ANTI-HCV ANTIBODIES DETECTION-A COMPARISON BETWEEN METHODOLOGY: ELISA VS DIPSTICK ASSAY

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Hepatitis C accounts for 70-95% cases of post-transfusion hepatitis in different parts of the world. The high prevalence of antibodies to hepatitis C virus (HCV) among frequently blood transfused patients (e.g. thalassaemic), those who receive blood products (e.g. haemophiliacs), and among intravenous drug abusers confirmed the parenteral route as the major route of HCV infection. To promote screening of blood donors for HCV antibodies, a Dipstick HCV assay was compared with third generation enzyme immunoassay. Fifty diagnosed cases of hepatitis C and twenty healthy control subjects were analyzed by both methods and hundred per cent same results were found.

INTRODUCTION

Chronic hepatitis and cirrhosis of the liver are common problems in Pakistan[1]. Formerly viral hepatitis was divided into hepatitis A, hepatitis B and hepatitis non-A, non-B. non-A. non-B hepatitis (NANBH) was first recognized in 1974 and it is the commonest type of hepatitis in Pakistan[3]. The causative agent for the great majority of cases of NANBH was discovered in 1989 and named hepatitis C virus (HCV)[4], which is a single stranded, enveloped RNA virus with a diameter of <50 nm[5]. It is well established now that HCV is the major etiological agent of parenterally transmitted non-A, non-B hepatitis (PTNANBH) and it has a worldwide distribution[6].

It has been reported that hepatitis C accounts for 70-95% cases of post-transfusion hepatitis in the western countries'. In the United States alone, it affects an estimated 200,000 to 300.000 people annually and caused chronic liver disease (CLD) in approximately 50% of post transfusion cases[8]. In Pakistan, the detection of anti-HCV has been reported to be between 20-75% of patients of CLD. whereas these antibodies are present in 43% of chronic hepatitis cases, 18% of cirrhosis and 61% patients of hepatocellular carcinoma (HCC)[9].

Hepatitis C virus infection, once a diagnosis of 'exclusion' based on the absence of markers of injections with hepatitis A virus (HAV) or hepatitis B virus (HBV) can now specifically be diagnosed by using serodiagnostic assays for virus-specific antibodies[11]'. A first generation anti-HCV enzyme-linked immunosorbent assay (ELISA) was developed by using recombinant Cl00-3 (derived from NS3/NS4 region of the genome) and antigen". The test, however, had some major drawbacks which included failure to discover all patients with HCV infection[1-] and long "window phase" before seroconversion[1']. A high rate of false positive reactions was also observed[14].

To circumvent the drawbacks of anti-HCV Cl00-3 test, recombinant or synthetic antigens derived from the other regions of HCV genome were included in the test and this is referred to as second generation anti-HCV ELISA test[11]. The additional antigens were C22 and C33[15] The second-generation test shortened the "window phase" to seroconversion and increased the sensitivity in diagnosing HCV infection, with a dramatic reduction in the number of false-positive reactions seen with the first generation tests[11]. Recently, third generation anti-HCV tests, which additionally detect 'non-structural region 5' (NS5) antibodies, have been developed. These third generation ELISA tests are more sensitive than second generation tests[16].

Several immunoblot assays have been developed, which unlike ELISA, detect separate antibody reactivities to several HCV antigens [16-17]. The "DIPSTICK HCV" test is a dot immunoassay designed for the rapid detection of anti-HCV antibodies. The test utilizes HCV core-derived synthetic peptides CP 9 (aa39-40) to capture anti-HCV antibodies in serum of plasma[18-19].

As performance of tests by an ELISA technique is time consuming and instruments needed are costly, therefore it is very difficult to perform individual tests of the donors for screening of anti-HCV antibodies in their blood before transfusion by ELISA method. On the other hand, the Dipstick test in easy to perform and is also cost effective. It does not need any instrument to perform the assay. The following study was undertaken to observe and compare the results for

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the detection of anti-HCV antibodies by Dipstick and ELISA third generation methods, with a view to promote screening for anti-HCV antibodies of blood donors, by Dipstick method.

MATERIALS AND METHODS

Fifty already diagnosed patients of chronic hepatitis C disease who had their anti-HCV test positive for the last six months or more were included in this study. Their relevant clinical details were recorded on a Performa. Ten ml blood was collected aseptically by a disposable syringe from all cases and sera separated by centrifugation were freezed in aliquots until analyzed for anti- HCV antibodies by the two different methods. Twenty age and sex matched normal healthy subjects were also included in the study as control group and dealt with in the same way as patients group. During performing anti-HCV test, both by Elisa and Dipstick methods, negative and positive controls w-ere included in batches and a cut off value calculated in ELISA method.

RESULTS

The mean age of the patients of chronic hepatitis C was 43.3±l 1.2 with a range of 24-73 years. Among them 28 (56%) were males and 22 (44%) females. The mean age of control subjects was 42.1±10.4 years that ranged from 22-70 years. Out of 20 control subjects 13 (65%) were males and 7 (35%) females.

The commonest complaints at the time of presentation were fatigue and general weakness (in 74% of patients) followed by abdominal discomfort, flatulence and pain in right hypochondrium.

The results of the anti-HCV antibodies detection were amazing. All the known patients of chronic hepatitis C (n=50) were detected positive for presence of IICV antibodies by both the methods i.e. ELISA III and Dipstick methods. In all the cases, the patient's titer was more than the cut off value determined in performing ELISA tests. On the other hand, a clear-cut pink dot was observed in all the cases during performing Dipstick method. Among normal healthy control subjects (n=20) no case was detected positive by any of the two methods except one which was the same very case detected positive by the both methods as shown in Table I.

Table-1: Comparison of Results of HCV Antibodies Detection by Two Methods

Method	Group A(n=50)		Group 13 (n=20)	
	Positive	Negative	Positive	Negative
ELISA III	50	-	1*	19
Dipstick Assay	50	-	I*	

DISCUSSION

At present, the most sensitive indicator of active HCV replication is reverse polymerase chain reaction (PCR) for viral RNA[20]. Using PCR assay, the data obtained shows that viremia can be detected within only a few days of exposure to the virus and many weeks before elevation of transaminases and antibody titres[-1]. However, the PCR assay is still neither universally standardized nor easily available in most of the developing countries and it cannot replace anti HCV ELISA screening test on a daily basis[11].

Third generation enzyme immunoassay is much more sensitive and specific then the first and second generation assays, which detected antibody to the non-structural antigen Cl 00-3 of the hepatitis C virus in case of first and C22 and 33 antigens in case of second generation assays respectively [(16-20)].

ELISA tests are time consuming and require costly instruments and it is very difficult to perform individual screening tests of blood donors before transfusion in emergency cases. The "DIPSTICK PICV" test is a dot immunoassay designed for the detection of anti-HCV antibodies which utilizes HCV case derived synthetic peptides CP9 and CP 14 to capture anti-HCV antibodies in serum or plasma[18-19].

The present study compares the efficacy for detecting anti-HCV antibodies by third generation enzyme immunoassay and Dipstick method. The kits used for this purpose were HCV antibody EIA by DRG Company Germany and Dipstick HCV by Immuno Chemical Lab Co. Ltd. Bangkok, Thailand respectively.

We found hundred per cent correlation in this regard. All the fifty known cases of chronic hepatitis C were detected positive by ELISA third generation as well as by Dipstick assay. On the other hand, among the twenty normal healthy control subjects, 19 cases were proved to be negative by both assays whereas only one and the same subject was detected positive by both of the methods. The tests were also repeated for confirmation of the results.

We conclude from the present study that the results obtained by the Dipstick HCV assay are equivalent to those obtained by third generation ELISA test, as far as detection of HCV antibodies are concerned, and we recommend that each and every blood donor should be screened to rule out the presence of HCV antibodies before transfusion by "Dipstick HCV Assay" as it takes only few minutes and does not require any special equipment.

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