4%), 59(57.3%) and 25(24.3%) for first trimester, second trimester and third trimester respectively, therefore seroprevalence of *Toxoplasma* IgMwas 01(5.3%), 01(1.7%), and 00 for first trimester, second trimester and third trimester respectively and the seroprevalence *Toxoplasma* IgG was 03(15.8%), 15(25.4%), and 08(32%) for first trimester, second trimester and third trimester respectively. differences in seroprevalence *T. gondii* IgM (P=0.47) and differences in seroprevalence *T. Gondii* IgG (P = 0.45) in relation to gestation age (first, second and third trimesters)were found statisticallynot significant, due to P- value more than 0.05($P > 0.05$)(Table 2).

Seroprevalence of *Toxoplasma* IgG^+IgM^- groupwas 03(15.8%), 15(25.4%), and 08(32%) for first trimester, second trimester and third trimester respectively. Seroprevalence of *Toxoplasma* IgG^+ IgM^+ group was 00, 02(3.4%), and 00 for first trimester, second trimester and third trimester respectively. Seroprevalence of *Toxoplasma* IgG^- IgM^+ group was 00, 00 and 00 for first trimester, second trimester and third trimester respectively. Seroprevalence of *Toxoplasma* IgG^- IgM^- group was 16 (82.2%), 44(74.6%), and 17(68%) for first trimester, second trimester and third trimester respectively (Table 3).

IgG seroprevalence in relation to age group of pregnant women indicated that 2 (20%) out of 10 pregnant women were less than or equal 20 years, and 14(21.9%) out of 64 pregnant women were ranged between 21-25 years, and 05(27.3%)

out of 22 pregnant women were ranged between 26-30 years, and 04(50%) out of pregnant women were ranged between 31-35 years,01(100%) out of 01 pregnant woman was ranged between 36-40 years, differences in seroprevalence *T. gondii* IgG P = 0.06 in relation to age were however statistically insignificant due to P-value more than 0.05 (P > 0.05). IgM seroprevalence in relation to age group of pregnant women indicated that 00 out of 10 pregnant women were less than or equal 20 years, and 02(3.1%) out of 64 pregnant women were ranged between 21-25 years, and 00 out of 22 pregnant women were ranged between 26-30 years, and 00 out of pregnant women were ranged between 31-35 years,00 out of 01 pregnant woman was ranged between 36-40 years, differences in seroprevalence *T. gondii* IgM P = 0.87 in relation to age were however statistically insignificant due to P-value more than 0.05 (P > 0.05) (table 4).

DISCUSSION

In this study the Seroprevalence of *Toxoplasma* $IgG^+ IgM^-$ group was in pregnant women were 03(15.8%),15(25.4%), and 08(32%) for first trimester, second trimester and third trimester respectively, these results indicate to past infection. Seroprevalence of *Toxoplasma* IgG^+IgM^+ group in pregnant women was 02(3.4%), for second trimester, this result indicates to recent infection. Seroprevalence of *Toxoplasma* $IgG^- IgM^-$ in pregnant women were 16 (82.2%), 44(74.6%), and 17(68%) for first trimester, second trimester and third trimester respectively, these a negative results indicate to no prior exposure to *Toxoplasma gondii* infection. These individuals are presumed to be susceptible to a primary infection (table 3). The results showed that the highest *Toxoplasma* IgG seropositive was 14 (21.9%) and IgM seropositive

Table 1. Anti *Toxoplasma* IgGandAnti *Toxoplasma*IgMin Pregnant women.

Microorganism	Total Number	Immunoglobulins IgG		IgM	
		Positive	Negative	Positive	Negative
	No (%)	No (%)	No (%)	No (%)	
Toxoplasma gondii	103	26 (25.2)	77 (74.8)	02 (1.9)	101 (98.1)

Table 2. Seroprevalence of *Toxoplasma gondii*IgG and IgM in Pregnant women according to Gestation Age

Gestational age at maternal	Total Number(%)	*T. gondii*IgG		*T. gondii*IgM	
		Positive No (%)	Negative No (%)	Positive No (%)	Negative No (%)
First trimester	19 (18.4)	03 (15.8)	16 (84.2)	01 (5.3)	18 (94.7)
Second trimester	59 (57.3)	15 (25.4)	44 (74.6)	01 (1.7)	58 (98.3)
Third trimester	25 (24.3)	08 (32.0)	17 (68.0)	00 (00)	25 (100)

P- value =0.47 P- value =0.47

Table 3. Distribution of four groups of Anti *Toxoplasma*IgG /IgM results in pregnant women according gestation age

Gestation age	Total	IgG^+IgM^-		IgG^+IgM^+		IgG^-IgM^+		IgG^-IgM^-	
		NO	%	NO	%	NO	%	NO	%
First trimester	19 (18.4)	03	15.8 %	00	00	00	00	16	84.2 %
Second trimester	59 (57.3)	15	25.4 %	02	3.4 %	00	00	42	71.2 %
Third trimester	25 (24.3)	08	32 %	00	00	00	00	17	68 %

was (02 (3.1%), both were among 64 (62.1%) of pregnant women with age range of 21-25 years (Table 4).

Toxoplasmosis commonly occurs in adults, usually in a mild or asymptomatic form. It can cause fetal infection if it is acquired during pregnancy, with unpredictable manifestation in the fetus and neonate (Foulon *et al.*, 1999). Therefore, the detection of specific antibodies in the patient's serum is considered the proper method to the diagnosis of toxoplasmosis (Lester, 1983).

Recently diagnosis had been done traditionally by detecting IgM or IgG antibodies or both (Suzuki *et al*., 2001; Rafil, 2008), IgM antibodies are considered to reflect active current\ recent infection (Sukthana, 2006).

Acute toxoplasmosis is usually diagnosed on the basis of IgM antibody detection, in acute infections, IgG and IgM antibodies levels generally rise within one to two weeks of infection (Montoya *et al.*, 2000). Detection of *T. gondii* specific IgM has been used as an aid in determining the time of infection: a negative IgM test result with a positive IgG result usually indicates infection at least six months previously (Wilson and McAuley, 1999).

A negative result indicates that there was no prior exposure to *Toxoplasma gondii*. These individuals are presumed to be susceptible to a primary infection. A positive result indicates that

Table 4. Seroprevalence of *T. gondii*IgG and IgM according to Age Group.

Age group (years)	Total Number(%)	*T. gondii*IgG		*T. gondii*IgM	
		Positive samples	Negative samples	Positive samples	Negative samples
e" 20	10 (9.7)	02 (20)	08 (80)	00 (0)	10 (100)
21—25	64 (62.1)	14 (21.9)	50 (78.1)	02 (3.1)	62 (96.9)
26—30	22 (21.4)	05 (22.7)	17 (77.3)	00 (0)	22 (100)
31—35	06 (5.8)	04 (66.7)	02 (33.3)	00 (0)	06 (100)
36—40	01 (0.97)	01 (100)	00 (0)	00 (0)	01 (100)

P-value= 0.06P- value = 0.87

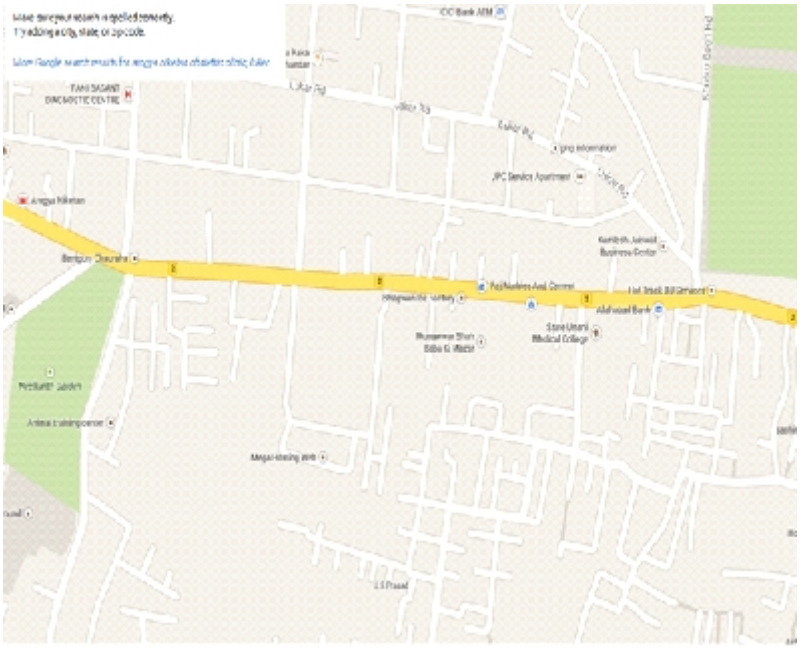

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Table 5. Seroprevalence of *Toxoplasma gondii* according to Risk Factors in 103 pregnant women

Risk factor	Number Sampled	Positive number of *T.gondii* IgM (%)	P-value	Positive number of *T.gondii* IgG (%)	P-value
Blood transfusion			Non		0.4263
Yes	17	00	determinable	01 (5.9 %)	No significant
No	80	00		01 (1.3%)	
No Response	06	00		00	
Miscarriage			Non		0.00000000023
Yes	03	00	determinable	02 (66.7 %)	significant
No	98	00		01(1.02 %)	
No Response	02	00		00	
Cats in household			0.000000061		0.000000061
Yes	02	01(50 %)	significant	02 (66.7 %)	significant
No	81	01(1.2 %)		01 (1.3%)	
No Response	20	00		01(5 %)	
Contact with dogs			Non		0.00000007
Yes	06	00	determinable	02(33.3 %)	significant
No	97	00		00	
No Response	00	00		00	
Outdoor gardening			Non		0.0184
Yes	23	00	determinable	03(13 %)	significant
No	64	00		00	
No Response	16	00		01 (6.3 %)	
Engage in Farming			Non		0.000002
Yes	05	00	determinable	02 (40 %)	significant
No	24	00		01 (4.2 %)	
No Response	74	00		00	
Uncooked vegetable			Non		0.70214
Yes	25	00	determinable	02 (8 %)	No significant
No	78	00		03(3.9 %)	
No Response	00	00		00	
Consume raw meat			Non		Non
yes	00	00	Determinable	00	determinable
No	103	00		01 (1 %)	
No response	00	00		00	
Not washing fruit			Non		Non
yes	00	00	determinable	00	determinable
No	103	00		02(1.9 %)	
No response	00	00		00	
Financial status(income)	Low			18 (17.5 %)	
	Middle			65 (63.1 %)	
	High			20 (19.4 %)	
Level of Education	Illiterate			00	
	Primary school			15 (14.6 %)	
	Intermediate school			11 (10.7 %)	
	Secondary school			40 (38.8 %)	
	High Education			37 (35.9 %)	
Pregnant women live in Urban Area.		80 (77.7 %)			
Pregnant women live in Rural Area.		23 (22.3 %)			

P-value < 0.05 is significant statistically.

there was a prior exposure at some undetermined time to *Toxoplasma gondii*. A highly positive result may indicate acute or recent disease (Deji-Agboola *et al*., 2011).

Then an IgG positive person should have an IgM test, If IgM negative test essentially excludes recent infection. However, a positive IgM test does not always mean a recent infection because *Toxoplasma* specific IgM antibodies may persist for months to year following primary infection therefore: - (i) Negative IgM with positive IgG always means past infection. (ii) Positive IgM with positive IgG indicate possibility of recent infection. (iii) Third situation is also possible when IgM test is positive but IgG test is negative. In this situation second sample should be taken after 2-4 weeks and should test. If second sample is still negative IgG with positive IgM, it indicates false positive IgM test (Arora, 2012).

Detection of antibodies is very important for pregnant women and women of child-bearing age. This is an effective way to find the infection, and then to provide treatment. It is also an efficient way to stop congenital toxoplasmosis in newborns (Zhou *et al*., 2011).

Women who have acquired *T. gondii* infection during pregnancy are treated with spiramycin to prevent transmission of *T. gondii* from the placenta to the fetus (amniotic fluid PCR negative) and with sulfadiazine and pyrimethamine to prevent fetal damage if the fetus is found to be infected (amniotic fluid PCR positive) (lone *et al*., 2004).

Recommendations

Measures to prevent primary *Toxoplasma gondii*infection during pregnancy.

- Cook meat to "well done" or thoroughly to 67°C (153°F). Meat should not be "pink" in the centre.
- Preservation of meat in deep freeze at –20ÚC.
- The meat is to be smoked, cured in brine, or dried may still be infectious.
- Mucous membrane contact must be avoided when handling raw meat.
- Hands must be carefully washed after contact with raw meat.
- Kitchen surfaces and utensils that have come in contact with raw meat should be washed wearing gloves.
- Refrain from skinning or butchering animals.
- Avoid contact with materials potentially contaminated with cat feaces, especially when handling cat litter or gardening.
- Wearing gloves is recommended when these activities cannot be avoided.
- Emptied cat-litter box have to be disinfected with near-boiling water for 5 min before refilling.
- Fruits and vegetables to be washed before consumption.
- Drinking of water potentially contaminated with oocysts to be washed.
- Health education for women of childbearing age should include information about preventing *T. gondii* transmission from food and soil.
- Pregnant women have to be educated about food hygiene and avoiding exposure to cat faeces.

Antenatal screening for *Toxoplasma* infection is now as important as VDRL, HIV, and HBV and HCV screenings in endemic areas, because toxoplasmosis is a preventable disease. Even when primary infection occurs during pregnancy, early diagnosis and treatment can reduce the frequency and severity of the disease in the neonates (Lone *et al*., 2003).

Ultrasound is recommended for women with suspected or diagnosed acute infection acquired during or shortly before gestation. Ultrasound may reveal the presence of fetal abnormalities, including hydrocephalus, brain or hepatic calcifications, splenomegaly, and ascites (Remington *et al.*, 2006).

CONCLUSION

In this study the positive seroprevalence of *Toxoplasmagondii* IgG and IgM in pregnant women were 26 (25.2%) and 02 (1.9%) respectively. Detection of IgM antibodies are considered to reflect active recent infection.Seroprevalence of *Toxoplasma* IgG^+ IgM^+ group was 02(3.4%) in pregnant women for second trimester so this result indicates the recent infection, therefore the pregnant women were more opportunity to morbidity consequence of congenital toxoplasmosis.Therefore, the assay of Antibodies *Toxoplasma gondii* during the period of pregnancy can identify *Toxoplasma* infection and facilitate appropriate care thereby effectively reducing the risk of birth defects and fetal demise.

To prevent toxoplasmosis, the food habits should be taken care by using peeled and thoroughly washed fruits and vegetables before use. The pregnant women should wear gloves when they touching or doing work in soil and not play or

touch the pates like cat. This transmission from food and soil, for health care providers should educate pregnant women about food hygiene and avoiding exposure to cat faeces.

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