Neopterin as a Predictor of Response to Pegylated Interferon Therapy in Egyptian Patients with Chronic Hepatitis C Virus

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Authors' contributions

This work was carried out in collaboration among all authors. Author HGA designed the study. Authors HGA, TMET and MMH performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors TMET, HAEMA and MMH managed the analyses of the study. All authors managed the literature searches and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Neopterin is a proinflammatorymarker which participates in cell-mediated immunity, and its elevated concentrations declare the presence of interferon-γ in body fluids. In thisstudy, neopterin concentrations were determined in patients with chronic hepatitis C virus (HCV)to assess whether its plasma level can be used to predict the response to antiviral therapy by pegIFNγ combined with ribavirin before starting the treatment.
Materials and Mehtods: A total of 54 subjects (all males with an average age of *46.12± 9.89*) were included in this study. Neopterin levels were evaluatedby Enzyme-linked immunosorbent assay (ELIZA) in HCV patients treated with peylated interferon combined with ribavirin. Liver function tests (Aspartate aminotransferase, Alanine aminotransferase), albumin and creatinine concentrations were also estimatied.
Results: Mean and median pretreatment neopterin levelsshowed no statistically difference between patients with sustained virological response (cured)and non-responders (relapsed) where the

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concentrations in both groups were≥*16 nmol/L*.On the other hand, a positive correlation was observed between neopterin level and creatinine at (p ≤ 0.05).

Conclusion: Our study suggests that the pretreatment level of neopterin might not be used in routine clinical practice as a marker to predict the response to antiviral therapy in HCVpatients.

1. INTRODUCTION

Hepatitis C virus (HCV) is an enveloped, single-stranded ribonucleic acid (RNA) virus with positive polarity, belongs to hepaciviruses genus in the Flaviviridae family [1]. This virus is one of the most important human pathogens thatcan cause mild to severe liver diseases and is considered one of a major blood-borne infection worldwide, with a silent epidemiology, that it has reached pandemic proportions [2]. The chronic infection with HCV, remains a troublesome health problem worldwide, which approximately 3% of the population suffering from it. Its prevalence is higher in some countries of Asia and Africa and approximately 14.5% in Egypt [3]. In developing countries, the chronic hepatitis C is the most prominent cause of liver cirrhosis and hepatocellular carcinoma [4]. According to the World Health Organization there are about 185 - 200 million people chronically infected with HCV worldwide and it contributes a significant cause of morbidity and mortality [5].In 2018,Egypt has one of the highest global burdens of HCV infection, with an estimated over 6 million people between 15-59 years, being chronically infected. Tragically, an estimated 150, 000 new people are being infected annually, and thousands die every year [6].

Over the past decades, the combination of pegylated interferon (PEG-INF) and ribavirin (RBV) for 48 weeks was the standard treatment of HCVpatient [7]. However,the efficacy of this combination to treat HCV patient was small where only 41% sustained virological response (SVR), defined as a viremia 24 weeks after completion of antiviral therapy [8]. As well as, between 2007 and 2014 the National program for control of HCV in Egypt has treated about 350, 000 patients with this combination [9] and the result showed SVR between 45% and 55% [10].

Interferon-stimulated genes (IFN-SGs) expression in the liver were suggested as a marker of response to anti-viral therapy as the guanosine triphosphate cyclohydrolase 1 (GTPCH), a rate-limiting enzyme of pteridines biosynthesis encoded by it. Neopterin, a stable byproduct of GTPCH-catalyzed reaction, is used as a marker of interferon-induced GTPCH activation [11]. Neopterin or 2-amino-4-hydroxy-6-(D-erythro-1′, 2′, 3′-trihydroxypropyl)-pteridine is produced from GTPCH-Iby activated monocytes, macrophages, dendritic cells, and endothelial cells and to a lesser extent in renal epithelial cells, fibroblasts, and vascular smooth muscle cells upon stimulation mainly by interferon gamma and to a lesser extent by interferon alpha and beta with its release being enhanced by tumor necrosis factor [12]. During acute viral infections increased neopterin levels have been observed, which correlate with the activity of disease [13] and subsequently neopterin elevations were noted in infections with hepatitis viruses, Epstein-Barr, measles, mumps, varicella zoster, rubella, and influenza viruses [14]. Elevated neopterin levels in body fluids were found at the end of the incubation period before the onset of clinical symptoms and just before specific antibodies against the virus become detectable, which is about two to four weeks after onset of increased neopterin production [15].

It was noted that in patients with acute viral hepatitis, most patients had elevated urinary neopterin levels with the highest levels found in patients with acute hepatitis A. While all patients with active hepatitis B had elevated neopterin serum levels and HbsAg carriers had normal urinary neopterin levels. A more recent investigation found that serum neopterin levels were significantly higher in patients with chronic hepatitis and cirrhosis than in control subjects as well as, cirrhotic patients had significantly higher serum neopterin levels than patients with chronic hepatitis [16]. We assessed neopterin concentrationsto define its availabilty as a marker to predict the response to antiviral therapy in chronic viral hepatitis C patients.

2. MATERIALS AND METHODS

The research protocol was reviewed and approved by the ethical committee of the ERMED (Railway Hospital)Hospital and faculty of pharmacy, Al-Azhar University, Cairo,

Egypt.Patients were informed about the study and informed consents were obtained.

Initially 85 chronic hepatitis C patients admitted to ERMED (Railway Hospital)were randomly approached to participate in the study. Out of 85 patients who were deemed fit to participate in the study, the number of patients had been decreased as some of them stopped treatment due to health problems(as neutropenia, intractable cough, flaring psoriasis, high blood sugar), others showed non-response to treatment after 12 weeks while, others stopped treatment by themselves. Consequently, a total of 54 HCV patients all males were finally enrolled in the study. The inclusion criterion for enrolment in the present study was all positive serum HCV RNA by Quantitative PCR. Evidence of chronic hepatitis in liver biopsy.

2.1 Treatment Regimens

All patients with chronic hepatitis C were treated with a weekly subcutaneous injection of peg IFN-α-2b at a dose of 1.5 µg/kg per week in combination with a weight-adjusted dose of oral RBV (1000 mg/day for<75 kg,1200 mg/day for>75 kg) for 48 weeks [17].

Every patient in this study was subjected to full histroy and clinical examination as well as laboratory investigatons for complete blood counts (CBCs as Hemoglobin, White blood cells and platelet counts). HCV-RNA detection was done using qualitative PCR.

Liver Biopsy was performed by experienced hepatologists under ultrasound guidance using 16G Menghini needles. Liver specimens were stained with haematoxilin/eosin standard procedures. Necroinflammatory activity and fibrosis were evaluated separately according to the Metavir score. Namely, fibrosis was divided into a five-point scale: F0 (no fibrosis), F1 (portal fibrosis without septa), F2 (portal fibrosis with few septa), F3 (numerous septa without cirrhosis) and F4 (cirrhosis). The necroinflammatory activity was graded in a four-point scale: A0 (none), A1 (mild), A2 (moderate) and A3 (severe) [18].

2.2 Methods

2.2.1 Sample collection

3ml of blood was drawn into a red capped evacuated glass collection tube; and allowed to clot for at least 30 minutes at room temperature. Following that,the serum was separated and stored in the dark at -20°C in a non- frost-free freezer before neopterin and liver biochemical parameters assay. Liver function tests (alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, creatinine, International Normalized Ratio (INR) and prothrombin time (PT))were measured using available commercial kitsand the normal range provided in the kit used as a reference values in the study.

2.2.2 Measurement of plasmaneopterin

Neopterin concentration was evaluatedusing (DRG® Neopterin (EIA-1476, USA) (ELISA) commercial kitby Enzyme-linked immunosorbent assayaccording to the manufacturer instructions.Theconcentrations were reported as nanomoles per liter (nmole/L).

2.3 Statistical Methods

Descriptive statistics including Mean, Median, Standard deviation, and different percentiles (25 and 75) as well as frequency of different categories within each variable were obtained using IBM SPSS ver.23 software. A non-parametric Mann-Whitney Rank Sum Test was used to compare any factor with two groups instead of the parametric t test while Kruskal-Wallis One Way Analysis of Variance on Ranks was used for comparing three or more groups instead of the parametric one-way ANOVA. All pairwise multiple comparisons were performed using Dunn's test. This test is used for comparisons that following a rank-based ANOVA and especially when group sizes are unequal (Systat Software, 2011). SigmaPlot® 12.5 and Microsoft™ Excel® 2013 were used for different comparison. Association between different variables was assessed using the non-parametric Spearman correlation. Degree of relationship and significance of association were considered only at p value < 0.05. Correlations were assessed using IBM SPSS ver. 23.

3. RESULTS

A total of 54 HCV patients with average age of 46.12± 9.89. Table (1) shows the demographic and biochemical data of participated patients; there were elevations in serum levels of ALT and AST with mean ± SD (93.46± 79.98 U//L and 76.52± 64.45U/L) respectively. As well as, there was high PCR value with mean ± SD (1253405.77± 2604964.55IU/ml) and an increase in WBC with mean ± SD (7.14± 3.42x1000/cm3). Moreover, there were increases in prothrombin

time and International Normalized Ratio (INR) with mean ± SD (12.28± 2.95 and 1.09± 0.27respectively). However, serum levels of albumin, hemoglobin and creatinine were at normal values with mean ± SD (4.21± 0.90 g/dl, 14.29±2.97 g/dl and 0.92± 0.22 mg/dl) respectively. Liver histology showed different degree of fibrosis, where 16.6% of patients were F1 and F2, 5.55% were F4, while most patients were F3 with 61.11%. On the other hand, most of patients had mild necroinflammtory activity (A1) with 70.37%; whilst 27.77% had moderate activity (A2) and 1.85% had severe necro-inflammtory activity (A3).

3.1 Spearman Correlation between Measured Varibale and Plasma Neopterin Level

In order to investigate the possible correlation among different parameters, non-parametric Spearman correlation was detected among different variables as shown in Table (2). We revealed that, there was a highly positive significant association between ALT and AST at P value <0.001. Moreover, neopterin level showed a highlypositive significant associations with serum creatinine and prothrombin (at P value <0.05).

3.2 Sustained Virologal Response Assessment (SVR)

Follow up was done after six months of finishing treatment, (SVR assessment) and we reveled that the percent of patients with negative PCR was 81.48% (cured) and positive PCR was 18.52% (relapsed) as shown in Table (3).

3.3 Initial PCR and Response to Treatment

To investigate the possible relationship between PCR and first response to the treatment, the 54 patients had been divided into three groups (response at four, 12 and 24 weeks). The differences statistically not significant between different groups. Median of PCR at different categories is shown in Table (4).

Table 1. Biochemical characteristics of HCV patients

Variable	Mean ± SD	Percentile 25	Median	Percentile 75
Age(yrs)	46.12± 9.89	42.0	49.5	54.25
Hemoglobin (g/dl)	14.29±2.97	13.50	14.40	15.62
Platelets (10^9/L)	190.02±60.17	159.75	188.50	224.75
WBCs (/cm^3)	7.14± 3.42	5.27	7.25	8.47
Prothrombin (sec)	12.28± 2.95	12.50	13.0	13.7
INR	1.09± 0.27	1.00	1.10	1.21
ALT(IU/L)	93.46± 79.98	44.0	54.50	76.5
AST(IU/L)	76.52± 64.45	40.0	55.0	74.25
Albumin (g/dl)	4.21± 0.90	4.00	4.30	4.60
Creatinine (mg/dl)	0.92± 0.22	0.80	0.90	1.000
PCR (HCV RNA) (IU/ml)	1253405.77± 2604964.55	55046.25	130988	419888.75
Fibrosis				
F0	0			
F1	9 (16.6%)			
F2	9 (16.6%)			
F3	33(61.11%)			
F4	3 (5.55%)			
Activity (degree of inflammation)				
A0	0			
A1	38 (70.37%)			
A2	15 (27.77%)			
A3	1 (1.85%)			

WBCs: white blood cells (normal range: 4,000-11,000/cm3), INR:international normalized ratio (normal range :0.8-1),prothrombin time (PT) (normal range :10-14sec), ALT: alanine amino-transferase (normal range: 5-35 IU/L), AST: aspartate transaminase(normal range: 5-45 IU/L), albumin (normal range: 3.2-4.5 g/dl), creatinine (normal range: 0.6-1.2 mg/dl) and PCR: polymerase chain reaction

Table 2. Spearman correlations among different recorded variables

	Age	Prothrombin	INR	ALT	AST	Albumin	Creatinine	PCR (HCV RNA)	Neopterin
Age	1.000	- 0.012	0.035	0.038	0.076	- 0.315	- 0.077	- 0.197	0.191
P value		0.930	0.798	0.781	0.584	*0.019**	0.575	0.152	0.165
Prothrombin	- 0.012	1.000	0.294	0.054	-0.075	- 0.190	0.081	0.005	0.300
P value	0.930		*0.030**	0.695	0.586	0.167	0.558	0.970	*0.027**
INR	0.035	0.294	1.000	-0.173	- 0.148	- 0.135	0.1622	0.012	0.222
P value	0.798	*0.030**		0.210	0.285	0.329	0.241	0.927	0.106
ALT	0.038	0.054	-0.173	1.000	0.788	- 0.079	- 0.149	- 0.062	-0.021
P value	0.781	0.695	0.210		*0.000***	0.566	0.279	0.655	0.876
AST	0.076	-0.075	- 0.148	0.788	1.000	- 0.096	- 0.183	- 0.157	- 0.033
P value	0.584	0.586	0.285	*0.000***		0.489	0.185	0.254	0.810
Albumin	- 0.315	- 0.190	- 0.135	- 0.079	- 0.096	1.000	0.145	0.148	- 0.119
P value	*0.019**	0.167	0.329	0.566	0.489		0.292	0.283	0.389
Creatinine	- 0.077	0.081	0.1622	- 0.149	- 0.183	0.145	1.000	0.204	0.336
P value	0.575	0.558	0.241	0.279	0.185	0.292		0.138	*0.012***
PCR	- 0.197	0.005	0.012	- 0.062	- 0.157	0.148	0.204	1.000	0.140
P value	0.152	0.970	0.927	0.655	0.254	0.283	0.138		0.310
Neopterin	0.191	0.300	0.222	-0.021	- 0.033	- 0.119	0.336	0.140	1.000
P value	0.165	*0.027**	0.106	0.876	0.810	0.389	*0.012***	0.310	

*, denote significant correlations at P value < 0.05 while **, denote highly significant correlations at P value < 0.001, empty cells are representing non-significant correlation

Table 3. Frequency of positive and negative PCR patients after six months of finishing treatment

PCR after 6months of finishing treatment	Frequency	Percentage (%)
+Ve	10	18.52
-Ve	44	81.48
Total	54	100.0

Table 4. Kruskal-wallis one way analysis of variance on ranks for the PCR titer at the different first response time of treatment (four, 12 and 24weeks)

Group	N	Median	25%	75%	*P* value
4 weeks	29	88166	44058	805973	0.242
12 weeks	22	485370.5	136690.3	970595.8	
24 weeks	3	1966826	686	3451142	

To examine the possible relationship between PCR and liver fibrosis, patient's liver biopsies before starting the treatment had been classified into four groups according to their degree of fibrosis (F1-F4). No significant differences were detected for PCR at different liver biopsy categories. Median of PCR at different liver biopsy categories is shown in Table (5)

Also, none significant differences were detected for PCR at different response groups. Median of PCR titer at different response categories is shown in Table (6).

3.4 Neopterin Level and Response to Treatment

The goal of our study is to reveal a relationship between neopterin level and response to treatment, so firstly, the 54 patients were divided according to first response to treatment into 4,12 and 48 weeks, neopterin level showed non-significant difference between the three groups. Table (7).

Secondly, after the follow up the participants divided into two groups according the PCR results to cured,and relapsed. We noticed that, there were none significant differences detected for neopterin level between the two groups with means ±SD (21.54± 40.40, 42.06± 65.34 respectively). Median of Neopterin level at different response categories is shown in Table (8).

4. DISCUSSION

Hepatitis C virus is an enveloped RNA virus which contains a single positive stranded RNA molecule. The virus is a causative agent of acute and chronic hepatitis as well as liver cirrhosis and hepatocellular carcinoma in humans [19].

During acute viral infections increased neopterin levels have been observed, and subsequently neopterin elevations were noted in infections with hepatitis viruses [14] and considered as a useful predictor of response to treatment of chronic HCV infection with pegylated interferon combined with ribavirin [20].

Table 5. Kruskal-wallis one way analysis of variance on ranks for the PCR titer between different liver biopsy groups

Group	N	Median	25%	75%	*P* value
F1	9	190726	13910	935138	0.943
F2	9	287632	81042	1343633	
F3	33	165199	45441	763303	
F4	3	627500	499735	4439985	

Table 6. Mann-whitney rank sum test for the PCR titer between the two groups of responses

Group	N	Median	25%	75%	*P* value
relapse	10	335256.5	20345.5	2586678	0.885
Cured	44	234205.5	59280.5	892179.3	

Table 7. Kruskal-wallis one way analysis of variance on ranks for the neopterin level at the different first response groups

Group	N	Median	25%	75%	P value
4 weeks	29	9.486	5.534	15.217	0.849
12 weeks	22	9.091	8.202	10.277	
24 weeks	3	8.3	4.743	13.439	

Table 8. Mann-whitney rank sum test for the neopterin level between the two groups of responses

Group	N	Median	25%	75%	P value
Relapse	10	9.684	8.202	50.198	0.321
Cured	44	9.289	5.534	10.672	

In chronic hepatitis, the IFN-gamma secreted from T lymphocytes activates macrophages and NP is released [21]. *Grüngreif et al*. [22] reported that, the serum NP levels of the patients with CHB and CHC were significantly higher than the serum NP levels of the control group. The high NP level in patients with chronic hepatitis C was linked to the stimulation of cellular immune system, where NP levels seem to increase in patients undergoing an immune-stimulator treatment. This may be caused by induction of the immune-regulatory cascade that stimulates IFN-γ secretion [23].

In this study we estimated the serum level of neopterin in HCV patients before treatment with pegylated interferon combined with ribavirin for 48 weeks for its evaluation as a marker of response to pegylated interferon therapy combined with ribavirin.

We revealed that, there were no statistically significance differences detected for neopterin level between cured and relapsed groups, as its concentration was higher in both groups ≥ 16 mmol/L. This result was in consistent with *Ozcimen et al*. [24] who measured serum level of neopterin (NP) before and after treatment in CHC and reported that the NP values at the end of 1-year pegylated interferon alpha and/or ribavirin therapy were found higher than the initial NP values without differentiating between the responders and non-responders (the number of responders were more).This highness was attributed to the immune-mod¬ulator effect of interferon as in most of infections, when a comparison is made before and after an infection treatment, a decrease is seen in NP levels because of the decline in immune system activation at the end of the treatment. However, when the disease is treated with an IFN therapy, which is a treatment that stimulates the immune system, the NP level remains high at the end of the treatment in connection with the IFN therapy.

On the other hand, neopterin level showed no statistical significance difference between chronic hepatitis C patients at different first response to treatment (Table 6). This result was in contract to *Gregory et al*. [25] who showed thatpretreatmentneopterin concentrations were lower in patients with sustained virological response than in relapsed.Also contradictory to Oxenkrug et al. [20] who reported that serum neopterin levels were found to be a useful predictor of response to treatment of chronic HCV infection with pegylated interferon combined with ribavirin, where mean and median pretreatment neopterin concentrations were lower in patients with sustained virological response than in non-responders. The rate of response was two-fold higher among patients with pretreatment neopterin levels <16 nmol/L than in patients with neopterin levels ≥16 nmol/L. Thus, they concluded that the neopterin levels measured before an antiviral treatment could be used for response to treatment estimates.

Our results revealed that there was a significant positive relationship detected between neopterin level and creatinine at P value <0.05. This result was in agreement with *Pihlstrøm et al.* [26] who reported that there was a high degree of correlation between neopterin and creatinine as neopterin is chemically inert and its elimination is solely through the kidneys, compromised renal function leads to a rise in serum neopterin that is not caused by increased inflammatory activity [27]. Subsequent studies had confirmed that elevation of serum or urinary neopterin precedes the rise in creatinine by up to several days in patients with acute early complications [28].

For liver function tests, our study revealed that there was a significant increase in the serum levels of liver function enzymes ALT and AST. On the other hand, highly significant positive relationship was detected between ALT and AST at P value <0.001. This result was in agreement *Rukiye and Fikriye* [29] who reported that higher levels of AST and ALT in HCV patients than healthy patients.

Our study showed that serum levels of Albumin was at normal value with mean± SD (4.2 ± 0.90), this result was in agreement with *Ge et al.* [30] who worked on patients with early nonalcoholic fatty liver disease, viral hepatitis, and cirrhosis and reported that no differences were observed in albumin levels among the healthy control and nonalcoholic fatty liver disease and hepatitis patients (p = 0.36). However, the albumin levels of cirrhotic patients were significantly lower than those of the non-cirrhotic patients (p<0.001). Furthermore, the albumin levels continued to decrease as the liver lost more function.

As well as our study showed that there was an increase in prothrombin time and International Normalized Ratio (INR) (Table 1). Also, there was a significant positive relationship between them at P value <0.05, and our results agreed with Sajjadieh et al. [31]whoreported that PTs and INR parameters were significantly different between patients and control group. Patients with elevated biochemical fibrosis markers had high PT values that differed significantly from those of control participants.

Also our study showed that there was a significant increase in PCR with mean± SD 1253405.77± 2604964.55; this result was in agreement with *Kato et al*. [32] who observed significantly higher HCV RNA titers in patients with chronic active hepatitis and cirrhosis compared to those with milder histological abnormalities such as persistent chronic hepatitis.

To investigate the relationship between PCR and response to treatment, the patients had been divided into two groups according to response to treatment into cured, and relapsed. There was no statistical significance difference within different response groups (p>0.05). This result agreed with *Shiffmanet al*. [33] who worked on patients with chronic HCV who completed a 6-month course of interferon therapy and reported that the mean serum HCV RNA titer before the start of interferon therapy was not significantly different for biochemical responders or non-responders.

To explore possible relationship between PCR and degree of fibrosis (F1-F4), patients had been divided into four groups according to their degree of fibrosis (F1-F4). Our study revealed that a non-significant correlation were detected between PCR and degree of fibrosis (F1-F4) p=0.94 and this result was in agreement with Noreldin et al. [34] who reported that there were no statistically significant difference between PCR with activity and fibrosis (p=0.37, p=0.22 respectively).This could be attributed to the fact that serum HCV RNA load is not a stable parameter because it fluctuates. In addition, a high amount of circulating HCV does not always imply a more active state of viral replication in the liver nor does it indicate a more severe degree of liver disease. HCV is known to replicate both within the liver as well as in extra-hepatic sites [35].

5. CONCLUSION

The evaluation of pretreatment concentrations of neopterin might not be a predictor for response to antiviral therapy (pegINF and ribavirin) in chronic hepatitis C patients. Some limitation was found, firstly, the study should be applied on large scale of patients to confirm our results. Secondly, evaluation of serumneopterin level in HCV patients before and after treatment in addition to healthy individuals as control group to assess the association between neopterin level and response to treatment.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Patients were informed about the study and informed consents were obtained.

ETHICAL APPROVAL

The research protocol was reviewed and approved by the ethical committee of the

ERMED (Railway Hospital)Hospital and faculty of pharmacy, Al-Azhar University, Cairo, Egypt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shakeri MT, Nomani H, Mobarhan MG, Sima HR, Gerayli S, Shahbazi S, Meshkat Z. The prevalence of hepatitis C virus in mashhad, iran: A population-based study. Hepatitis Monthly. 2013;13(3).
2. Feldman M, Friedman LS, Brandt LJ. Sleisenger and fordtran's gastrointestinal and liver disease e-book: pathophysiology, diagnosis, management, expert consult premium edition-enhanced online features. Elsevier Health Sciences. 2010;1).
3. MohdHanafiah K, Groeger J, Flaxman AD, Wiersma ST. Global epidemiology of hepatitis C virus infection: New estimates of age-specific antibody to HCV seroprevalence. Hepatology. 2013;57(4): 1333-1342.
4. Jahanbakhsh SF, Keyvani H, Monavari SH, et al. Distribution of hepatitis C virus genotypes in Iranian chronic infected patients. Hepat. 2013;13(1).
5. Flynn JK, Sacks-Davis R, Higgs P, Aitken C, Moneer S, Suppiah V, George J. Detection of HCV-specific IFN-γ responses in HCV antibody and HCV RNA negative injecting drug users. Hepatitis Monthly. 2014;14(1).
6. El-Zanaty F, Way A. Egypt Demographic and Health Survey Cairo, Egypt: Ministry of Health, El-Zanaty and Associates, and Macro International; 2018.
7. Abdel-Razek W, Waked I. Optimal therapy in genotype 4 chronic hepatitis C: Finally cured? Liver International. 2015;35:27–34.
8. Marcellin P, Cheinquer H, Curescu M, Dusheiko GM, Ferenci P, Horban A andOuzan D. High sustained virologic response rates in rapid virologicresponsepatients in the large real-world Prophesys cohort confirm results from randomizedclinical trials. Hepatology. 2012;56(6):2039–2050.
9. Doss W, Mohamed MK, Esmat G, El Sayed M, Fontanet A, Cooper S, ElSayed N. Egyptian national control strategy for viral hepatitis 2008–2012. Arab Republic of Egypt, Ministry of Health and Population, National Committee for the Control of Viral Hepatitis; 2008.
10. Esmat G, El Kassas M, Hassany M, Gamil M, El Raziky M. Optimizingtreatment for HCV genotype 4: PEG-IFN alfa 2a vs. PEG-IFN alfa 2b; The debatecontinues. Liver International. 2014;34(1):24–28.
11. Foster GR, Hézode C, Bronowicki JP, Carosi G, Weiland O, Verlinden L, Beumont M. Telaprevir alone or with peginterferon and ribavirin reduces HCV RNA in patients with chronic genotype 2 but not genotype 3 infections. Gastroenterology. 2011;141(3):881-889.
12. Hoffmann G, Wirleitner B, Fuchs D. Potential role of immune system activation-associated production of neopterin derivatives in humans. Inflammation Research. 2003;52(8):313–321.
13. Wachter H, Hausen A, Grassmayr K. Erhöhte Ausscheidung von Neopterinim Harn von Patientenmitmalignen Tumoren und mit Viruserkrankungen. Hoppe Seylers Z Physiol Chem. 1979;360(12): 1957-60.
14. Huang A, Zhang YY, Chen K, Hatakeyama K, Keaney JF. Cytokine-stimulated GTP cyclohydrolase I expression in endothelial cells requires coordinated activation of nuclear factor-κB and Stat1/Stat3. Circulation Research. 2005;96(2):164-71.
15. Nyamweya S, Townend J, Zaman A, Steele SJ, Jeffries D, Rowland-Jones S, Whittle H, Flanagan KL, Jaye A. Are plasma biomarkers of immune activation predictive of HIV progression: a longitudinal comparison and analyses in HIV-1 and HIV-2 infections?.PloS One. 2012;7(9):44411.
16. Gulcan EM, Tirit I, Anil A, Adal E, Ozbay G. Serum neopterin levels in children with hepatitis-B-related chronic liver disease and its relationship to disease severity. World Journal of Gastroenterology. 2008; 14(44):6840–6843.
17. Kamal S, Nasser I. Hepatitis C genotype 4: what we know and what we don't yet know. Hepatology. 2008;47:1371-1383.
18. Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. Hepatology. 1996;24:289–293.
19. Kchayama, Hayes CN, HCV diversity. Journal of Gastroenterology and Hepatology Journal of Gastroenterology and Hepatology Foundation and Blackwell

Publishing Asia Pty Ltd. 2011;26(1):83–95 91

20. Oxenkrug GF, Requintina PJ, Mikolich DL, Ruthazer R, Viveiros K, Lee H, Summergrad P. Neopterin as a marker of response to antiviral therapy in hepatitis C virus patients. Hepatitis Research and Treatment, 2012;4.

21. Schennach H, Hessenberger G, Mayersbach P, Schonitzer D, Fuchs D. Acute cytomegalovirus infections in blood donors are indicated by increased serum neopterin concentrations. Med MicrobiolImmunol (Berl). 2002;191:115-8.

22. Grüngreif K, Reinhold D, Ansorge S. Serum concentra- tions of sIL-2R, IL-6, TGF-beta1, neopterin, and zinc inchronic hepatitis C patients treated with interferon-alpha. Cytokine. 1999;11:1076-80.

23. Hamerlinck FFV. Neopterin: a review. Exp Dermatol. 1999;8:167-76.

24. Ozcimen S, Bitirgen M, Kandemir B. Role of neopterin in determining the efficacy of interferon therapy in chronic hepatitis B and C. European Journal of General Medicine. 2015;12(1).

25. Gregory F. Oxenkrug, Pura J. Requintina, Dennis L. Mikolich, Robin Ruthazer, Kathleen Viveiros, Hannah Lee, Paul Summergrad. Neopterin as a marker of response to antiviral therapy in hepatitis C virus patients. Hepatitis Research and Treatment. 2012;619609:4.

26. Pihlstrøm H, Mjøen G, März W, Olav Dahle D, Abedini S, Holme I, Fellström B, JardineA, Pilz S, Holdaas H. Neopterin is associated with cardiovascular events and all cause mortality in renal transplant patients. Clinical Transplantation. 2014;28(1):111-9.

27. Aulitzky WE, Tilg H, Niederwieser D, Riccabona G, Obendorf L, Margreiter R, Pfaller W, Huber C. Comparison of serum neopterin levels and urinary neopterin excretion in renal allograft recipients. Clinical Nephrology. 1988;29(5):248-52.

28. Chin GK, Adams CL, Carey BS, Shaw S, Tse WY, Kaminski ER. The value of serum neopterin, interferon-gamma levels and interleukin-12B polymorphisms in predicting acute renal allograft rejection. Clinical & Experimental Immunology. 2008; 152(2):239-44.

29. Rukiye NAR, Fikriye MS. The relationship between the serum RNA titers of hepatitis C virus and biochemical parameters in chronic hepatitis C patients. Viral Hepat. J. 2016;22:28-33.

30. Ge P, Yang H, Lu J, Liao W, Du S, Xu Y, Xu H, Zhao H, Lu X, Sang X, Zhon. Albumin binding function: The potential earliest indicator for liver function damage. Gastroenterology Research and Practice; 2016.

31. Sajjadieh M, Viunytska L. Prothrombin time in patients with and without fibrotic chronic liver disease. The Internet Journal of Pathology. 2008;8(1).

32. Kato NK, Hosoda K, Ito Y, Ohto M, Omata M. Quantification of hepatitis C virus by competitive reverse transcription – polymerase chain reaction: increase of the virus in advanced liver disease. Hepatology. 1993;18:16-20.

33. ShiffmanMitchell L, Charlotte m. Hofmann, Velimir AC. Luketic, Arunj. Sanyal, melisa J. Contos, Scott Mills A. Improved sustained response following treatment of chronic hepatitis C by gradual reduction in the interferon dose. Hepatology. 1996; 24(1):21-26.

34. Noreldin AKA, Mohamed AS, Abdelaziz AH, Ali GAM. Correlation between liver function tests and polymerase chain reaction in chronic hepatitis C patients. J Hepatol Gastroint Dis. 2015;1:110.

35. Fouad SA, Esmat S, Omran D, Rashid L, Kobaisi MH. Noninvasive assessment of hepatic fibrosis in Egyptian patients with chronic hepatitis C virus infection. World J Gastroenterol. 2012;18:2988-2994.