Different Treatment Modalities for Improving HCV Response

Ghada A Salem, Nahla E El-Gamal, Maged B Abd El-Aziz , Rashed Hassan

Tropical medicine Department , Faculty of Medicine, Zagazig University,Egypt

Corresponding Author

Ghada A. Salem
Mobile: +201110810100

E mail:ghadasalem21 @yahoo.com

Received :6 / 7 /2012
Accepted after revision: 19 / 8 /2012

Key words:Treatment, HCV, response .

Background and study aim: Hepatitis C virus is a major health problem throughout the world .Interferon (INF) was the only therapeutic opinion until the mid 1990s.Ribavirin(RBV) when added improve the SVR rate (8 to 42%) in patients with genotype 4 infection. Nitazoxanide induces a naturally occurring antiviral intracellular protein and a key mediator of host cell defense against viral infection, it is also believed that it inhibits viral glycoproteins at the post translational level. We aimed to study the impact of NTZ in addition to PEG-INFα 2a and RBV on virological response in patients with chronic hepatitis C.

Patients and Methods: In this work, we studied 100 HCV patients who met the inclusion criteria of age, BMI, normal laboratory findings of liver and kidney functions, CBC, blood glucose level, thyroid functions and with absence of immunological disease. Quantitative PCR for HCV RNA and liver biopsy were done for each patient. Any patient showed more than F3 or A3 in this biopsy was excluded. All patients are followed clinically and by laboratory throughout the period of the study. All patients were divided into three groups: Group (A) received the SOC: PEG-IFN α2a 180µg and RBV (1000, 1200mg), group (B): received SOC and Nitazoxanide and group (C): received NTZ alone.

Results: EVR in group (A) patients was 83.3% compared to 86.6% in group B patients . 24 week PCR negativity was 76.6% for group A and 80% for group B . As regard NTZ as a monotherapy ; four patients (10%) showed pEVR (>2log drop in HCV RNA) but they failed to achieve – ve HCV RNA at the end of treatment, nineteen patients showed <2log drop in HCV RNA at week 12. Out of these nineteen patients, 15 patients showed further decrease in HCV viral load at weeks 24. Abdominal pain 7%, nausea 5% vomiting 2.5%, urine discoloration 2.5% were the most side effects of NTZ.

Conclusion: We can conclude that treatment modalities with PEG- INF, RBV and NTZ is associated with increase in virological response rates and monotherapy of CHC patients with NTZ decreases HCV RNA viral load in some patients, there was mild side effects attributable to NTZ.

INTRODUCTION

Hepatitis C virus is a major health problem throughout the world. The WHO estimates about 200 million people of those infected with HCV, 80% develops chronic hepatitis C infection [1].

More than 90% of HCV isolates from Egyptian patients are of the genotype 4 variant Egypt has the highest world wide prevalence of HCV (10-20%) [2].

Antiviral therapy for CHC has many goals, the primary goal is durable viral decrease as evidenced by the absence of HCV RNA in the serum (virological response), the second goal is the reduction of liver damage as determined by either persistently normal ALT (biochemical response) or improved liver biopsy. (Histological response) . Antiviral therapy will delay or prevent cirrhosis, HCC, liver transplantation and death, as well as to prevent the viral spread to other persons and improving the patient's quality of life [3].

INF was the only therapeutic opinion until the mid-1990s. RBV added to INF resulted in improvement of SVR

rates (8 to 42%) in patients with genotype 4 infection [4].

Modification of the therapeutic molecules of INF through the attachment of polyethylene glycol (PEG) moieties (pegylation) is a common approach to optimize delivery of the drug and to avoid large fluctuating serum concentrations and the inconvenient dosing regimens associated with the standard INF-α. Two PEG-modified INFs have been approved for the treatment of CHC which are (PEG-INFα$_{2a}$ and $_{2b}$). the current treatment of CHC is the combination of PEG-INF and RBV [5].

Nitazoxanid is the first member of thiazolides, anti-infective role which has an oral agent with no major side effects and licensed in the USA in treatment of cryptosporidium parvum and giardia lamblia [6].

A serendipitous observation during drug development revelaed that some patients with cryptosporidium and AIDS who are coinfected with HCV or HBV, had a reduction in serum ALT during therapy. NTZ induces double stranded RNA activated protein kinase (PKR) phosphorylation, which results in increased intracellular concentration of phosphrylated factor 2 [7].

Aim of the work :

The aim of this work is to study impact of NTZ in addition to PEG-α$_{2a}$ and RBV on virological responses in patients with CHC.

PATIENTS AND METHODS

The present study was conducted in Tropical Medicine Department Zagazig University Hospital. This work comprised 100HCV patients attending the out patient clinics.

All patients were divided into the following groups:

Group (A): 30 patients received the SOC, PEG-INFα-2a 180/µg once weekly and RBV (1000-1200mg) based on body weight .

Group (B): 30 patients received NTZ 500mg tablet twice daily for 4 weeks lead-in phase followed by NTZ of the same dose plus PEG INFα-2a (180µg once weekly) and RBV (1000-1200mg) for another 24 weeks. Patients in group A and B stopped treatment when EVR is not achieved as cost-effectiveness in crucial to maximize the health gain achieved in Egypt with the highest prevalence of HCV which is considered a heavy economic burden.

Group (C): 40 patients received NTZ montherapy 500mg tablet twice daily for 24 weeks.

All patients fulfilled the inclusion criteria which are age 18 years or older normal complete blood picture, normal kidney function tests, thyroid stimulating hormone is within normal level, positive HCV antibody and HCV-RNA with no contraindication to liver biopsy, if liver biopsy >F1 with elevated liver enzymes, if > F2 with normal liver enzymes, alpha fetoprotein <100 IUml, female patients participating adequate contraception. All patients who are pregnant female, lactating, decompensate liver cirrhosis, active epileptic fits, ischemic heart disease, chronic renal failure, autoimmune disease, retinal abnormality severe psychiatric condition or with BMI> 35 were excluded from the study. All patients underwent the following test:

- Full history taking and through clinical examination.
- BMI was calculated as weight / height (m2) <30
- Complete blood picture.
- Liver, kidney function tests, fasting, post prandial blood glucose level.
- Abdominal ultrasonography using aloka SSD-500.
- αFeto protein, pregnancy test, antinuclear antibody, thyroid stimulating hormone, ECG, funds examination, HCV antibody by Eliza method.
- Quantitative PCR for HCV at week, 0, 12, 24 was done by cobas amplicor HCV monitor test (HCV, V2.0 Roche Brungburg USA).
- Liver biopsy and fibrosis was evaluated using the metavir scoring system a scale f0-F4: f0 = no-fibrosis, F1= portal tract expansion, F2=less than 50% bridging fibrosis, F3=more than 50% bridging fibrosis without cirrhosis, F4= established cirrhosis .
- The grading of activity was classified as: A0, no histological activity, A1= mild activity, A2= moderate activity, A3= severe activity.

Statistical analysis:

The data were statistically analysed using microstate soft ware program (8) and the following statistical tests were applied :

- Studied "t" test for comparison of means two independent groups.

- ANOVA or f-test for comparison of means of more than two groups.
- Description of quantitative and qualitative variable.
- Chi-square test was to compare qualitative variables.
- Correlation co-efficient test (r-test) was used to rank different variables against each other directly or indirectly.
- P value >0.05 insignificant and P< 0.01 highly significant.

RESULTS

Table (1): Comparison of baseline demographic and laboratory characteristics of the three studied groups.

Variable	Group (A) (n=30)		Group B (n=30)		Group C (n=40)		F	P Value
Age (year)								
Mean ± SD	41.8 ± 9.5		40.3 ± 8		41 ± 7.5		0.246	0.78
Range	23-57		26-55		28-56			
Sex,	N	%	N	%	N	%		
male	20	66.7%	20	66.7%	30	75%		0.593
Female	10	33.3%	10	33.3%	10	25%		
BMI (Kg/m^2)								
Mean ± SD	28 ± 2		27 ± 3		28 ± 1		2.448	0.09
Range	27-30		26-30		27-29			
Total bilirubin (mg/dl)								
Mean ± SD	0.7 ± 0.2		0.6 ± 0.2		0.6 ± 0.2		2.625	0.07
Range	0.4 – 1.5		0.3-1.4		0.3-1.4			
ALT)IU/L)								
Mean ± SD	73 ± 41		70.7 ± 36		61.4 ± 21.5		13.50	0.264
Range	15-153		30-140		15-109			
AST (IU/L)								
Mean ± SD	69.1 ± 45.9		58 ± 43		63.7 ± 36.3		0.540	0.585
Range	23-99		26-90		20-130			
PT (seconds)								
Mean ± SD	11.6 ± 0.9		11.5 ± 0.9		12.1 ± 0.8		4.996	0.09
Range	10-14.5		10-14		11-14			
S. creatinine (mg/dl)								
Mean ± SD	0.79 ± 0.19		0.7 ± 0.2		0.7 ± 0.2		0.000	1
Range	0.2 – 1.1		0.1 – 1		0.1 – 1.1			
WBC (x10^{3}cell/cmm)								
Mean ± SD	6 ± 1.4		5.8 ± 1.7		6.2 ± 1.3		0.648	0.525
Range	4.1 – 9.2		4-9		4.6 – 8.9			
HB (gm/dl)								
Mean ± SD	13.6 ± 1.1		13 ± 0.9		12.9 ± 1.6		2.829	0.06
Range	12-16		11.9-15		11-15			
Platelets (10^3/cmm)								
Mean ± SD	191 + 46		196 ± 42		204 ± 33.4		0.939	0.3
Range	118-294		115-290		168-350			

Table (2): Baseline virological characteristics of three studied groups:

Variable	Group A (n=30)	Group B (n=30)	Group C (n=30)	F	P
HCV RNA (IU/ml x 10^3) Mean ± SD	895 ± 230	836 ± 218	800 ± 356	0.958	0.387

Table (3): Frequency of virological response rates of the three studied groups.

Virological response	Group A (n=30)		Group B (n=30)		Group C (n=30)	
	N	%	N	%	N	%
EVR	25	83.3%	26	86.6%	6	15%
-ve HCV RNA after 24 week	23	76.6%	24	80%	0	0%

Table (4): Comparison of demographic, biochemical and histopathological characteristics of responders of group A and B.

Variable	**Group A n=(23/30) 76.6%**		**Group B n=(24/30) 80%**		**t**	**P**
Age (year)	44.6 ± 9.8 25-57		43.3 ± 8.4 26-51		0.4890	0.627
Sex,	No.	%	No.	%		
Male	15	50%	17	56.7%		0.760
Female	8	26.65	7	23.3%		
BMI (kg/m^2)						
Mean ± SD	27.8 ± 2.2		27.2 ± 1		1.2122	0.231
Range	27-29		26-29			
ALT (IU/ml)						
Mean ± SD	48.5 ± 37.9		56.7 ± 36.9		0.7515	0.456
Range	30-85		30-102			
AST (IU/ml)						
Mean ± SD	42 ± 37.4		51.2 ± 37.2		0.8453	0.402
Range	25-99		26-98			
Baseling viral load (IU/ml)x10^3						
Mean ± SD	850±240		782±238		0.9751	0.334
Range	410-1.200		344-1.820			
Histopathologiocal fibrosis	No.	%	No.	%		
F1	14	46.6	14	46.6%		
F2	7	23.3	9	30%		
F3	2	6.7	1	3.3%		
Activity						
A1	13	43.3	14	46.6%		1
A2	7	23.3	9	30		
A3	3	10	1	3.3%		0.347

Table (5): Comparison of biochemical and hematological characteristics before and at the end of the study (24 weeks) for group C.

Variable	Before the start of the study	At the end of the study	t	P
ALT (IU/L)				
Mean ± SD	61.4 ± 21.5	29 ± 11.8	2.916	0.007*
Range	15-109	13-52		
AST (IU/L)				
Mean ± SD	63.7 ± 36.3	59 ± 40.7	1.021	0.316
Range	20-136	19-160		
Total bilirubin (mg/dl)				
Mean ± SD	0.6 ± 0.2	0.7 ± 0.3	-0.953	0.349
Range	0.3-1.4	0.4-1.7	-1.467	1.53
S. creatinine (mg/dl)				
Mean ± SD	0.7 ± 0.2	0.8 ± 0.21	-2.015	0.053*
Range	0.1-1.1	0.4-1.2		
WBC (x10^{-3}cell/cmm)				
Mean ± SD	6.2 ± 1.3	6.4 ± 1	-1.467	0.153
Range	3.6-8.9	4-8		
HB (gm/dl)				
Mean ± SD	12.9 ± 1.6	12.8 ± 0.8	0.930	0.360
Range	11-15.2	11-14.3		
Platelets (x10^{3}/cmm)				
Mean ± SD	204 ± 33.4	213 ± 37	-1.845	0.076
Range	168-350	182-340		

Table (6): Quantitative serum HCV RNA over time for group C (n=40)

	Week 12		Week 24	
	N	%	N	%
Negative HCV RNA	2/40	5%	0	0%
>2 log drop of HCV RNA	4/40	10%	The four patients showed increase in HCV RNA viral load in comparison to week 12. however, it did not exceed baseline HCV RNA.	
< 2 log drop of HCV RNA	19/40	47.5%	* Fifteen patients (15/40) 37.5% showed further decrease in HCV RNA compared to week 12 and the mean reduction of HCV RNA is log. * Four patients (4/40) 10% showed increase in HCV RNA compared to week 12, however, it did not exceed baseline HCV RNA.	
Stationary viral load	5/40	12.5%	Stopped treatment	
Increased HCV RNA	10/40	25%	Stopped treatment	

Table (7): Comparison of biochemical and hematological characteristics at the end of the study (24 week) between the three studied groups.

Variable	Group A	Group B	Group C	t	P
ALT (IU/L) Mean ± SD	30.7 ± 9	31.9 ± 7.3	29 ± 11.8	0.849	0.431
AST (IU/L) Mean ± SD	28.6 ± 16.4	29.6 ± 15.8	59.407	13.363	0.001*
Total bilirubin (mg/dl) Mean ± SD	0.8 ± 0.2	0.8±0.23	0.8 ± 0.21	2.307	0.105
WBC (x10^{3}cell/cmmm) Mean ± SD	4.2 ± 2	4.2 ± 1.9	6.4 ± 1	21.894	0.0001*
HB (gm/dl) Mean ± SD	11.4 ± 1.5	11.2 ± 1.8	12.8 ± 0.8	14.379	0.001*
Platelets (x103/cmm) Mean ± SD	168 ± 76	167.4 ± 73.9	213 ± 37	6.299	0.003*

Table (8): Demographic and biochemical characteristics of 6 patients achieved negative and >2 log reduction of HCV RNA at week 12 and non responders of group C.

Variable	Negative and >2 log reduction of HCV RNA at week 12 (n=6)		Non responders (n= 34)		t	P
Age (years) Mean ± SD Range	45 ± 4.2 32-56		43 ± 3 28-52		1.814	0.164
Sex	**N**	**%**	**N**	**%**		
Male	4	10%	26	65%		0.628
Female	2	5%	8	20%		
BMI (kg/m^2)	27.2 ± 1		28 ± 1		1.563	0.132
Range	27.2 ± 1		28 ± 1		1.536	0.132
ALT (IU/L) Mean ± SD Range	66.7 ± 32.1 20-114		62 ± 30 26-156		0.350	0.727
AST (IU/L) Mean ± SD Range	66.7 ± 32.1 20-114		62 ± 30 26-156		0.350	0.727

DISCUSSION

The WHO estimates that about 200 million people were infected with HCV of those infected with HCV 80% develops chronic infection [1].

INF was the only therapeutic opinion until the mid 1990s RBV was added to INF resulted in improvement of SVR rates (8.42%) in patients with genotype 4 infection [4].

Nitazoxanide induces double stranded RNA activated protein kinase (PKR) phosporylation, which results in increased intracellular concentration of phosphrylated eukaryotic irritation factor 2 (eIF2α), a naturally occurring antiviral intracellular protein and a key mediator of host cell defences against viral infection [7]. It is also believed that it inhibits viral glycoproteins at the posttranslational level. This would prevent the final assembly of the virus before it can exit and affect another.

A serendipitous observation during nitazoxanide development revealed that some patients with cryptosporidiosis and are co-infected with HCV or HIV had a reduction in serum level of ALT during therapy. This observation led to studies of the antiviral activity of NTZ [7].

This work revealed that patients of group A showed an EVR of 83.3% and 24 week HCV RNA negativity of 76.6%. This is in accordance with the results of El-Makhzangy et al.,[10] who conducted a prospective trial to study the response to PEG-INFα-2a and RBV in 95 patients with CHC genotype 4 for 48 weeks with

results of an EVR of 82% and negative HCV RNA at week 24 achieved in 78% .

El-Zayadi et al. [11] studied the response of CHC patients with genotype 4 to 24 weeks of PEG INFα2b and he reported as slightly lowered response rate than those reported in our study. This difference between these results may be attributed to the difference in the demographic characteristics of the patients and the difference between PEG-INFα-2a and 2b.

The mechanisms underlying in efficacy between the two PEG-INF-α foundation is not clear. However, the structure and the size of the polyether glycol moiety and the means of covalent attachment might affect the pharmacokinetics and parmakodynamics of the two formul-ations, their antiviral activity and the therapy outcome [12].

Our results are contraverry to those of Dimitroulepoulos [13] who studied the effect of ethnic origin on the treatment outcomes in patients chronically infected with HCV-4 (30 Europeans and 30 Egyptians) and with elevated baseline HCV RNA7800.U/ml. They reported a RVR, EVR and HCV RNA at week 24 in Europeans and Egyptians were (RVR 26.7% vs, 30%) (23.3% vs 17.6%, 13.3% vs 16.7% respectively). This can be explained by the that patients selected in this study were with high baseline HCV RNA and advanced stage of liver histology.

As regard treatment modalities results in our study (group B), the EVR (86.6%, vs 83.3%) for group A) and 24 week PCR negative was (80% vs 76.6% for group A). These results shows that NTZ increase EVR and PCR negativity at week 24.

Rossignol et al. [14] reported a cEVR for the SOC and treatment modalities with NTZ of 70% and 86% respectively while cEVR reported in our study was 80.3% vs 86.6% for the SOC and triple arm respectively while cEVR reported in our study was 80.3% vs 86.6% for the Soc and triple arm respectively.

A possible explanation to the difference between the two studies may be due to that Rossignal et al., conducted a 12 weeks lead in phase compared with a 4 weeks lead in phase with NTZ in our study.

Although, the required duration of NTZ lead-in phase is unknown and 12 weeks was selected as an initial conservative estimate to optimize the potential benefit of NTZ pretreatment. A subsequent study has shown that a 4-week lead-in phase may be satisfactory [15,16].

Bacon et al. [17] reported a cEVR of 62% which is lower than the present study (86.6%) when studied a 4 weeks lead in phase of NTZ followed by NTZ, PEG-INF-α2a and RBV for 48 weeks. This may be attributed to that genotype 1 is associated with poorer response to antiviral therapy. The most important clinical property of HCV genotype is different susceptibility to INF [18]. It is also possible that demographics, disease related characteristics of the populations that have been studied or pharmacokinetics might account for difference is responses to therapy.

The differences in INF response could be secondary to either differences in the viral virulence and/or replication rate among HCV types, or different in the host immune response to the different HCV genotype .

The poorer response of genotype 4 in Egypt possible because of various subtypes, to different forms of INFs or probably related to an intrinsic resistance to direct antiviral effect of INF [19].

Egyptian patients infected with HCV genotype 4 after IFN/RBN combination therapy have a high frequency (about double) and significant difference of BCL-2 genotype and allele for non-responds patients compared with responders as well as healthy controls. This suggests that polymorphism in BCL-2 gene can be augment the current array of predictors of therapeutic response to INF and RBV in HCV type 4 infected patients [20].

As regard NTZ monotherapy for treatment of CHC in this study two patients (5%) with very low viremia 3.000 and 70.000IU/ml showed cEVR and was instructed to continue on NTZ till the end of 24 week. However, these patients experienced viral breakthrough with positive HCV RNA at the end of the week 24. Four patients (10%) showed pEVR (>2log drop in HCV RNA) but they failed to achieve negative HCV RNA at the end of the treatment, nineteen patients showed <2log drop of HCV RNA at 12 week. Out these nineteen patients, fifteen patients showed further decrease in HCV viral load at week 24. So this study demonstrated that some patients experienced a partial virological response with NTZ.

Rossignol et al. [16] showed that treatment with NTZ montherapy for patients with CHC genotype 4 at a dose of 500mg orally with food compared with placebo was associated with an ETR at week 24 of (7/23) 30.4% and SVR at week 48 of (4/23) 17.4% compared to 0% for placebo. Whereas, our study reported on EVR of (6/40) 15% and ETR (week 24) of 0%.

These results also confirmed by Mederake [21] who showed that during treatment with NTZ as lead-in phase for 12 week before PEG-INF and RBV, two of 53 patients had a decline of more than 1 Log10 and just one patient achieved a cEVR.

We could find a significant correlation between high baseline viral load and treatment response these results, are in concordance with Kamel et al. [22] Zekri et al. [23].

Patients who responded to IFN treatment had statistically less number in both transitions and the genetic distances between the quasipseices. So, viral genetic complexity and variability may play a role in the response to IFN treatment. The fibrosis score negatively affected the response to IFN. Treatment and pretreatment viral load didn't affect the outcome of treatment [24].

Conclusion:

We can conclude that, treatment modalities with PEG-INF, RBV and NTZ is associated with increase in the virological response rates but with no statistically significant difference, monotherapy of CHC patients with NTZ decrease HCV RNA viral load in some patients. Mild side effects were present to NTZ as abdominal pain, nausea, vomiting and urine discoloration and we can recommend double dose of NTZ for improving the response or study the response of NTZ with STAT-S antiviral drugs as teleprevir & bociprevir when they are in our hand.

Funding: Non .

Conflicts of interest: Non .

Ethical approval: Informed consents were routinely obtained from patients.The study was performed in accordance with the ethical standards on human experimentation and with the Helsinki Declaration of 1964.

REFERENCES

1. Derbala M, El-Dweik N, Al- Kaabi S, Al-Marri AD, Pasic F, Bener AB et al. Viral kinetic of HCV genotype-4 during pegylated infterferon alpha 2a: Ribavirin therapy. *J. Viral Hepat*. 2008 (Equb ahead of print).
2. Kamal S. Hepatitis C virus genotype 4 therapy: progress and challenges. *Liver international* 2008; 31(1): 45-52.
3. Manns M, Zeuzem S, Sood A, Lurie Y, Cornberg M, Klinker H et al., Reduced dose and duration of peiginterferon alfa-2b and weight-based ribavirin in patients with genotype 2 and 3 chronic hepatitis C. *J. Hepatol*, 2011; DOI: 10.1016/j. jep. 12.024.
4. Koshy A, Madda J, Marcellin P, De`Leede, Humphries J, Bechet, A et al. Treatment of hepatitis C virus genotype 4-related cirrhosis: Ribavirin and interferon combination compared with interferon alone. *J Clin Gastroenterol*. 2009; 33: 82-85.
5. Fried M, Shiffman, M. Reddy K, Smith **C**, Marinos G, Gonçales FL et al., Peginterferon alpha-2a plus ribavirin for chronic hepatitis C virus infection. *N. Engl. J. Med*. 2002; 347: 975-82.
6. Anderson V and Curran M, Nitazoxanide: A review of its use in the treatment of gastrointerstinal infection. *Drugs* 2009; 67; 1947-1967.
7. Elazar M, Liu M, McKenna SA, Liu P, Gehrig EA, Puglisi JD , et al., Nitazoxanide (NTZ) is an inducer of eLF2a and PKR phosphorylation. *HEPATOLOGY* 48: 1151A.
8. Nourusis MJ, Package for social science (SPSS) base 8.0 for windows user's guide Chicago, III: SPSS, 1997.
9. Rossignol J, Elfert A, El-Gohary M, Dhillon S, Kaker A, Dosanjh A. Improved virolgoic response in chronic hepatitis C genotype 4 treated with nitazoxanide, peginterferon, and ribavirin. *Gastroenterology* 2009; 136: 856-862.
10. El-Makhzangy H, Esmat G, Said M, Elraziky M, Shouman S, Refai R et al., Response to pegylated interferon alfa-2a and ribavirin in chronic hepatitis C genotype 4. *J. Med. Virol*.; 81: 1576-1583.
11. El-Zayadi AR. Hepatitis C comobidities affecting the course and response to therapy. *World J. Gastroenterol* 2009; 15(40): 4993-4999.
12. Kamal S, El-Kamary S, Shardell M, Hashem M, Ahmed IN, Muhammadi M et al., Pegylated interferon alpha-2b plus ribavirin in patients with genotype 4 chronic hepatitis C: the role of rapid and early virologic response. *Hepatology* 2011; 46: 1732-1740.
13. Dimitroulopoulos D, Elefsiniotis I, Pavlidis C. Xinopoulos D, Tsamakidis K.European vs. Egyptian HCV-4 patients with elevated baseline HCV RNA, treated with PEG-INF-α2a and

Ribavirin: The role of rapid and early virologic response. *Hepat Man*; 10(3): 193-198.
14. Rossignol J, Kabil S, El-Gohary M, Diago M, Hassanein T, Rodes J. Clinical trial: randomized, double-blind, placebo controlled study of nitazoxanide montherapy for the treatment of paitents with chronic hepatitis C genotype 4. *aliment pharmacol ther* 2009; 28: 574-580.
15. Korba B, Glenn J, El-azar M, Enomoto N, Sakuma I, Asahina Y. A role of nitazoxanide in combination with STAT-C agents for inhibition of HCV replications and the potential for prevention of viral resistance. *Antiviral research* 2009; (82)(A): A20.
16. Rossignol J, Elfert A, Keefee E, Farafonova T, Olenina L, Kolesanova E, et al. evaluation of 4 weeks lead-in phase with nitazoxanide (NTZ) prior to peginterferon (PEGINE) plus. NTZ for treatment of chronic hepatitis C: Final report. *Hepatology* 2008; 48: 344A-345A.
17. Bacon B, Shiffman M, Lim J, Fishman S, Factor S, Balestrieri C, et al. Phase 2 randomized, double-bind, placebo-controlled study of nitazoxanide plus peginterferon and ribavirin in HCV genotype 1 naïve patients: Interim analysis shows increase in EVR. *Journal of Hepatology* 2009; 50: S381.
18. Zein N. Clinical significance of hepatitis C virus genotypes. Clin. Microbiol. Rev.2010; 13(2) : 223-235.
19. Derbala M, Amer A, Bener A, Lopez AC, Omar M, El Ghannam M. Peylated interferon-alpha 2b-ribavirin combination in Egyptian patients with genotype 4 chronic hepatitis. *Journal of viral hepatitis* 2005; 12, 180-385.
20. Shaker O, Eskander E, Yahya S. Mohamed MS, Abd-Rabou AA. Genetic variation in BCL-2 and response to interferon in hepatitis C virus type 4 patients. *Clinica Chimica Acta* 2011; 412: 593-598.
21. Mederacke I & Wedemeyr H., Nitazoxanide for the treatment of chronic hepatitis C new opportunities but new challenges? *Annals of Hepatology* 2011; 8(2): 166-168.
22. Kamal S, El Tawil A, Nakano T, He Q, Rasenack J, Hakam SA et al., Peginterferon alpha-2b and ribavirin therapy in chronic hepatitis C genotype 4: impact of treatment duration and viral kinetics on sustained virological response. *Gut* 2005; 54: 858-866.
23. Zekri A, Alam El-Din H, Bahnassy A. Khaled MM, Omar A, Fouad I et al., Genetic distance and heterogenecity between quasispescies is a critical predictor to IFN response in Egyptian patients with HCV genotype-4. *Virology J*2009; 4: 16. http://www.virologyj.com/contents/4/16.
24. Poynard T, Bedossa P, Opolon, P, Flisiak R, Horban A, Kierkus J. et al. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The obsvirc, metavir clinivir, and dosvirc groups. *Lancet* 2006; 349: 825-32.