COMPARISON OF SD ICT KIT AND ARCHITECT FOR THE SCREENING OF HEPATITIS B VIRUS

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ABSTRACT

Objective of study: To compare ICT HBsAg with Architect i1000 for the diagnosis of HBsAg.

Material & Methods: The study was carried out at RMI laboratory from healthy blood donors from 2nd May 2017 till 1st July 2017.After taking history and excluding already known patients of any chronic illness and high risk people such as sex workers and I/V drug abusers, 257 samples were collected for the study. The samples were then tested on both & SD HBsAg ICT kit and architect.The result were analyzed on using SPSS version 15.0.

Results: Out of 257 donors 97.7% were males and 2.3% were females. B+ve was the blood group in the highest frequency according to our study followed by O+ve,A+Ve&AB+ve. Architect showed that 10 donors i.e 3.9% were positive for HBsAg.Whereas HBsAg ICT showed reactivity of 7 people that is 2.7% so, it missed three cases which is positive in Architect. And the sensitivity came out to be 70%.

Conclusion: The sensitivity of the best available SDICT kit in Peshawar used by most of the laboratories is very less according to our study. So, missing out a large numbers of positive hepatitis B cases. Therefore although they can be used as a screening test. But should be confirmed by a more sensitive method as EIA or NAAT.

Key words: HBsAg, ELISA, ARCHITECT, ICT, TTI's

INTRODUCTION

Hepatitis B is estimated to have a prevalence of three hundred million people worldwide. [1]Hepatitis B virus is a DNA virus belonging to the hepadnaviridae family.[2]There are eight genotypes of hepatitis B virus with different geographical distribution.[3] The infectious type of hepatitis B virion has a double shell, is spherical and forty two nm in diameter. It is composed of an envelope havingHBsAg which encircles nucleocapsid which is hepatis B core antigen (HBcAg), the enzyme RTPolymerase and DNA of the virus. [4]Depending upon the heterogenecity of Hepatitis B surface antigen there are four major subtypes adw, ayw, adr and ayr.[5] The hepatitis B core antigen is on the surface of core particles and does not circulate in the blood. Its only an integral part of the virus .the sequence of core antigen and hepatitis B envelope antigen has quite similarities. DNA of HBV is partially double stranded with a size of 3.2 (kb) pairs. [1]

Polymerase is a protein present in the virus having three domains. The first domain also known as terminal protein domain participates in ecapsidation and has a role in minus strand synthesis. The second domain is the RT domain which plays arole in synthesizing RNA from DNA and the last H domain which plays its role in modifying RNA and promotes replication. [6]

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The replication process of Hep B virus has been studied in great detail. [7].it involves entry of virus into the hepatocyte leaving out the envelope, through carboxypeptidase D receptor. [8]. After uncoating the viral genome enters the nucleus and the gaps in the viral DNA are repaired by the Pol protein and viral DNA is then converted into covalently closed circular ccc DNA [1]thiscccDNA is then transcribed into several types of RNAs and it is this cccDNA which has a role in persistence of hepatitis B virus infection.[9].The RNAs of different sizes are formed and then translated into different structures as well as functional proteins. [7].

Hepatitis B virus infection can result in acute hepatitis with complete recovery, non progressive chronic hepatitis, progressive chronic disease culminating in cirrhosis and fulminant hepatitis with massive necrosis. It can also result in asyptomatic carrier state. [10](robins). The chronic liver disease induced by hepatitis B virus is an important cause of hepatocellular carcinoma. [11]. Incubation period of HBV has an average of two to three months followed by a short prodromal period with flu like symptoms. It is during this preicteric phase that serum ALT levels rise and HBsAg and HBV DNA can be detected in serum. This is followed by the icteric phase which can last for an average period of two weeks. This is followed by convalescence during which icterus disappears but flu like symptoms may persist even till months. It is during this phase that HBsAg declines HBV DNA disappears and Hepatitis B surface antibody starts appearing in serum(12). Chronic Hepatitis B has a somewhat different course. HBeAg, HBsAg and HBV DNA are usually high during early infection. However with the passage of time the disease may either persist

with high levels of HBeAg and HBV DNA or there may be very low levels of HBeAg and HBV DNA. [13]. The outcome of patientswith chronic Hepatitis Bvirus infection is directly connected to the intensity of infection. And in even in severe cases five year survival rate is fifty percent. [14]. Rarely patients with chronic hepatitis can get acute manifestations with very high aminotransferases.[15].Prevalence of hepatitis B throughout the world is variableranging from approximately 8% in Africa and Asia to round about 2% in Australia, Northern America and Western Europe.(16). Frequent studies have been done to find out the frequencies of hepatitis B virus serological positivity in healthy blood donors in Pakistan. for example a study at railway hospiatal Rawalpindi the percentage of HBV was found to be 5.86% from March 2001 to May 2002. [16,17]. Another study carried out in LRH Peshawar from healthy blood donors showed the percentage of HBsAgseropositivity to be 2.68%. [18] According to another study carried out at RMI from January 2008 to December 2014 showed the frequency of Hep B seropositivity to be 961 out of 41003 healthy blood donors and a percentage of 2.3%. [19]

AIM OF THE STIUDY

Aim of the study is to compare ICT SD HBsAg device with Architect i 1000 for the diagnosis of Hepatitis B virus.

MATERIAL AND METHODS

Study design

It is a descriptive, cross sectional study. Carried out at RMI laboratory blood donors. From 2nd May, 2017 till 1st of July 2017.

All the samples counting for blood donation after taking history were studies and those already having a history of HBV, HCV, HIV or any other serious illness were excluded from the study. High risk people such as sex workers and in drug abusers were also excluded from study. After taking history and excluding samples based on exclusion criteria the samples were there pasted on both SD HBsAg ICT kit and architect.

Sample size

A total of 257 samples were included in the study and the sample size was calculated using the estimated prevalence of Hepatitis B infection in Pakistan.

Statistical analysis was carried out using SPSS version 15.0

A)Rapid method

SD BIOLINE HBsAg

It is an immuno chromatographic single step test used for detection of HBsAg in serum or plasma. SD BiolineHBsAg is used in many laboratories as a diagnostic test whereas the literature of kit suggests that it should be used only as a screening test and positive samples should be confirmed by a superior method [R20]

Principle

The principle of test is that the test device has a membrane strip coated with monoclonal anti-HBS Antibody at the band region. The serum or plasma moves along the membrane by chromatography to test region. And combines with the monoclonal anti HBs colloid to form a complex which forms a line if the sample is positive for the virus.

B) Architect system HBsAg Qualitative II

The architect HBsAg qualitative II is a chemiluminescent microparticle immunoassay (CMIA), intended to detect HBsAg in human serum /plasma. As it a qualitative test it can be used for screening of HBV infection in apparently healthy donors. It is a one-step immunoassay using CMIA immunotechnology using flexible protocols also known as chemiflex. An architect HBsAg Qualitative II assay, the anti-HBs coated paramagnetic micro particles and anti-HBs acridinium labelled conjugate are combined to create a reaction mixture with the sample. If HBsAg is present in the sample it binds to anti-HBs coated microparticles and to anti-HBs acridinium labelled conjugates.

After washing ancillary washed buffer is added to the reaction mixture following another wash cycle. Pre trigger and trigger solutions are added to the reaction mixture and reactive chemi luminescent reaction is measured as relative light units (RLUs)

The presence or absence of HBsAg can be found out by comparing the chemi luminescent signal in the reaction to the cut off signal obtained by active calibration.

RESULTS HBV ICT AND ARCHITECT

Table 1 Frequency distribution according to age. Table 2 Frequency distribution according to gender. Table 3 Frequency distribution according to blood groups. Table 4 Frequency of positive patients according to Architect.Table 5 Frequency of positive patients according to IC. Table 6Cross tabulation of positive patients according to Architect and ICT. Table 7Correlation and p value. Table 8Correlation and p value.

DISCUSSION

Out of the total 257 subjects in the study, ten subjects ie 3.7 % were positive for HBsAg according to Architect and seven subjects were reactive according to the SD ICT kit. So according to our study there is a significant difference between Architect when compared with ICT results. P value is found less than 0.000. Similar studies have been done throughout the world. A study carried out at department of hematology

Age in years

	Frequency	Percent	Valid Pervent	Cumulative Percent
Valid 0-10	4	1.6	1.6	1.6
11-20	36	14.0	14.0	15.6
21-30	76	29.6	29.6	45.1
31-40	44	17.1	17.1	62.3
41-50	26	10.1	10.1	72.4
51 and above	71	27.6	27.6	100.0
Total	257	100.0	100.0	

Gender

	Frequency	Percent	Valid Pervent	Cumulative Percent
Valid Male	251	97.7	97.7	97.7
Female	6	2.3	2.3	100
Total	257	100.0	100.0	

blood groups

	Frequency	Percent	Valid Pervent	Cumulative Percent
Valid B+ive	77	30.0	30.0	30.
B-ive	9	3.5	3.5	33.3
O+ive	72	28.0	28.0	61.5
O-ive	19	7.4	7.4	68.9
A+ive	55	21.4	21.4	90.3
A-ive	3	1.2	1.2	91.4
AB+ive	21	8.2	8.2	99.6
AB-ive	1	.4	.4	100.0
Total	257	100.0	100.0	

HBs'Ag Architect

	Frequency	Percent	Valid Pervent	Cumulative Percent
Valid Positive	10	3.9	3.9	3.9
Negative	247	96.1	96.1	100.0
Total	257	100.0	100.0	

HBSAg ICT

	Frequency	Percent	Valid Pervent	Cumulative Percent
Valid reactive	7	2.7	2.7	2.7
non reactive	250	97.3	97.3	100.0
Total	257	100.0	100.0	

HBSag ICT * HBs'Ag Architect Cross tabulation

			HBs'Ag Architect		Total
			Positive	Negative	Positive
HBSag ICT	reactive	Count	7	0	7
		% within HBs'Ag Architect	70.0%	.0%	2.7%
	non reactive	Count	3	247	250
		% within HBs'Ag Architect	30.0%	100.0%	97.3%
Total		Count	10	247	257
		% within HBs'Ag Architect	100.0%	100.0%	100.0%

Correlations

		HBSag ICT	HBs'Ag Architect
HBSag ICT	Pearson Correlation	1	.832(**)
	Sig. (2-tailed)		.000
	N	257	257
HBs'Ag Architect	Pearson Correlation	.832(**)	1
	Sig. (2-tailed)	.000	
	N	257	257

** Correlation is significant at the 0.01 level (2-tailed).

OlabisiOnabanjo University Nigeria in which ICT was compared with ELISA.5.7% out of the total 660 subjects were positive for HBsAg on ICT where as 10.8% were positive for HBsAg using ELISA.[21]

In another study carried out in Uganda rapid ICT strips for HBsAg, most commonly used in sub-Sahara Africa were compared with EIA and then even more advanced Nucleic Acid Amplification test.According to the study out of the total 102 patients 8% were positive for HBsAg by ICT 13% were positive by EIA. Whereas when patients were tested by NAT HBV DNA was positive in 54% of patients.[22]

A similar cross sectional study comparing SD ELISA, Rapid ICT VikiaHBsAg, Acumen HBsAg and Determine HBsAg was done in Yaounde-Cameroon

A total of 360 random blood donors were selected out of 360 blood donors 180 were positive by SD ELISA HBsAg,179 were positive by VikiaHBsAg,177 were positive by Determine HBsAg and 176 were positive by ACUMEN HBsAg.[23]

Another study carried out in India compared "HEPACARD" a rapid one step immunochromatographic assay with third generation EIA. (Enzyme immunoassay). The sensitivity of HEPACHARD was 79% as compared to the EIA assay.[24]

A study carried out in Pakistan compared rapid ICT test with fourth generation ELISA. ICT kits used were Membrane Canada, Nobis Germany and Acon USA.Which showed sensitivities of 89%, 86% and 93% respectively.[25]

Keeping in view this situation and the facilities in all primary and secondary health care units in Pakistan, these simple one step ICT kits are being used, due to their easy use and cheaper cost and this caters for 70% to 80% of the population. This is an alarming situation as it can be seen by all these studies that one step ICT kits are not 100 % sensitive for detecting HBsAg. So it can be concluded that it cannot be used as an initial screening test, professional blood donation centers should use more advanced techniques as EIA or NAAT in order to prevent these transfusion transmitted infections.

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