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The role of IL-28, IFN-γ, and TNF-α in predicting response to pegylated interferon/ribavirin in chronic HCV patients

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The primary goal of HCV therapy is to achieve a sustained virological response (SVR). Many host and viral factors influence the treatment response. Cytokines play an important role in the defense against viral infections, where successful treatment of hepatitis C depends on a complex balance between pro- and anti-inflammatory responses. In the present study, we investigated the relationship between the presence and percentage of some cytokines (IL-28, IFN-γ, and TNF-α) regarding different clinicopathological parameters including response to therapy in chronic HCV patients using immunohistochemical technique. This study was carried out on 64 chronic HCV patients (34 responders and 30 non-responders). Of cases, 54% showed IL-28 expression, which was associated with low AST ($p = 0.002$) and low HAI score ($p = 0.006$). Of cases, 67 and 45% showed IFN-γ and TNF-α expression, respectively, where the median percentage of TNF-α expression was higher in grade II spotty necrosis compared to grade I. Some inflammatory cytokines expressed by intrahepatic inflammatory cells in chronic HCV patients promote inflammation and injury (pro-inflammatory) such as TNF-α. Other cytokines aid in resolving inflammation and injury (anti-inflammatory) such as IL-28. The balance between these cytokines will determine the degree of inflammatory state. None of the investigated cytokines proved its clear cut role in affecting response to therapy, however, their levels varied between responders and non-responders for further investigations to clarify.

Key words: IL-28; IFNγ; TNF-α; chronic HCV; response to therapy; pegylated interferon/ribavirin; immunohistochemistry.

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About 150 million people worldwide are chronically infected with hepatitis C virus (HCV) and more than 350,000 people die every year from hepatitis C-related liver diseases (1). The primary goal of HCV therapy is to achieve a sustained virological response (SVR), in which HCV RNA remains undetectable for 24 weeks after the end of therapy. The current standard therapy is based on a combination of pegylated interferon (Peg-IFN) and ribavirin (RBV) (2). Many host and viral factors including the genotype of HCV and variation in certain genes influence the treatment response to Peg-IFN and RBV combination therapy (3).

Moreover, side effects from the therapy such as hematological abnormalities could result in dose reduction or even premature discontinuation of the treatment (2). So, it is necessary to predict an individual's response before or at an early stage of the treatment, to increase treatment success rate and avoid potential adverse events in patients who do

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not benefit from the treatment and also to reduce the cost of therapy (4).

The outcome of therapy may be influenced by a dynamic complex relationship that exists between the pharmacological characteristics of the therapeutic regimen, viral kinetics, and host immune responses (5).

Cytokines play an important role in the defense against viral infections, determining the pattern of host immune response and inhibiting viral replication (6). Both Peg-IFN and RBV have not only antiviral but also immunomodulatory properties such as alteration of immune functions and Th1/Th2 cytokine balance (7, 8). Increased Th2 and altered Th1 cytokine production have been associated with viral persistence and failure of antiviral treatment in chronic HCV patients (9, 10). Th1 cytokines such as IL-2 and interferon gamma are required for host antiviral immune responses, while Th2 cytokines (IL-4, IL-10) can inhibit the development of these effector mechanisms (11).

Cytokines are key mediators of inflammation, apoptosis, necrosis, and fibrosis, and they are actively involved in the regeneration process of liver tissue after injury. It has been hypothesized that successful treatment of hepatitis C depends on a complex balance between pro- and anti-inflammatory responses (12, 13).

In the present study, we investigated the relationship between the presence and percentage of some cytokines (IL-28, IFN-γ, and TNF-α) regarding different clinicopathological parameters including response to therapy in chronic HCV patients. IL-28, IFN-γ, and TNF-α had been evaluated by means of immunohistochemical staining.

MATERIALS AND METHODS

This retrospective study was carried out on 64 chronic HCV patients who were submitted to pretreatment liver biopsy and were eligible to receive Peg-IFN plus RBV for 48 weeks. Clinical and follow-up data were retrieved from medical records of HCV outpatient Clinic, National Liver Institute, Menofiya University. The patients were divided into two groups:

Group A: Responders to interferon therapy, who had negative HCV RNA by polymerase chain reaction (PCR) 6 months following completion of a 48-week course.

Group B: Non-responders to interferon therapy who were one of the followings:

1. Non-responders with no disappearance of HCV RNA at the end of the 12th week.
2. Partial responders at the end of 12th week, but null response at 24th week.
3. Breakthrough patients who had complete response at 12th week, but detectable HCV RNA at any time during the course of treatment (48 weeks).

Inclusion criteria used to choose chronic HCV patients were:

1. Positive for HCV only by ELIZA and PCR.
2. Liver biopsy done within 3 month before initiation of therapy.
3. Age is not less than 18 years.
4. Patients eligible to interferon therapy depending on clinical and laboratory data [ANA titer less than 1/60, normal TSH and kidney functions, no evidence of decompensated liver disease or thrombocytopenia (PLT $< 75{,}000/mm^3$), moderate to severe anemia (Hb < 10 g/dL), or neutropenia (neutrophil count $< 2000/mm^3$)].
5. Complete follow-up data at least 6 months after cessation of therapy.
6. Treatment naïve patients

All patients received combined Peg-IFN and RBV therapy (Peg-IFN2α at a dose of 180 μg once weekly plus RBV). The dose of RBV was adjusted according to body weight (less than 75 kg: 1000 mg per day, 75 kg or more: 1200 mg per day).

The following data were collected from patients' medical records and included age (years), gender, ALT, AST, alpha fetoprotein, and serum HCV RNA level by quantitative PCR, which was divided into mild (viremia <106 copies/mL), moderate (viremia 106–108 copies/mL), and severe (viremia >108 copies/mL).

Pretreatment liver biopsies were retrieved from Pathology Archives for suitable biopsy length (12–22 m) and re-evaluation for further histopathological analysis of the followings.

1. Evaluation of grade of inflammation was performed according to the criteria described by Ishak et al. (14). Grades of necroinflammatory changes (HAI grading) were grouped into three categories for statistical purposes as follows: Minimal activity score (1–3), mid activity score (4–8), and moderate/severe activity score (9–18). The degree of portal inflammation (Identified by mononuclear infiltration of portal tracts) (15) was divided into scores 0, 1,2,3,4 and for statistical purposes, scores 1 and 2 were lumped as a grade I and scores 3 and 4 as a grade II. The degree of interface hepatitis was grouped for statistical purposes, where score 1 was alone (I) and scores 2 and 3 were lumped (II) (no cases showed score 4). The degree of spotty necrosis was also grouped, where score 1 was alone (I) and scores 2 and 3 were lumped (II) (no cases showed score 4). Confluent necrosis was assigned as present or absent.
2. Staging of fibrosis was performed according to the criteria described by Ishak et al. (14). Stages of architectural changes (fibrosis Ishak score) were grouped for statistical purposes as follows: Mild portal fibrosis {scores 1 and 2}, bridging fibrosis {scores 3 and 4}, and cirrhosis {scores 5 and 6} (no cases showed cirrhosis). Degree of fibrosis was also assessed by METAVIR score (16).
3. Steatosis was graded according to the Brunt's grading system, based on percentage of involved hepatocytes in the biopsy specimen as follows: Grade 0 = none involved, grade 1 (mild) = up to 33%, grade 2 (moderate) = up to 66%, and grade 3 (severe) = more than 66% (17). These grades were grouped into three categories for statistical purposes as follows: grade 0, grade I (1, 2), and grade II (3).

4. Lymphoid aggregates, bile duct injury, and vascular changes were identified by their presence or absence.

Immunohistochemistry

Several paraffin sections, each one was 4 μm in thickness, were cut from each case, one section for hematoxylin and eosin staining and the others for immunohistochemical process. The method used for immunostaining was streptavidin–biotin amplified system. Paraffin-embedded tissue sections were deparaffinized in xylene, rehydrated in a graded series of ethanol, and then incubated with 3% hydrogen peroxide. Slides were rinsed in phosphate-buffered saline (PBS) and then exposed to heat-induced epitope retrieval in citrate buffer solution (pH 6) for 20 minutes. After cooling, the slides were incubated overnight at room temperature with rabbit polyclonal anti-interleukin-28 antibody (SIGMA, St. Louis, USA) (100 μg concentrated and diluted by PBS in a dilution 1:100), mouse monoclonal anti-IFN-γ antibody (Biomedical laboratories, Florida, USA) (100 μg concentrated and diluted by PBS in a dilution 1:100) and rabbit polyclonal anti-TNF-α (BIOTEC, Washington, USA) (1.0 mL concentrated and diluted by PBS in a dilution 1:50). Positive tissue controls were tissue containing activated T cells (active autoimmune hepatitis) for IL-28 and IFN-γ and hepatocellular carcinoma for TNF-α. Detection of immunoreactivity was carried out using the ultravision detection system, ready-to-use anti-polyvalent horseradish peroxidase/diaminobenzidine (NeoMarkers, LabVision, California, USA). Finally, the reaction was visualized by an appropriate substrate/chromogen (diaminobenzidine) reagent. Counter stain was carried out using Mayer's hematoxylin. The staining procedure included negative controls obtained by substitution of primary antibodies with phosphate-buffered saline.

Interpretation of IL-28, IFN-γ, and TNF-α immunostaining

The cases were assigned positive for IL-28, IFN-γ, and TNF-α when cytoplasmic expression was seen in lymphocytes either in portal tract or in adjacent parenchyma. The extent of immunoreactivity for each cytokine was assessed as a percentage of positivity of lymphocytes out of total infiltrate. Percentage of positivity was expressed as mean, median, and range.

Statistical analysis

Data were collected, tabulated, and statistically analyzed using a personal computer with "Statistical Package for the Social Sciences (SPSS) version 16" program. Chi square and Fisher's exact tests were used in comparison between qualitative variables, while Mann–Whitney and Kruskal–Wallis tests were used in comparison between quantitative variables. Corrected $p \leq 0.002$ was considered significant.

RESULTS

Clinical data of studied cases are presented in Table 1 and histopathological data are presented in Table 2.

Table 1. Demographic and routine laboratory data of the studied cases

	Total (n = 64)	
	n	%
Age (years)		
Mean ± SD	35.8 ± 10.0	
Median	34	
Range	19–57	
Sex		
Male	41	64.1
Female	23	35.9
Viremia		
Mild	35	54.7
Moderate	26	40.6
High	3	4.7
AST (μ/L)		
Mean ± SD	49.8 ± 39.1	
Median	38	
Range	20–256	
ALT (μ/L)		
Mean ± SD	56.5 ± 39.5	
Median	42	
Range	10.3–231	
αFP (ng/mL)		
Mean ± SD	3.6 ± 5.2	
Median	2.0	
Range	0.4–37	
Response to therapy		
Responder	34	53
Non-responder	30	47

Immunohistochemical results

IL-28 expression in studied cases – IL-28 immune positive lymphocytes were seen distributed in portal tract, interface hepatitis, and hepatic parenchyma (Fig. 1). Thirty five cases of 64 cases (54%) were positive for IL-28, 17 of them were responders (10.8%) and 18 were non-responders (11.52%). Percentage of expression ranged between 2 and 70% of inflammatory cells with a mean ± SD of 34.5 ± 21.5 and a median of 32.5.

There was no significant difference between responders and non-responders regarding IL-28 expression, however, mean and median percentage of IL-28 were higher in responders in comparison to non-responders (Table 3). Positive IL-28 expression was significantly associated with low AST level ($p = 0.002$) and low HAI score ($p = 0.002$) (Fig. 2). Furthermore, grade I interface hepatitis and absence of confluent necrosis and vascular changes were all in favor of IL-28 expression, although the significance could not be reached.

IFN-γ expression in studied cases – Forty three cases of 64 cases (67%) showed positive expression of IFN-γ in lymphocytes (Fig. 3) either in portal tract and hepatic parenchyma, 23 were responders and 20 cases were non-responders. Percentage of expression ranged between 2 and 80% of inflamma-

Table 2. Histopathological data of studied cases

	Total (n = 64) n	%
Portal tract inflammation		
I	53	82.8
II	11	17.2
Nature of infiltrate		
Lymphocytes only	28	43.8
Lymphocytes+plasma cells	17	26.6
Lymphocytes+eosinophils	19	29.7
Lymphoid aggregates		
Present	35	54.7
Absent	29	35.4
Interface hepatitis		
0	1	1.6
I	39	60.9
II	24	37.5
Spotty necrosis		
0	1	1.6
I	33	51.6
II	30	46.9
Confluent necrosis		
Present	27	42.2
Absent	37	57.8
HAI score		
Mean ± SD	5.4 ± 1.8	
Median	5	
Range	2–11	
HAI grade		
Minimal (1–3)	10	15.6
Mild (4–8)	44	68.8
Moderate (9–12)	10	15.6
Fibrosis stage (IShak)		
Mild	49	76.6
Bridging	15	23.4
Fibrosis stage (METAVIR)		
I	50	78.1
II	10	15.6
III	4	6.2
Steatosis		
Present	28	43.8
Absent	38	56.2
Steatosis grade		
0	36	56.2
I	22	34.4
II	4	6.2
III	2	3.1
Bile duct injury		
Present	19	29.7
Absent	45	70.3
Vascular changes		
Present	11	17.2
Absent	53	82.8

tory cells with a mean ± SD of 24.4 ± 22.5 and a median of 20.

There was no significant difference between responders and non-responders regarding IFN-γ expression ($p > 0.05$), however, mean and median percentage of IL-28 were higher in responders in comparison to non-responders (Table 3).

There was no significant association between IFN-γ positivity or percentage of expression and the studied parameters in all investigated cases ($p > 0.05$). However, 62.2% (33/53) of grade I portal tract inflammation showed IFN-γ expression, but the association was away of significance.

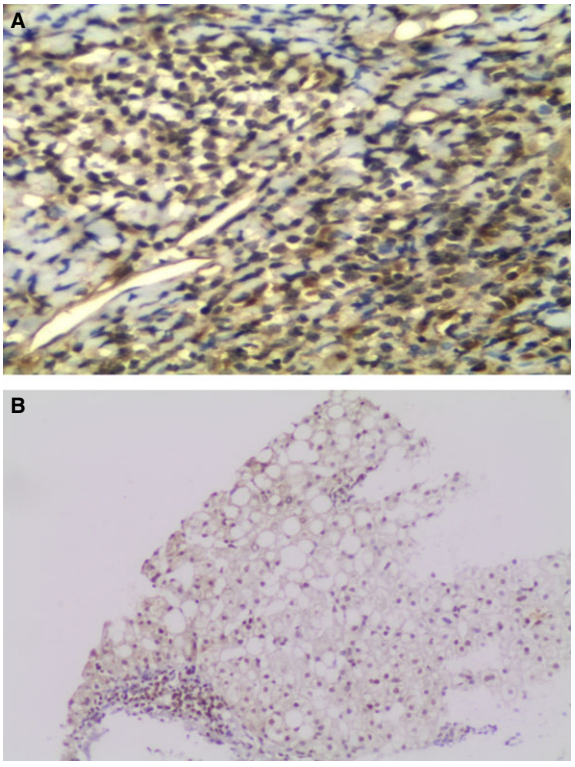

Fig. 1. IL-28 immunoreactivity in inflammatory cells of portal tract (A) with adjacent steatosis (B) in chronic HCV patient (Immunohistochemical staining x 400 in A and x 100 in B).

TNF-α expression in studied cases – TNF-α positive expression was localized to lymphocytes in portal tract, interface hepatitis, and hepatic parenchyma (Fig. 4). Twenty nine cases of 64 (45%) were positive for TNF-α, 15 were responders and 14 were non-responders (11.52%). Percentage of expression ranged between 2 and 50% of inflammatory cells with a mean ± SD of 18.1 ± 18.0 and a median of 12.5.

There was no significant difference between responders and non-responders regarding TNF-α expression ($p > 0.05$), however, the median value was higher in non-responder compared to non-responder (Table 3).

There was no significant association between TNF-α positivity or percentage of expression and the studied parameters ($p > 0.05$). However, the percentage of TNF-α was of higher median value (20%) in grade II spotty necrosis compared to grade I (5%) (Fig. 5)

Table 3. Differences between responders and non-responders with regard to IL-28, IFN-γ and TNF-α

	Cases 64		Groups: Responders (n = 34)		Non-responders (n = 30)		Test	p-value
	n	%	n	%	n	%		
IL-28								
Mean ± SD	34.5 ± 21.5		29.6 ± 25.8		24.0 ± 16.4		U = 0.08	0.943
Median	32.5		40.0		32.5			
Range	2–70		4–70		2–65			
IL-28								
Positive	35	54.3	17	50.0	18	60.0	χ^2 = 0.64	0.423
Negative	29	45.7	17	50.0	12	40.0		
IFN-γ								
Mean ± SD	24.4 ± 22.5		22.3 ± 18.9		19.0 ± 18.3		U = 0.38	0.703
Median	20		17.0		7.5			
Range	5–80		2–70		5–80			
Positive	43	67.2	23	67.6	20	23	χ^2 = 0.01	0.934
Negative	21	32.8	11	32.4	10	11		
TNF-α								
Mean ± SD	18.1 ± 18.0		15.1 ± 15.5		13.5 ± 12.4		U = 0.44	0.658
Median	12.5		10.0		15.0			
Range	2–50		2–50		4–50			
TNF-α								
Positive	29	45.7	15	44.1	14	46.7	χ^2 = 0.04	0.838
Negative	35	54.3	19	55.9	16	53.3		

χ^2 = chi square U, Mann–Whitney.

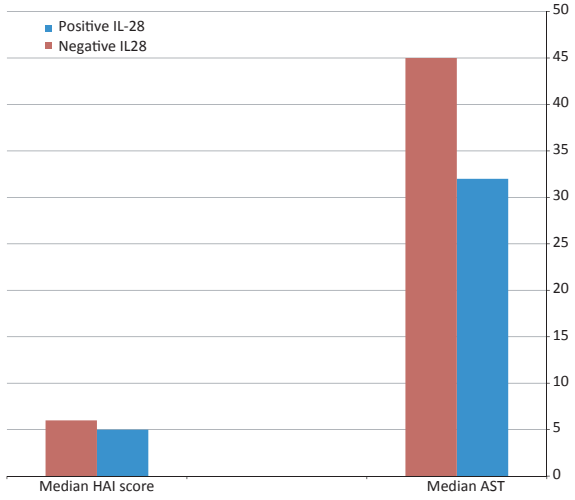

Fig. 2. The association of IL-28 expression with parameters of less inflammatory states such as low HAI score and low AST level in chronic HCV patients.

The cases were divided according to profile of IL-28, IFN-γ, and TNF-α into:

Positive group: positive for the three markers (10 cases, 15.6%)

Mixed group: positive for one or two markers (48 cases, 75%)

Negative group: negative for the three markers (6 cases, 9.4%)

Of cases, 79% (42/53) showing grade I portal tract inflammation was seen in mixed group compared to 11% (6/53) in positive group, but the association was not significant (Fig. 6).

Characteristics of mixed group

Twenty nine of 48 cases of the mixed group (60%) showed either IL-28 or IFN-γ or both expressions. While TNF-α was expressed alone in one case and in combination with either IFN-γ or IL-28 in 18 cases (37.5%). In addition, the mean value of TNF-α in the group showing positivity for the three markers was higher than its corresponding value in the mixed group where the latter mean ± SD was 4.2 ± 11.34 in comparison to 18.10 ± 18.0 in positive group.

The cases that showed positive expression for any of the studied markers were 58 cases. They were further classified according to TNF-α expression into two groups: group showing positive expression for TNF-α either alone or in combination with other markers (IL-28 or IFN-γ) (29 cases) and another group that lacked TNF-α expression, but showed positivity to other markers (IL-28 or IFN-γ or both) (29 cases). Group positive for TNF-α was in favor of grade II portal tract inflammation (8/11) in comparison to those lacked TNF-α expression (1/11) (Fig. 7).

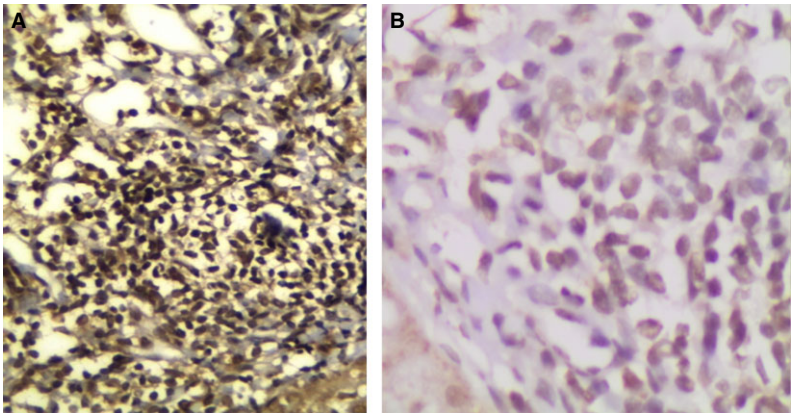

Fig. 3. Inflammatory cells of portal tract in a case of chronic HCV showed strong IFN-γ expression in A and moderate expression in B (Immunohistochemical staining x 200 in A and x 400 in B).

The relationship between the studied markers (IL-28, IFN-γ, and TNF-α)

Positive cases for IFN-γ showed high percentage of IL-28 expression in responders and in all patients, as the median percentage in positive cases was 30% (responder) and 32% (all patients) in comparison to 10% in negative group (either responder or all patients). Also, positive cases for TNF-α was associated with high median percentage of IL-28 (30%) compared to negative group (10%) expression in non-responder group only.

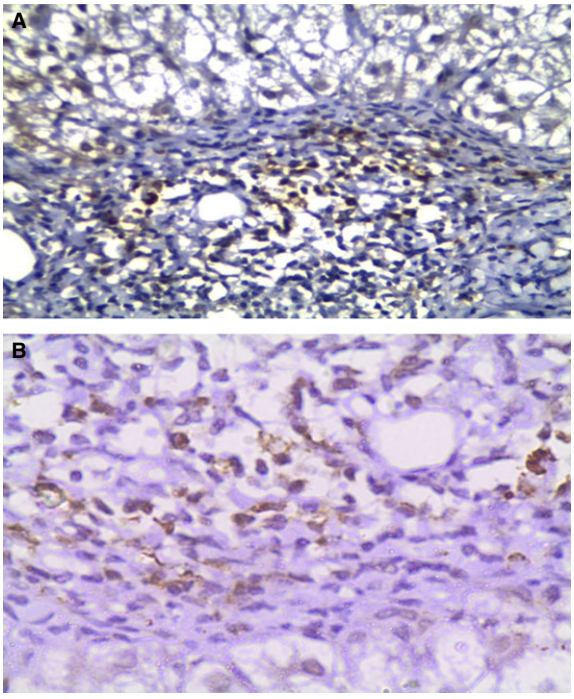

Fig. 4. Low and high power views of scattered expression of TNF-α in inflammatory cells in a case of chronic HCV (Immunohistochemical staining x 200 for A and x 400 for B).

DISCUSSION

Recent studies focused on IL-28 as a new cytokine for predicting response to therapy in chronic HCV patients. Previous studies focused on IL-28 polymorphism such as Agúndez et al. (18) or its level in the serum such as Langhans et al. (19). However in the present study, we tried to detect its expression in tissue by immunohistochemistry. Thirty five cases of 64 were positive for IL-28 (54%), 17 were responders (10.8%) and 18 cases

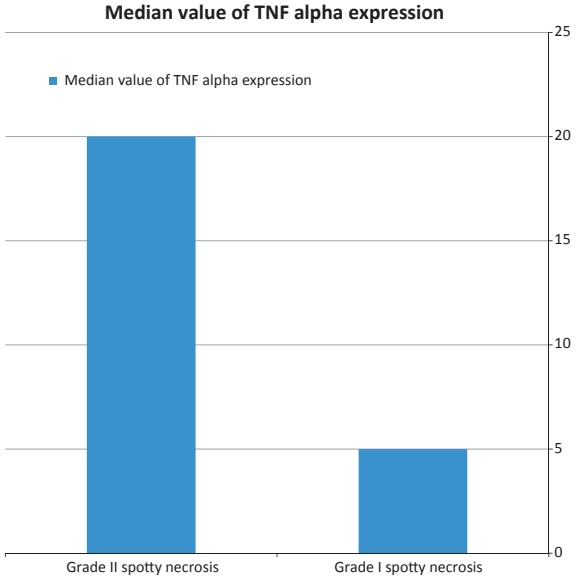

Fig. 5. The association between TNF-α expression and grade II spotty necrosis.

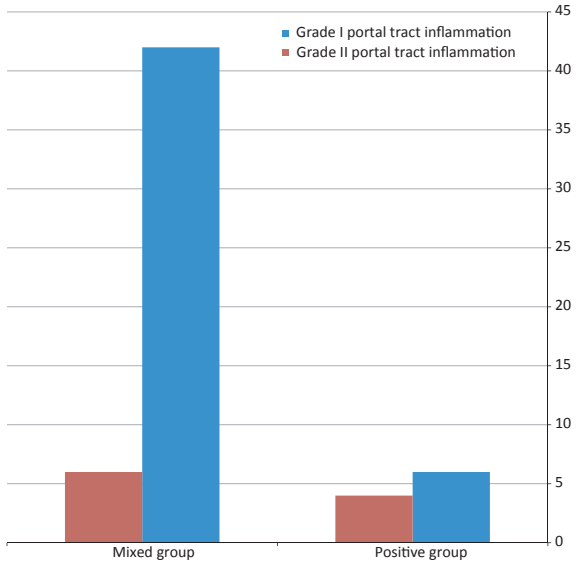

Fig. 6. The mixed group (positive expression for one or two markers) tended to show less degree of inflammation compared to the positive group for the three markers.

were non-responders (11.52%). The expression was localized to the mononuclear inflammatory cells.

IL-28B polymorphism has been associated with spontaneous HCV clearance (20, 21) and with SVR in patients with chronic HCV genotype 1 who are treated with Peg-IFN plus RBV in the non-transplant setting (22, 23). The present study showed absence of significant difference between responders and non-responders regarding IL-28 expression ($p > 0.05$), however, the percentage of IL-28

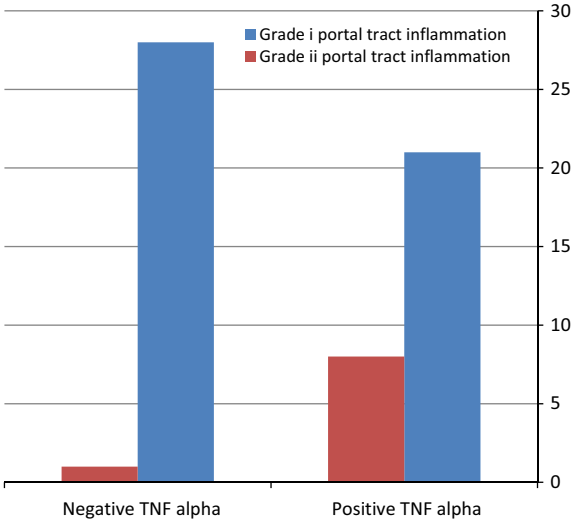

Fig. 7. The group that showed TNF-α expression was in favor of higher grade of portal tract inflammation compared to the group lacking TNF-α expression.

expression was higher in responders compared to non-responders. This finding agreed with Langhans et al. (19) who found that high IFN-lambda (IL-28 A/B and IL-29) levels measured by ELIZA predisposed to spontaneous clearance of HCV infection.

The present study demonstrated an association between IL-28 expression and low HAI Ishak score of necroinflammation ($p = 0.002$) and low AST level ($p = 0.002$) referring to its anti-inflammatory role. According to Abe et al. (24) and Thompson et al. (25), there was a significant relationship between the IL-28B gene polymorphism and the histological necroinflammatory activity, however, this association was not proved by other reports (18).

TNF-α is a cytokine with pleiotropic properties that is expressed in a variety of pathological conditions including viral infection (26). TNF-α production is an early event in the pathogenesis of liver damage, triggering the synthesis of other cytokines. It is required for the proliferation of normal hepatocytes in liver regeneration, but it also mediates hepatocyte death (27). The present study demonstrated TNF-α expression in lymphocytes of 29 cases of 64 (45%), 15 were responders and 14 cases were non-responders. Previous studies have shown that serum TNF-α levels were elevated in all chronic HCV patients (26). While other investigators reported that detectable serum TNF-α was found to be elevated in 74% of HCV patients affected by diabetes and in 64% not affected by diabetes (28).

IFN-γ, or type II interferon, is a cytokine that is critical for innate and adaptive immunity against viral and intracellular bacterial infections and for tumor control. Aberrant IFN-γ expression is associated with a number of autoinflammatory and autoimmune diseases. The importance of IFN-γ in the immune system stems in part from its ability to inhibit viral replication directly (29). The present study demonstrated IFN-γ expression in mononuclear inflammatory cells of 43 cases of 64 cases (67%), 23 were responders and 20 cases were non-responders.

The present study revealed that TNF-α and INF-γ immunohistochemical expression did not correlate with response to therapy. However, higher levels of INF-γ and lower levels of TNF-α were found in responder compared to non-responders. Par et al. (30) found that patients with RVR (rapid viral response) showed an increased baseline TNF-α production by TLR-4 activated monocytes and increased IFN-γ. On the other hand, Dumoulin et al. (31) semiquantify mRNA of IFN-γ and TNF-α on pretreatment liver biopsies by reverse transcription/competitive polymerase chain reaction. They found that higher intrahepatic mRNA

levels of IFN-γ and TNF-α may reflect interferon resistance of HCV strains and may contribute to tissue damage in patient's refractory to antiviral treatment. According to Lio et al. (32), low TNF-α and high IL-10 were associated with resolved HCV infection. This finding was supported by Larrea et al. (26) who found that high levels of TNF-α may play a role in resistance to interferon therapy. That controversy about the impact of these cytokines (IFN-γ and TNF-α) on response to therapy in chronic HCV patients may be due to differences in methodology as previous studies measured these cytokines in serum by PCR (31) or in peripheral blood cells by cell sorting techniques (30), while in the present study, these cytokines were assessed in tissue by immunohistochemical technique.

The present study demonstrated that high percentage of TNF-α was seen in higher grades of spotty necrosis. TNF-α is an inflammatory cytokine secreted mainly by activated macrophages known to upregulate adhesion molecules and to provoke hepatocyte apoptosis (33). HCV infection enhanced TNF-α-induced cell death by suppressing of NF-kB. Therefore, TNF-α contribute to immune-mediated liver injury in chronic HCV patients (34). This may explain increased presence of TNF-α in lobular inflammation like spotty necrosis as liver macrophages (kupffer cells) are mainly localized in sinusoids. Also TNF-α system is not only involved in the elimination of viral pathogens but also has been linked to the severity of HCV-associated liver disease (35, 36). Dumoulin et al. (31) detected a significant correlation in intrahepatic TNF-α mRNA levels between viral load and histological fibrosis scores (indicator of severity of inflammation). Fasting serum TNF-α in chronic HCV patients was higher than normal persons and it is associated with the severity of disease, but it did not correlate with antiviral therapy (37).

The present study showed that positive cases for IFN-γ demonstrated high percentage of IL-28 expression in responders and in all patients and this may be explained as both markers belong to the same group of cytokines (Interferons). IFN-γ is a dimerized soluble cytokine which is the only member type II class of interferons. The recently classified type III interferon group consists of three IFN-λ (lambda) molecules called IFN-λ1, IFN-λ2, and IFN-λ3 (also called IL-29, IL-28A, and IL-28B respectively) (38, 39).

The current study demonstrated that in non-responders group, TNF-α was associated with high median percentage of IL-28 (30%) compared to negative group (10%). As TNF-α expression may reflect severe inflammatory state (its association with spotty necrosis), so the increased level of IL-28 in these cases is to counteract action of TNF-α. This again reflects the presence of an interaction between pro-inflammatory cytokines represented by TNF-α and anti-inflammatory cytokines represented by IL-28.

In this study, cases were further classified with regard to the expression of the three cytokines into positive group (positive for the three cytokines), mixed group (positive for one or two cytokines), and negative group (negative for the three cytokines). Twenty nine of 48 cases of the mixed group (60%) showed either IL-28 or IFN-γ or both expressions. While TNF-α was expressed alone in one case and in combination of either IFN-γ or IL-28 in 18 cases (37.5%). So, the mixed group tended to show more expression toward anti-inflammatory cytokines (IL-28 and IFN-γ) than pro-inflammatory cytokines (TNF-α). This may explain why the mixed group showed more association with grade I portal tract inflammation (42/53) compared to positive group (6/53). In addition, the mean value of TNF-α in the group showing positivity for the three markers was higher (18.10 ± 18.0) than its value in the mixed group (4.2 ± 11.34). Therefore, we suggested that the pro-inflammatory cytokines were highly reduced in the mixed group, which explained its association with less inflammatory state.

Therefore, based on the results of comparison between mixed and positive groups, we further divided our cases according to presence or absence of TNF-α. This division showed that the cases demonstrating TNF-α were in favor of higher grade of portal tract inflammation compared to the group lacked this cytokine.

Finally, the present study demonstrates that some inflammatory cytokines expressed by intrahepatic inflammatory cells in chronic HCV patients promote inflammation and injury (pro-inflammatory) such as TNF-α. However, other cytokines aid in resolving inflammation and injury (anti-inflammatory) such as IL-28. The balance between these cytokines will determine the degree of inflammatory state. None of the investigated cytokines proved its clear cut role in affecting response to therapy, however, their levels varied between responders and non-responders for further investigations to clarify.

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