Original Article

Viral Load Among the Sera of Iraqi Hepatitis C Virus Patients

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Fac Med Baghdad 2007; Vol.49, No.4
Received Sept. 2006
Accepted Oct .2007

Summary:

Background:
Hepatitis C Virus is the main causative agent of hepatitis among blood transfused patients, in which most chronic cases result in liver carcinoma.

Materials & Methods:
Enzyme-Linked Immunosorbent Assay for HCV, FNF- and IL-12p40 estimation with Recombinant Immunoblotting Assay (RIBA) as a confirmatory test for HCV, have been applied for HCV detection in 80 HCV patients' samples in comparison with 30 samples for apparently healthy control, while viral load has been estimated using Branched-DNA (b-DNA) technique for 32 randomly selected positive cases for HCV. Liver function test has been applied for patients' sera in comparison with control.

Results & Conclusions:
This study reveals highly specificity & sensitivity of ELISA technique for HCV detection which results in 100% positivity by RIBA methods. Moreover, Viral load estimation shows that (71.9%) of HCV sera samples with viral load >615 IU /ml and only (28.1%) with viral load <615 IU/ml with highly significant difference between them (P = 0.013). Furthermore, there is a highly significant variations between liver function test in comparison with control group (P< 0.01), while SGPT is the only parameter which significantly affected by viral load (P= 0.01 1). Beside that, neither INF- nor IL-12p40 level has been affected by viral loads more or less 6151U /ml. It was concluded that ELISA technique is still the best accurate reliable method for viral detection and SGPT is a good marker for highly viral loaded samples.

Introduction:

Viral hepatitis is a systemic disease primarily involving the liver and is caused by many viral agents which are etiologically, immunologically and epidemiologically distinct [1].

Hepatitis C was first recognized in early 1970, after the discovery of hepatitis A virus (HAV) and hepatitis B virus (HBV). It was noticed at that time that most cases of transfusion-associated hepatitis were not caused by either these viruses, this virus was called "non A non B" [2].

In 1989, the responsible virus for most transfusion-associated non A-non B hepatitis was identified through molecular biological techniques and Hepatitis C virus (HCV) was cloned [3, 4].

Hepatitis C is an enveloped virus, with a diameter of about 50-60 nm, positive sense, single stranded RNA virus [5, 6]. The HCV genotype is an intrinsic characteristic of the transmitted HCV strains(s) and does not change during the course of the infection. HCV genotypes from six clades or types (number from 1-6) and are themselves subdivided into number of subclades or subtypes identified by lower case letter (1a, 1b, 1c, … etc) [7].

Probably as many as 70%-90% of infected people fail to clear the virus during the acute-phase of the disease and become chronic carrier [8, 9].

Chronic hepatitis C is highly heterogenous and many patients present with mild form of

liver disease. However, progression of liver fibrosis can be observed at long-term (>5-7 years) follow-up particularly in those cases with elevated and/or fluctuating transaminase level [10]. In 1990 it was become possible to screen for HCV infection with antibody test or direct amplification assays for the nucleic acid [i.e. reverse transcription polymerase chain reaction (RT-PCR)] HCV has been found to be the causative agent of most post-transfusion NANBH (PT-NANBH) [11].

A new test has recently been developed by Ortho-clinical diagnostics detect the HCV core protein (HCV core antigen) which is coded by the most conserved regions of the virus genome [12].

The assay has better sensitivity and permits the detection of HCV infection earlier than the HCV antibody screening tests and an average of only two days later than quantitative HCV-RNA detection in individual specimens. The performance of the assay correlates well with those of molecular HCV-RNA detection methods, but the lower level of detection (20,000 IU/ml) is significantly higher [13].

Materials and Methods:

Eighty Iraqi patients complained from hepatitis C were involved in this study during the period from November 2005 to August 2006, with ages ranged between (8-80) years. They were sequentially selected from cases referred to the Hepatology and Gastro-enterology Teaching Hospital in Baghdad at first presentation. Their diagnosis based upon the patients medical history, physical examination and laboratory tests which include biochemical tests (bilirubin (TSB) [Spin react, Spain], Alanine amino-transferase, (AIT), Asparate amino-transferase (AST), Alkaline Phosphatase (AIP) [Biomeriex, France; for both enzymes]; and virological testes include {Anti-HCV screening (ELISA, Rand ox, UK) and HCV confirmatory RIBA, Chiron, Irland} and detected HCV RNA by branched DNA assay (b-DNA), [Bayer Healthcare, USA].

From those patients, 60 cases were confirmed by using test by (RIBA) and 32 cases were measure for viral load by branched DNA (b-DNA) assay because no material available on all cases.

After these tests; determination of some serological markers include (IL-12p 40 and INF-) [Bio source, Europe; for both] in the sera of these patients have been performed.

Statistical Analysis:

All the results have been statistically analyzed for comparison of significance using:

A. Binomial test.
B. Krushal Wallis test.
C. Matched paired t-test for repeated measurements.
D. Pearson correlation coefficient (r) [14]

Results:

I. Some Clinical and Demographical Picture of Patients:

It is clear from Table 1 that the majority of patients are the males [55 out of 80] (i.e. 68.8%) rather than the females [25 out of 80 (i.e. 31.2%) with highly significant differences between both frequencies (P<0.001). The ratio between male to female was 2.2:1.

Table 1: Demographical Picture of HCV patients

Age groups (years)	*Patients' No.*	*Frequency*	*P value*
1-20	9	11.3	0.001
21-40	24	30	
41-60	33	41.3	
61-80	14	17.5	
Gender:	**Patients' No.**	**Frequency**	
Male	55	68.8	
Female	25	31.2	
Clinical Presentation	**Patients' No.**	**Frequency**	
Acute	24	30	
Chronic	47	58.8	
Unknown	9	11.3	
Total	80	100	

Moreover, Table 1 revealed the frequency of patient's distribution of among different age groups. According to this table, the majority of patients are at the age between 41-60 years (41.3%), while children elicit less frequency of infection (11.3%). There are highly significant differences between the incidences of the different age groups (P<0.01). The chronicity of HCV is very important finding which determines the treatment plane of the disease. Table 4.2 reveals the frequency of different clinical presentations of the disease among 80 patients whom they were studied. It was observed that 47 patients had undergone chronic HCV infection (58.8%) and only 24 patients were at acute stage of the disease (30%). Only 9 patients were miss-diagnosed (11.3%) if the are at acute or chronic stage, it may be explained that they were in between or at the transitional stage of the disease. There was a highly significant differences between acute and chronic frequencies (P<0.001).

II. Methods for HCV Detection:

There are several methods for HCV diagnosis. The first step is the screening method by using ELISA technique for detection of anti-HCV antibody. All the sera samples of the 80 hepatitis patients were found to be positive for this test.

Table 2: Distribution of Chronic HCV patients according to Anti-HCV Screen Test, RIBA Test and Viral Load by (b-DNA) assay (IU/ml) results

Anti-RNA HCV	RIBA* No.=60	b-DNA No.=32	No. of patients (%)	P value
No. of (+)ve = 80	60 (100%)	> 615 IU/ml	23 (71.9)	0.013#
No. of (-)ve = 0	0 (0.0%)	< 615 IU/ml	9 (28.1)	
Total = 80 (100)	60 (100)	32	32 (100	

* out of 80 randomly selected 60 samples and from those 60 only 32 samples tested for viral load.
# P value for comparison of difference between the frequencies of less and more than 615 IU/ml.

Another advanced method as a confirmative test such as Immuno-blotting (RIBA) has been applied. All these specimens gave positive results. These findings reflected highly sensitivity and specificity of ELISA technique detection of HCV infection as a screening accurate method while branched-DNA (b-DNA) assay method applied for studying HCV viral load. The last method applied on randomly selected samples from chronic HCV. It was observed that 23 out of 32 had > 615 IU/ml (71.9%) and only nine had viral load < 615 IU/ml (28.1%) with significant difference between them (P=0.013) as shown in Table 4.3. All the control groups, samples gave negative results for all the above tests.

III. The Correlation Between Liver Function and Viral Load:

The correlation between liver function tests and viral load had been listed in table 3. This table revealed that there were significant differences in TSB, S.GPT and S.GOT for those with less or more than 615 IU/ml viral load in comparison with control group.

Serum GPT was observed to be the single marker elicits significant difference between samples with < 615 IU/ml viral load and those with > 615 IU/ml viral load. This means that S.GPT is an important inverse indicator for the viral load.

Table 3: Correlation of liver function with viral load among HCV patients and apparently healthy control

Studied Groups	Liver Function Tests			
	TSB (mmol/l)*	**S.GPT (IU/ml)**	**S.GOT (IU/ml)**	**S.ALP (IU/ml)**
< 615 IU/ml No.=9	128.83 ± 44.14	119.33 ± 74.27	48.00 ± 24.37	104.83 ± 44.03
> 615 IU/ml No.=23	130.94 ± 61.85	74.47 ± 37.71	55.12 ± 20.77	85.12 ± 41.18

Control Group No.=30	9.5 ± 1.73	9.25 ± 2.31	9.40 ± 2.95	66.20 ± 13.36
P value vs Control	0.001	0.001	0.001	0.013
P value < 615 vs > 615 IU/ml	0.917	0.011	0.35	0.198

* Mean ± SD [Standard Deviation]

IV. Correlation Between HCV Viral Load and Some Cytokines:

A. The Correlation between INF-γ and viral load:

The association between INF- and viral load had been listed in table 4.8. This table showed that there was a highly significant association between the samples with viral load > 615 IU/ml and that for sample with < 615 IU/ml in comparison with control group.

Table 4: Association between INF- level and viral load among HCV patients

Studied Group	Number	Mean ± SD	P value
Control	30	1.585 ± 0.24	-
< 615 IU/ml	9	0.324	-
> 615 IU/ml	23	0.3322 ± 0.256	0.001
Total	62	<615 IU/ml vs. >615 IU/ml = 0.87	

SD = Standard Deviation

B. The Correlation between IL-12p40 and viral load:

The correlation between IL-12 level in the sera of HCV patients and the viral load appeared in table 4.9. This table showed that there is no significant difference between viral load less and more than 615 IU/ml and the level of IL-12 (P=0.157).

Table 5: The association between IL-12p40 level and viral load among HCV patients

Studied Group	Number	Mean ± SD	P value
Control	30	22.934 ± 7.284	-
< 615 IU/ml	9	187.004 ± 179.329	0.007
> 615 IU/ml	23	103.416 ± 97.335	0.083
Total	62	< 615 vs. > 615 IU/ml = 0.157	

SD = Standard Deviation

Discussion:

Most studied denoted to the prevalence of HCV was among men rather than women which revealed that the male:female ratio is (2:1) [15-19]. Some observed an equal ratio of males:females (1:1) in Iraq [20, 21]. The explanation for these variations may be attributed to the difference in sample's size in addition to the different time of blood collection.

Concerning the effect of age on the incidence of HCV the result of the current study is comparable to that of the others which mentioned that there was high infectivity among Iraqi patients at age range between 27-60 years (65.9%) [17] as well as for the others which was between 40-60 years (70%) [15, 22]. On the other hand, aboard one declared that the highly infected age range between 13-82 years (62%) [14]. This may be due to so many drug abusers among these communities in comparison with Iraqis. Moreover many bad costumes are prevalent among teenagers such as tattooing [23], besides intra-familial sharing razors, tooth brushes which enhances the disease transmission [24].

The current study revealed that (58.8%) of HCV cases are chronic. This frequency is lower than that of Europeans (70%) [25], though it is higher than the last report for WHO (20%) [26]. While the local studies denoted to similar frequency (58.9%) [27, 28]. These variations may be attributed to the geographical and environmental differences between Iraq and other countries.

Studies referred to an increase in the viral load of patients' sera [29], though it was observed that only (25%) of patients showed

High viral load in comparison with this study (71.9%) revealed High viremia. This variation related to the genotype of HCV particularly, genotype 3 HCV which observed to elicit lower viremia. Furthermore the difference in methods used for viral load estimation added another reason for this variation between this study which detected viral load by Taqmqn PCR and the other one [29, 30]. Moreover the cut-off value may vary according to the method and company [31-32]. Additionally, viral load was found to be varied according to the duration of the disease; highest viremia was achieved within 4-12 weeks of infection [33]. Most of Iraqi patients, who involved in this study had chronic infection and had HCV infection before months.

Although, there was no correlation between viral load and disease severity since the cytopathic effect attributed to the immune mechanisms rather than the viral pathogenicity. These findings are analogue to some extent with that of other studies which denoted that no correlation between viral load and S.GPT, S.GOT levels [34-35]. While it was well-inversely associated with the cytotoxic T-lymphocyte [36].

There was no significant difference between the levels of INF- according to the viral load of the patients' specimens. These findings were disagree to some extent with another study which referred to an inversely correlation between viral load and INF- in chronic cases of HCV [37]. Perhaps the reason was related to the disease duration and small sample size.

Obviously, serum IL-12p40 concentration inversely related to the viral load. This fact was confirmed by others [38].

References:

1. *Alter HJ, Purcell RH, Shih JW, et al., "Detection of antibody to hepatitis C virus in prospectively followed transfusion with acute and chronic non-A, non-B hepatitis." N Engl. J. Med. (1989) 321: 1494-1500.*
2. *Feinston SM, Kapikian AZ, Purcell RH, et al., "Transfusion-associated hepatitis not de to viral hepatitis type A or B". N. Engl. J. Med. (1975) 292: 767-770.*
3. *Houghton M. "Hepatitis C viruses" In: "Fields Virology", by fields BN, Knipe DM, Howley PM [Eds.] 3rd ed. Philadelphia, Lippincott Raven, (1996) PP: 1035-1058.*
4. *Purcell RH "Hepatitis C virus". In "Encyclopedia of Virology" by Webster RG, Granoff A, [eds.] London, Academic Press Ltd (1994) PP: 569-574.*
5. *Walkder CM "Hepatitis C virus." In: "Persistent viral infections"; by Ahmed R and Chen L (eds.) Chi Chester, Wiley (1999) PP: 93-115.*
6. *Ockner RK Acute Viral Hepatitis in Hepatobiliary Disease." In "Cecil Textbook of Medicine." 20th (Ed.) Published by Saunder's WB (1996) West Philadelphia, USA PP: 769.*
7. *Pawlotsky JM "Use and interpretation of virological tests for hepatitis c." Hepatology (2002) 36 (5 Suppl): S65-S73.*
8. *World Health Organization "Expanded Programme on Immunization, weekly Epidemiological Report (1997) 67: 671.*
9. *World Health Organization "Global surveillance and control of hepatitis C." Report of a WHO consultation organized in collaboration with the viral Hepatitis prevention Board, An. Werp, Belgium, J. Viral Hepatol. (1999) 6: 35-47.*
10. *Alberti A. "What's the mild chronic hepatitis C?" Hepatology (2005) 19-23.*
11. *Klaus R, Huber CS, and Kurt B "Detection of common HCV types with a third generation enzyme immunoassay" J. Hepatol (1996) 24: 3.*
12. *Reddy AK, Dakshinamurty KV and Lakshmi V. "Utility of HCV core antigen ELISA in the screening for hepatitis C virus infection in patient on hemodialysis." Ind. J. Med. Microbiol. (2006) 24: 55-57.*
13. *Berry V, Arora R, and Paul P. "Hepatitis C – Clinical outcome and diagnosis." Ind. J. K. Science (2005) July-September, 7(1): 129-32.*
14. *Sorlie, DE "Medical biostatistics and epidemiology: Examination and board review." 1st ed. Appleton and Lange, Norwalk, Connecticut (1995) PP: 47-88.*
15. *Anne F, Celona MC, Macnish P, et al "Hepatitis C in Los Angeles public hepatitis clinic: Demographic and biochemical differences associated with Race-Ethnicity." Clin. Gastroenterol. Hepatol. (2004) 2: 459-462.*
16. *Sabri HJA. "The diagnostic role of liver biopsy in grading, staging and etiology of chronic hepatitis." A thesis fellowship (2003) Iraq commission for Medical Specialization in Pathology.*
17. *Douraid KS. "Prevalence of hepatitis C virus infection in a sample of Iraqi patients with chronic liver disease." A thesis fellowship (1995). Iraqi commission for Medical Specialization in Medicine.*
18. *Shigenobu K Osamu Y, Tatsuo K, et al., "Quantification of hepatitis C virus by taqman PCR. Comparison with amplicor Monitor Assay." Journal of Medical virology, (1999) 58: 121-126.*
19. *Derek P, Norah A, Terraul T, et al. Cost-effectiveness of Hepatocellular carcinoma surveillance in patients with hepatitis C virus-*

related cirrhosis clinical gastro-enterology and Hepatology (2005) 3: 75-84.

20. Rahi SJ. "Cytokines profile in Iraqi patients infected with HCV." M. Sc. Thesis (2003). College of Medicine / University of Baghdad.

21. Moatter T, Hussainy AS, Hamid S, Ahmed Z, & Siddiqui S. "Comparative analysis of viral titers and histologic features of Pakistani patients infected with hepatitis C virus type 3." Int. J. Infect. Dis. (2002) 6: 272-276.

22. Vandelli C, Renzo F, Braun HB, Tisminetzky S, Albrech M, et al., "Prediction of successful outcome in a randomized controlled trial of the long-term efficacy of Interferon alpha treatment for chronic hepatitis C. " J. Med. Vir. (1999) 58: 26-34.

23. Sun DX, Zhang FG, Geng YQ, et al. "Hepatitis C virus transmission by cosmetic tattooing in women." LANCET (1996) 347: 541-42.

24. Dienstag JL, "Viral hepatitis: A mini-symposium in which recognized experts critically review clinically relevant aspects of acute and chronic." Semin. Liver Dis. (1991) 11 May.

25. Lau GKK, Davis GL, Wu SpC, Gish RG, Balart LA, and Lau JYN. "Hepatic Expression of Hepatitis C virus RNA in chronic Hepatitis C: A study by in situ Reverse-Transcription Polymerase Chain Reaction." Hepatology (1996) 23(6): 1318-1323.

26. Poynard T, Yuen M-f, Ratziu V & Lai CL. "Viral hepatitis C." LANCET (2003) 362: 2095-100.

27. Al-Waysi SA. "Effectiveness of interferon-alpha and antiviral drugs in the treatment of chronic viral hepatitis (B and C) among Iraqi patients." Ph.D. thesis (2005) College of Medicine / University of Baghdad.

28. Omer AR and Mohammed DAM. "Hepatitis C virus in Iraq". Bull. End. Dis. (2001) 31(1):38.

29. Schirf DJ. "Viral load level at week 8 may be as accurate as 12-week rule for predicting failure to achieve SVR in HCV patients." Viral Load Articles, http://www.janis/ Janis and Friends Hepatitis C Support Website (2005).

30. URL: http://www.astrohep.com. "Viral load measurements in Hep C with antiviral therapy J. Viral. Hepatol. (2005) 12(5): 465.

31. Anad BS and Velez M. "Assessment of correlation between serum titers of hepatitis C virus and severity of liver disease." World J. Gastroenterol. (2004) 10(16): 2409-11.

32. Franciscus A. "The latest viral load tests." http://www.questdiagnostics.com. (2005).

33. Koziel MJ, Wong DKH, Dudley D, Houghton M, and Walker BD. "Hepatitis C virus-specific Cytolytic T lymphocyte and T helper cell responses in seronegative persons." J. Infect. Dis. (1997) 179: 859-66.

34. Henry Ford Health Organization (MHI) "Interleukin-12." Med. Help. International A Not-For-Profit Organization (1999): 1-3.

35. Zeniya M, Ohkawa Y, Takahashi H, Aizawa Y, Watanabe F, et al., "Increased serum level of interleukin-1 receptor antagonist correlates with efficacy of interferon therapy and HCV-RNA level in chronic hepatitis C." American Association for the Study of Liver Disease (1996) Annual Meeting.

36. Nainan OV, Alter MJ, Moran-Kruszon D, et al., "Hepatitis C virus genotypes and viral concentrations in participants of a general population survey in the United States." Gastroenterology (2006) 131(2); 478-84.

37. Eisen-Vandervelde AL, Waggoner SN, Yao ZQ, Cale EM, Hahn CS, and Hahn YS. "Hepatitis C virus core selectively suppresses interleukin-12 synthesis in human macrophages by interfereing with AP-1 activation." J. Boil. Chem. (2004) Oct. 15: 279(42): 43479-86.

38. Ernst A. "What is the viral load?" Deutsches Hepatitis C Forum e.V., Hepatitis Weekly (2001) 5:2.