Molecular Markers Predicting the Efficacy of Interferon Based Therapies in Patients with Chronic Hepatitis C

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Abstract: Egypt has an exceeding high prevalence of cirrhosis, liver failure, hepatocellular carcinoma and death attributable to hepatitis C virus (HCV) infections. Although the best standard treatment of chronic hepatitis C involves a 48-week course of peg-interferon-α 2β, it is well known that many patients will not be cured by treatment. For these reasons, identification of the determinants of response to treatment is a high priority. Osteopontin (or secreted phosphoprotein 1, SPP1) is a cytokine produced by macrophages and activated T cells. Overexpression of osteopontin gene is associated with various inflammatory liver diseases**.** Four single-nucleotide polymorphism (SNPs) in the promotor region of the osteopontin gene, at nucleotide (nt)-155, -443, -616, and -1748, were detected and suggested that the SNP at nt-443, was a marker reflecting hepatitis activity in patients with HCV. **The aim of this study** is to detect the SNP in the promotor region of the osteopontin gene at nucleotide -443 and its protein level in the blood of chronic hepatitis C patients under treatment with interferon(responders and non-responders) and their value as pretreatment predictor of responsiveness to treatment. Also, to compare the results with non infected patients. **Subjects and Methods** the study included 99 patients with chronic viral hepatitis C and 20 healthy persons serving as control. Osteopontin protein was measured before treatment **Only**, SNP at nt -443 in promoter of osteopontin gene detected, PCR, liver enzymes (ALT, AST, ALK), Albumin, bilirubin and alpha fetoprotein(AFP)all are measured before treatment, three months after receiving treatment and after treatment completion by six months. Biopsy was done pre-treatment to all patients to determine the fibrosis grade. **This study showed that**: there was insignificant difference between hepatitis C patients and controls in the distribution of SNP -443 genotypes (T/T, T/C and C/C). There was a significant difference in pretreatment osteopontin serum protein level between patients and controls (P=<0.0001) and between non-responders and responders 3 months (mo.)after treatment (P =0.01) with high level in non-responders (37.08±5.49) than responders (34.20±5.49). Also, there was a significant difference between non-responders and responders 6 mo. after ending treatment (P =<0.001) with high level in non-responder (37.56±5.19) than responders (33.20±4.67). **Conclusion:** SNP in the promotor region of the osteopontin gene (OPN) at nucleotide (nt) -443 and serum OPN protein level are predictors of responses to combination therapy of HCV.
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1.Introduction:

The hepatitis C virus (HCV) in Egypt is unique in the world as there are many publications suggesting that over 15% of the people in Egypt are infected, this is ten times greater than in any other country in the world ***(El-zanaty et al., 2009).*** HCV genomic analysis by means of gene sequencing of many viruses has lead to the division of HCV into 6 genotypes based on homology ***(Bonkovsky et al., 2001).*** Molecular differences between genotypes are relatively large, and they have a difference of at least 30% at the nucleotide level. The major HCV genotype worldwide is the genotype 1, which accounts for 40-80% of all isolates ***(Mukherjee et al., 2011).*** However***, El Zayadi et al. (2005)*** found that genotype 4 is the principle one in Egypt and Middle East.

Treatment of HCV is to prevent complications of HCV infection; this is mainly achieved by elimination of the virus. For patients with HCV genotype-4 infection, combination treatment with pegylated interferon (PEG-IFN) plus weight based ribavirin administered for 48 weeks appears to be the optimal regimen ***(Kamal et al., 2007).*** The efficacy of the IFN-based therapies is dissimilar between patients with HCV. This indicates that genetic polymophisms may play an important role in the mechanisms by which the body response to interferon treatment ***(Naito et al., 2005).***

Response to therapy in chronic hepatitis C can be categorized as biochemical as shown by normal alanine aminotransferase (ALT) levels, or virological as shown by the absence of detectable HCV RNA in the serum and histologically (<2 point improvement in necroinflammatory score with no worsening in fibrosis score) ***(Jeffers et al., 2004).*** Typically, multiple end points of a combined response are used to describe results of trials of antiviral therapy.

Responses can also be categorized by timing of the measurements of success, as early during treatment (initial response), at the end of therapy (end of treatment response), or 6 months after therapy (sustained response) ***(Radkowski et al., 2005).***The virologic response has many phases:

Sustained Virologic Response (SVR) which is defined by the absence of detectable HCV RNA in the serum as shown by a qualitative HCV RNA assay with lower limit of detection of 50 IU/ml or less at 24 weeks after the end of treatment ***(NIH consensus, 2002).*** Rapid Virologic Response (RVR) is defined as undetectable HCV RNA by polymerase chain reaction [PCR] after 4 weeks of treatment and patients who achieve RVR have an excellent chance of achieving SVR ***(Shiffman, 2008).*** Early Virologic Response (EVR) is defined as reduction in the HCV RNA level > 2-logl0 drop or loss of HCV RNA 12 weeks into therapy. Among those with an EVR, the likelihood of SVR is > 70% ***(Shiffman, 2008)***. End of Treatment Response (ETR)**;** it is indicated by non-delectability of HCV RNA at the end of therapy. While, Breakthrough is the reappearance of HCV RNA in serum while still on therapy.

Relapse is the reappearance of HCV RNA in serum after therapy is discontinued and an ETR was documented.

Patients should be monitored during therapy to assess the response to treatment ***(Ghany et al., 2009)***. Laboratory monitoring should include measurement of the complete blood count, serum creatinine and ALT levels, and HCV RNA by a sensitive assay. Thyroid function should be monitored. Patients who achieve an SVR usually have improvement in liver histology and clinical outcomes ***(Bruno et al., 2007).***

Osteopontin (OPN) is a multi-faced protein; ***(Plumer et al., 2008).*** It is biosynthesized by a variety of tissues and is secreted in the body fluids. OPN exists both as a component of the extracellular matrix and as a soluble cytokine ***(Wesson et al., 2003).***

Over expression of OPN was associated with various inflammatory liver diseases ***(Morimoto et al., 2004).*** Pretreatment with a neutralizing OPN antibody attenuated the inflammatory liver injuries ***(Kwon et al., 2009).***

Its levels were elevated in patients with fulminant hepatitis. Probably reflecting production of osteopontin in Kupffer cells and hepatic macrophages ***(Matsui et al., 2004).***

Osteopontin hepatic expression and serum levels are increased in patients with alcoholic hepatitis and correlated with disease severity. ***(Patouraux et al., 2012)***. Moreover, it can be used as a prognostic marker for HBV related hepatocellular carcinoma HCC ***(Xie et al., 2007),*** as increased plasma OPN concentration is predictive of cirrhosis and HCC in patients with HBV infection ***(Zhao et al., 2008)***. The plasma OPN level is correlated with the severity of liver fibrosis and inflammation, suggesting OPN could be used a biomarker to evaluate the severity of liver damages in HCV subjects ***(Huang et al., 2010).***

Four single nucleotide polyrmophism SNPs in the promoter region of OPN gene (-155, -443, -616 and -1748) may be useful as a marker to predict the efficacy of IFN-based therapies in patients with chronic hepatitis C ***(Naito et al., 2005).***

Mochida et al. (2004) evaluated four SNPs in the promoter region of OPN at (-155, -616, -1748 and -443). In patients with chronic hepatitis C. They concluded that SNP at nt -443 may be a useful marker reflecting hepatitis activity in chronic hepatitis C patients which was based on serum ALT levels measured at intervals between 1 and 3 months at least for 2 years in this study.

Naito et al. (2005) showed that the SVR rate differed depending on the alleles of the four SNPs in the promoter region of OPN. Such differences were particularly evident in patients with genotype 1b and 1a high titer. And they concluded that the SNP at nt -443 and the three SNPs at nt-155, -616, and -1748 with linkage disequilibrium were useful as a marker to predict the therapeutic efficacy of IFN alone or IFN plus ribavirin. And so this point should be investigated more.

Aim of study, is to investigate SNP at -443 in the promoter region of OPN gene and OPN protein level and their effect on response to interferon treatment in patients with chronic hepatitis C.

2.Subjects and Methods:

This study was conducted on 119 subjects with their ages ranging between 18-54 years, fifty-three of them are females. They were classified into 2 groups: **Group I:** Included 99 HCV chronically infected Egyptian patients, with their ages ranging between 18-54 years with mean (39.41±7.571). All of them attended the liver unit of Tropical Medicine Department, at Kasr El-Aini Hospital, Cairo University outpatient's Clinic to receive combined treatment of Interferon and Ribavirin. **Group II:** Included 20 healthy individuals without HCV infection, they were collected as control samples, with their ages ranging between 18-47 years with mean (33.10±8.169). All patients were subjected to clinical examination and ultrasonography, serological, virological and histological diagnosis of chronic HCV. Pretreatment and post-treatment histopathological examination of percutaneous needle liver biopsy were also done.

All had elevated ALT level above the upper limit of normal within 6 months prior to entry to the study.Patients had not been previously treated with

interferon based therapy.

During study patients were treated with PEG-IFN α 2b 1.5 µg /kg weekly and ribavirin (800-1000mg /day) for 48weeks. For evaluating the response to treatment, the response was monitored at 3 months from the start of treatment to detect the early virological response and at 6 months after the end of treatment to detect the sustained virological response, as well as, normalization of aminotransferases (ALT and AST) levels and clearance of the virus. According to response to treatment, patients were either responders or non responders.

The following laboratory work was done for all subjects:-

1-Liver Function Tests.

Including determination of total and direct serum bilirubin, serum albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALK) [by spectrophotometry using Hitachi 902] alpha fetoprotein (AFP) [by chemiluminescence using Immulite 2000] **2- Markers of Hepatitis virus:** HBsAg, Anti-HBc, Anti-HCV were assessed by routine methods using commercially available assays.

3- HCV-RNA titer:

HCV –RNA titer was measured before and after treatment by Real time PCR.

4- Autoantibodies:

Anti-Nuclear antibody (ANA), Anti-DNA was done using immunoflurescence kits.

5- Serum urea and creatinine: by spectrophotometry using Hitachi 902**.**

6. Quantitation of Osteopontin (OPN) protein in serum:

Human osteopontin in serum was measured using ELISA kit provided by RayBio (RayBiotech, Inc., NY, USA).

7-Analysis of osteopontin gene polymorphism, DNA was extracted from whole blood samples using the QIAamp® DNA minikit (Qiagen, USA) following the manufacturer's instructions. One set of primers were used for amplification of the osteopontin gene that contains the nucleotide -443. The primer sequences were as follows: Forward 5'-TGTCACTAGTGCCATTTGT3' and reverse5'-TGTACCTTGGTCGGCGTTTG-3'.

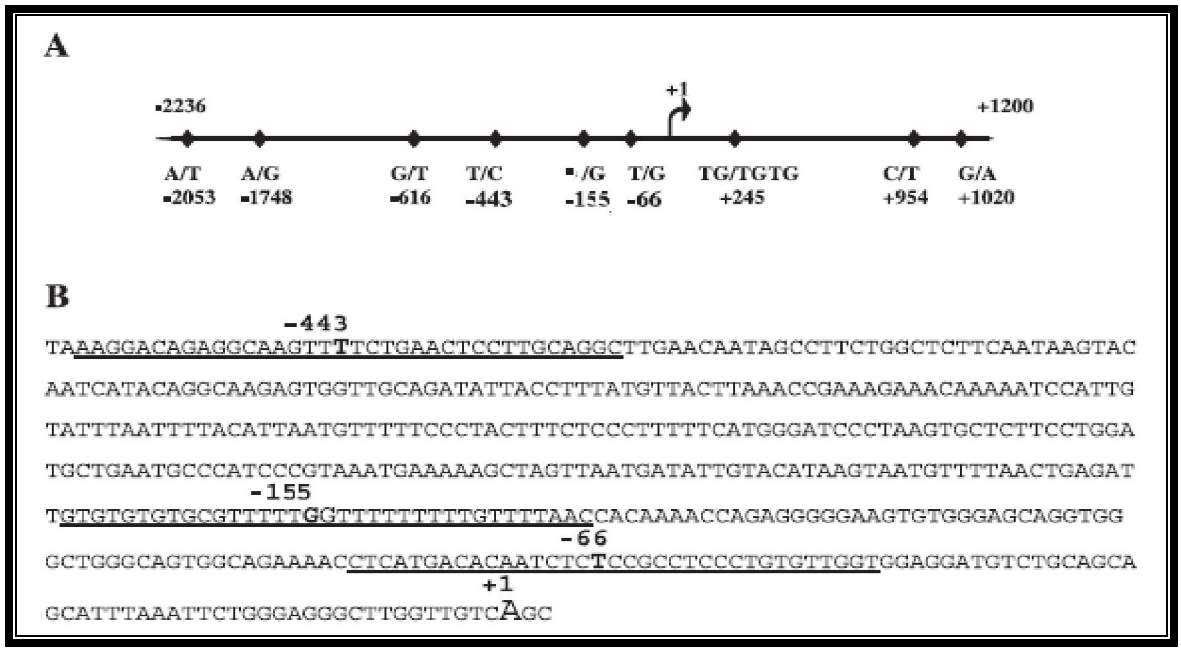


Figure (1): A: represents different sits of polymorphisms on osteopontin gene. B: represents the DNA sequence of PCR product including 3 polymorphisms (-443, -155, -66 nt)

The PCR amplification product was detected at 630 bp. demonstrates different nucleotide polymorphisms among the osteopontin gene. Then by using gel electrophoresis: The gel was taken for viewing on an ultra-violet transilluminator. The PCR products were subjected to direct sequencing using both forward and reverse primers. 5'-TGTCACTAGTGCCATTTGT- 3' and reverse primer 5'-TGTACCTTGGTCGGCGTTTG -3'. The Big Dye Terminator mix V 3.1 system and ABI 310

sequencer (ABI, Lincoln Center Drive Foster City, USA) was used for sequencing. Homology searches using BLAST was carried out to confirm the nucleotide sequence (http:// blast.ncbi.nlm.nih.gov/ Blast.cgi). Computer program Chromas 2.33 was used to interpret the result and determine the genotype.

All the collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 15.

3.Results:

This study showed that:

1. There was insignificant difference between hepatitis C patients and controls in the distribution of SNP -443 genotypes (T/T, T/C and C/C) Table(1) and Fig. (2). The relation between response at 3 months after treatment and genotypes shows statistically insignificant difference between responders and non responders regarding different genotypes distribution Table(2) and Fig. (3). While, there was statistically significant increase of T allele in responders when compared to distribution of T allele in non responders (68.5% vs 60.3%) Table(3) and Fig. (4). As regard relation between response at 6 months after ending treatment and genotypes, there was statistically significant difference between responders and non-responders as regard genotype distribution CC genotypes was more presented in non responders (28.8%) in comparison to responders (7.40%), while TT genotype was more presented in responders (59.25%) in comparison to non responders (35.6%)Table(4) and Fig. (5).
2. There was statistically significant difference between responders and non responders as regard allele distribution (T allele represents 75.93% of responders compared to 53.3% of non responders Table(5) and Fig. (6); i.e., T allele is associated with increasing response to therapy. While, C allele respresent 24.07% of responders compared to 46.7% of non responders Table(5) and Fig.(6); i.e., C allele is associated with increasing non response to therapy
3. There was a significant difference in pretreatment osteopontin serum protein level between patients and controls (P=<0.0001) Table(6a) and between non-responders and responders 3 mo. after treatment (P =0.01) with high level in non-responders (37.08±5.49) than responders (34.20±5.49). Also, there was a significant difference between non-responders and responders 6 mo. after ending treatment (P =<0.001) with high level in non-responder (37.56±5.19) than responders (33.20±4.67) Table (6b).
4. There was significant difference of mean level of osteopontin protein in non responders in comparison to responders 3 mo. after treatment regarding TC and TT genotypes, while this difference is insignificant in CC genotypes Table(7). There was statistically insignificant difference between different genotypes in patients group as regard the mean osteopontin levels. There was insignificant difference of mean level of osteopontin protein in non responders in comparison to responders 6 mo. after ending treatment Table(8).
5. Univariate logistic analysis revaled that osteopontin protein level. Tbil, Dbil, ALK, ALB, PL, fibrosis, viraemia OPN protein and OPN SNP at nt (-443) represent significant predictor for treatment responsiveness. Table(9)
6. Multivariate analysis represent that only fibrosis, PT, OPN protein and OPN SNP at nt (-443) were found to be significant predioctors for treatment responsiveness. Table(10)

Table (1): Genotype frequency for SNP (-443) in osteopontin gene in hepatitis C patients and controls

Group	OPN (- 443)						*P*
	CC		TC		TT		
	N	r %	N	r %	N	r %	
Case (99)	17	17.2	34	34.3	48	48.5	0.406(NS)
Control (20)	3	15.0	10	50.0	7	35.0	
Total	20	16.8	44	37.0	55	46.2	

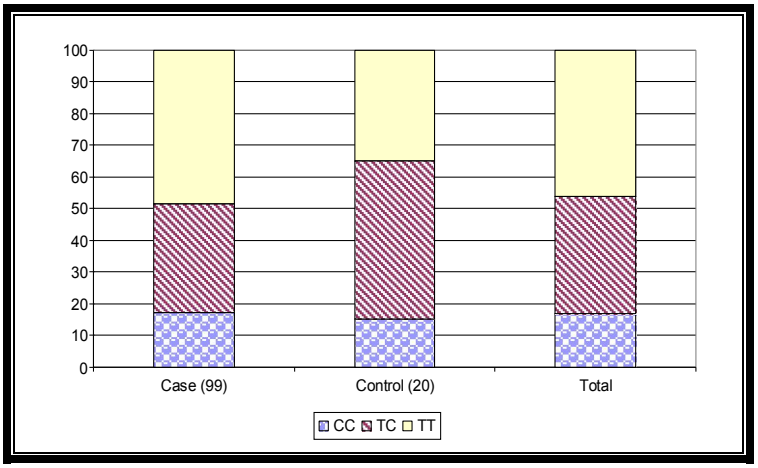


Figure (2): Group distribution of study subjects in relation to OPN (- 443)

Table (2): Relation between response at 3 months after treatment and OPN gene SNP (-443)

		Responders		Non responders		*P*
		No	**%**	**No**	**%**	
Genotypes	CC	11	16.9	6	17.6	0.27(NS)
	TC	19	29.2	15	44.1	
	TT	35	53.8	13	38.2	
Total		65	100.0	34	100.0	

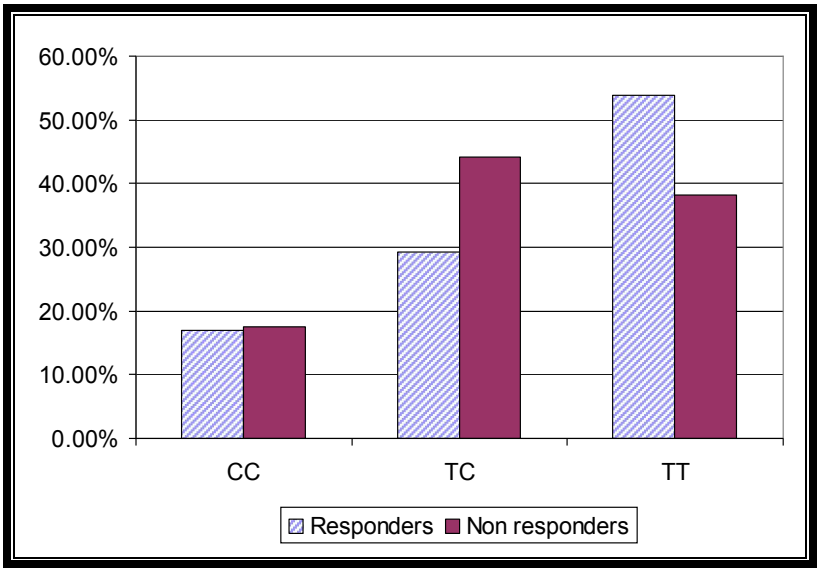


Figure (3): Relation between response and genotyping at OPN gene SNP (-433) at 3 months

Table (3): Relation between response at 3 months after treatment and OPN allele of SNP (-443) gene

		Responders		Non responders		*P*
		No	%	No	%	
Alleles	C	41	31.5	27	39.7	<0.001*
	T	89	68.5	41	60.3	
Total		130	100.0	68	100.0	

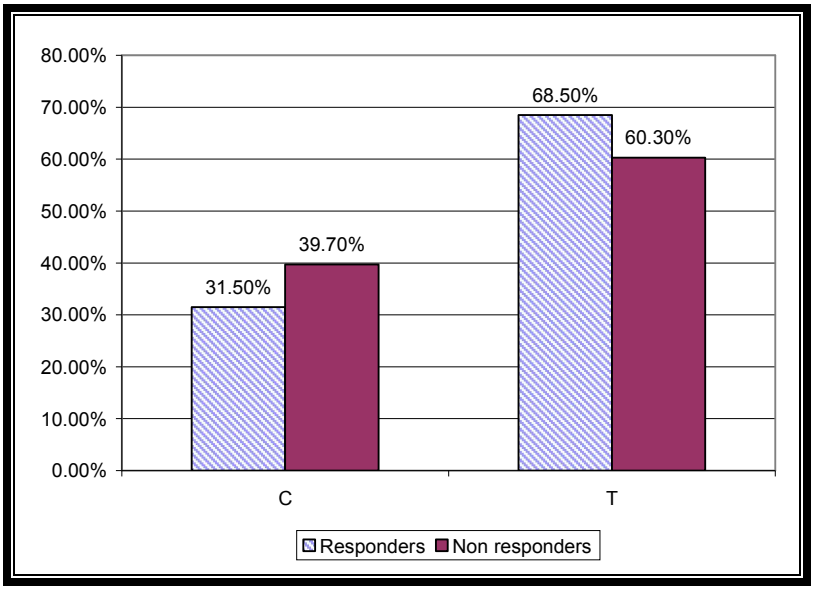


Figure (4): Relation between response at 3 months after treatment and OPN allele of SNP (-443) gene.

Table (4):Relation between OPN gene SNP (-443) and response at 6 months after end of treatment

OPN (-443)	Response				*p*
	Responders		Non-responders		
	N	%	N	%	
CC (17)	4	7.40	13*	28.8	0.009(S)
TC (34)	18	33.33	16	35.6	
TT (48)	32*	59.25	16	35.6	
Total	54	100.0	45	100.0	

(*) = statistically significant difference

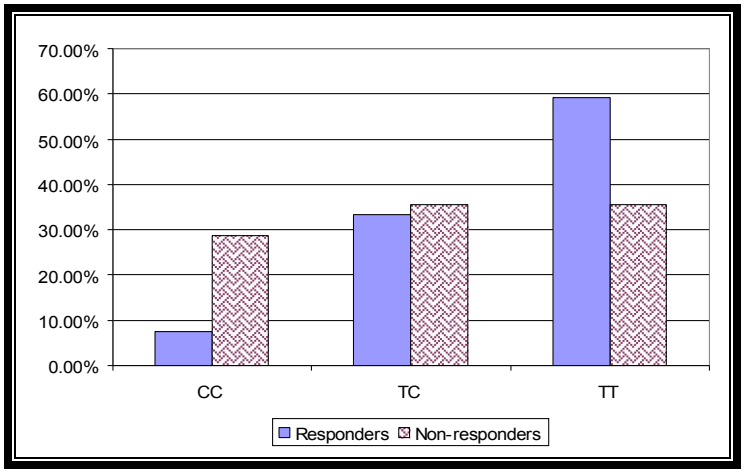
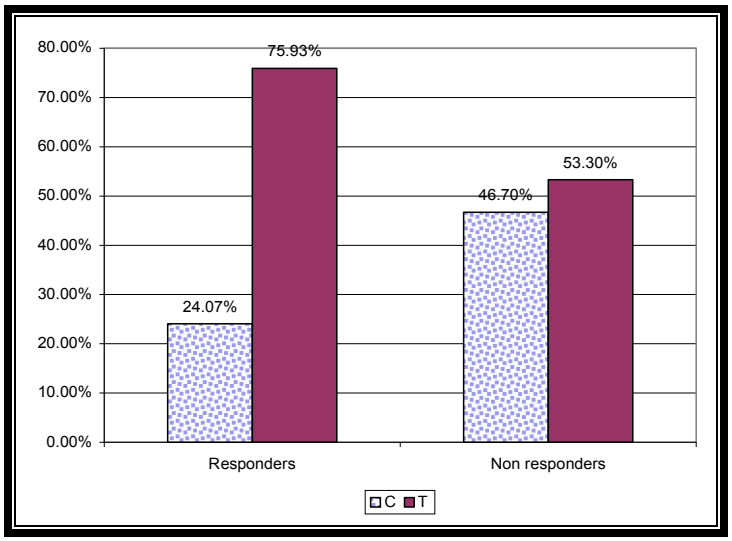


Figure (5): Relation between Response and genotyping at OPN gene SNP (-433) at 6months.

Table (5): Relation between alleles of SNP (-443) of OPN gene and response at 6months after end of treatment.

OPN (-443)	Response				*P*
	Responders		Non-responders		
	N	%	N	%	
C	26	24.07	42	46.7	<0.001*
T	82	75.93	48	53.3	
Total	108	100.0	90	100.0	



Figure(6): Relation between response and OPN alleles SNP (-443) and response at 6 months after end of treatment.

Table (6a): Relation between osteopontin serum protein level of hepatitis C patients before treatment in comparison to control group

Group	HCV patients Mean ± SD	Control Mean ± SD	*P*
OPN protein	68.05±6.70	20.18±0.37	< 0.0001*

Table (6b): Relation between response and osteopontin levels at 3 and 6 months.

		Mean	±S.D	Minimum	Maximum	*p*
At 3 months	Responders	34.20	5.04	22.90	48.30	0.01(S)
	Non responders	37.08	5.49	26.00	45.30	
At 6 months	Responders	33.20	4.67	22.90	41.00	< 0.001(S)
	Non responders	37.56	5.19	26.00	48.30	

Table (7): Compariosn between responders and non responders in different genotypes as regard osteopontin levels at three months.

		N	Mean	±S. D	t	*p*
CC	Responders	11	35.71	4.22	0.7	0.49(NS)
	Non responders	6	34.16	4.55		
TC	Responders	19	32.54	5.53	2.08	0.045*
	Non responders	15	36.58	5.71		
TT	Responders	35	34.62	4.89	2.70	0.009
	Non responders	13	39.01	5.27		

Table (8): Compariosn between responders and non responders at six months in different genotypes as regard mean osteopontin levels.

		N	Mean	±S. D	t	*p*
CC	Responders	13	34.85	4.29	0.51	0.59(NS)
	Non responders	4	36.20	4.66		
TC	Responders	25	34.70	6.31	0.61	0.53(NS)
	Non responders	9	33.26	4.66		
TT	Responders	16	37.13	5.67	1.21	0.22(NS)
	Non responders	32	35.15	5.09		

Table (9): Univariate regression analysis of several variables.

	B	Sig.	Odds ratio	95.0% C.I. (for odds ratio)	
				Lower	Upper
Sex	.152	.711	1.164	.523	2.590
Constant	-.398	.518	.671		
Age	-.005	.859	.995	.944	1.049
Constant	.005	.996	1.005		
ALT	-.001	.733	.999	.990	1.007
Constant	-.054	.900	.948		
AST	.004	.157	1.004	.998	1.010
Constant	-.657	.092	.518		
T. bil	1.819	.000	6.164	2.640	14.393
Constant	-2.538	.000	.079		
D. bil	4.475	.000	87.815	10.234	753.528
Constant	-1.806	.000	.164		
ALK	.017	.050	1.017	1.000	1.035
Constant	-2.125	.039	.119		
ALB	-2.033	.001	.131	.041	.416
Constant	6.956	.001	1048.952		
AFP	.060	.016	1.062	1.011	1.114
Constant	-1.145	.009	.318		
PT	.556	.000	1.744	1.305	2.332
Constant	-7.298	.000	.001		
PCR	.000	.321	1.000	1.000	1.000
Constant	-.252	.238	.778		
Fibrosis	2.773	.000	16.000	5.388	47.509
Constant	-4.747	.000	.009		
Viremia	-.937	.031	.392	.167	.916
Constant	1.102	.076	3.010		
OPN (-443)	-.969	.020	.379	.168	.858
Constant	1.246	.053	3.475		
OPN	.178	.000	1.195	1.090	1.310
Constant	-6.491	.000	.002		

Table (10): Multivariate regression analysis of several variables.

Variables in the Equation	B	Sig.	Odds ratio	95.0% C.I (for odds ratio)	
OPN (ng/ml)	0.383	0.001	1.466	1.182	1.819
T. bil	1.086	0.524	2.963	0.105	83.882
D. bil	3.166	0.516	23.716	0.002	332521.196
ALB	1.530	0.333	4.617	0.209	102.009
AFP	-0.068	0.126	0.934	0.856	1.019
PT	0.654	0.032	1.924	1.058	3.499
Fibrosis	6.502	0.008	666.396	5.439	81647.769
Viremia	-1.375	0.154	0.253	0.038	1.671
OPN(-443)	-5.847	0.017	0.003	0.000	0.346

4. Discussion:

Chronic HCV infection is a major public health problem due to late complications as increasing the risk for hepatic steatosis, cirrhosis, and hepatocellular carcinoma ***(Choi, 2012).*** The gold standard treatment of chronic hepatitis C (CHC) is PEG-IFN in combination with ribavirin. Being expensive, involving severe side effects and requiring long treatment duration,therefore accurate prediction of response to the therapeutic regimen is of great interest ***(Saludes et al., 2010).*** Osteopontin is shown to be essential for initiation of T helper 1 immune response at the upstream of IL-18 and IL-12 in a cytokine network. The T helper 1 immune response is involved

in the development of inflammation in chronic hepatitis C, and the hepatocytes infected with HCV are eradicated by Th1 response during IFN-based therapies ***(Ashkar et al., 2000).***

Polymorphisms in the osteopontin gene may provoke diverse immunological response through regulation of osteopontin expression in macrophages. Also, Osteopontin single nucleotide polymorphisms (SNPs) was reported to be associated with a virological response to IFN-based therapy ***(Naito et al., 2005).***

Four single-nucleotide polymorphisms (SNPs) in the promoter region of the osteopontin gene (OPN), at nucleotide (nt) -155, -443, -616, and 1748 were identified. Those at nt -155, -616, and -1748 had already been registered in a database of Japanese single-nucleotide polymorphisms (JSNP) and/or in the dbSNP (National Center for Biotechnology Information) ***(Haga et al., 2002),*** the SNP at nt -443 was identified by ***(Mochida et al., 2004)***.

In this study, no significant difference observed as regard different genotypes distribution between hepatitis C patients (TT 48.5%, TC 34.3% and CC 17.2%) in comparison to control (TT 7%, TC 10% and CC 15.0%).

As regard relation between genotyping and response at 3 months, there was statistically insignificant difference between responders and non responders regarding different genotypes distribution. While, there was statistically significant increase of T allele in responders when compared to distribution of T allele in non responders (68.5% vs 60.3%).

As regard relation between response at 6 months after ending treatment and genotypes, there was statistically significant difference between responders and non-responders as regard genotype distribution (CC genotypes was more presented in non responders (28.8%) in comparison to responders (7.40%), while TT genotype was more presented in responders (59.25%) in comparison to non responders (35.6%). There was statistically significant difference between responders and non responders as regard allele distribution (T allele represent 75.93% of responders compared to 53.3% of non responders; i.e., T allele is associated with increasing response to therapy. While, C allele represent 24.07% of responders compared to 46.7% of non responders; i.e., C allele is associated with increasing non response to therapy).

Mochida et al. (2004) designed their study to examine if the SNP in the promoter of OPN gene could be used as a marker predicting the activity of hepatitis C. And as regard the distribution of SNP genotypes at nt (-443) they found that CT is the predominant (CC 16.8%, CT 50.9% and TT 32.4%) in patients.

Moreovr, ***Naito et al. (2005) studied*** the distribution of genotypes of SNP at nt (-443), they found that CC (19.5%), CT (51.9%) and TT (28.67%). The study of ***Mochida et al. (2004)*** **and** ***Naito et al. (2005)*** was done on Japanese patient with CT genotype more prevalent (51.9% - 50.9% of patients) and CC less prevalent (19.5% - 16.8% of patients) while the present study was done on Egyptian patients with less prevalence of CC (23.8% of patients) and more prevalence of TT (48.5% of patients). This may be due to the different races,but this observation should be investigated over more larger groups to be confirmed. ***Natio et al. (2005)*** reported that the SNP at nt -443 is located 13 base pairs (bp) upstream of the cis-acting enhancing element of human OPN. Considering that the Th1 response is involved in the development of inflammation in chronic hepatitis C and that hepatocytes infected with HCV are eradicated by T helper 1 (Th1) response during IFN-based therapies, the SNP in OPN at nt -443 may be crucial in provoking diverse Th1 immune reactions against HCV through the regulation of osteopontin expression in the liver.

Natio et al. (2005) concluded that the sustained virological response rate in patients with the TT genotype in the SNP at nt -443 (86.4%) was also significantly higher than the sustained virological response rate in those with the CC or CT genotypes (47.3%).

The results of the current study revealed that, the sustained virological response (SVR) to PEG-IFN alpha-2b plus ribavirin therapy was 60 %. This is comparable with response rate (63%) predicted by ***Al Ashgar et al. (2009)*** but is higher compared to that reported by ***kamal et al. (2005)*** which was 48 % and ***EL zayadi et al. (2005)*** which was (47%). And is lower compared to that reported by ***Al Ashgar et al. (2008)*** which was (75.6%).

The result of the current study revealed that, by simple univariate analysis single nucleotide polymorphism (SNP) in the promotor region of the osteopontin gene (OPN) at nucleotide (nt) 443 show significant difference between responders and non responders (p =.02) and was a predictor to response to interferon therapy of chronic hepatitis C by multivariate logistic regression analysis.

This finding agrees with ***Naito et al. (2005)*** who reported that by simple univariate analysis the SVR rate differed depending on different genotypes of the SNP in the promoter region of OPN at nucleotide nt (-443). ***Mochida et al., (2004)*** evaluates SNP in the promoter region of OPN and its association with hepatitis activity in hepatitis C patients. They concluded that, OPN at nt -443, show significant differences between low, moderate and high activity groups by both univariate and multivariate logistic regression analysis and may be a useful marker

reflecting hepatitis activity in chronic hepatitis C patients.

In the present study a clear relationship between baseline of OPN protein level in blood of chronic hepatitis C patients and response to PEGIFN alpha-2b plus ribavirin was observed. Lower pretreatment plasma OPN protein were correlated with better response (p=.000). By both univariate and multivariate logistic regression analysis pretreatment plasma OPN protein level was a good predictor of response to hepatitis C therapy (p =.000) and (p =.001) respectively.

This finding is further verified from ***Huang et al. (2010)*** study who found that the OPN concentrations were significantly increased in the HCV individual with extensive fibrosis and inflammation. The OPN concentrations were significantly correlated with the liver fibrosis and HAl (Histology Activity Index) score in subjects with the HCV infection. They provide evidence that the surveillance of plasma OPN concentration may be a non-invasive biomarker for the evaluation of severity of liver damage in HCV-infected subjects.

In the present study, analyzing prognostic viral and host factors (other than the SNP of the promoter region of osteopontin gene nt-443 & OPN protein level in blood) for the therapeutic efficacy of PEG-INF By univariate analysis revealed that, patients with SVR were significantly had higher serum albumin (p =.00), lower serum alpha-fetoprotein levels (p =.015), lower pretreatment serum total bilirubin levels (p =.000), lower pretreatment serum direct bilirubin levels (p =.000), lower pretreatment serum alkaline phosphatase levels (p =0.05), lower prothrombin time (p =.000) and lower stage of fibrosis (p =.000). Both groups were similar in age distribution, sex distribution, pre-treatment viral load, alanine transaminase and aspartate transaminase levels.

This finding is consistent with a study done by ***Al Ashgar et al. (2009)*** who reported that good indicators for response were young age, non-diabetic status, high serum albumin, low aspartate aminotransferase (AST), low alpha fetoprotein and being treatment naive. However, in multivariate analysis only young age, low AST and treatment naive status were associated with SVR. ***Al Ashgar et al. (2009)*** demonstrates for the first time that lower baseline serum AST and not ALT is an independent predictor of SVR to PEG-INF and Ribavirin in patients with chronic HCV-4. they believe that these lower AST levels reflect less severe histological parameters in the sustained responders.

In addition ***Sarwar and Tarique (2010)*** identified three variables i.e. platelet count <180 x 10^9 /L, serum albumin less than 4 grams/dl and duration between diagnosis and treatment of hepatitis C more than 11 months to be associated with non-response to interferon therapy in chronic hepatitis C.

Furthermore, ***Gad et al. (2008)*** reported that among genotype 4: chronic hepatitis C patients, severe fibrosis, severe steatosis, treatment with standard interferon and a high serum AFP level were all negatively associated with SVR. In the present study, both univariate and multivariate logistic regression were performed. Factors entered into univariate logistic regression analysis were age, sex. ALT, AST, serum total bilirubin, serum direct bilirubin, ALK, ALB, AFP, PT, HCV RNA, Fibrosis grades, Viremia grades distribution, plasma OPN protein level and SNP nt (-443). Univariate logistic regression analysis identified the following predictors of response: Tbil, Dbil, ALK, ALB, PT, fibrosis, viraemia OPN protein and OPN SNP at nt-443.

The independent predictive value of factors that were significally associated with achievement of response by univariate logistic regression analysis (p < 0.05) was determined by multiple logistic regression analysis. The factors significally associated with response were pretreatment fibrosis grades, PT pretreatment serum OPN protein level and OPN SNP at nt-443.

The result of current study revealed that, fibrosis stage in the pretreatment liver biopsy was found to be statistically different between responders and those who not respond to interferon treatment. Also univariate and multivariate regression analysis revealed that pretreatment fibrosis stage was a good predictor of response (p=.000), (p =.008) respectively.

This is consistent with what was previously reported by ***Fried et al. (2002), Hadziyannis et al. (2004) and Zeuzem et al. (2009)*** in patients infected with genotype 1, ***Parise et al. (2006)*** in patients infected with genotype 2,3 and ***Hasan et al. (2004) and Kamal et al. (2007)*** in patients infected with genotype 4.

In the present study, pretreatment viral load was not found to be a predictor of response by univariate logistic regression analysis (p =.32) This finding is consistent with ***Hu et al. (2001)*** who reported that viral load fluctuates and a single reading of HCV quantification may not reflect the actual viral load at the time of treatment, especially if we know that viral load was assessed at varying intervals from the onset of treatment. It has also been reported that the differences in interferon response could be secondary to either a difference in the viral virulence and/or replication rate among different HCV genotypes and not the absolute viral load.

Our results are not consistent with ***Aziz et al. (2011)*** who reported that, there is a close relationship between baseline viral concentration and response to

PEG-IFN alpha-2b plus ribavirin. This finding is further verified from several studies ***Idrees and Riazuddin (2009), Galocsy et al. (2010) and Mauss et al. (2011).***

In short, the results of the present study proved that, osteopontin level is a good predictor of response to interferon therapy in patients with HCV infection.

Conclusion:

SNP in the promotor region of the osteopontin gene (OPN) at nucleotide (nt) -443 and serum OPN protein level are predictors of response to combination therapy of HCV.

Physicians may then use the available data on predictionesponse to interferon-based therapy to better direct the choice between the various treatment options in order to tailor therapy to individual patients both, in relation to the type of therapy used and its duration.

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