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Full Length Research Paper

The Seroprevalence and Co-Existence of *Chlamydia Trachomatis* and *Herpes Simplex Virus* Antibodies among Students in a Tertiary Institution

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Abstract

Sexually transmitted infections (STI) occur worldwide with more than 340 million cases occurring annually in adults aged 15-49 years. The aim of the study is to determine the prevalence and co-existence of *Chlamydia trachomatis* and *Herpes simplex virus* (HSV) antibodies among students in a tertiary institution in Anambra State, Nigeria. This is a cross sectional descriptive study of the co–existence of *Chlamydia trachomatis* and HSV antibodies among students in a tertiary institution in Anambra State Nigeria carried out using enzyme immunoassay kits for determination of IgG class antibodies. The seroprevalence of HSV antibodies, (27.3%), was slightly higher than that of *Chlamydia trachomatis*, (21.6%) among the students. Also more of the students, (18.2%) were positive to HSV antibodies only, when compared to 12.5% of them that were positive to *Chlamydia trachomatis* antibodies only, while 9.1% of them were seropositive to both organisms. The antibody titres to both organisms were found to be significantly higher among students with high risk sexual behaviours. The seroprevalence and co-existence of these organisms were high in both populations thus there is need to institute appropriate control measures to reduce the prevalence of these infections.

Key words: Seroprevalence, Co-existence, *Chlamydia trachomatis*, *Herpes simplex virus*, Tertiary, Students.

INTRODUCTION

Sexually transmitted infections (STI) are a group of communicable infections that are transmitted predominantly by sexual contact and caused by a wide range of bacterial, viral, protozoa, fungal agents, and ectoparasites. (Anderson, 1991). Worldwide more than 340 million cases of sexually transmitted infections occur annually in adults aged 15-49 years. These infections are common in sexually active adolescents and young adults (WHO, 1981). Women and infants bear the brunt of complications such as still birth, ectopic pregnancy, infertility and ophthalmic neonatorium (WHO, 2001). Most infected women are asymptomatic and therefore fail to seek timely treatment (Bogaerts et al., 1999; Hawkes et al., 2000; Wassertheit et al., 1989; Wassertheit, 1989). The true incidence of STIs will is often not known not only because of inadequate reporting systems in most developing countries but because of the secrecy that surrounds them and many of them are not even notifiable (WHO, 1981).

Chlamydia infection is a common STI in humans caused by the bacterium *Chlamydia trachomatis*. *Chlamydia trachomatis* is gram-negative, obligate intracellular bacterium pathogens of prokaryotic cells and are differentiated from other bacterial by their morphology and a unique developmental cycle involving

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two morphological forms; one adapted to extracellular survival by multiplication within cytoplasmic vesicles. They are widely distributed in nature and are responsible for a variety of ocular, genitor-urinary and respiratory diseases in man (Jawetz et al., 2004). So far, 15 immunotypes of *Chlamydia trachomatis* have been identified, eight of which are responsible for genital tract infections, three others for *lymphogranuloma venerum* associated with genital ulcer disease in tropical countries and the remaining four produce trachoma (McGregor and French, 1987). The prevalence of *Chlamydia* infection varies from place to place but there was an estimated 90 million cases of infection globally in 2001 and approximately 4 million cases of *Chlamydia* infection are reported per year in the United States of America (USA), with an overall prevalence of 5 percent (WHO, 2001; Wein et al., 2004; CDC, 2009). In Ethiopia the prevalence rate for *Chlamydia* infection of the cervix was 5.9% and also a prevalence rate of 13.3% was noted among unsuspecting women attending antenatal clinic in Benin City Nigeria (Buve et al., 2001; Isibor et al., 2005). Nwanguma et al., (2009) reported a prevalence of 33% in asymptomatic volunteers in a population of Nigerians living in two cities in the South Eastern part of the country. Also in Anambra State, South East, Nigeria, the prevalence among tertiary school students and that found among infertile couples were 6.0% and 28.2% respectively, while it was 29.4% in both students and non-students in another study done in Enugu State Nigeria (Chukwuma, 2005; Anahalu, 2006; Ikeme et al., 2011). Genital *Chlamydia* infections are often chronic and asymptomatic, leading to severe complications including pelvic inflammatory disease, ectopic pregnancy and infertility (Daville, 2000).

Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) are enveloped DNA viruses of the viral family herpesviridae. Genital herpes may be caused by either herpes virus type 1 or type 2 but globally, the large majority of cases are caused by HSV-2. The estimated number of people aged 15-49 years who were living with HSV-2 worldwide in 2003 was 536 million, or roughly 16% of the world population in this age group. More women than men were infected with an estimated 315 million infected women compared to 221 million infected men. The estimated number of new HSV-2 infections among 15-49 year olds worldwide in 2003 was 23.6 million, of which 12.8 million were women and 10.8 million were men. The number of new infections was highest in the youngest age groups and declined thereafter due to decline in the number of susceptibles (Katharine et al., 2008).

In the USA about 50 million people have genital HSV and 26% of women have antibodies to HSV-2 (Fleming et al., 1997; CDC, 2007). The prevalence estimates by region were highly heterogeneous. The prevalence of HSV-2 was generally higher in developing region than in developed regions. It is a common infection in many countries, with prevalence in some regions such as sub-Saharan Africa ranging from 30 to 80% in women and from 10% to 50% in men (Weiss, 2004). Most data in Central and South America are from women, in whom HSV-2 prevalence ranges from about 20-40%. Prevalence in the general population in developing Asian countries appears to be lower, (10-30%). The lowest prevalence was in Western Europe, where prevalence reached a maximum of around 18% among women and 13% among men. Historically most genital infections have been caused by HSV-2 but however there is considerable overlap and the virus can be transmitted by masturbation, oro-genital contact, or poor hygiene. It has also been reported that 30-40% of genital infections and up to 80% of new genital infections are caused by HSV-1 (Lafferty et al., 2000). Factors that contribute to differences in prevalence by region for herpes are likely to be similar to those for *Human immunodeficiency virus* (HIV). These may include regional differences in the frequency and pattern of sexual risk behavior including rates of oral versus vaginal sex, differences in age at first sex, differences in the prevalence of sexually transmitted infections, co-factors for HSV-2 and transmission such as HIV and differences in the structure of sexual networks (Anderson et al., 1991; Garnet and Anderson, 1993; Morris et al., 1996; Morris and Kretzschmar, 1997; Wald and Link, 2002; Drain et al., 2004; Pettifor et al., 2004; Kaestle et al., 2005) In contrast, genital herpes due to HSV-1 is of significant public-health importance but the inability of seroprevalence studies to distinguish between the two infection sites means it is much more difficult to generate estimates of the burden of genital HSV-1 infection (Christie et al., 1997; Löwhagen et al., 2002; Scouler et al., 2002; Roberts et al.,2003; Scrutt et al.,2003, Lafferly et al., 2003). Despite the typically asymptomatic nature of HSV which facilitate its spread in the population, it is a useful marker of sexual behavior, and it is associated with considerable morbidity and even mortality especially in pregnancy and in infancy. Neonatal infection can be very serious (Cowan et al., 1994; Brown et al., 1997; Brown, 2004). In general HSV seroprevalence is high in populations whose behavior leads to a high risk of acquiring STIs, such as STI clinic attendees and sex works, with some studies reporting greater than 80% HSV seropositivity in sex workers (Weiss, 2004). Moreover, areas with high HSV-2 prevalence commonly have a high prevalence of other sexually transmitted infections, such as *Chlamydia* and *gonorrhea* (WHO, 2001). Just as reported among other STIs, several studies have shown that *Chlamydia trachomatis* and HSV co-infections occur in vivo (Raavonen et al., 1985, Tait et al., 1985). Reports on *Chlamydia trachomatis* co-infection have indicated that HSV-2 co-infection alters *Chlamydia* development leading to persistence in host cells with subsequent attendant problems; however reviewed studies did not examine the co-infection process in details (Pontefract et al., 1989; Chiarini, et al., 1996; Superti et al., 2001).

Thus the aim of this study is to assess if the co-existence between the two organisms exist in our environment. There is dearth of such findings in our study area. Therefore this study could help guide other researchers in conducting further studies on the prevalence and co-existence of both organisms especially in our environment.

MATERIALS AND METHODS

Study area

This study was carried out among students in a tertiary institution in Anambra State, South East, Nigeria. Anambra State has a total population of about 4.7 million and invariable one of the most populous states in the south eastern part of Nigeria with a population growth rate of 3.0 from 1999 till date. The state is the commercial hub of the south east, and also one of the top most commercial states in Nigeria. There are many tertiary institutions in the state which consist of four Universities (two public and two private), two colleges of education, two polytechnics and five recognized school of nursing and midwifery among others. Nnamdi Azikiwe University is a federal institution with its main campus located at the state capital, Awka and has two other campuses in Nnewi and Agulu in the same state.

Study design and sample size estimation

This study is a cross sectional descriptive study of the seroprevalence and co-existence of *Chlamydia trachomatis* and HSV antibodies among students in a tertiary institution in Anambra State, Nigeria. The study population consisted of both male and female students from the selected tertiary institution. Using the Cochrane formula for sample size estimation for cross sectional studies and the sero-prevalence of *Chlamydia trachomatis* among tertiary students reported earlier in the state, 6.0%, the sample size that was used for this study was 88 students (Chukwuma, 2005)

$$n = \frac{Z^2pq}{d^2}$$

Where n= sample size to be estimated, z= the standard normal deviate corresponding to level of significance at 95% (≈1.96), p=the prevalence rate=6.0%, d=the degree of accuracy desired, set at 0.05 or 95%.

Sampling technique

The sampling technique was multistage sampling technique;

Stage 1 involved the selection of Nnamdi Azikiwe University from the list of tertiary institutions in Anambra State, using simple random sampling by balloting.

Stage II involved the selection of faculties to be studied of which faculties of education and social sciences were selected using simple random sampling by balloting

Stage III which was the last stage involved the selection of respondents to be studied. Forty four students each from the faculties were selected using quota sampling.

Materials used and test procedure

A semi structured questionnaire was used to obtain information on the socio-demographic characteristics of respondents and their sexual behaviours. Blood was collected aseptically from consenting individuals who met the inclusion criteria by venepuncture, was allowed to cloth and serum was separated by centrifugation at room temperature and capped in non-sterile serum containers, stored at -20^{0}C. Materials used for the study included; IgG serodiagnostic kit for both organisms, a micro plate calibrated reader, manual and automatic equipment for rinsing, vortex tube mixer, deionized water, timer, absorbent paper springs, gloves, serum collection bottles and test tubes. The *Chlamydia trachomatis* and HSV IgG kits (by DRG instruments GmBH, Germany) were used.

Limitations

This study was conducted in only one tertiary institution in the state, so caution should be taken in generalizing the findings. This can be regarded as a baseline study as only few works have been done in this sub-region to assess the co-existence of *Chlamydia trachomatis* and HSV

RESULTS

Most of the participants were more than 24 years of age (51.1%) with a mean age of 24.2±5.6 years. Seventy percent of the respondents were females with majority of them being singles, (88.6%) and living off campus, (80.7%) Table1.

Table 2 showed that about eighty-two percent of the respondents have had penetrative vaginal/penile sexual intercourse before, of this number, 84.7% of students claimed to have had at least an unprotected sexual intercourse in the last one year preceding the survey. Most of them, 68.1%, had sex within the same period with at least 2 or more sexual partners. Almost seventy percent of students have had symptoms suggestive of sexually transmitted infections, with forty one percent of them having multiple symptoms at presentation. The

Table 1. Socio-demographic characteristics of participants

Socio demographic characteristics	Frequency (n=88)	%
Age group (years)		
15-24	43	48.9
> 24	45	51.1
Total	88	100.0
Mean age 24.2±5.6yrs		
Sex		
Male	26	29.5
Female	62	70.5
Total	88	100.0
Marital Status		
Married	10	11.4
Single	78	88.6
Total	88	100.0
Place of Residence		
Off campus	71	80.7
School Hostel	5	5.7
Living with parents/relatives	12	13.6
Total	88	100.0

Table 2. Sexual behaviours of participants and symptoms suggestive of STI

Variable	Frequency	%
Ever had penetrative sexual intercourse before (Vaginal/penile) n=88		
Yes	72	81.9
No	16	18.1
Total	88	100.0
Had unprotected sexual intercourse in the last 1 year n=72		
Yes	61	84.7
No	17	15.3
Total	72	100.0
Number of sexual partners in the last 1 year n=61		
1	19	31.1
>2	42	68.8
Total	61	100.0
Ever had symptoms suggestive of STI n=88		
Yes	61	69.3
No	27	31.7
Total	88	100.0
Had more than one symptom at presentation n=61		
Yes	25	41.0
No	36	59.0
Total	61	100.0
Types of symptoms mentioned (multiple response) n=61		
Itching	39	63.9
Lower abdominal pain	23	37.7
Rashes	20	32.8
Discharge	18	29.5
Sores	8	13.3
Dysuria	4	6.5
Severe menstrual pain	3	4.9
Others	3	4.9
Others inability to conceive, low sexual performance.		

Table 3. Seroprevalence prevalence of *Chlamydia trachomatis* and HSV antibodies

Test Result	Frequency (n=88)	%
***Chlamydia trachomatis* antibody seroprevalence**		
Positive	19	21.6
Negative	69	78.6
Total	88	100.0
HSV antibody seroprevalence		
Positive	24	27.3
Negative	64	72.7
Total	88	100.0

commonest symptoms of STI that the students had were; itching of the private part, (63.9%), lower abdominal pain, (37.7%), rashes, (32.8%), and vaginal/penile discharge, (29.5%).

The seroprevalence of HSV infection, 24(27.3%) was found to be slightly higher than that of *chlamydia trachomatis*, 19(21.6%), among the study participants, this difference was not statistically significant, (p=0.380, p>0.05). Table 3

Also more students were positive to HSV antibodies only, 16(18.2%) when compared to *Chlamydia trachomatis* antibodies only, 11(12.5%), with eight participants, 8(9.5%) being seropositive for both organisms. (Figure 1)

The seroprevalence of *Chlamydia trachomatis* in those that were positive to HSV antibodies was slightly higher than their counterpart with negative HSV antibodies, (33.3% versus 17.2%), similarly the seroprevalence of HSV antibodies was higher in those with positive *Chlamydia* antibodies, than their counterparts with negative *Chlamydia trachomatis* antibodies, (42.1% versus 23.2%). These differences were not statistically significant, p>0.05. Table 4

Table 5 showed that antibody titres to both *Chlamydia trachomatis* and HSV were higher among those who have had sexual intercourse before, (26.4% and 33.3%), had unprotected sexual intercourse in the last 1 year preceding the study, (29.5% and 37.7%), and also among those who have 2 or more sexual partners one year prior to survey, (35.7% and 42.9%) than their counterparts without such sexual history. These differences were statistically significant, p<0.05.

DISCUSSION

The mean of participants in this study was 24.2±6.6 years which fall within the age of youths and young adults (WHO, 1981, 2001). Sexual activity and risky sexual behaviours were found to be high among the students studied with a high proportion of them having penetrative vaginal/penile, unprotected sexual intercourse, and sex with multiple partners. This pattern of risky sexual behaviours has been widely reported among adolescent and young adults in many studies (WHO, 2001; Hardick et al., 2003; Duru et al., 2010). Despite wide spread enlightenment campaigns on safe sex, these risky behaviours have remained prevalent and there is decreasing age at initiation of sex in most countries of the world (Isibor, 2005). This could likely account for large burden of sexually transmitted infections reported in this age group. The issue is that the world has been made a global village due to easy accessibility to information technology that had made communication very easy and a large proportion of our young adults' access wrong information about sexuality and related practices from many sites in the internet. This coupled with their risky behaviours is posing a threat to this group of people and the world at large.

The prevalence of HSV was found to be 27.3%, this is within the range reported in sub-Saharan African which ranges from 30-80% in women and 10-50% in men (Weiss, 2004). It also within the range found in South America, (20-40%) and Asia, (10-30%) but lower than the average in Europe, 13% and 18% for men and women respectively (Lafferty et al 2000). These wide variations are due to differences in frequency and pattern of risky sexual behaviours including rate of oral versus vaginal sex, differences in age at first sex, differences in sexually transmitted infection and the structure of sexual networks. Also the general HSV seroprevalence has been reported to be high in population whose behaviour leads to a high risk of acquiring other sexually transmitted infections, and this was noted in our study where risky sexual behaviours among respondents were prevalent (Weiss, 2004).

The prevalence of *Chlamydia trachomatis* antibodies in this survey was 21.6%. There is a wide variation in the incidence and prevalence of *Chlamydia trachomatis* globally and this variation depends on the age, marital status, clinical condition, sensitiveness of the methods used and other various factors (Ikeme et al., 2011; Buve et al., 2001). This finding was higher than earlier report from students in this same institution, which was 6.0%, (Chukwuma, 2005) also higher than reports from Benin city, Nigeria, 13.3%, (Isibor, 2005) among unsuspecting women attending ANC in Ethiopia, 5.9%, (Buve et al., 2001) and reports from USA, which stood at an overall

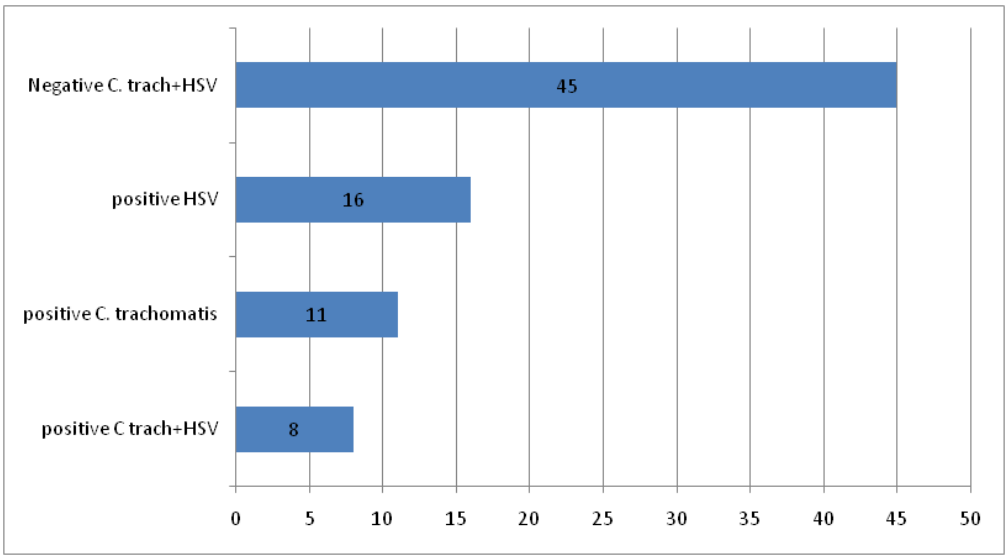

Figure 1. Co-existence of antibodies to both organisms

Table 4. Comparison of seroprevalence of both organisms in those with positive and negative results to each of them.

Chlamydia trachomatis	**Positive (%)**	**Negative (%)**	**Total (%**
HSV			
Positive	8(33.3)	16(66.7)	24(100.0)
Negative	11(17.2)	53(82.8)	64(100.0)
Total	19(21.6)	69(78.4)	88(100.0)
HSV	**Positive (%)**	**Negative (%)**	**Total (%)**
Chlamydia trachomatis			
Positive	8(42.1)	11(57.9)	19(100.0)
Negative	16(23.2)	53(76.8)	69(100.0)
Total	24(27.3)	64(72.7)	88(100.0)
χ^2=2.69, df=1, p=0.101			

Table 5. Sexual behavior of partcipants and pattern of seropositivity

	Chlamydia trachomatis			**HSV**		
Variable	**+ve(%)**	**-ve(%)**	**Total(%)**	**+ve(%)**	**-ve(%)**	**Total(%)**
Ever had sexual intercourse before						
Yes	19(26.4)	53(73.6)	72(100.0)	24(33.3)	48(66.7)	72(100.0)
No	0(0.0)	16(100.0)	16(100.0)	0(0.0)	16(100.0)	16(100.0)
Total	19(21.6)	69(78.4)	88(100.0)	24(27.3)	64(72.7)	88(100.0)
		F=5.38, df=1, p=0.013*			F=7.33, df=1, p=0.003*	
Had unprotected sexual intercourse in the last 1 year						
Yes	18(29.5)	43(70.5)	61(100.0)	23(37.7)	38(68.3)	61(100.0)
No	1(3.7)	26(96.3)	27(100.0)	1(3.7)	26(96.3)	27(100.0)
Total	19(21.6)	69(78.4)	88(100.0)	24(27.3)	64(72.7)	88(100.0)
		F=7.36, df=1, p=0.004*			F=10.90, df=1, p=0.000*	
No of sexual partners in the last one year						
0-1	4(8.70)	42(91.3)	46(100.0)	6(13.0)	40(87.0)	46(100.0)
≥2	15(35.7)	27(64.3)	42(100.0)	18(42.9)	24(57.1)	42(100.0)
Total	19(21.6)	69(78.4)	88(100.0)	24(27.3)	64(72.7)	88(100.0)
		F=9.46, df=1, p=0.002*			χ^2=9.83, df=1, p=0.003*	

*= Significant. F= Fishers exact.

prevalence of 5.0% (Wein et al.,2004, CDC, 2009). It was within the range found among patients attending family planning clinics in correctional facilities in the UK and another report from Ibadan Nigeria (Darogar et al., 1990; LaMontagne et al., 2004). It was observed to be lower than figures reported from Anambra state among infertile couples, 28.3%, (Anahalu, 2006) and Enugu among students and non-students, 29.4% (Ikeme et al., 2011). It is also lower than figures reported by Nwanguma et al., (2009) in two Nigeria cities which showed the seroprevalence of Chlamydia trachomatis to be 33.0% among volunteers with unknown HIV status, and 50% among HIV positive patients. The prevalence of *chlamydia trachomatis* has been reported globally to be high among young population; this is consistent with findings in this study. This may indicate high sexual activity, multiple sexual partners and low use of barrier (condom) methods of contraception which is prevalent in this group and was shown in this survey.

There was evidence of co-infection of *Chlamydia trachomatis* and HSV infection antibodies in this study as shown by, 9.6% of the total having antibodies to both organisms. Also found in the study is the complimentary nature of antibodies to both organisms. Co-existent of sexually transmitted of sexually transmitted disease have been widely reported (Raavonen et al., 1985; Tait et al., 1985; Wasserheit, 1989; Bogarts et al., 2000; WHO, 1981, 2001; Superti et al., 2001; Wein et al., 2004). The postulated reason are simple, they all have one main route of transmission which is sexual contact, so risky behaviours can expose one to multiple infections easily, also some of the organism cause inflammation of the mucosal layers of the genital tract, distorting its integrity, thereby enhancing access into the genital tract tissues by other organisms. Also some molecular explanations have been suggested for chronicity and persistence of infections. In this survey, the simple explanation is likely that of risky sexual behaviours noticed among participants and this finding was strengthened by higher prevalence rates found among those with known risky sexual behaviors. This finding of higher seroprevalences to STIs among individuals with high risk sexual behaviours have been reported in several studies (Buve et al., 2001; CDC, 2009; Nwanguma et al., 2009; Ikeme et al., 2011). Despite wide spread public enlightenment from several sources, sexually transmitted diseases are on the rise, especially in under developed countries where the burden of the disease is worse.

CONCLUSION

This study revealed a high prevalence of antibodies to both organisms with some level of co-infection noticed, and prevalence to both organisms was significantly higher among those with some form of risky sexual behaviours. This is of grave public health importance to the nation and region at large as sexually transmitted diseases are communicable and can cause both acute and chronic complications even in the absence of obvious symptoms. In-depth research papers on this co-existence in the country and region are low when compared to volume of research papers from developed countries. Infection by these organisms can be reduced to the barest minimum if adequate control measures are strictly implemented as this has been achieved in most developed countries. Thus, there is need to sustain, improve and expand the existing mass enlightenment campaigns on the consequences of unprotected sexual intercourse. Evidence based research even at molecular and genetic levels should be financed in poorly developed nations, to ascertain the true picture of the burden of these diseases and their existence together with others, and proffer the appropriate treatment needed even in resistance cases. The tests for these organisms should be made available in our hospitals by authorities concerned and free of charge especially to the sexually active adolescent and young adults.

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Competing Interest

The authors hereby declare that there is no competing interest.

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