Distinct cytokine patterns in Occult Hepatitis C and Chronic Hepatitis C Virus Infection

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Background&Aim: The immunopathogenesis of chronic hepatitis C virus (HCV) infection is a matter of great controversy. The imbalance of T-helper lymphocyte cells cytokine production were believed to play an important pathogenic role in chronic viral hepatitis. Occult hepatitis C infection is regarded as a new entity that should be considered when diagnosing patients with a liver disease of unknown origin. The aim of this study was to determine serum T-helper 1 and T-helper 2 cytokine production in patients with occult HCV infection and its role in its pathogenesis versus chronic viral hepatitis C infection.

Methods: Serum levels of cytokines of T-helper 1 (IL-2, IFN-γ) and T-helper 2 (IL-4) were measured in 27 patients with occult HCV infection and 50 patients with chronic hepatitis C infection.

Results: The levels of the T-helper 1 cytokines, IL-2 and IFN-γ, were highly significant increased in patients with chronic HCV infection as compared with occult HCV infection ($p < 0.001$). The T-helper 2 cytokine IL-4 was highly significant increased in occult HCV infection as compared with chronic HCV infection ($p < 0.001$). Necroinflammation ($P < 0.001$) fibrosis ($P < 0.001$) and cirrhosis ($P = 0.03$) were significantly increased in chronic HCV than occult HCV.

Conclusion: Patients with occult HCV infection exhibited a distinct immunoregulatory cytokine patterns; favoring viral persistence in the liver in spit of its absence from peripheral blood and explaining the less aggressive course of this disease entity than chronic hepatitis C virus infection.

Key words: Occult HCV, Chronic hepatitis C, Cytokines.

Introduction:

 Hepatitis C virus (HCV) is a commonly encountered pathogen in medical practice. It is estimated that 2-3% of the world is affected by HCV, with a prevalence of 170 million people (3% of the world's population) and incidence of 3-4 million per year (1). Chronic HCV infection is a progressive disease that may lead to liver cirrhosis and hepatocellular carcinoma. The hallmark of chronic hepatitis C is the presence of anti-HCV and HCV RNA in serum for more than 6 months after acute infection (2, 3).

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Recently a new form of HCV infection called occult HCV infection has been described. Occult HCV infection is defined by the presence of HCV-RNA in liver cells but with undetectable anti-HCV and serum viral RNA. Patients with occult HCV infection have abnormal liver function tests and 35% of them have histological damage, including liver cirrhosis (4).

Histological evaluation of the liver biopsies of patients with occult HCV infection documented different degrees of necroinflammatory activity and fibrosis (including cirrhosis) as reported for chronic hepatitis C (5).

Patients with occult HCV infection presents a milder disease than patients with chronic hepatitis C. The low number of infected hepatocytes found in patients with occult HCV infection may be related with less liver damage. Furthermore, immune response of these patients may be fine-tuned better than that of patients with chronic hepatitis C, leading to a more effective control of the infection (6).

There is suggestive evidence that T-cell immunoregulatory cytokines may play a key role in influencing the persistence of HCV infection and the extent of liver damage (7-11). Activated CD4+ T cells can be divided into 2 subsets based on their cytokine secretion profiles (12-14). The T helper type 1 (Th1) subset produces interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α, and interleukin (IL)-2, and participates in cell-mediated immune responses (13, 14); the T helper type 2 (Th2) subset produces IL-4 and IL-10, and mediates humoral immune responses (15). The Th1/Th2 cytokine balance is likely important in determining the rate of HCV infection chronicity and HCV-induced liver injury (16,17).

The aim of this work is to study the Th1/Th2 cytokine profiles in patients with occult HCV infection versus patients with chronic hepatitis C.

Materials and Methods:

A total of 27 out of 45 untreated patients with elevated transaminases of unknown etiology were proved to be occult HCV infection (HCV-RNA in liver as detected by reverse transcriptase-polymerase chain reaction (RT-PCR) and by in situ hybridization, but anti-HCV and serum HCV-RNA were negative) were included in this study. The Cytokine patterns, biochemical and histological characteristics of these patients were compared with those of a group of 50 untreated anti-HCV and serum HCV-RNA-positive patients with histologically proved chronic hepatitis C matched to age, gender, estimated duration of abnormal liver function tests and body mass index (BMI) to avoid statistical differences that could affect the characteristics of the disease. All patients attending the outpatient clinic of Tropical Medicine Unit, Mansoura University Hospital during the period from June 2007 to December 2009.

All known causes of liver diseases were excluded on the basis of analytical, clinical, and epidemiological data: infection by HBV (i.e., subjects were negative for hepatitis B surface antigen and for serum HBV-DNA), autoimmunity, metabolic and genetic disorders, NASH, alcohol intake, and drug toxicity and all cases were negative for anti-HIV antibodies. RT PCR was performed to detect HCV RNA in hepatocytes of all patients with occult HCV.

The study was conducted following the guidelines of the 1975 Declaration of Helsinki, and all patients gave their written informed consent to participate in it.

Samples:

1. *Cytokines assay*:   From each patient two ml venous blood was collected into   clean dry plastic tube and allowed to clot, the yielded serum was used for quantitative detection of IL-2, IL-4 and IFN-γ serum levels which were measured with enzyme-linked immunosorbent assay (ELISA) kits (Bender   Med Systems, Vienna, Austria).

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2. Liver biopsy

The patients underwent an ultrasound-guided liver biopsy (using Tru-cut needles) for diagnostic purposes. Liver biopsy specimens were divided into 2 portions. One portion was fixed in 10% formalin and was paraffin embedded for routine histological diagnosis. Histological evaluation was performed by a pathologist who was blinded with respect to the HCV RNA status of the liver biopsy specimens. Necroinflammatory activity and fibrosis were scored according to the METAVIR score system (18,19). A minor (4–5mm to make an average weight of 15-20mg) fragment of the specimen, was immediately immersed in dry ice until extraction of RNA in the lab within minutes The extracted RNA was immediately reverse transcribed and stored at -20°C until used for detection of HCV RNA by RT-PCR.

2.1. Preparation of liver cell lysate for total RNA isolation

The amount of tissue was determined by weighing (all samples weighed 20mg each) according to the recommendation of NORGEN Biotek corporation (ON, Canada). Tissues were then slowly ground while still in dry ice. Then 600 µl of lysis solution was added to the tissue samples and grinding was continued until homogenization of the samples. Homogenization was insured by passing the lysate 5-10 times through a 25 gauge needle attached to a syringe. Lysate was then transferred to an RNase-free microfuge tube and spun down for 2 minutes to pellet any cell debris. Supernatant was then transferred to another RNase-free microfuge tube and an equal volume of 70% ethanol was added and vortexed for proper mixing.

2.2. Total RNA extraction and purification

Lysate (600 µl) with ethanol from the previous step was applied to the assembled column and collection tube and centrifuged for 1 minute. The flowthrough was discarded and the spin column and collection tube was reassembled and the process was repeated until the lysate finishes. After this 400 µl of wash solution was added to the column and centrifuged for one minute, the flowthrough was discarded and the process repeated twice. Then RNA was eluted by adding 50 µl of elution buffer to the column and centrifugation for a minute. The purified RNA was immediately reverse transcribed into cDNA and stored at -20°C until used for PCR.

3. Blood samples

Blood samples for separating peripheral blood mononuclear cells (PBMCs) were collected from all patients on the same day that the liver biopsy was performed. PBMC were obtained by Ficoll-Hypaque density gradient of EDTA anti-coagulated blood according to the manufacturer's instructions (Lymphoflot, Biotest, 63303 Dreleich, Germany). Cells were washed three times with Mg^{2+}- and Ca^{2+}-free PBS and resuspended to $1x10^{6}$ cell ml^{-1} in PBS.

3.1. Peripheral-blood mononuclear cell lysate for total RNA isolation

Up to 100µl of PBMCs suspension in PBS was transferred to an RNase-free microfuge tube. To them was added 350 µl of lysis solution, cells were then lysed by vortexing for 15 seconds (or until the mixture becomes clear). After this 200 µl of 95% ethanol was added to the lysate and mixed by vortexing for 10 seconds. The lysate was used for RNA extraction following the same protocol for liver cell lysate.

REVERSE TRANSCRIPTION AND NESTED PCR

The synthesis of cDNA and the two PCR rounds were performed using oligonucleotide primers from the highly conserved 5' untranslated region (UTR) of the genome; external primers [P1 (sense, -GCGACACTCCACCATAGAT-; nucleotides 10–28) and P4 (antisense, -ACTCGCAAGCACCCTATCA-; nucleotides 303–285)] for the first-round PCR and internal primers [P2 (sense, -CTGTGAGCAACTACTGTCT-; nucleotides 36–55) and P3 (antisense, -CGGTGTACTCACCGGTTCC-; nucleotides 161–143)] for the second-round PCR (20).

Amplification of the cDNA was performed using 15 μL of the cDNA solution and 50 pmol of the outer primers (P1 and P4). Thirty cycles of DNA amplification were carried out, followed by an extension step for 10 min at 72°C. Each cycle of PCR consisted of 95 °C for 45 s, 50 °C for 45 s and 72°C for 45 s. The second PCR was carried out in the same way with 5 μL of the first PCR mixture and 50 pmol of each inner primer (P2 and P3). The amplified DNA was visualized by 2% agarose gel electrophoresis and ethidium bromide staining. The size of the second product generated by PCR was 126 bp.

All PCR assays were performed according to the recommendations of Kwok and Higuchi (21).

Furthermore, several negative controls (no-RNA) were included in each PCR step, to assure the specificity of the results.

Statistical analysis:

The statistical analysis of data done by using *excel* program and *SPSS* program statistical package for social science version 10. The description of the data done in form of mean (+/-) SD for quantitative data and

frequency & proportion for qualitative data. The analysis of the data was done to test statistical significant difference between groups. For quantitative date student t-test was used to compared between 2 gp paired sample t-test was used to compare one gp at different measurements. A 2-tailed P value <0.05 was considered to denote statistical significance.

Results:

Table (1) showed characteristics of patients with occult HCV infection and those with chronic hepatitis C infection where no significant difference regarding age, gender and BMI was observed. Significant increase were found in classic HCV than occult HCV infection regarding bilirubin (P <0.001), ALT (P =0.009), AST (P =0.013), AFP (P <0.001), while serum albumin was significantly higher in occult HCV than chronic HCV (p <0.001).

Table (2) showed histological characteristics of both studied groups. Hepatic necroinflammation (P <0.001) fibrosis (P <0.001) and cirrhosis (P =0.03) were significantly increased in chronic HCV than occult HCV.

Table (3) showed the Th1/Th2 cytokine patterns of the studied patients' groups. The levels of the Th1 cytokines, IL-2 and IFN-γ, were increased in patients with chronic HCV infection versus those with occult HCV infection (p < 0.001). The Th2- cytokine, IL-4 was significantly increased in occult HCV infection versus those with chronic HCV infection (p < 0.001).

Discussion:

Several studies have suggested that T-cell immunoregulatory cytokines play a key role in both HCV viral persistence and in the extent of liver damage, while some cytokines may exert a pro-inflammatory activity, such as IFN-α, IL-8, TNF-α and IL-2, which can prime T-cells towards a Th-1 type immunity,

others have a predominantly anti-inflammatory activity, as is the case for IL-4 and IL-10, which are involved in Th-2 immunity. Some of these cytokines may have a fibrogenic (e.g., TGF-β) or an antifibrogenic (e.g., IFN-α) role (22). Some authors have suggested that a preferential shift towards either Th1 or Th2 response may influence the clinical outcome and disease progression (23-27). Patients with occult HCV infection present a milder disease than patients with chronic hepatitis C (6).

The aims of the present study were (1) to evaluate the Th1/Th2 cytokine profiles in patients with occult HCV infection compared to chronic HCV infection patients, and (b) to clarify the histological characteristics of occult HCV infection versus chronic HCV infection patients.

This study demonstrated that there was a significant increase in Th1 cytokine IL-2 and IFNγ in patients with chronic HCV infection compared to the occult HCV infection. This significant shift of cytokine pattern in chronic HCV towards Th1 pattern more than occult HCV infection may suggest that, the clinical and histological differences observed between occult and chronic hepatitis C may be a consequence of the hosts immunological system and its derived cytokines as shift of cytokine towards Th1 is associated with more progression of disease. These data are supported by the following. The expression of some cytokines is more closely related to the severity of the disease. IFN-γ is clearly augmented in the serum of chronic hepatitis C patients (28-30) and its increase has been

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correlated with an increase in the severity of disease (31). In contrast of our result Pham et al found that, PBMC from individuals with occult infection transcribed significantly greater levels of IFN-α, IFN-γ and TNF-α, but less interleukin (IL)-10 than those from CHC (32). IL-2 is considered to be a Th1 type cytokine and is involved in enhancing the proliferation and activation of most T lymphocytes, NK cells and B-lymphocytes. Liver sinusoidal and inflammatory cells have been reported to be sources of IL-2 and no consensus exists on the predictive value of this cytokine. Apparently, expression of IL-2 is associated with a more advanced stage of disease, as previously reported (33-35). Napoli et al in 1996 (23) demonstrated that, as liver injury worsens there is an increase in intrahepatic Th1-like cytokine m RNA levels. In particular there was a positive correlation between IFN- γ and IL-2 mRNA expression and the severity of both the inflammatory (portal tract) and fibrotic components. In contrast to our suggestion of the responsibility of predominance of Th1 cytokines (IFN- γ and IL-2) about the high degree of histological activity in chronic HCV than occult HCV is that of Abayli et al who found no difference in the serum levels of IFN-γ between patients with chronic HCV infection and normal controls. On the other hand, Th2 cytokines, IL-4 and IL-10, were found to be higher in patients with chronic HCV infection than in healthy controls. None of the cytokines was correlated with the histological activity score (36).

Our data show a significant increase in serum IL-4 in patients with occult HCV infection in comparison to chronic HCV infection. IL-4 is a 15- KD protein that has been shown to regulate a wide spectrum of function of B cells, monocytes/ macrophages and other non-haematopoietic cells (37). Among T cells clones. IL-4 is produced by Th0 and Th2 cells, but not by Th1, and this now been demonstrated both in mice and humans (38). Beside T cells, mast cells and basophils can produceIL-4 and mRNA for IL-4 has been found in eosinophils (39).

Increased level of Th2 cytokineIL-4 may be responsible for the decrease in IFN-γ and IL-2 production among occult HCV infection in the present study. This finding is concordant with other study (36) who found that, Th2 cytokines regulate the antibody secretion by B cells and have suppressor functions. Increased levels of Th2 cytokines, IL-4 and IL-10, may be responsible for the decrease in IFN-γ production.

In spit of less aggressive course of occult HCV and absent of HCV-RNA in peripheral blood the body immune system can not eradicate the infection. This could possibly be due to predominant Th2 IL-4 in occult HCV that is may be responsible for persistence of infection in patients with occult HCV. These could be supported by reports from other authors which proposed that the inability to terminate HCV infection may result from the inappropriate release of some T-cell and monocytemacrophage derived cytokines, such as interleukin

(IL-4) and IL10 (40, 41). These cytokines may inhibit cellular mediated antiviral responses by interfering with T cell activation and function (42 45).

 Undetectable serum HCV-RNA in case of occult HCV infection can explain the low level of Th1 cytokines in our study among occult infection than chronic HCV infection. The existence of a relationship between chronic HCV replication and Th1 predominance was further supported by the finding of marked decrease in the Th1 response in patients in whom the HCV-RNA titre dropped to undetectable levels following IFN-α abolishing the antigenic stimulus to derive a Th1 response. This could also explain the apparent contradiction that IFN- α, which has been reported to promote development of Th1 cells (46-47), induced a strong reduction of Th1 cells in vivo.

In conclusion: Occult HCV infection has predominant Th2 cytokine pattern that may responsible for viral persistent in the liver in spit of its absence from peripheral blood. Lack of Th1 cytokine response in occult HCV may explain the less aggressive course of occult HCV than chronic HCV infection.

References

1. Antonelli A, Ferri C, Galeazzi M, et al. HCV infection: pathogenesis, clinical manifestations, and therapy. 2008. Clin Exp Rheumatol. 26(1 SuppI 48):S39-47.

2. Chen, S. L., and T. R. Morgan. 2006. The natural history of hepatitis C virus (HCV) infection. Int. J. Med. Sci. 3:47–52.

3. Seeff, L. B. 2002. Natural history of chronic hepatitis C. Hepatology 36 (Suppl. 1):S35–S46.

4. Castillo I, Pardo M, Bartolome´ J, et al. 2004. Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. J Infect Dis. 189: 7–14.

5. Yano M, Kumada H, Kage M, et al. 1996. The long-term pathological evolution of chronic hepatitis C. Hepatology 23: 1334–1340.

6. Pardo, M., J. M. López-Alcorocho, E. Rodríguez-Iñigo, et al. 2007. Comparative study between occult hepatitis C virus infection and chronic hepatitis C. J Viral Hepat 14: 36-40.

7. Koziel MJ. Cytokines in viral hepatitis. Seminar Liver Dis 1999; 2:157-168.

8. Gramenzi A, Andreone P, Loggi E, et al. 2005. Cytokine profile of peripheral blood mononuclear cell from patients with different outcomes of hepatitis C virus infection. J Viral Hepat .12:525-530.

9. Kempuraj D, Donelan J, Frydas S, et al. Interleukin-28 and 29 (IL-28 and IL-29): 2004. new cytokines with anti-viral activities. Int J Immunopathol Pharmacol . 17: 103-106.

10. Petrarca C, Frydas S, Donelan J, et al. 2005. A novel pleiotropic cytokine involved in T cell differentiation and T cell response modulation. Int J Immunopathol Pharmacol. 18:191-194.

11. Huang SH, Frydas S, Conti P, et al. 2004. Interleukin-17: a revisited study. Int J Immunopathol Pharmacol. 17: 1-4.

12. Masmann TR, Cherwinski H, Bond MW, et al. 1986. Two types of murine helper T cell clone. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 136:2348-2357.

13. Gioia C, Horejsh D, Agrati C, et al. 2005.T-Cell response profiling to biological threat agents including the SARS coronavirus. Int J Immunopathol Pharmacol. 18:525-530.

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14. Lanzilli G, Falchetti R, Tricarico M, et al. 2005. In vitro effects of an immunostimulating bacterial lysate on human lymphocyte function. Int J Immunopathol Pharmacol. 18:245-254.

15. Clerici M, Shearer GM. 1994. The Th1-Th2 hypothesis of HIV infection: new insights. Immunol Today. 15:575- 581.

16. Vecchiet J, Dalessandro M, Travasi F, et al. 2003. Interleukin-4 and interferon-gamma production during HIV-1 infection and changes induced by anti-retroviral therapy. Int J Immunopathol Pharmacol. 16:157- 166.

17. Perrella A, Borgia G, Borrelli F, et al. 2005. TNF-alpha serum level elevations in chronic hepatitis C patients with diabetes mellitus. Int J Immunopathol Pharmacol. 18:189-193.

18. Bedossa P. 1994. Intraobserver and interobserver variations in liver biopsy interpretation in patients with chronic hepatitis C. METAVIR Cooperative Study Group. Hepatology. 20: 15-20.

19. Bedossa P, Poynard T. 1996. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. Hepatology. 24: 289–93.

20. McOmish F, Yap PL, Dow BC, et al. 1994. Geographical distribution of hepatitis C virus genotypes in blood donors: an international collaborative survey. J Clin Microbiol. 32: 884–92.

21. Kwok S, Higuchi R. 1989. Avoiding false positives with PCR. Nature . 339: 237–8.

22. Tilg H, Kaser A, Moschen AR. 2006. How to modulate inflammatory cytokines in liver diseases. Liver Int. 26: 1029-1039.

23. Napoli J, Bishop A, Mc Guinness PH, et al. 1996. Progressive liver injury in chronic hepatitis C infection correlates with increased intra-hepatic expression of Th1-associated cytokines. Hepatology. 24:759-765.

24. Tsai SL, Liaw YF, Chen MH, et al. 1997. Detection of type 2-like helper cells in hepatitis C virus infection: implications for hepatitis C virus chronicity. Hepatology. 25:449-457.

25. Sarih M, Bouchrit N, Bebslimane A. 2000. Different cytokine profiles of peripheral blood mononuclear cells from patients with persistent and self-limited hepatitis C virus infection. Immunol Lett. 74:117-120.

26. Sobue S, Nomura T, Ishikawa T, et al. 2001. Th1/Th2 cytokine profiles and their relationship to clinical features in patients with chronic hepatitis C virus infection. J Gastroenterol. 36:544-551.

27. Yamaki K, Uchida H, Li X, et al. 2005. Effect of varying types of anti-arthritic drugs on Th1 and Th2 immune responses in mice. Int J Immunopathol Pharmacol. 18:133-144.

28. Tilg H, Wilmer A, Vogel W, et al. 1992. Serun levels of cytokynes in chronic liver diseases. Gastroenterology. 103: 264-274.

29. Cacciarelli TV, Martinez OM, Gish RG, et al. 1996. Immunoregulatory cytokines in chronic hepatitis C virus infection: pre- and post-treatment with interferon alfa. Hepatology. 24: 6-9.

30. Kamal SM, Graham CS, He Q, et al. 2004. Kinetics of intrahepatic hepatitis C virus (HCV)-specific CD4+ T cell responser in HCV and Schistosoma mansoni coinfection: relation to progression of liver fibrosis. J Infect Dis .189: 1140-1150.

31. Gonzalez-Peralta RP, Fang JW, Davis GL, et al. 1994. Optimization for the detection of hepatitis C virus antigens in the liver. J Hepatol. 20: 143-147.

32. Pham TNQ, Mercer SE and Michalak TI. 2009. Chronic hepatitis C and persistent occult hepatitis C virus infection are characterized by distinct immune cell cytokine expression profiles. Journal of Viral Hepatitis. 16, 547–556

33. Makris M, Preston FE, Ralph S. 1994. Increased soluble IL-2 receptor levels in HCV-infected haemophiliacs: a possible indicator of liver disease severity. Br J Haematol. 87: 419-421.

34. Bozkaya H, Bozdayi AM, Aslan N, et al. Circulating IL-2 and IL-10 in chronic active hepatitis C with respect to the response to IFN treatment. Infection 2000;28: 309-313.

35. Gramenzi A, Andreone P, Loggi E, et al. 2005. Cytokine profile of peripheral blood mononuclear cells from patients with different outcomes of hepatitis C virus infection. J Viral Hepat. 12: 525-530.

36. Abayli B, Canataroğlu A, Akkiz H. 2003. Serum profile of T helper 1 and T helper 2 cytokines in patients with chronic hepatitis C virus infection. Turk J Gastroenterol. 14 (1): 7-11.

37. Paul WE. Interleukin4: 1991. A prototypic immunoregulatory lymphokine. Bllod. 77; 1859-70.

38. Romagnani S. 1992. Human Th1and Th2 subsets: Regulation of differentiation and role in protection and immunopathology.INt Arck Allergy Immunol. 98:279-85.

39. Moqbel R, ying S, Barkans J. 1995. Identification of messenger RNA for IL-4 in human eosinophilis with granule localization and relase of the translated product. J Immunol. 155:4939-47.

40. Salgame P, Abrams JS, Clayberger C et al. 1991. Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. Science. 254:279±82.

41. Mosmann TR, Subash S. 1996. The expanding universe of T cell subsets. Th1, Th2 and more. Immunol Today. 17:138-46.

42. Sher A, Gazzinelli RT, Oswald IP, et al. 1992. Role of T-cell derived cytokines in the downregulation of immune responses in parasitic and retroviral infection. Immunol Rev. 127:183-204.

43. Scott P, Kaufmann SH. 1991. The role of T-cell subsets and cytokines in the regulation of infection. Immunol Today. 12:346-8.

44. Ada GL, Blanden RV. 1994. immunity and cytokine regulation in viral infection. Res Immunol. 145:625-9.

45. Del Prete G, Maggi E, Romagnani S. 1994.Human Th1 and Th2 cells: functional properties, mechanisms of regulation, and role in diseases. Laboratory Invest. 70:299-306.

46. Brinkmann V, Geiger T, Alkan S, et al. 1993. Interferon α increases the frequency of interferon gamma-producing. Cd41 T Cells. J Exp Med. 178:1655-62.

47. Parronchi P, De Carli M, Manetti R, et al. 1992.IL-4 and IFN (alpha and gamma) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones. J Immunol. 149:2977-83.

Table (1): Characteristics of patients with occult HCV infection and those with chronic hepatitis C:

	Occult HCV (n=27)	Chronic HCV (n=50)	P-value
Age/ye	49.96±2.64	51.07±2.55	0.07
Albumin (gm/dL)	4.05±0.07	3.69±.0..25	<0.001
Bilirubin(mg/dl)	0.8±.0.15	1.2±0.29	<0.001
ALT (u/L)	54.4±14.7	62.1±10.5	0.009
AST (u/L)	50.6±13.3	57.4±9.7	0.013
AFP(ng/mL)	4.3±1.7	13.5±4.3	<0.001

Table (2): Histological characteristics of patients with occult HCV infection and Classic chronic hepatitis C.

	Occult HCV (n=27)	Chronic HCV (n=50)	P-value
Necroinflammation	10 (37%)	50 (100%)	<0.001
Fibrosis	6 (22.2%)	40 (80%)	<0.001
Cirrhosis	1 (3.7%)	11 (22%)	0.03

Table 3: Th1/Th2 cytokine patterns of occult and chronic HCV infection.

Cytokines	Occult HCV	Chronic HCV	P value
IL2 (pg/L)	102.1±6.7	193.6±15.4	< 0.001
IFNγ (pg/L)	28.6±4.2	64.8±6.76	< 0.001
IL4 (pg/L)	120.3±9.2	68.9±2.15	< 0.001