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Epidemiological, serological and molecular analysis of hepatitis C virus infection in different risk groups

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ABSTRACT

Hepatitis C is an infectious disease of liver caused by the hepatitis C virus (HCV). This study was to examine the epidemiological, serological and molecular analysis of hepatitis C virus infection in different risk groups. Fifty patients were selected in which thirty two were females and eighteen were males. All patients were tested by different methods such as immunochromatographic method, ELISA and PCR. Among them, 23 females (71%) & 9 males (50%) were HCV infected. It was also determined that patients at the age of 31-40 years of age group had high risk of HCV infection.
Keywords: HCV Infections, Epidemiological, Serological, Molecular Analysis.

INTRODUCTION

Hepatitis is an inflammation of the liver, most commonly caused by a viral infection. There are five main types of hepatitis viruse, referred to as types A, B, C, D and E (Wasley & Alter, 2000). Hepatitis C is an infectious disease affecting the liver, caused by the hepatitis C virus (HCV) (Sherris, 2004). The infection is often asymptomatic, however, once established, chronic infection can progress to scarring of the liver (fibrosis). Acute hepatitis C refers to HCV infection during the first 6 months (Kamal., 2008). Symptoms of acute hepatitis C infection include decreased appetite, fatigue, abdominal pain, jaundice, itching, and flu-like symptoms (Caruntu & Benea, 2006). Chronic hepatitis C is defined as infection with the hepatitis C virus persisting for more than six months. Generalized signs and symptoms associated with chronic hepatitis C include fatigue, flu-like symptoms, joint pains, itching, sleep disturbances, appetite changes, nausea and depression. It is estimated that Hepatitis C has infected nearly 200 million people worldwide, and infects 3 -4 million more people per year. It is currently a leading cause of cirrhosis, a common cause of hepato-cellular carcinoma, and as a result of these conditions, it is the leading reason for liver transplantation. The Hepatitis C Virus particle consists of core of genetic material (RNA), surrounded by an icosahedral protective shell of protein which is further encased in a lipid (fatty) envelope of cellular origin. Two viral envelope glycoprotiens E1 and E2 are embedded in the lipid envelope (DeBeeck & Dubuisson, 2003).

The viral genomic RNA is single- stranded, approximately 9379 nucleotides and is further divided into the core, envelope and along with at least four nonstructural (NS) proteins. This property of the virus particularly indicates its relationship to flaviviridae (Family of HCV). Structural proteins are coded by

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the 5'-end, and nonstructural proteins coded by the 3'-end of RNA (Miyamoto H, 1992).

MATERIALS & METHODS

Collection of Blood Sample

Blood samples from 50 patients were taken by using sterilized syringes and transferred to a vial containing EDTA which prevents clotting. The blood samples were centrifuged for 5 minutes, and then the serum was separated.

Three techniques were applied on HCV infected patients, such as Immunochromatographic, ELISA and PCR.

Immunochromatographic Method was a qualitative, membrane based immunoassay for the detection of antibody to HCV in serum or plasma (Wilber, 1993).

ELISA is an Immunoenzymatic method in which the wells of a microplate are coated with recombinant antigens representing epitopes of HCV: core of virus, NS3, NS4, and NS5. Serum or plasma samples are added in the wells. If antibodies specific for HCV are present in the sample, they will form stable complexes with the HCV antigens on the wells (Bradle, 1983).

PCR (polymerase chain reaction) is a technique in molecular genetics that permits the analysis of any short sequence of DNA (or RNA) even in samples containing only minute quantities of DNA or RNA. PCR is used to amplify selected sections of DNA or RNA for analysis. Three major steps are involved in a PCR technique, which are repeated for 30 or 40 cycles. The main steps are denaturation, annealing and extension. These cycles are done on an automated cycler, a device which rapidly heats and cools the test tubes containing the reaction mixture (Sambrook & Russel, 2001).

RESULTS AND DISCUSSION

This study was carried out in INMOL hospital during December 2009 to July 2010. Clinical features of patients were shown in table 1. Out of 50 patients, 32 were females and 18 were males.

The study indicated that, 32 females and 18 males who were studied, among them 71% females and 50% males have HCV infection was checked by Immunochromatographic method, ELISA and by PCR. Females were more infected with HCV infection (Fig. 1). In previous report, Bakr *et al*, (2006) reported that females were more infected with HCV compared with males (44.6% vs. 33.7%) respectively. These results correlated with our present study that females were more infected with HCV than males.

In present study the age group-2 (30-40 years) had high risk of HCV infection (12%) as showed in table 2. When these results was correlated with the previous reports of USA and Australia, most of the HCV infection cases are between 30-49 years old people due to rise in liver enzyme such as rise in ALT AST, SGPT and SGOT which cause HCV infection (Dienstag& Isselbacher , 2005).

Table 1: Clinical features of Patients.

Characteristics	N	(%)
Total Patients:	50	
Females	32	64
Males	18	36
Age:		
10-29 years	14	28
30-40 years	18	36
41-65 years	18	36
Median age	37.5	
Range	10-65	
ALT Level: (50 lU/ml)		
High	7	14
Normal	9	18
Median value	209.5	
Range	16-403	
ELISA:		
Positive	45	90
Negative	5	10

Characteristics	N	(%)
Anti-HCV:		
Positive	32	64
Negative	18	36
PCR:		
Positive	26	52
Negative	24	48
Symptoms of patients:		
Lower limb pain	1	2
Loss of appetite	19	38
Weakness	4	8
Headache	5	10
Mild fever	4	8
Dizziness	2	4
Vomiting	5	10
Nausea	5	10
Fever	6	12
Jaundice	2	4

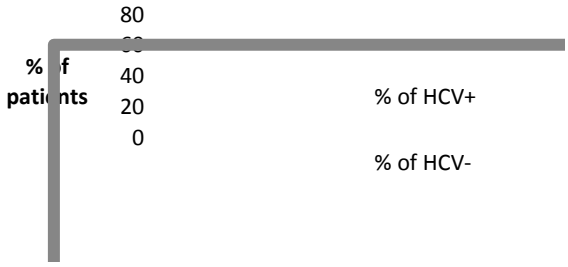

Fig., 1: Prevalence of HCV infection among females and males.

Table 2: Prevalence of HCV among different age groups.

Age groups	% of HCV infection among patients
G-1 (10-29 years)	9
G-2 (30-40 years)	12
G-3 (41-65 years)	11

Table 3: Comparison of ELISA results.

Test Results	Females (%)	Males (%)
ELISA+	90	88
ELISA-	9	11

In present study ELISA method was performed for the detection of HCV infection by using biokit. 88% males were ELISA positive while 90% females were ELISA positive. ELISA method was very sensitive method for the evaluation of HCV as shown in table 3. There was a correlation between present study and

previous report that positivity of HCV infection in patients is related to the ELISA kit results (Alter *et al.*, 2002).

In present study there was also a correlation between the PCR results and HCV infection PCR is a very accurate method to find out anti-HCV. In present study 19 females and 7 males were HCV positive by using PCR method. PCR positive mean HCV positive and HCV positive mean rise in ALT level in the liver of infected patients. PCR positive results were shown in Fig., 2.

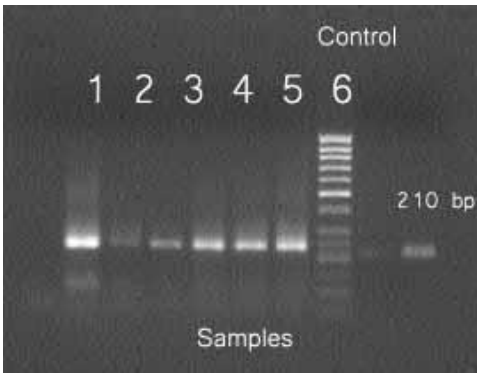

Fig., 2: Presence of 210 bp shown as amplified product

Results of the present study resemble with previous report showing that there was a correlation between PCR positive and HCV positive. Huma *et al.*, (2003) reported that 50 patients were HCV positive, 21 of whom had raised ALT,16 were PCR positivity 42% showing raised ALT indicates the PCR positive results. Screening was also done on chronic liver patients. Out of 45 patients, 44% were also PCR positive as showed in table 4.These results are being supported by the reports, which stated that almost 45% of patients with HCV were chronically infected, detected by PCR method (Stanley *et al.*, 1996).

Conclusion

Hepatitis C was studied in INMOL hospital. 50 patients were analyzed out of which 23 females and 9 males were HCV infected. It was observed that females were more infected with HCV as compared to the male. Most of patients had normal ALT level with HCV infection and some had elevated ALT level (due to injury in liver) with HCV infection. Among different age groups, groups-2 (30-40 years) was more infected with HCV. HCV screening was done for the detection of HCV infection. Immunochromatographic, ELISA and PCR method were applied for the detection of HCV infection among patients. PCR was applied on 50 patients, among them 45 were chronically infected and 44% were PCR positive patients mean presence of chronic HCV infection. Therefore, it was concluded that PCR was found to be very specific and sensitive method to evaluate the presence of HCV infection.

Table 4: HCV Screening among chronic Liver patient.

Sr. No	AGE	ALT	ALT↑/ ALT↓	HCV Screen	Anti-HCV	PCR +	PCR -
1	17	18		+IVE	+IVE		-IVE
2	24	44	H	+IVE	+IVE	+IVE	
3	24	48	H	+IVE	-IVE		-IVE
4	24	230	H	-IVE	+IVE	+IVE	
5	25	25	H	-IVE	+IVE		-IVE
6	26	16		+IVE	+IVE	+IVE	

Table 4: continued…

7	26	17		-IVE	-IVE	+IVE	
8	27	74	N	+IVE	-IVE		-IVE
9	28	53	N	-IVE	+IVE		-IVE
10	28	78		+IVE	+IVE		-IVE
11	30	70		-IVE	+IVE	+IVE	
12	33	72		+IVE	-IVE		-IVE
13	35	206	N	-IVE	+IVE	+IVE	
14	35	36		+IVE	+IVE	+IVE	
15	36	25		-IVE	-IVE		-IVE
16	36	40		+IVE	+IVE	+IVE	
17	38	24		-IVE	+IVE	+IVE	
18	40	62		+IVE	+IVE	+IVE	
19	40	50	N	+IVE	+IVE	+IVE	
20	40	75		+IVE	+IVE	+IVE	
21	44	95		+IVE	+IVE	+IVE	
22	45	43		+IVE	+IVE	+IVE	
23	45	255	N	-IVE	-IVE	+IVE	
24	45	72		-IVE	-IVE		-IVE
25	46	150	N	+IVE	+IVE		-IVE
26	55	28		-IVE	-IVE	+IVE	
27	55	162	N	+IVE	+IVE		-IVE
28	55	50	N	-IVE	-IVE		-IVE
29	56	29	N	+IVE	+IVE		-IVE
30	57	101		+IVE	+IVE	+IVE	
31	57	36		+IVE	+IVE	+IVE	
32	65	36		-IVE	+IVE	+IVE	
33	18	42		+IVE	-IVE		-IVE
34	21	403		-IVE	+IVE	+IVE	
35	27	29		+IVE	-IVE		-IVE
36	28	38		-IVE	+IVE	+IVE	
37	30	137	H	+IVE	-IVE		-IVE
38	32	87		+IVE	+IVE	+IVE	
39	33	45		+IVE	+IVE		-IVE
40	35	46	H	+IVE	+IVE		-IVE
41	36	25		+IVE	-IVE		-IVE
42	38	137		-IVE	-IVE		-IVE
43	40	93		-IVE	-IVE	+IVE	
44	40	33	H	+IVE	+IVE	+IVE	
45	45	72		+IVE	-IVE	+IVE	
46	45	34		IVE	-IVE	+IVE	
47	50	38		-IVE	-IVE		-IVE
48	53	88		+IVE	+IVE		-IVE
49	54	70		-IVE	+IVE		-IVE
50	55	70		+IVE	+IVE		-IVE

N: Normal (Below 50 lU/ml); **H:** High (Above 50 lU//ML);

REFERENCES

Alter, M.J. Hutin, Y.J. & Armestrong, G.L., 2002. Epidemiology of hepatitis C. In: Liang, TL. Hoofnagle, JH. eds. Biomedical Research Reports of Hepatitis C. *1st Ed. New York: Academic Press*. pp:169-183.

Bakr, I., Rekacewicz, C., Hosseiny, M., Ismai, l. S., Daly, M., Kafrawy, S., Esmat, G., Hamid, M.A. & Mohamed, M.K., 2006. Higher clearance of hepatitis C virus infection in females compared with males. *J. Gast.,* **55:** 1183-1187.

Bradle, D.W., Maynard, J.E., Popper, H., Cook, E.H., Ebert, J.W., McCaustland, K.A., Schable, C.A. & Fields, H.A., 1983. Post transfusion Non-A, Non-B hepatitis: Physiochemical properties of two distinct agents. *J. infects. Dis*., **148**(**2**): 254-265.

Caruntu, FA. & Benea, L., 2006. Acute hepatitis C virus infection: Diagnosis, Pathogenesis, treatment. *J. Gastrointestinal and Liver Disease,* **15**(**3**): 249-256.

DeBeeck, A. & Dubuisson J., 2003. Topology of Hepatitis C virus envelope glycoprotein. *Rev. Med. Virol.,* **13** (**4**): 233-241.

Dienstag, J.L. & Isselbacher, K.J., 2005. Chronic hepatitis. In: Kasper, DL. Braunwald, E. Fauci, AS. eds. Harrison's Principles of Internal Medicine. *16th Ed. New York: Mc Graw-Hill.* 1844-1855.

Huma, Q. Ambreen, A. Waquaruddin, A. & Ejaz, A S., 2003. HCV exposure in spouses of the index cases. *J. Pak. Med. Association*. **102**(**2**): 79-85.

Kamal, R., 2008. Acute hepatitis C a Sysmatic Review. *Am. J. Gastroenterology,* **103**(**5**), 1283-97.

Miyamoto, H., 1992. Extraordinary low density of Hepatitis C virus estimated by Sucrose density gradient centrifugation and the polymerase chain reaction. *J. G. Virol.*, **73**: 715-718.

Sambrook, J. & Russel, D.W., 2001. Molecular cloning a laboratory manual. *3rd Ed. Cold Spring Harbour Labs, 2342-2344.*

Sherris, 2004. Medical Microbiology. 4th Ed. *McGraw Hill*. pp:551-552.

Stanley, A.J., Haydon, G.H., Piris, J., Jarvis, L.M. & Hayes, P.C., 1996. Assessment of liver histology in patients with hepatitis C and normal transaminase levels. *Eur. J. Gastroenterology Hepatol*., **8**: 869-872.

Wasley, A. & Alter, M., 2000. Geographic difference and temporal trends, Semin Liver Dis. *J. Epidmol hepatitis C*., **20** (**1**): 1-16.

Wilber, J. C., 1993. Development and use of laboratory test for hepatitis C infection: a review. *J. Clin. Immunoassay,* **16:** 204.- 210