ORIGINAL RESEARCH

Seroprevalance of HBsAg, anti-HIV 1&2 and anti-HCV by chemiluminescence method - A hospital based study from 2007 to 2014

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Abstract

Introduction: Hepatitis B virus (HBV), human immunodeficiency virus 1&2 (HIV1&2) and hepatitis C virus (HCV) are major public health problem. Detection of hepatitis B surface antigen (HBsAg), antibodies to human immunodeficiency virus 1&2 and hepatitis C virus in patients (both inpatients and outpatients) can help in patient management. Screening of patient samples for HBsAg, anti-HIV 1&2 and anti-HCV is important in managing surgical and medical care. The objective is to know prevalence of hepatitis B surface antigen (HBsAg), antibodies to HIV 1&2 and HCV inpatient and outpatient, their gender, age wise distribution and department wise distribution.

Material & methods: The present study was conducted in the Department of Microbiology, Krishna Institute of Medical Sciences (KIMS), Secunderabad, Telangana, during a study period of 8 years from January 2007 to December 2014. It is a cross sectional study. It is a laboratory based study, no clinical correlation has been done. The positive/reactive samples were studied for their gender, age group and department. Analysis was done on a yearly basis. A cumulative data of 8 years is being presented. The samples were screened by enhanced chemiluminescence (ECLIA) technology by using an instrument, Vitros ECiQ(Ortho Clinical Diagnostic).

Results: 1,00,590 patients were screened 1,805 (1.79 %) were positive for HBsAg. 92,819 patients screened 501(0.53%) were reactive for HIV 1&2 antibodies. 61,072 screened 744(1.21%) were reactive for HCV infection. The seroprevalence of all viral markers is more in males than females. In the present study seropositivity of HBsAg was higher in 41-50 year & 51-60 years followed by 31-40 years & 61-70 years. Highest prevalence of HIV was observed in 31-40 years followed by 41-50 years. Seropositivity of HCV was higher in 51-60 years followed by 41-50 years & 61-70 years.

Conclusion: Emphasizes the need of screening of viral markers for inpatients and out patients. Adherence to universal precautions

Keywords: Seroprevalence of HBsAg; anti-HIV 1&2; anti-HCV; chemiluminescence method

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Received 12 November 2014; *Revised* 10 December 2014; *Accepted* 17 December 2014;.Published.27.December.2014

Citation: Anil Kumar B, Madhavi Latha B, Gopal Reddy S, Vanaja S, Imtiyaz Ahmed MD, Radhika Chowdary D. Seroprevalance of HBsAg, anti-HIV 1&2 and anti-HCV by chemiluminescence method - A hospital based study from 2007 to 2014. J Med Sci Res. 2015; 3(1):1-8. DOI: http://dx.doi.org/10.17727/JMSR.2015/3-001

Introduction

Hepatitis B virus (HBV), human immunodeficiency virus 1&2 (HIV1&2) and hepatitis C virus (HCV) are major public health problem. They are important among the blood borne viruses transmissible through the parenteral route, blood transfusion and sexual intercourse. They are known to cause asymptomatic persistent infections causing morbidity and mortality. Early diagnosis and treatment of infection is important in reducing its transmission.

HBV is a major public health problem globally and is tenth leading cause of death. There are 350 million carriers [1]. In India, HBsAg prevalence among general population ranges from 2 to 8% [2]. The prevalence of HIV is 38.6 million. India has 5.2 million cases of HIV infection, the second highest of these patients in the world. The estimated number of HIV infected people in India has been revised downward from 5.7 million to 2.5 million [3]. Hepatitis C virus causes chronic hepatitis C in 200 million people worldwide. In India, antibodies against hepatitis C virus are present in approximately 15 million people with a prevalence rate of 2% [4]. Detection of hepatitis B surface antigen (HBsAg), antibodies to HIV 1&2 and HCV in patients (both inpatients and outpatients) can help in patient management. Screening of patient samples for HBsAg, anti-HIV 1&2 and anti-HCV is important in managing surgical and medical care.

Several screening tests for HBV, HIV1&2 and HCV are routinely done. In emergency situations, the assay results are expected to be reported early. Screening of these viral markers has been made easy with user friendly enhanced chemiluminescence (ECLIA) technology. This technology based tests can be performed with rapidity, sensitivity and specificity [5].

In the current knowledge, the information of use of chemiluminescence (ECLIA) technology for the detection of seroprevalence of HBsAg, anti-HIV 1&2 and anti HCV in the patients attending to a corporate hospital in India is lacking.

An attempt has been made to know prevalence of HBsAg, antibodies to HIV 1&2 and HCV inpatient and outpatient, their gender, age wise distribution and department wise distribution. In the present study, the instrument Vitros ECiQ (Ortho Clinical Diagnostics & Johnson & Johnson) based on chemiluminescence technology, is used to screen patients samples for HBsAg, anti-HIV 1&2 & anti HCV [6] (Table 1).

Material and methods

The present study was conducted in the Department of Microbiology, Krishna Institute of Medical Sciences (KIMS), Secunderabad, Telangana, during a study period of 8 years from January 2007 to December 2014. The hospital is a tertiary care, multi super specialty health facility with a referral status. It has NABL accreditation for laboratory since 2007.

The samples from inpatients and out patients were collected and the information was documented in the computer. It is a cross sectional study and laboratory based study, no clinical correlation has been done. The patients are from various super specialty departments of medicine and surgery. A pretest voluntary informed consent was taken as per NACO guidelines for HIV testing. Posttest counseling was done to the HIV reactive patients as per NACO guidelines.

The patient samples for which the result was negative/ non-reactive in screening test were reported. The patient samples for which the result was positive/ reactive were further tested by using different technologies. The data was collected from the computer. The positive/reactive samples were studied for their gender, age group and department. Analysis was done on a yearly basis. A cumulative data of 8 years is being presented.

Blood samples were collected aseptically by veni-puncture from the patients and were analyzed for HBsAg, anti-HIV 1&2 and anti-HCV. The samples were screened by enhanced chemiluminescence (ECLIA) technology by using an instrument, Vitros ECiQ (Ortho Clinical Diagnostic). Details of the instrument are given in table 1.

Showing the details of Vitros ECiQ (Ortho Clinical Diagnostic)

The Vitros ECiQ imunodiagnostic system uses sophisticated hardware and software, including intellicheck technology and enhanced chemiluminescence detection technology, to process

samples and assays and report results (Table 1). Vitros ECi/ECiQ Imunodiagnostic System consists of five main areas, which are operational unit, system command center, e-Connectivity center, output centre and power unit.

Intellicheck technology

It is designed to detect critical errors. Intellicheck is integrated. The Process Control, a series of patented and unique technologies that perform, monitor and verify diagnostic checks throughout sample and assay processing and results reporting. When exceptions are detected, Intellicheck technology provides immediate operator notifications and prevents results that may be affected from being reported.

Managing reagents and supplies: The Vitros ECiQ imunodiagnostic system uses reagents and supplies that can be loaded, unloaded and replaced. It continuously monitors their inventory, including reagent levels and expiration dates: Integrated reagent packs, signal reagent (that generates the chemiluminescence reaction in the coated wells after they have been washed with universal wash reagent). Tips and wells must be periodically removed from the solid waste container.

Maintenance procedure: can be categorized as: Daily, weekly, monthly and as required.

In daily maintenance: the following procedures are done like empty solid and liquid waste, remove outdated and empty reagent packs and universal wash reagent, clean the signal reagent probe assembly, inspect universal sample trays, micro collection container adapters, verify inventory and load reagent packs and SR pack, universal wash reagents, verify that QC fluids have been processed.

In weekly maintenance: the following procedures are done like clean the sample metering proboscis, clean the tip disposal chute/cup retainer, clean the sample supply subsystem, clean the touch screen monitor, clean the key board and key board cover, perform subsystem cleaning and clean the proboscis center.

In monthly maintenance: the following procedures are done like back up QC, calibration and configuration files, inspect the reagent cooler filter, change the vapor adsorption cartridge and change the universal wash reservoir filter.

Quality control

Calibrations: Calibration is lot specific; reagent packs and calibrators are linked by lot number. A master calibration is established for each new reagent lot by performing multiple assays. This is the process by which a lot-specific parameter (a) which links the cut-off value to the calibrator signal is determined [7-9]. Cut-off value = (a x Signal of CAL 1).

The lot specific parameter, the expected calibrator signal and the data enables a system to calculate the cut-off value. Scanning the lot calibration loads the encoded data onto the system. The validity of the calibrator is assessed against the quantity parameter which compares the actual signal of the calibrator with the expected signal. If the calibration is acceptable the cut-off value is calculated and stored to use with any reagent pack of that lot. The quality of calibration cannot be completely described by a single parameter. The calibration report should be used in conjunction with control values to determine the validity of the calibration. Recalibration is required after pre-determined calibration interval or when a different reagent lot is loaded.

Controls: The use of controls has been mentioned in the table 1. The instrument uses the Levey-Jennings (LJ) graphs of control records and results. These graphs plot the result data points against the mean and standard deviation. The characteristics of screening assay of HBsAg, anti HIV 1&2 and anti HCV by Vitros ECiQ are given in table1.

Results

The table 2 shows the details of screening HBsAg, anti HIV1&2, anti HCV from Jan 2007- Dec 2014 (8 years). Overall 1,00,590 samples were tested for HBsAg, 92,819 samples were tested for anti-HIV 1&2 and 61,072 were tested for anti-HCV respectively. The overall seroprevalance of HBsAg was 1.79%, anti HIV1 & 2 was 0.53% and anti-HCV was 1.21% respectively.

The table 3 shows the details of gender and age wise distribution of HBsAg Positive, HIV and HCV reactive patients. In the present study seropositivity was more in males followed by females. Considering the age group, a higher seroprevalence was observed in 41-50 year and 51-60 years followed by 31-40 years and 61-70 years among HBsAg positive patients.

Table 1: Characteristics of screening assay of HBsAg, HIV and HCV by Vitros ECiQ.

S.No		*HBsAg*	*HIV*	*HCV*
1	Intended use of detection	Qualitative detection of HBsAg	Qualitative detection of antibody to HIV 1&2. Antibody to HIV 1 subtype O	Qualitative detection of antibody to c22-3, c200 & NS5
2	Principle	Immunometric -1stage reaction	Immunometric -2 stage reaction	Immunometric -2 stage reaction
3	Solid phase	Mouse monoclonal antibody	Recombinant HIV-1 & HIV-2 antigen.	Recombinant HCV antigen
4	Signal reagent [10-12]	Luminol, a substituted acetanilide & peracid salt		
5	Controls	2 controls HBsAg negative HBsAg positive	3 controls HIV negative HIV1positive HIV2 positive	2 controls HCV negative HCV positive
5a	Frequency for processing controls	24 hours	24 hours	24 hours
5b	Preparation of controls	Reconstitute lyophilized control as per instructions mentioned.		
6	Sample volume (µL)	80	80	20
7	Time of reaction - duration of test (min)	37	40	56
8	Result			
8a	Negative	0.00 to 0.89	0.00 to 0.89	0.00 to 0.89
8b	Borderline	0.90 - 0.99	0.90 - 0.99	0.90 - 0.99
8c	Positive/reactive	1.00 or > 1.00	1.00 or > 1.00	1.00 or > 1.00
9	Sensitivity	100%	100%	100 %
10	Specificity	99.98 %	100% in clinical population	99.76 %

Table 2: Showing the details of screening HBsAg, anti HIV1 & 2, anti HCV from Jan 2007- Dec 2014 (8 years).

MONTH	*HBsAg*		*HIV*		*HCV*	
	Total number of samples screened	*Positive*	*Total number of samples screened*	*Reactive*	*Total number of samples screened*	*Reactive*
2007	6,324	149 (2.3%)	5,652	30 (0.53%)	2,089	27 (1.2%)
2008	8,762	152 (1.73%)	8,768	46 (0.52%)	2,058	35 (1.7%)
2009	10,074	157 (1.55%)	10,015	47 (0.46%)	1,872	58 (3.0%)
2010	11,491	192 (1.6%)	11,154	61 (0.54%)	3,558	90 (2.5%)
2011	13,075	250 (1.91%)	11,455	82 (0.71%)	8,739	133 (1.52%)
2012	14,856	272 (1.8%)	13,095	74 (0.56%)	12,151	141 (1.16%)
2013	16,904	293 (1.7%)	15,085	59 (0.39%)	14,211	129 (0.90%)
2014	19,104	340 (1.77%)	17,595	102 (0.57%)	16,394	131 (0.7%)
Total	1,00,590	1,805 (1.79%)	92,819	501 (0.53%)	61,072	744 (1.21%)

Table 3: Gender and age wise distribution of HBsAg positive, HIV and HCV reactive patients from 2007 to 2014 (8 years).

	HBsAg		***HIV***		***HCV***	
Total numbers of samples screened	1,00,590		92,819		61,072	
Total negative/ non-reactive	98,785		92,318		60,328	
Total positive / reactive	1,805 (1.79%)		501 (0.53%)		744 (1.21%)	
	In patient	***Out patient***	***In patient***	***Out patient***	***In patient***	***Out patient***
Total number of positive/ reactive (n)	960	845	293	208	437	307
Male (n)	746	638	223	159	297	215
Female (n)	214	207	70	49	140	92
Age wise distribution (years) (n)						
0-10	02	00	03	03	06	00
11-20	18	23	03	04	08	05
21-30	87	163	32	30	25	26
31-40	162	176	93	61	34	52
41-50	221	198	81	59	98	71
51-60	255	163	56	37	138	80
61-70	161	97	23	14	84	51
71-80	48	23	02	00	41	21
81-85	06	02	00	00	03	01
Total	960	845	293	208	437	307

A higher seroprevalence was observed in 31-40 years followed by 41-50 years in anti HIV 1&2 reactive patients. A higher seroprevalence was observed in 51-60 years followed by 41-50 years and 61-70 years among HCV reactive patients.

The table 4 shows the details of the department wise distribution of the HBsAg positive, HIV & HCV reactive patients. Some of the important points from the table 4 are i) Majority of the HBsAg positive Inpatients belonged to the following departments: Cardiology, neurosurgery, orthopedics, oncology, gastroenterology and nephrology and internal medicine, ii) Majority of the HBsAg positive out patients belonged to the following departments: Cardiology, gastroenterology, internal medicine, surgical gastroenterology, orthopedics, nephrology and health checkup, iii) Majority of the HIV reactive inpatients belonged to the following departments: Cardiology, neurosurgery, orthopedics, neurology, internal medicine and pulmonology, iv) Majority of the HIV reactive out patients belonged to the following departments: Internal medicine, cardiology, casualty, pulmonology, orthopedics and nephrology, v) Majority of the HCV reactive inpatients belonged to the following departments: Cardiology, nephrology, orthopedics, neurosurgery, gastroenterology and cardiothoracic unit, vi) Majority of the HCV reactive out patients belonged to the following departments: Gastroenterology, nephrology, cardiology, surgical gastroenterology, infertility clinic, crthopedics and neurosurgery.

Discussion

The study was carried out to document the seroprevalence of HBsAg, anti HIV 1&2 & anti HCV in patients attending to KIMS hospital, Secunderabad, Telangana, 1,00,590 patients were screened 1,805 (1.79 %) were positive for HBsAg. 92,819 patients screened 501 (0.53%) were reactive for HIV 1&2 antibodies. 61,072 screened 744 (1.21%) were reactive for HCV infection.

In the screening method, when HBsAg was found to be positive, it was confirmed by one more method of testing with enzyme linked immuno fluorescence

Table 4: Department wise distribution of HBsAg positive, HIV and HCV reactive patients from 2007 to 2014 (8 years).

Department wise distribution of patients.	*HBsAg n=1805*		*HIV n=501*		*HCV n=744*	
	In patient n=960	*Out patient n=845*	*In patient n=293*	*Out patient n=208*	*In patient n=437*	*Out patient n=307*
Cardiology	273	158	54	27	110	60
Internal Medicine	38	131	32	55	20	05
CT	31	12	07	01	27	03
Ortho	127	49	38	10	48	12
Neuro surgery	145	28	40	07	42	12
Neurology	37	13	37	08	06	07
Pulmonology	08	02	20	11	05	01
Urology	34	08	09	05	09	05
Nephrology	56	42	14	10	75	65
Surgical GE	50	60	09	09	17	14
GE	62	156	06	08	36	66
Dermatology	-	01	01	-	-	-
Casualty	03	26	01	14	03	04
Plastic surgery	06	05	03	-	02	01
Vascular surgery	-	10	02	-	02	-
ENT	05	12	-	09	02	03
Gynecology	03	11	01	04	-	02
Rheumatology	01	05	-	01	-	02
Oncology	69	18	17	02	23	08
KTP	07	07	-	-	08	07
Ophthalmology	-	-	-	-	-	-
Endocrinology	-	03	-	-	-	-
Breast unit	-	11	-	01	01	02
Infertility clinic	-	29	01	04	-	13
Self	-	08	-	13	-	10
External	-	07	01	03	-	03
Pediatrics	01	-	-	02	-	-
Health check	-	31	-	04	-	-
Liver transplant unit	02	02	-	-	-	-
Radiology	02	-	-	-	01	02
Total	960	845	293	208	437	307

assay (ELFA). A sample which was tested positive in two different screening methods was reported as positive for HBsAg. The ELFA is qualitative test 100% sensitivity, 100% specificity.

In this study, HBsAg seroprevalence was 1.79 %. In a previous study, Lodha et al. (2001) in their review article on hepatitis B epidemiology have suggested the true prevalence rate in India as 1-2% [13]. The

prevalence of HBsAg in different regions of our country varies widely, and the highest prevalence has been reported from the aborigine population of Andaman as well as from Arunachal Pradesh [21]. The prevalence of hepatitis B varies from country to country and depends upon a complex mix of behavioral, environmental, and host factors. Sood et al. has noted 0.87% prevalence in a study of HBsAg prevalence in hospital based population [14]. Our data confirms the endemicity of HBV infection in Telangana region; and shows that this is a problem that poses a risk to health care workers.

The seroprevalence of HBsAg among males and females was 1.8% (1,384/72,869) and 1.5% (421/27,721) respectively. Most of the studies have reported higher prevalence among males which was also found in our study. Sood et al. has reported the prevalence of 1.04% and 0.58% respectively for males and females. Dutta et al. has reported prevalence of 35.3% in males and 19.3% in females [22]. Singh et al. has noticed prevalence to be 0.65% and 0.25% respectively in males and female subjects [23]. No plausible explanation has been given for the higher prevalence in males in the general population but it is hypothesized that females probably clear the HBV more efficiently in comparison to males [24]. In the present study seropositivity was higher in 41-50 year and 51-60 years followed by 31-40 years and 61-70 years. Similar findings were found by Sood et al. [14].

In the screening method, when anti HIV 1&2 was found to be reactive, it was confirmed by two more methods of testing with enzyme linked immuno fluorescence assay and immunochromatography (ICT) rapid visual test (HIV TRI-DOT) in a step wise order. A sample which was tested reactive in three different methods was reported as reactive for anti-HIV 1&2.

The WHO & UNAIDS recommends three criteria for choosing an HIV testing strategy: i) Objective of the test (transfusion/ transplant , safety, surveillance, diagnosis of HIV infection), ii) Sensitivity and specificity, iii) HIV prevalence in the population being tested.

Strategy III requiring 3 test for use in diagnosis in population with an HIV prevalence ≤10% among asymptomatic. The selection of testing technologies and the order in which they are used are important for obtaining valid results. First, the test should contain different antigen. Second the sensitivity of the first test should be as high as possible. The first test is the screening test, so it is necessary to use a highly sensitive test to detect all positives. Because a few false positives will occur, the second test (confirmatory test) needs to be highly specific to ensure that all truly negative test results are identified as negative.

Based on ELFA technology, VIDAS DUO Ultra was used. It is a screening test for the combined detection of anti HIV 1 (group M&O) and anti HIV 2 total immunoglobulins and HIV 1 p24 antigen. It is qualitative test 100% sensitivity, 100% specificity. The immunochromatography is a qualitative test and visual read results and differentiates between HIV1 & HIV 2 with 100% sensitivity and 100% specificity.

In this study, the antibodies to HIV prevalence were 0.53 %. The seroprevalence of antibodies to HIV in tertiary care hospital in Jaipur was reported to be 0.35% [14]. The seroprevalence of HIV antibodies among 183,912 persons screened in a teaching tertiary care hospital in Haryana was reported to be 0.64% [15].

In the present study, seropositivity was more in males 0.59%) (382/63,720) compared to females 0.40% (119/29,099) and age wise highest prevalence was observed in 31-40 years followed by 41-50 years which was comparable with Sood et al. [14]. In a study on HIV seroprevalence in a hospital-based population in Secunderabad (Andhra Pradesh), 4.3% males and 2% females tested positive for HIV and the highest seroprevalence was reported in males and females of age group 21–30 years [26].

In the screening method, when anti HCV was found to be reactive, it was confirmed by one more method of testing with chemiluminescent microparticle immunoassay (CMIA)/ enzyme linked immuno fluorescence assay. A sample which was tested reactive in two different methods was reported as reactive for anti-HCV.

In this study, the HCV prevalence of 1.21% was observed. This seroprevalence is lower than the 1.7% seroprevalence reported in an earlier study from Jaipur (Rajasthan) in 2007 [16]. In India, the seroprevalence of HCV varies among hospital-based populations with 1.57% reported from Cuttack

(Orissa) [17], 4.8% from Pondicherry [18], and 2.46% from Jodhpur (Rajasthan) [19].

Geographical variation in the seroprevalence of HCV has also been documented [20]. The seroprevalence of HCV among males and females was 1.29% (512/39,441) and 1.07% (232/21,631) respectively. In the present study seropositivity was higher in 51-60 years followed by 41-50 years and 61-70 years. Similar observations were made by Sood et al [14]. However, Ramarokoto et al., in their study on the seroprevalence of hepatitis C in urban areas of Madagascar reported that the prevalence did not differ significantly according to the gender but it increased with age [25].

Conclusion

Our study gives us the observed rates of the blood borne transmissible viruses among the patients attending to a tertiary care hospital and the importance of screening the viral markers. This study serves a basis of knowledge for the possibility of viral transmission to the community as well as health care workers (HCW). This emphasizes all that HCWs should adhere to universal precautions, including the appropriate use of hand washing, protective barriers, and care in the use and disposal of needles and other sharp instruments and should also comply with current guidelines for disinfection and sterilization of reusable devices used in invasive procedures.

Acknowledgements

We would like to acknowledge KIMS hospital for giving us the opportunity to carry out the study.

Conflict of Interest

The authors declare no conflict of interest.

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