Special article on viral hepatitis 2015

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INTRODUCTION

Viral hepatitis caused by hepatitis viruses A through E remains a major public health problem in India. Hepatitis viruses B and C (HBV and HCV) have the potential to cause persistent infection in a subset of those infected, which may progress to cirrhosis or hepatocellular carcinoma. Hepatitis viruses A and E (HAV and HEV) are important causes of acute viral hepatitis and acute liver failure in India and have led to several outbreaks.[1]

Diagnosis of HBV and HCV infections is a key tool to identify acute and chronic cases of infection to determine the severity of the disease, to define preventive measures and to initiate antiviral treatment.[2] It is vital in monitoring the response to therapeutic interventions in HCV infection.

Detection of HBV and HCV infections and diagnosis is mainly based on immunological assays among which enzyme-linked immunosorbent assay (ELISA) and rapid tests are the most commonly used methods. For proper diagnosis of infection as well as disease management and prevention, identification of appropriate test kit is necessary. Hence, a need was felt to compile, share and analyse the data on viral hepatitis and the methods and kits used in the diagnosis.

Data for a special article on viral hepatitis for the year 2015 sent from 12 centres in Kerala were analysed [Table 1]. No data was received from centres outside Kerala for comparison and analysis.

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The total number of samples tested for serodiagnosis of viral hepatitis (A, B, C and E) during the year 2015 in the different centres varied from 3695 to 70,970. In two centres, total number of samples was <10,000; three had samples from 10,000 to 20,000 and the remaining seven had samples above 20,000 [Tables 2 and 3].

The percentage of samples positive for viral hepatitis (A, B, C and E) ranged from 0.1% to 1.7% except for one centre (Centre no. 1), which had a positivity rate of 4.5% [Figure 1]. This could be because the sample load was less and the samples were all from clinically suspected cases of hepatitis [Figure 1].

HEPATITIS B VIRUS

The percentage of samples positive for hepatitis B surface antigen (HBsAg) ranged from 0.4% to 1.3% except for Centre no. 5 (2.7%) [Figure 2]. It could probably be due to larger percentage of patients on haemodialysis in this group. This compares well with point prevalence of 3.7% in India.[1]

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HBsAg ELISA was done at all 12 centres. Different centres have utilised ELISA kits from varied manufacturers.

The various ELISA kits used to detect HBsAg included Hepalisa J. Mitra, ERBA Lisa Transasia and Microscreen Span Diagnostics (three centres); VIDAS ELFA Biomerieux, SD, Merilisa Meril Diagnostics (two centres); Qualisa, Monolisa HBsAg Ultra Bio-Rad, Ortho Clinical Diagnostics Vitros (one centre each) [Table 4].

Immunochromatographic (ICT) kits were used by eight centres.

Card/ICT kits used to detect HBsAg were Hepacard, J. Mitra (four centres), Hepaview and QualPro (four centres) and SD Bioline, ECI ES card and Crystal Span Diagnostics (one centre each) [Table 4].

Three centres have used ICT kits from two different manufacturers during this period.

Polymerase chain reaction for hepatitis B virus DNA

Quantitative HBV polymerase chain reaction (PCR) was carried out on samples from chronic HBsAg-positive patients in two centres. Out of 32 tested, two (37.5%) were positive in Centre no. 5 and out of 415, 232 (56%) were positive in Centre no. 8. Geno-sense HBV quantitative PCR kit (Genome Diagnostics, New Delhi, India) was used in Centre No. 8. Centre No. 5 and

Table 1: Centres which participated in the study

Name of the centres
Government Medical College, Kozhikode
Pushpagiri Institute of Medical Sciences and Research Centre, Thiruvalla
Government Medical College, Thrissur
Jubilee Mission Hospital and Research Institute, Thrissur
Government Medical College, Thiruvananthapuram
Government T.D. Medical College, Alappuzha
SCTIMST, Trivandrum
Amala Institute of Medical Sciences, Thrissur
Government Medical College, Kottayam
KMCT Medical College, Manassery, Mukkam, Kozhikode
Believers Church Medical College Hospital, Thiruvalla
Baby Memorial Hospital, Kozhikode

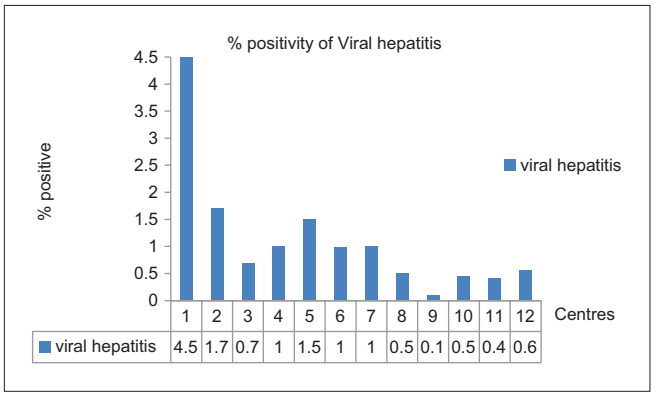


Figure 1: Percentage positivity of serum samples tested for hepatitis viruses (hepatitis B surface antigen, anti-hepatitis C virus antibody, hepatitis A virus, IgM, hepatitis E virus, IgM)

Table 2: Serodiagnosis of viral hepatitis and analysis of samples for diagnosis of Hepatitis B

	Centres											
	1	2	3	4	5	6	7	8	9	10	11	12
Total number of samples tested for serodiagnosis of viral hepatitis (A, B, C, E) during the year 2015	3695	4983	10,427	20,955	12,194	36,061	25,356	70,970	56,490	29,873	67,925	43,833
Total positives (A + B + C + E)	167	86	70	224	183	322	258	347	550	134	283	244
Total positives %	4.50	1.70	0.70	1	1.50	0.90	1	0.50	0.10	0.45	0.42	0.56
Analysis of samples for diagnosis of hepatitis B												
Total samples tested	2235	4920	4878	13,360	6333	18,371	14,788	35,953	30,955	9971	37,219	20,963
Total positives	22	60	29	175	173	170	84	153	202	101	212	81
Total positives %	1	1.20	0.60	1.30	2.70	0.90	0.60	0.40	0.70	1	0.60	0.40
Card test : Number of samples tested	ND	4612	4499	200	ND	170	70	33,487	22,814	ND	212	ND
Card test: Number of samples detected positive	ND	44	23	160	ND	145	53	153	195	ND	212	ND
Card test: Number of samples detected positive %	ND	1	0.50	80	ND	85.30	75.70	0.50	0.90	ND	100	ND
ELISA: Number of samples tested	2235	308	379	20,955	ELFA 6333	18,371	14,788	29,987	8141	ELFA 9971	37,219	20,963
ELISA: Number of samples detected positive	22	16	6	175	ELFA 173	170	84	153	7	ELFA 101	212	81
ELISA: Number of samples detected positive %	1	5.20	1.60	0.80	2.70	0.90	0.60	0.50	0.09	ELFA 1.01	0.60	0.39
Quantitative PCR (HBsAg positives)	NA	NA	NA	NA	12 (37.5%)	NA	NA	232 (56%)	NA	NA	NA	NA
Total samples tested	ND	ND	ND	ND	32	ND	ND	415	ND	ND	ND	ND

ELISA: Enzyme-linked immunosorbent assay; ELFA: Enzyme-linked fluorescent assay; ND: Not done; NA: Not applicable; PCR: Polymerase chain reaction; HBsAg: Hepatitis B surface antigen

Table 3: Analysis of samples for diagnosis of Hepatitis C, E and A

	Centres											
	1	2	3	4	5	6	7	8	9	10	11	12
Analysis of samples for diagnosis of hepatitis C												
Total samples tested	1102	4754	5475	7286	5525	17,478	10,080	34,663	25,032	9925	30,573	22,194
Total positives	14	7	21	29	37	140	86	69	43	33	47	22
Total positives %	1.30	0.15	0.40	0.40	0.70	0.80	0.90	0.20	0.20	0.30	0.20	0.10
Card test: Number of samples tested	ND	4504	5475	100	ND	ND	76	29,487	16,891	126	47	ND
Card test: Number of samples detected positive	ND	4	21	20	ND	ND	22	69	43	33	47	ND
Card test: Number of samples detected positive %	ND	0.09	0.40	20	ND	ND	29	0.20	0.30	26.20	100	ND
ELISA: Number of samples tested	1102	250	ND	7286	5525	17,478	10,080	34,663	8141	ELFA 9925	30,573	22,194
ELISA: Number of samples detected positive	14	3	ND	29	37	140	86	69	Nil	ELFA 33	47	22
ELISA: Number of samples detected positive %	1.27	1.20	ND	0.40	0.67	0.80	0.85	0.20	Nil	0.33	0.15	
Quantitative PCR (hepatitis C virus positives)	NA	NA	NA	NA	4 (26.6%)	NA	NA	41 (50%)	NA	NA	NA	NA
Total samples tested	Nil	ND	ND	ND	15	ND	ND	82	ND	ND	ND	ND
Analysis of samples for diagnosis of hepatitis E												
Total samples tested	20#	ND	ND	85	80	ND	204	26	ND	ND	23*	233
Total positives	1	ND	ND	10	4	ND	6	Nil	ND	ND	Nil	33
Total positives %	5	ND	ND	11.80	5	ND	3	Nil	ND	ND	Nil	14.20
Analysis of samples for diagnosis of hepatitis A												
Total samples tested	338	63	74	224	256	212	284	328	503	ND	110*	443
Total positives	130	19	20	10	69	12	82	125	305	ND	24	108
Total positives %	39%	30	27	4.50	27	5.70	29	38	61	ND	22	24.40

*Samples less as kits not available, #Not done after 13/7/15. ND: Not done; NA: Not applicable; ELISA: Enzyme-linked immunosorbent assay; ELFA: Enzyme-linked fluorescent assay; PCR: Polymerase chain reaction

No. 8 seem to be specialised for viral hepatitis diagnosis [Table 2].

HEPATITIS C VIRUS

The percentage of samples detected positive for anti-HCV antibody ranged from 0.1% to 1.3% [Figure 2]. This is in keeping with Indian data of 1% population prevalence.[1]

ELISAs were used by all centres.

The various ELISA kits used to detect anti HCV antibody:

Microlisa, J. Mitra, ERBA Transasia and SD (three centres); Monolisa plus version 2 Bio-Rad, Ortho Clinical diagnostics Vitros by one centre each [Table 4].

ICT kits were used by eight centres. For anti-HCV antibody detection, some of the centres have utilised enzyme-linked fluorescent (ELFA) kits from different manufacturers. VIDAS ELFA Biomerieux and Architect ELFA were the most common [Table 4].

Card/Immunochromatographic kits that were used to detect anti-hepatitis C virus antibody

HCV Tridot, J. Mitra and Signal Immunodot Span Diagnostics (two centres).

The other kits were used by only one centre each were SD Bioline, ECI OCD card and Flavi Screen Rapid QualPro Tulip [Table 4].

Polymerase chain reaction for hepatitis C virus RNA

Quantitative HCV PCR was carried out only in two centres to differentiate recent/past infection, to decide on initiation of treatment and to evaluate response to treatment. Positivity in Centre no. 5 was 4 of 15 (26.6%) and 41 out of 82 (50%) in Centre No. 8 [Table 3].

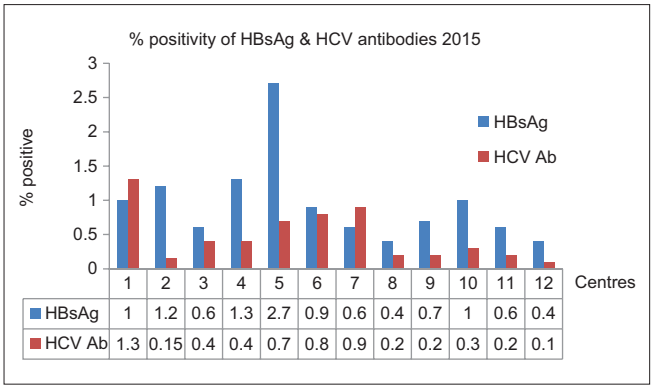
HEPATITIS E VIRUS

The percentage of samples positive for anti-HEV IgM ranged from 3% to 14.2% in various centres. As per the available data, HEV IgM positivity varies from 10.54% in Mangalore[3] to 41.8% in Kolkata [Figure 3].[4]

Table 4: Details of kits used

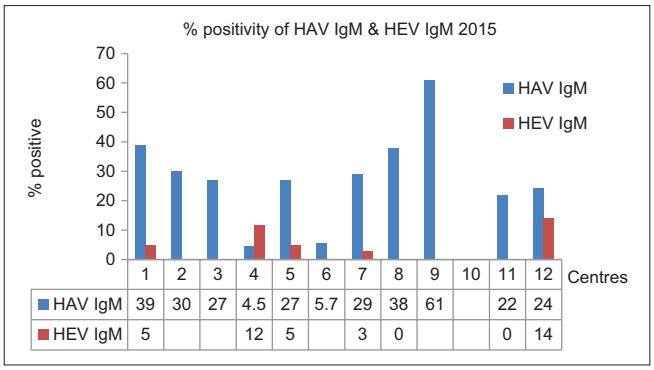
	Centres											
	1	2	3	4	5	6	7	8	9	10	11	12
HBsAg card kits used	NA	Hepacard J. Mitra	SD Bioline ICT	Hepaview Hepacard J. Mitra	NA	Hepaview crystal	Hepacard	Hepaview Hepacard	ECI HBsAg ES card test	NA	QualPro Hepaview	NA
HBsAg ELISA kits used	Hepalisa J. Mitra	Hepalisa J. Mitra	Qualisa	Hepalisa J. Mitra	VIDAS HBsAg ultra ELFA Biomerieux	ERBA Microscreen	OCD Vitros	SD, Meril Diagnostics, Span Diagnostics, Microscreen, ERBA	Monolisa HBsAg ultra Bio-Rad	VIDAS	Microscreen Span Diagnostics	ERBA Merilisa SD
HCV-Ab card kits used	NA	HCV Tridot Mitra	Signal immunodot	Bioline SD	NA	NA	HCV Tridot	Signal	ECI OCD card test		Flavi Screen Rapid QualPro	NA
HCV-Ab ELISA kits used	Microlisa J. Mitra		ND	Microlisa J. Mitra	VIDAS ELFA Biomerieux	ERBA SD	OCD Vitros	SD	Monolisa Plus version 2 Bio-Rad	Architect	ERBA SD	SD, ERBA, Microlisa
HEV IgM kits used	Wantai Bio-pharm	NA	NA	DSI	DiaPro	NA	Amar Immuno Diagnostics	DSI	NA	ND	Rapid Tulip	ELISA, DSI-EIA
HAV IgM kits used	Wantai Bio-pharm	ELFA-VIDAS Biomerieux	Insight	DSI and Wantai	VIDAS HAV IgM ELFA Biomerieux	Rapid CTK Biotech Recombilisa	DS-EIA-Recombilisa	DSI	ECi Dxl Beckman Coulter	NA	Insight rapid ELISA CTK	Recombilisa

HBsAg: Hepatitis B surface antigen; HCV: Hepatitis C virus; HEV: Hepatitis E virus; HAV: Hepatitis A virus; ELISA: Enzyme-linked immunosorbent assay; ELFA: Enzyme-linked fluorescent assay; OCD: Ortho clinical diagnostics



	1	2	3	4	5	6	7	8	9	10	11	12
HBsAg	1	1.2	0.6	1.3	2.7	0.9	0.6	0.4	0.7	1	0.6	0.4
HCV Ab	1.3	0.15	0.4	0.4	0.7	0.8	0.9	0.2	0.2	0.3	0.2	0.1

Figure 2: Percentage positivity of serum samples tested for hepatitis B surface antigen and hepatitis C virus antibodies



	1	2	3	4	5	6	7	8	9	10	11	12
HAV IgM	39	30	27	4.5	27	5.7	29	38	61		22	24
HEV IgM	5			12	5		3	0			0	14

Figure 3: Percentage positivity of serum samples tested for hepatitis A virus IgM and hepatitis E virus IgM

The ELISA kits used to detect anti-HEV IgM antibody were Wantai Bio-pharm ELISA, DiaPro ELISA, DSI ELISA, Amar Immunodiagnostics ELISA [Table 4].

Card/ICT kit used was INSIGHT Rapid Tulip [Table 4].

HEPATITIS A VIRUS

The percentage of samples positive for anti-HAV IgM ranged from 22% to 39% in most centres. However, it was 61% at one centre (No. 9); and 4.5% and 5.7% at two centres (Centre no. 4 and no. 6, respectively) [Figure 3].

The high percentage of positivity in some of these centres was probably due to the occurrence of an outbreak during this period. HAV IgM positivity varies from 12.76% in Puducherry[5] to 19.31% in Mangalore.[3]

Card/ICT kits used were Rapid CTK Biotech Recombilisa, Insight Rapid HAV IgM [Table 4].

The ELISA kits used were Wantai, Insight, ELFA VIDAS Biomerieux, DSI, Recombilisa, CTK (each by two centres), ECI Beckman Coulter and DS EIA (one centre each) [Table 4].

In some centres, the serum samples which tested positive by ELISA kits were further confirmed by Card/ICT kits for the same parameter. This explains the high rate of positivity with card tests in these centres. However, in other centres, those tested by ELISA kits are probably different from those tested by the card/ICT kits for the respective parameter; in some centres, they are a subset. Hence, analysis could not be done whether there was agreement between the ELISA and card results for a particular parameter.

CONCLUSION

Rapid card/ICTs are simple without much instrumentation requiring minimum training and can be carried out at room temperature. The latest rapid tests using synthetic antigens have increased specificity, a high positive predictive value and less false negatives. Although ELISA kits have a high degree of sensitivity, they are expensive and time-consuming. Therefore, commercially available ELISA kits are good for screening but rapid tests may be used for further confirmation. Improvement of sensitivity of the rapid kit will help laboratories without an ELISA/CLIA set-up.

An evaluation of some HBsAg and HCV rapid and ELISA kits was carried out by the National Reference Laboratory at National Institute of Cholera and Enteric Diseases, Indian Council of Medical research, Kolkata. The HBsAg rapid kits evaluated were Hepacard, J. Mitra, Crystal Span and SD Bioline, all of which were recommended. The HCV rapid kits evaluated included HCV Comb, J Mitra, Signal Ver 2.0 Span, and SD Bioline, of which only the latter two were recommended. The HBsAg ELISA kits evaluated were Microscreen Span, Hepalisa J Mitra and ERBA Lisa Transasia, of which the latter two were recommended. The HCV kits tested were HCV Microlisa J Mitra, Innova Span and ERBA Lisa Transasia. However, only ERBA Lisa Transasia has been recommended.[6]

Evaluation of kits used on a regular basis will help ensure availability of quality commercial kits and reliable reporting.

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Conflicts of interest
There are no conflicts of interest.

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