Original research article

Seroprevalence of Human Immunodeficiency Virus, Hepatitis-B Virus and Syphilis in Antenatal Cases in and Around Bihar

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Abstract

Background: HIV, HBV and syphilis in pregnancy are associated with adverse pregnancy outcomes including spontaneous abortion, preterm birth, still birth, low birth weight, congenital infections etc.

Objectives: The present study was undertaken to know the seroprevalence of HIV, HBV and syphilis in and around bihar.

Methods: The study was conducted in the Department of Microbiology, IGIMS, Patna. The study included 300 blood samples from antenatal women attending outpatient and inpatient and also private hospital antenatal cases, whose blood samples were sent for routine blood investigations to our Laboratory. All the test sera were tested for anti-HIV IgG antibodies by a DOT immunoassay, if reactive was confirmed by TRIDOT and ELISA test. They were also tested for HBV by HBsAg ELISA and syphilis by VDRL test, if reactive was confirmed by TPHA test.

Conclusion: Prevalence of HIV, HBV are high from this study, so it is important to screen all the antenatal mothers for both, so as to provide proper antenatal, intranatal andpost natal care to seropositive women and prevent MTCT. Eventhough the seroprevalence of syphilis is low from the present study, it is advisable to screen for syphilis also, as the disease is treatable and thus helps to eliminate the adverse effectof untreated syphilis both in mother and foetus.

Keywords: HIV; HBV; syphilis; Seroprevalence; ELISA; TRIDOT; DOTImmunoassay; VDRL; TPHA.

Introduction

The worldwide burden of sexually transmitted infections (STIs) is estimated at over 350 million cases yearly, most of which occurs in less developed countries.[1] Sexually transmitted diseases (STD) in pregnancy are associated with adversepregnancy outcomes

including spontaneous abortion, preterm birth, low birth weight, congenital infections, congenital abnormalities and long term neurologic disability.[2] As women and their offsprings carry the major burden of complications and serious sequelae, the development of STI control measure for this segment of the population is of particular importance.[3] Prevalence assessment and monitoring are important components of STI surveillance. With such testing, pregnant women are screened to obtain data for purposes of programme planning.[4] Antenatal screening for Human Immunodeficiency Virus (HIV) was added to the antenatal screening package in June 2003 by the Gujarat state acquired immunodeficiency syndrome (AIDS) control society. Universal screening of all pregnant women for HIV is cost effective and has clearly demonstrated reductions inMaternal to child transmission (MTCT).[5] In India, about 28 million deliveries occur annually of which 84,000 deliverieswould occur in HIV-positive women considering a national average of 0.3% prevalence of HIV in pregnant women. Without any intervention, about 30-45% of these babies will become infected with HIV.[6] Worldwide, World Health Organization (WHO) estimates that maternal syphilis is responsible for 4,60,000 stillbirths or abortions, 2,70,000 cases of congenital syphilis and 2,70,000 low birth weight or premature babies.[7] Spread of infection from Hepatitis-B virus (HBV) carrier mothers to their babies is the major mode of transmission of hepatitis B. If pregnant women who arehepatitis B surface antigen (HBsAg) positive are identified before delivery, it is possible to prevent HBV infection in their neonates by passive or active immunizationor both.[8]

Objectives

The seroprevalence of anti-HIV antibodies in antenatal women.
The seropositivitiy of HbsAg in the pregnant women.
The seroprevalence of syphilis in the pregnant women.

Review of Literature

First indication of AIDS came in the summer of 1981, with reports from New York and Los Angeles, of a sudden unexplained outbreak of two very rare diseases – Kaposi's sarcoma and Pneumocystis Carinii pneumonia in young adults who werehomosexuals or intravenous drug abusers. In 1983, Luc Montagnier and Colleagues from the Pasteur Institute, Paris isolated a retrovirus from a West African patient with persistent generalizedlymphadenopathy and called it lymphadenopathy associated virus (LAV). In 1984, Robert Gallo and colleagues from National Institute of Health, USA, called it human T cell lymphotropic virus – III. In 1986, International Committee on virus nomenclature decided the name HIV for these viruses. In 1985, Serological tests became available for detection of anti-HIVantibodies (Abs). In 1986, First case of HIV infection in India was found in femalesex worker in Chennai and the first AIDS patient was from Mumbai. Human infection could have come from Chimpanzees. The Simian immunodeficiency virusmay have taken root in humans by becoming HIV through mutation or recombination. HIV, the etiological agent of AIDS, belongs to the Family Retroviridae and subfamily Lentivirus. Morphology of HIV[10]HIV is a spherical enveloped virus, about 90-120 nm in size with icosahedral symmetry. The nucleocapsid has an outer shell and an inner core, enclosing the genome. The genome is diploid, made of two identical single stranded positive sense RNA, which is associated with reverse transcriptase (RT) enzyme. It has lipoprotein envelope, which consists of lipid derived from host cell membrane and glycoproteins(seen as projecting spikes on the surface and transmembrane pedicles) which are virus coded. Viral Genes[9,10] Species specific T-pallidum tests use the virulent Nichol's strain. These tests include Treponema Pallidum

immobilization test (TPI), fluorescent treponemal antibody absorption test (FTA-Abs), T.pallidum haemagglutination test (TPHA), microhaemagglutination test for T.pallidum (MHA-TP) and enzyme immunoassay (EIA). TPI test (1949) is based on the ability of patient's antibody and complement to immobilize treponemes (Nichol's strain), as observed by dark field microscopy. It is complicated difficult, expensive and time consuming, so not in use now. FTA Abs test is a indirect immunofluorescence test, where Nichol's strain fixed on glass slides is used. The patient's serum is first diluted in sorbent (Reiter's treponeme) to remove non specific treponemal antibodies. The serum is added to the slides. If it contains antibody, it coats the treponemes. Now add fluorescene isothiocyanate labelled anti-human Ig to the slide and examined under a fluorescent microscope. It is first to become positive in early syphilis and has higher sensitivity than non-treponemal tests in late syphilis. However it can be done only in suitably equipped laboratories. TPHA test uses tanned erythrocytes sensitized with T.pallidum, as antigen. Itis as specific as FTA-ABS test except in primary stage. It is much simpler, and economical and kits are available commercially, hence TPHA is used as standard confirmatory test in many labs. EIA have been developed using T.pallidum antigens. These are available commercially. Specific treponemal tests are of no value in monitoring the treatment, as they remain positive in spite of treatment.

Detection of IgM antibodies can be used as a indicator of active syphilis for diagnosis of congenital syphilis and reinfection. It appears by 2 weeks of infection and disappears within 6 months.[11] Penicillin is the most effective drug. A single injection of 2.4 million units of benzathine penicillin G is adequate in early syphilis, except in pregnant women who requires 2 doses given one week apart. For late syphilis, it is given weekly once, for 3 weeks. Pregnant women with late syphilis require a full 15 day course of aqueous procaine penicillin once daily. In patients allergic to penicillin, doxycycline, ceftriaxone, tetracyclines, erythromycin can be used.[12,13] Prevention and Control[14]: Enhanced surveillance, screening, partner notification as well as collaboration with NGOs are important components. Presumptive treatment of all contacts of patients who were exposed, within 90 days. Public education about sequelae andprevention. Routine screening in high risk groups and use of physical barriers (condom) has been found effective in control of the disease. Around 12 million cases occur worldwide annually of which 4 million occur in Africa. A cross sectional survey of reproductive age women in Mumbai, showed seropositivity of 0.5% while a study in Delhi showed seropositivity of 4%.[15] The national goal of syphilis elimination is to reduce syphilis to <1000 cases per 1,00,000 population.[16]

Material and methods

The study was conducted in the Department of Microbiology, Indira Gandhi institute of medical sciences, Patna, Bihar. Study duration of two years.

Inclusion Criteria

Antenatal cases attending outpatient and inpatient department of IGIMS, Patna, Private Hospital antenatal cases, whose blood samples were sent for routine blood investigations to our laboratory.

Exclusion Criteria

Women who are not pregnant.

Specimen Collection

About 5 ml of blood was collected by venepuncture with all aseptic precautions. Sera was

separated by centrifugation (3000 rpm for 10 minutes) and stored at -20^{o}C.

Methods Used:

All the test sera were tested for anti-HIV IgG antibodies by a Dot immunoassay (COMBAIDS-RS Advantage), if reactive was confirmed by TRIDOT& ELISA test. Test sera were also tested for HBV by HBsAg ELISA and syphilis by VDRL test, if reactive was confirmed by TPHA test. It has same principle as EIA whereby the immobilized antigen-antibody complex is visualized by chromogenic reaction. In EIA the colour is developed by a coupled reaction between enzyme, substrate and chromogen whereas in this colour is developed by a colloidal Gold-Protein A signal reagent. Each tooth of the comb has 2 spots, one near the tip (Test spot) with an optimally standardized blent of HIV 1 and 2 recombinant antigens and synthetic peptides and the other spot, just above the Test Spot with "Control Reagent" (Control spot). When incubated with a specimen containing antibodies, they form a immune complex which is directly visualized after incubation with signal reagent as a pink coloured dot in the test area.

TPHA Test (IMMUTREP TPHA)

It is a specific, sensitive, passive haemagglutination test for the detection of specific antibodies to T.pallidum in the serum / CSF.

Principle: When diluted positive samples are mixed with sensitized fowl erythrocytes, antibody to the sensitized Ag causes agglutination of erythrocytes to form characteristic carpet pattern in the bottom of microtitre well. In the absence of antibody, they form a compact button in the well.

Initial procedure is as above. After the above procedure, 25 µl diluent to be added to the required number of wells for different dilutions. To the first wellof diluent add 25 µl of diluted sample, mix and transfer 25 µl from this well to next well. Continue this for the required number of dilutions, to produce doubling dilutions of the sample (1:80, 1:160, 1:320, 1:640, 1:1280 etc). Cover the plate and incubate at room temperature for 45-60 minutes.

Interpretation of Results:

Carpet pattern of agglutination – Positive result, Button pattern – Negative result, Quantitative procedure – Highest dilution of serum showing carpet pattern of agglutination is taken as titre.

Results

A total of 300 serum samples from antenatal women were screened for anti- HIV antibodies, HBsAg and anti-treponemal antibodies. All were within the age group of 17-32 years, out of which 289 were married, 9 were unmarried and 2 were divorce

Table 1: PREVALENCE OF HIV, HBV AND SYPHILIS IN ANTENATAL CASES

STDs	Prevalence Rate (%)	95% Confidence Interval
HIV	1.0%	0 – 2.2%
HBV	1.7%	0.1 – 3.1%
Syphilis	0.3%	0 – 0.6%

it is evident that, out of 300 antenatal cases, 3 were positive for anti-HIV antibodies accounting for 1% prevalence rate (95% CI- 0 to 2.2%), 5 were positive for HBsAg accounting for 1.7% prevalence rate (95% CI – 0.1 to 3.1%) and 1 was positive for anti-treponemal antibodies accounting for 0.35prevalence rate (95% confidence interval – 0 to

0.6%).

Table2: SEROPREVALENCE IN RELATION TO AGE

Age Group (Years)	No. Studied	HIV +ve n (%)	HBsAg+ve n (%)	Syphilis (R) n (%)
17 – 20	77	-	1 (1.3)	-
21 – 25	182	2 (1.1)	4 (2.2)	1 (0.5)
26 – 32	41	1 (2.4)	-	-
Total	300	3 (1.0)	5 (1.7)	1 (0.3)

out of 300 antenatal cases, 77 were between age group of 17-20 years, among them, none were positive for HIV and syphilis, one was positive for HBsAg accounting for 1.3%. 182 cases were between 21-25 years age group, out of which 2 were positive for HIV (1.1%), 4 were positive for HBV (2.2%) and 1 was positive for shyphilis (0.5%). 41 cases were between age group of 26-32 years, out of which one was positive for HIV (2.4%) and none was positive for HBVor syphilis. So for HBV and syphilis, highest prevalence was observed between age group of 21-25 years but for HIV, highest prevalence was between age group of 26-32 years.

Table 3: SEROPREVALENCE IN RELATION TO MARITAL STATUS.

Marital Status	No. of Women	HIV +ve n (%)	HBsAg+ve n (%)	Syphilis (R) n (%)
Unmarried	9	1 (11.1)	-	-
Married	289	2 (0.7)	5 (1.7)	1 (0.3)
Divorced	2	-	-	-

highest prevalence rate of HIV i.e. 11.1% wasseen among unmarried women. In married women prevalence of HIV was 0.7%, HBsAg was 1.7%, syphilis was 0.3%.

Table 4: PARITY WISE DISTRIBUTION OF SEROPREVALENCE

Parity	No. of Women	HIV +ve n (%)	HBsAg+ve n (%)	Syphilis (R) n (%)
Primi	145	2 (1.4)	5 (3.4)	-
Multi	155	1 (0.6)	-	1 (0.6)
Total	300	3 (1.0)	5 (1.7)	1 (0.3)

145 women were Primi gravida, out of which 2 were positive for HIV (1.4%), 5 were positive for HBsAg (3.4%). 155 were multigravida, out of which 1 was positive for HIV (0.6%) and 1 for syphilis (0.6%). So highest prevalence rate of HIV and HBV was seen among primigravida.

Table 5: SEROPREVALENCE IN RELATION TO RISK FACTORS

H/o	No. of Women	HIV + n (%)	HBsAg+ n (%)	Syphilis (R) n (%)
Blood Transfusion	60	-	2 (3.3)	-
Husband +ve for HIV	2	2 (100)	-	-
Multiple sex partners	3	1 (33.3)	-	1 (33.3)
Occupational exposure to HBV	1	-	-	-
No known risk factors	234	-	3 (1.3)	-
Total	300	3 (1.0)	5 (1.7)	1 (0.3)

it is evident that if husband was positive for HIV prevalence rate was 100%. Among women with multiple sexual partners, prevalence of HIV was 33.3% and of HBsAg was 33.3%. HBsAg seropositivity was highest i.e. 3.3% among women with history of blood transfusion followed by 1.3% among women with no known risk factors. From all the above tables and graphs, it is evident that there is no any co- infection of HIV, HBV or syphilis.

Discussion

Prenatal identification of HIV infected women is crucial to the delivery ofoptimal care to both mother and fetus. Therefore several studies have been carried out all over the world to know the seroprevalence of HIV in antenatal women. Amaral et al[15] (1996) reported 0.4% prevalence rate. Datey et al[3] (1997) reported prevalence rate of 4.5% from Mumbai, 0.5% from Calcutta and Pune, 0.3%from Pondicherry and 0% from Chandigarh with overall prevalence rate of 1.2%. Miranda et al[18] (1999) reported 0.8% in Brazil. Aggarwal et al[17] (2000) reported 0.6% from Amritsar, Punjab. Shymala et al[19] (2001) reported 1.1% from Manipal, Karnataka. Ragini et al[20] (2003-2004) reported 0.4% from Allahabad, UP. Nandita et al[5] (2003-2004) reported 1.09% from Surat, Gujarat. The NACO Sentinel Surveillance data for the state of Delhi reported HIV prevalence of 0.25% in 2003, 0.38% in 2004, 0.25% in 2005. Tohon et al[21] (2006) reported 1.3%, from Nigeria. Gupta et al[22] (2006) reported HIV prevalence of0.8% from New Delhi. A total of 300 serum samples were screened for anti-HIV antibodies.

Out of which 3 were positive, accounting for seroprevalence of 1%. The results of the present study are comparable with the studies conducted by Miranda et al,[18] Shymala et al,[19] Nandidaet al[5] and Gupta et al.[22] Spread of infection from HBV carrier mothers to their babies is the majormode of transmission of HBV. Hence several studies have been reported from different areas to know the seropositvity of HBsAg among antenatal women. Datey et al[3] (1997) reported a prevalence of 6% from Mumbai, 4.3% from Calcutta, 3% from Pondicherry, 2.3% from Pune, 1.3% from Chandigarh with overallprevalence of 3.4%. Anvikar et al[8] (1998) from Aurangabad reported 2.7% seropositivity. Miranda et al[22] (1999) reported 1.1% at Brazil. Martinez et al[27] (2000) reported 1.6% in Mexico. Jindal et al[23] (2004) from Amritsar, Punjab reported prevalence of 5%. Akaniet al[25] (2004) reported 4.3% in Nigeria. Liu et al[25] (2006) reported 3.5% from Indonesia. Single et al (2007) reported 1.73% from North India and he also statedthe seropositivity of HBsAg to be in the range of 2.2 – 7% in India. Syphilis is a serious cause of maternal and infant morbidity and mortality.Maternal syphilis is associated with still births, abortions, prematurity, low birth weight babies etc. Hence screening for syphilis during pregnancy is important. Several studies have been carried out throughout the world to know the prevalence of syphilis in pregnant women. Datey et al[3] (1997) reported the prevalence of 2.8% in Mumbai, 0.8% in Pune,0.7% in Pondicherry, 0.3% in Calcutta and Chandigarh with overall prevalence of 1%. Miranda et al[18] (1999) reported prevalence of 3% in Vitoria, Brazil. Chen et al (2002) from Fuzhou, China reported 0.2% prevalence rate. Potter et al (2002) reported a high prevalence rate of 6.6% in Sub-Saharan Africa. China reported a prevalence of 0.3% in 2002, 0.5% in 2003, 2004 and2005.. He also stated thatrates of seropositive pregnant women in Nigeria is in the range of 0.6 – 2.3% while inAfrica its between 3-18%. Olokoba et al[26] (2008) reported 0.4% prevalence in NorthEastern Nigeria. In the present study, the seroprevalence of syphilis in pregnant women was 0.3% and it can be compared with the studies conducted by Datey et al[3] from Calcuttaand Chandigarh, Olokoba et al[26] from

Nigeria. This high prevalence rates, suggest the need to improve syphilis screening and treatment.

Conclusion

Seroprevalence of HIV was found to be 1% (95% CI – 0-2.2%) HBV was found to be 1.7% (95% CI - 0.1 – 3.1%) and syphilis was found to be 0.3% (95% CI – 0 – 0.6%). There was no co-infection. Prevalence was highest for HIV in the age group of 26-32 years, HBV and Syphilis in 21-25 years age group. Prevalence of HIV (1.4%) and HBV (3.4%) was highest in primigravida whereas for syphilis (0.6%) in multigravida.

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