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Assessment of Hepatitis C Virus RNA Levels in Patients with Chronic Hepatitis C Infection

تقييم مستويات الحمض النووي الريبي لفيروس التهاب الكبد الوبائي سي في المرضى المصابين بعدوى التهاب الكبد الوبائي المزمن

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Abstract.

Background Hepatitis C virus (HCV) is characterized by persistent viremia and is the leading cause of chronic liver disease worldwide and the leading indication for liver transplantation. The hallmark of the disease is its propensity to evolve into chronicity, probably because viral heterogeneity allows the virus to escape immune-mediated neutralization. **Aim**: This study aimed to assess the level of serum viral load in relation to chronic hepatitis C infection **Methods:** Thirty patients (twenty-seven males, three females, mean age 40 □ 3 years) with chronic hepatitis C infection referred from outpatient clinic of internal medicine department at Al-Gamhuria Modern Hospital. Ten Healthy volunteers, age and sex matched were included in this study as a control group. All underwent HCV serologic testing with quantitation of HCV-RNA on Cobas Amplicor analyzer. **Result:** The result of common sign observed on the patients were 14 patients (46.7.0%) with hepatosplenomegaly and 6 patients (20.0%) with hepatosplenomegaly and ascites. AST and ALT levels showed highly statistical significant elevation in patient groups when compared to control ($P < 0.001$). AST levels in HCV patients with moderate viraemia showed statistical significant elevation when compared to patients with viral load of less than 600 IU/ml ($P < 0.05$).**Conclusion:** Measurement of serum HCV RNA titer and noninvasive biochemical parameters can be a mirror to predict the severity of liver injury in chronic HCV infection.
Keywords: chronic HCV infection, HCV- RNA.

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أحمد مثنى ناصر البيشي

الملخص العربي

الخلفية: يتميز فيروس التهاب الكبد الوبائي سي بالفيروس المستمر وهو السبب الرئيسي لمرض الكبد المزمن في جميع أنحاء العالم والمؤشر الرئيسي لزرع الكبد. السمة المميزة للمرض هي ميله للتطور إلى مزمن ، ربما لأن عدم التجانس الفيروسي يسمح للفيروس بالهروب من تحييد المناعة بوساطة.

الهدف: هدفت هذه الدراسة إلى تقييم مستوى الحمل الفيروسي في المصل فيما يتعلق بطرق الإصابة بالتهاب الكبد C المزمن: ثلاثون مريضًا (سبعة وعشرون ذكورًا ، ثلاث إناث ، متوسط العمر 40 3 سنوات) مصابين بعدوى التهاب الكبد سي المزمن المحولين من العيادة الخارجية قسم الباطنة بمستشفى الجمهورية الحديث. تم تضمين عشرة متطوعين أصحاء ، العمر والجنس المتطابق في هذه الدراسة كمجموعة ضابطة. خضعت جميع الاختبارات المصلية لفيروس الكبد الوبائي سي مع تقدير كمية الحمض النووي الريبي على محلل كوباس امبيلكور.

النتيجة: كانت نتيجة العلامة المشتركة التي لوحظت على المرضى 14 مريضا (46.7.0٪) مصابين بضخم الكبد و 6 مرضى (20.0٪) يعانون من تضخم الكبد والطحال والاستسقاء. أظهرت مستويات إنويمات الكبد AST و ALT ارتفاعًا ذا دلالة إحصائية عالية في مجموعات المرضى بالمقارنة مع مجموعة التحكم (P 0.001).أظهرت مستويات AST في مرضى التهاب الكبد سي في الدم المتوسط ارتفاعاً ذا دلالة إحصائية عند مقارنتها بالمرضى الذين يعانون من حمولة فيروسية أقل من 600 وحدة دولية / مل (P 0.05).الاستنتاج: يمكن أن يكون قياس عيار الحمض النووي الريبي التهاب الكبد الوبائي سي في الدم والمعايير الكيميائية الحيوية غير الغازية مرآة للتنبؤ ب شدة إصابة الكبد في العدوى المزمنة بفيروس التهاب الكبد الوبائي.

الكلمات المفتاحيه: عدوى التهاب الكبد سي ا لمزمن ، الحمض النووي الريبي

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Introduction:

Hepatitis C virus (HCV) is a blood borne pathogen that is endemic in most parts of the world, the highest burden of disease is in the Eastern Mediterranean Region and European Region.(1) Approximately 80% patients with hepatitis C virus develop chronic infection, and progression to cirrhosis occurs in nearly 20% of these subjects. (2) HCV infection, a leading cause of cirrhosis, hepatocellular carcinoma, and liver transplantation. (3) Most infected persons are asymptomatic, and fewer than 25% with chronic HCV are aware of their status.(4) A limited understanding of asymptomatic infection has led clinicians to underappreciate HCV as an explanation for chronic liver disease.(5) Physicians may not ask patients about, or patients may hesitate to disclose, behaviors that increase risk of HCV. (6) In adults, the course of HCV infection is slowly progressive, and many individuals are asymptomatic for decades. (7) HCV has been associated with various extrahepatic manifestations such as mixed cryoglobulinemia, membranoproliferative glomerulonephritis and cutaneatarda.(8) Blood born non A non B (NANB) hepatitis was first recognized in the mid–1970, (9) but identifying the major responsible agent by conventional methods proved to be difficult, in 1989, Choo and colleagues used molecular techniques to clone a viral genome from chimpanzee that were experimentally infected with a contaminated human factor VIII concentrate. (10) The development of an immunoassay based on the detection of circulating antibodies to a recombinant epitope proved that this virus, designated hepatitis C virus (HCV), was the etiologic agent in most cases of post-transfusion NANB hepatitis. (11) HCV has been classified as the prototype of a third genus of the flaviviridae family because it has been found to be distantly related to members of the other two genera, pestiviruses (which include bovin viral diarrhea virus and veterinary pathogenus of substantial importance) and flaviviruses (i.e. viruses that include dengue and yellow fever virus). All member of this family contain a positive polarity, single – stranded Ribonucleic acid (RNA) genome. (12) HCV infection is diagnosed by the presence of antibody to HCV and/or HCV RNA,(13) screening test for HCV antibodies (anti-HCV) by methods of enzyme-linked immunosorbent assay (ELISA) and chemilum inescence immunoassay (CIA), and further confirmed by HCV RNA qualitative or quantitative testing. (14) Detection of HCV-RNA by polymerase chain reaction (PCR) is considered the gold standard to confirm active HCV infection. Anti-HCV titer with different cut-off values has been proposed to predict HCV viremia. (15) A quantitative competitive RNA polymerase chain reaction (QC-PCR) assay was developed for measuring absolute levels of hepatitis C virus HCV - RNA. Although

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demonstrated benefit from the treatment of asymptomatic HCV infection is lacking, clinical outcomes seem to be improved when therapy is initiated in younger patients and at earlier stages of disease. (16)

Objective:

General objective:

To assess the relationship between the serum viral load and liver injury in patients with chronic hepatitis C.

Specific objective:

- Study the Common presenting features in patient group.
- Observed Clinical signs of patients found on examination
- To study the Mean values of liver enzymes AST and ALT in control and patients groups.
- Investigate Liver enzymes AST, ALT in different patients groups (according to the viral load).
- To find the relationship between viral load and clinical sign.

Method:

In a hospital –based case control study, comprised thirty patients (twenty-seven males, three females, aged between 20-60 years old with mean age 40 ± 3 years) with chronic hepatitis C referred from outpatient clinic of Internal Medicine Department (Gastero-entrology Unit) AL-Gamhuria Modern Hospital – Aden governorate –Yemen during a 14month period (Novermber 2020 – December 2021).

Ten Healthy volunteers, age and sex matched were included in this study as a control group. All the participants were tested for HCV–RNA which measured on COBAS AMPLICOR Analyzer by the Cobas Amplicor HCV monitor test version 2.0 (U 2.0) supplied from Roche Diagnostics based on nucleic and amplification test for quantitation of Hepatitis C virus RNA in human serum. The quantitation of HCV viral RNA was performed using the HCV quantitation standard. Patients were included if they had a Diagnosis of chronic hepatitis C based on detection of HCV antibodies by Micro-particle Enzyme Immunoassay (MEA).General Laboratory Investigations were included and Liver function test (serum total protein, albumin,

ISSN: 2410-7727

bilirubin, ALT, AST, ALP and GGT) were done on B.M Hitachi 911 autoanalyzer. Peripheral hemogram were done on cell Dyin 3500.Kidney function tests (serum urea, creatinine) were done on B.M Hitachi 911 auto analyzer. HCV-anti bodies were performed on Aysym by Abbots based on microparticle enzyme immunoassay (MEIA)/version 3 .HBs-Ag were performed on AXSYM by Abbots based on microparticle enzyme immunoassay .

RESULTS:

This study was conducted on thirty patients with chronic HCV infection (27 male and 3 Female with mean age 40 ± 3 years)

, in addition, ten healthy person age and sex matched were included as a control group.

Table (1): Common presenting features in patient group.

Presenting feature	No.	(%)
Accidental discovering	12	40.0%
Right hypochondrial pain	11	36.7%
Fatigue	7	23.3%

The common presenting features of the studied patients 12 patients (40%) were accidentally discovered as hepatitis C liver disease patients, 11 patients (36.7%) were presented by right hypochondrial pain and 7 patients (23.3%) were presented by fatigue only as showed in table (1) .

Table (2): Clinical signs observed on examination of the patients

Signs	No.	(%)
No palpable organs	10	33.3%
Hepatosplenomegaly	14	46.7.0%
Hepatosplenomegaly with ascites	6	20.0%

ISSN: 2410-7727

The common signs observed on examination, 10 patients (33.3%) had no palpable organs, 14 patients (46.7.0%) with hepatomegaly only, 6 patients (20%) with hepatospleenomegaly and ascites.

Table (3): Mean values of liver enzymes AST and ALT in control and patients groups.

	No.	AST (I.U)	ALT (I.U)
Control group:	10		
Range		4 – 12	4 – 17
Mean ± SD		7.75 ±2.5	8.5 ±3.8
Patients group:	30		
Range		15 – 120	11 – 134
Mean SD		73.5 ±30.4	76 ±31.6
P		< 0.001	< 0.001

AST and ALT levels showed highly statistical significant elevation in patient groups when compared to control (P < 0.001) as showed I tale (3).

Table (4): Liver enzymes AST, ALT in different patients groups (according to the viral load).

Viral load / Enzyme	Less than 600 n = 8	Low n = 4	Moderate n = 11	High n = 7
AST (i.u)				
Mean ±SD	39.2±24.5	87±18.67	88±32.1	57±25.1
Range	15 – 80	50 – 88	22 – 120	22 – 88
P	0 .311	0.471	(0.05)	≤ 0.05
ALT (i.u):				
Mean ± SD	45.5 ±31.32	87±10.03	81.9±31.9	61.70±25.21
Range	11 – 102	72 – 93	31 – 134	11 – 87
P	0.053	≤ 0.05	≤0.05	≤ 0.05

ISSN: 2410-7727

Low 20.000 IU/ml **Moderate** 20.000 – 2.000.000 IU/ml
High 2.000.000 IU/ml **(Chi-squared test** ≤ 0.05 statistically significant**)**

AST levels in HCV patients moderate viraemia showed statistical

significant elevation when compared to patients with viral load of less than 600 IU/ml ($P < 0.05$).

ALT levels in low and moderate viral load HCV patients showed

significant elevation when compared to those with viral load of less than 600 IU/ml ($P < 0.05$) as showed in table (4).

Table (5): The relation between viral load and clinical sign.

	No	**Viral load**			
		Less n = 8	**Low n = 4**	**Moderate n = 11**	**High n = 7**
No palpable organs	10	5 (50%)	3 (30%)	2 (20%)	
Hepatosplenomegaly	15	3 (20. %)	1 (13.3%)	7 (40%)	4 (26.7%)
Hepatosplenomegaly with ascites	5			2 (40%)	3 (60%)

Most Patients with no palapale organ had less than 600IU/ml or low viral load and most of Hepatosplenomegaly madmoderate and high viral load,while most of Hepatosplenomegaly with ascites had high viral load as showed in table (5).

Discussion

The spectrum of liver disease in patients infected with hepatitis C virus (HCV) ranges from minimal lesions in HCV asymptomatic carriers to chronic hepatitis of variable severity, cirrhosis, and hepatocellular carcinoma (17).

The pathogenesis of HCV–induced hepatic injury remains unclear

and could be attributable to either direct cytopathic damage by HCV or to the host immune response against infected hepatocytes (18).

In the current study, we demonstrated that anti-HCV titer provided excellent diagnostic performance for HCV viremia**,** in which HCV RNA levels were estimated in 30 patients with chronic HCV infection and compared with the degree of clinical manifestation.

In this work it was observed that, 40% of HCV patients were

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Asymptomatic and 33.3% with no palpable organs had elevated serum aminotransferases and variable degree of viremia. This finding was similar to that found by Persico et al who found 38.3 of studied patients with HCV were asymptomatic (19) and Chalermra et al who estimated the prevalence of 30.1% among asymptomatic population and demonstrated that, many subjects with HCV infection have persistently normal ALT level and considered asymptomatic carrier, and most of these patients have some degree of viral load. (20)

We found that 22 of 30 patients 73.3% were presented with low,

moderate and high viral load, had significant elevation of serum aminotransferases. In these patients positive correlation was observed between levels of viraemia and serum aminotransferases activity.

These results were in agreement with Barbara et al. (21) who

found that 61.1% of their studied group, had relation between viral HCV load levels and serum aminotransferase elevation and also in agreement with that reported by Kuo et al. (22) who found a strong correlation between HCV RNA loads present in serum and level of serum aminotransferases.

Our results also consistent with those found by Kumar et al. (23) and Bhupinder and Maria ,(24) who reported a significant positive

Correlate ion between alanine aminotransferases (ALT) and high HCV

RNA.On the other hand, ninety-three Chinese patients with chronic

HCV infection were enrolled for study by Luo et al. (25), showed no significant relationship between the serum HCV RNA and serum

aminotransferase levels and Elena *et* al. (26) who found that 81.4% of their patients with considerable levels of serum HCV RNA had not associated with elevated serum aminotransferases, this may be related to that, HCV is an RNA virus and its genetic diversity is constantly, were our study depend on the most common type in Yemen, HCV genotype 4, (27) while Elena *et* al his study was based on HCV genome type 1.

Moreover, Subodh *et al.* (28) studied eighty-nine patients with different virus C genotypes; they proved no correlation between available levels

of viraemia, genotypes or serum alanine aminotransferases activity. Janice et al. (28) compared the median HCV RNA levels for patients co-infected with human immunodeficiency virus and HCV with patients infected only with HCV who were in the same age range, and they found no correlation between HCV RNA levels and AST or ALT level for either monoinfected or co-infected patients. Peignoux et al. (30) evaluated the significance of HCV RNA determination by analyzing a group of hospitalized patients with abnormal liver function tests. They found only slight

ISSN: 2410-7727

increase of serum aminotransferases above the upper limit of normal value in patients with detectable level of serum HCV RNA.

Conclusion:

Measurement of HCV concentration in serum can be a mirror of

Liver injury in chronic HCV infection and there was positive correlation of viral load and serum markers with clinical manifestation.

Recommendation:

- Application of this on a large number of patients to determine the association between viral load and grade of inflammation.
- Determination of the relationship between HCV and other factors such as cell mediated immunity, viral replication in the hepatocytes, degree of viral genetic heterogeneity and direct cytotoxicity of the virus.
- The future direction is to expand research and biopsy work.

Limitation:

- Small sample size thus, our findings need to be further validated in larger teaching hospital.
- There was minimal evidence in the included studies surrounding the impact of the viral load on activity of liver enzyme.
- No college or university funding resource helps in the advancement of research,

ISSN: 2410-7727

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ISSN: 2410-7727

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ISSN: 2410-7727

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