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Efficacy of Pegylated INF α-2a in Combination with Ribavirin in the Treatment of Naïve Egyptian Chronic HCV Hepatitis with Genotype 4

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Abstract: Objective: The aim of this work is to study the efficacy of Pegylated INF α-2a in chronic hepatitis patients with genotype 4. As well as the significance of estimating the viral load, serum alpha glutathione-S-transferase (αGST) as a marker of hepatocellular damage and pseudo-choline esterase (PCHE) activity which serves as a sensitive index of the synthetic capacity of the liver, on predicting the outcome of interferon alpha 2a therapy in patients with chronic HCV infection. As most of Egyptian patients with chronic HCV were infected with genotype 4. The viral genotype, the degree of inflammation, fibrosis and the viral load prior to treatment are considered the strongest predictors of response to antiviral therapy. HCV genotype 4 was poorly responding to treatment by standard INF therapy. Good response of other genotypes (2 & 3) to Pegylated INF therapy pushed us to study its efficacy on genotype 4. Material and Methods: 53 compensated liver disease CLD patients infected by HCV genotype 4 , with normal TSH, T4 and T3 and negative ANA and AMA antibody to exclude autoimmune disease and 10 healthy individuals as a reference group were enrolled in this study. Patients were classified into two groups according to the liver histology. Group A=43 chronic HCV patients and group B=10 chronic HCV patients with established cirrhosis. Patients in group A were treated by 180 µg Pegylated INF α-2a once weekly & 1200 mg Ribavirin/ day in two doses and patients of group B were treated by 135 µg Pegylated INF α-2a once weekly & 1200 mg Ribavirin/ day in two doses. Results End of treatment response (ETR) and sustained virological response (SVR) were 37/ 53 (69.8%) and 34/ 53 (64.1%) respectively through estimating serum ALT, serum αGST , serum PCHE and HCV RNA by RT- PCR. Patients with established cirrhosis had significantly lower SVR rate compared to non-cirrhotic group (30% vs. 72%), however patients with pretreatment high viral load, low PCHE level and high serum αGST are of significantly lower SVR rate compared to those with moderate and low viral load and serum αGST, and normal PCHE level. Dose reduction from group A was necessary in two patients for the PEG INF (4%) and in 3 patients for the Ribavirin (6%). Conclusion Alpha GST and PCHE are good sensitive markers in choosing and following up the HCV infected patients and could be used as important factors for predicting efficacy of IFN-alpha and ribavirin combination therapy in Egyptian patients. Pegylated INF α-2a and Ribavirin are effective combination in treatment of chronic HCV genotype 4, however this treatment has a disappointing outcome in cirrhotic patients.

Key words:

INTRODUCTION

Hepatitis C virus (HCV) infection is one of the most important diseases with high chronic rate (50-80%) leading to end-stage cirrhosis and its sequel in 20-30% of these individuals. Chronic HCV is a major public health problem in Egypt and, more than 90% of patients were genotype 4 (Zekry AR *et.,al* 2001). Early treatment of chronic HCV patients will markedly reduce progression to cirrhosis, de-compensated disease and hepatocellular carcinoma (Attia MA *et.,al* 1998).

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Interferon α, the currently available therapy for chronic HCV infection, usually results in an initial response in about half of the patients, but a sustained biochemical and virological response with histological improvement occurs in only 15-20% of treated patients. Until recently, INF and Ribavirin were the combination of choice in treatment of HCV hepatitis, however less than 40% had sustained virological reaction (SVR) (Mc Hutchinson JG *et., al* 1998 and Poynard T *et., al* 1998).

Sustained biochemical and virological response, defined as persistently normal ALT values and absence of circulating virus for at least 6 months after discontinuing treatment (Mc Hutchinson JG *et., al* 1998). However, serum ALT in chronic HCV infection has some shortcomings as a marker of response to IFNα therapy because normalization of serum ALT sometimes occurs in patients who still have active viral replication (Nelson D R *et., al* 1995). Measurement of pseudo-choline esterase (PCHE) activity serves as a sensitive index of the synthetic capacity of the liver if the patient's normal baseline level is known (Abdel Rahman H 1990). Alpha glutathione S–transferase (αGST) has been proposed as an alternative marker of hepatocellular damage (Mannervik B, *et., al* 1992). It is relatively a small enzyme (MW 50,000) present in high concentration in the hepatocyte cytosol (Hayes PC *et., al* 1991). Thus, it is rapidly detectable in the circulation following hepatoceullar damage. In addition, it has a short plasma half-life (<90 minutes), which provides a more accurate reflection of the activity of hepatocellular damage (Mulder TP *et., al* 1996).

Modification of the pharmacokinetic profile of IFN α-2a through pegylation has resulted in a marked improvement of drug efficacy for the treatment of infection due to hepatitis C virus, as it has longer half-life than standard INF, more reduced distribution and lower elimination rate than the respective non- pegylated IFN α (Manns MP *et., al* 2001, and Krawitt EL *et., al* 2006). Therefore, it could be administered once weekly and its combination with Ribavirin resulted in SVR in 82% of patients with genotype 2, 3, 42% of patients with genotype 1 (Mulder TP *et., al* 1996 , Krawitt EL *et., al* 2006, Dusheiko G *et., al* 2008 and .Hanouneh IA; *et., al* 2008). This combination also resulted in a marked improvement of drug efficacy in the treatment of infection due to hepatitis C virus with genotype 4 in Egyptian patients (El-Zayadi A *et., al* 1999).

The number of patients in genotype 4 who were enrolled in the European studies was too small to be included in statistical analysis subsequently the response of genotype 4 to Pegylated INF α 2a was not properly studied in the Middle East and North Africa (Frank C *et., al* 2000).

Aim of the Work: The aim of this work was to study the efficacy of the Pegylated INF α-2a and Ribavirin in patients with chronic HCV of genotype 4. As well as to study the reflection of viral load and the estimation of serum PCHE and αGST as a markers of hepatocellular damage prior to and after treatment and the presence of cirrhosis on the response rate.

Patients and Methods: This study was conducted on 53 naïve patients with chronic HCV genotype 4. With exclusion Criteria:: clinical or biochemical evidence of hepatic decompensation, history of severe Psychotic disorder, disturbed thyroid function, Hb >11mg /dl, platelets >100000/ cmm, WBCs > 1500/ cmm, the presence of autoimmune disease affecting the liver by negative ANA and AMA and history of organ transplantation, viral therapy or immunosuppressive drugs.

Table 1: Shows characteristics of the 53 genotype 4 patients

Gender:	Count	%
Male	44	83.00%
Female	9	17.00%
Total	53	100.00%
Age:	20 - 70	
From 20 to 39	20	37.70%
From 40 to 54	31	58.50%
From 55 to 70	2	3.80%
Total	53	100.00%
Non- cirrhotic	43	81.10%
Cirrhotic	10	18.90%
HCV RNA load > 2 $X10^6$ copies/ml	13	24.5%
HCV RNA load < 2 $X10^6$ copies/ml	40	75.5%
ALT before treatment U/L	101.5± 8.5	100%
PCHE before treatment U/ml	5.2±2.39	100%
α GlutathioneS transferas before ttt mg/l	22.3±9.1	100%

Table 2: Shows the biochemical value and response in the 53 patients during the 72 weeks follow up.

	Reference	Basal	Week 12	Week48	Week 72
ALT (U/l)	28.8± 5.25	101.5± 8.5	63.25±22.37	32.5±4.65	33.25±5.06
P value			0.023	0.006	0.0087
Response% of gp A			31(72%)	28 (65%)	27 (62%)
Response% of gp B			4 (40%)	3 (30%)	3 (30%)
PCHE U/ml	11.79±2.84	5.2±2.39	10.10±2.35	10.90±2.60	11.29±1.83
P value			0.01	0.01	0.01
Response% of gp A			34 (79%)	33 (76.7%)	31 (72%)
Response% of gp B			5 (50%)	4 (40%)	3 (30%)
αGST (mg/l)	4.93±1.75	22.3±9.1	5.85±3.41	3.9±0.84	6.22±1.83
P value			0.04	0.024	0.035
Response% of gp A			34 (79%)	33 (76.7%)	31 (72%)
Response% of gp B			5 (50%)	4 (40%)	3 (30%)

P value compared to the basal value

All Patients Had:

- Detectable HCV RNA by RT- PCR (Amplicor Molecular System, F Hoffmann - La roche, Basel - Switzerland).
- Genotype 4 infecting virus detected by the Inno Lippa HCV II assay (innogenetics inc., GA, USA).
- Elevated serum alanine amino transferase (ALT) were done by autoanalyzer dimension expressed as U/L.
- Elevated serum α-GST, determined by ELISA biotrin HEPKIT-alpha Human GST-alpha 15 - Biotrin, the enzyme activity was expressed as ug/l the normal range is less than 1.2 ug/l (Nelson D.R 1995 and Rees, J.W. 1995).
- Low serum PCHE activity determined by using spectrophotometrically butrylthiocholine substrate by colourimetric test at 37^{o}C, the enzyme activity was expressed as U/ml the normal range is 5.4-13.2 U/ml.
- TSH was done by using ELISA and expressed as uIU/ml only less than 4 uIU/ml were chosen.
- Free T4 was done by using ELISA and expressed as pg/ml only less than 1.9 pg/ml were chosen.
- Free T3 was done by using ELISA and expressed as ng/ml only less than 4.2 ng/ml were chosen.
- ANA and AMA were done by the indirect immunofluorescence antibody test for detection and quantitation of ANA and AMA (The Binding site LTD, Birmingham, England) . Significant positive titre for ANA was > 1:40. While significant positive titre for AMA was >1:80. Negative results were seen as dull green staining in all reactions without fluorescence, only negative results were enrolled in our study.
- Liver biopsy providing pathological criteria of chronic HCV hepatitis or cirrhosis.

Study Design: The study was done over a period of 30 months from Jan 2003. The initial response (IR) was defined as the clearance of the virus or reduction of the viral load by two logs after treatment for 12 weeks. Patients who failed to achieve IR discontinued treatment. Only 53 patients were chosen from a group of patients who continue with us until the end of the study. They were classified into two groups:

Group A: 43 patients with chronic HCV hepatitis received 180 µg Pegylated INF α-2a subcutaneously once weekly & 1200 mg Ribavirin/ day in two doses by oral route.

Group B: 10 patients with liver cirrhosis received 135 µg Pegylated INF α-2a once weekly & 1200 mg Ribavirin/ day in two doses by oral route.

ALT, PCHE, α GST and CBC were done every two weeks. The end of treatment response (ETR) was defined as clearance of the virus at 48 weeks of treatment.

Reassessment of ALT, PCHE, α GST and HCV RNA were done 6 months (72 weeks) after stopping therapy. Patients who had absent HCV after these 6 months were defined to have sustained virological response SVR.

The dose of Pegylated INF α-2a was reduced to 135 µg in two patients from group A when the WBCs count dropped below 1500/cmm and rose again in one of them after improvement of the count.

The dose of Ribavirin was reduced to 600 mg / day in 3 patients from group A when the Hb level dropped below 10 mg/dl.

Statistical Analysis: Medians were compared using the median test, continuous variables, expressed as mean

± SD, were compared by using student's t-test or correlated by using simple regression done by Excel program. Differences were considered significant if P< 0.01.

RESULTS AND DISCUSSION

Results: The initial response (IR) was high in both cirrhotic and non Cirrhotic groups then it decreased until it became 71.7% in non cirrhotic and 30% in cirrhotics, same as the biochemical response which was initially high then decreased in both groups.

Table 3: Shows the virological and biochemical response in the 53 studies patients during the 72 weeks follow up.

Virological response:	Group A	Group B
Week 12	34 (79%)	5 (50%)
Week 48	33 (76.7%)	4 (40%)
Week 72	31 (72%)	3 (30%)
ALT response:		
Week 12	31 (72%)	4 (40%)
Week 48	28 (65%)	3 (30%)
Week 72	27 (62%)	3 (30%)
PCHE response:		
Week 12	34 (79%)	5 (50%)
Week 48	33 (76.7%)	4 (40%)
Week 72	31 (72%)	3 (30%)
α Glutathione –S- transferase response:		
Week 12	34 (79%)	5 (50%)
Week 48	33 (76.7%)	4 (40%)
Week 72	31 (72%)	3 (30%)

The difference was significant between both groups concerning virological response and the biochemical response. (P < 0.01)

The low viral load group was statistically higher in response rate 75% than the group with high viraemia 30.7% P< 0.01

Table 4: It compares the SVR in patients with high pre- treatment viral load versus patients with low viraemia

viral load	Count	%
≤ 2 X10^6 copies/ml	30/40	75%
> 2 X10^6 copies/ml	4/13	30.7%

Table 5: It compares the serum α-GST level in patients with high pre-treatment serum level versus patients with low serum level

serum α-GST level	Count	%
≤ 22.3± 9.1/ml	30/40	75%
> 30.17± 7.91/ml	4/13	30.7%

Table 6a: It compares the serum PCHE activity level in patients with low pre-treatment serum level versus patients with normal serum level

serum PCHE activity level	Count	%
≥ 5.2±2.39U/ml	30/40	75%
>5.2±2.39U/ml	4/13	30.7%

Table 6b: Compares the SVR in relation to age, it showed that the response is significantly higher in young age group (P value 0.012)

	Count	%
<40	17/20	85%
>40	17/33	51%

Table 7: Success Rate According to Degree of Histopathologic Severity

	Cured		Uncured		Count
	Count	Row %	Count	Row %	
Non cirrhotic	30	69.80%	13	30.20%	43
Cirrhotic	3	30.00%	7	70.00%	10
Total	33	62.30%	20	37.70%	53

The low serum α-GST level group was statistically higher in response rate 75% than the group with high serum α-GST level 30.7 % P< 0.01

Discussion: The Egyptian ministry of health estimated that national prevalence rate of HCV in 1999 to be 25-30% of the population (Mannervik B *et., al* 1992). It has been reported that 90% of the chronically infected patients with HCV harbor genotype 4 (Hayes PC, 1991).

Autoantibodies are biological probes that may reflect the underlying cellular immune response, and are useful in identifying putative, pathogenetically relevant autoantigen (Manal Diab *et., al* 2001). The distinction between autoimmune diseases caused by viruses and viral diseases with autoimmune features must be carefully investigated (Czaja A.J *et., al* 2000). Serum ANA and AMA were done to all patients; only patients with negative results were chosen to exclude any autoimmune effect on hepatic T lymphocytes. In addition, normal T3, T4 and TSH were done to all patients to exclude their effect on hepatic T lymphocytes.

Serum ALT, PCHE, aGST levels and negative HCV by PCR are the standard markers to assess liver disease and to monitor response to therapy in most patients with chronic HCV hepatitis. However, other factors as viral load, genotype and grade of fibrosis are also, used to predict the mean treatment outcome for chronic HCV patients (Mulder TP *et., al* 1996). In 1995 Martinot *et al.,* proved that viral genotype is a major predictor of SVR and patients with genotype 4 were considered as "difficult to treat" by the standard interferon (Manns MP *et., al* 2001).

In this study, 53 naïve patients with chronic HCV infection genotype 4 were treated by Pegylated INF α-2a and Ribavirin for 48 weeks. The end of treatment response ETR and sustained virological response SVR were 37/53 (69.8%) and 34/53 (64%) respectively. The end of treatment response ETR in our study was compared with Thakeb *et al.* (2003), who reported response rate of 68.6% (Frank C *et., al* 2000 and Thakeb F *et., al* 2003) and Sherif *et al.* (Hassan M, *et., al* 2001) who reported ETR of 73.2% (Thakeb F *et., al* 2003).

In our study, the SVR was 64% compared with Hassan *et al.* (Thakeb F *et., al* 2003), who reported a sustained biochemical and viral response of 68% (Hassan M, *et., al* 2001). SVR was significantly lower (30%) in cirrhotic patients in our study compared to 72% in non- cirrhotics and this finding was observed in many other trials (Martinot-Pegnoux M *et., al* 1995, Frank C *et., al* 2000, Hassan M, *et., al* 2001, Diago M *et., al* 2002, Krawitt EL *et., al* 2006 and Dusheiko G *et., al* 2008).

In our study, high plasma level of ALT was unchanged in non-responders while normalization observed in responders, which was reported by Hassan *et al.,* (Hassan M, *et., al* 2001). In addition, we noticed that patients with pre-treatment low viraemia, normal PCHE activity and low serum level of aGST showed significantly higher SVR rate 75.5% compared with those with pre-treatment high viral load, low PCHE activity and high serum aGST level (30.7%). In addition, both viral load and serum aGST show positive correlation, while both have negative correlation with serum PCHE activity. Serum PCHE activity level was within normal range at the base line and after treatment with a significant statistical increase after treatment (Abdel Rahman H 1990). This was also, reported in other trials (Nelson D.R. 1995 and Martinot-Pegnoux M *et., al* 1995). Abdel Fattah A *et al.,* (1997) proved that the measurement of the hepatic isoenzyme aGST was highly sensitive and early marker of hepatocellular integrity (Propert DN, *et., al* 1976). As a GST is readily and, rapidly released into the circulation following hepatic damage. Their short plasma half-life (<90 min), allows early detection of hepatic damage and its resolution (Abdel Fattah A. 1997)]. Mostafa I. *et..,al.* (2000), concluded that serum αGST level in combination with ALT and /or AST may serve as a better indicator of histologic activity. It is possible that combination with other markers, aGST may play a complimentary role in the assessment of active hepatocellular damage (Mostafa I *et., al* 2000).

Concerning, the age it has been noticed that 17/20 patients less than 40 years had been cured 85% compared to 17/33 patients 51% over 40 years. However, this significant difference was explained by, the slow course of chronic HCV hepatitis and the more pathologic hepatic injury, which can occur with age (Propert DN 1976, Hayashi J *et., al* 1998 and Krawitt EL *et., al* 2006).

Concerning safety, the combination of both studied drugs were well tolerated by all patients and none of them stopped treatment due to serious or severe adverse events 63% of the patients experienced flu like symptoms during the first month of treatment.

Concerning the dose of PEG-INF, it was reduced to 135 µg in 2 patients from group A when there WBCs count dropped to 1500/ cmm and, raised again to 180 µg after improvement of the count. The other patients continued therapy with the use of leucogen beside the standard study combination therapy to maintain the WBCs count above 1500/cmm. During the last month of treatment Ribavirin dose was reduced to 600 mg/day

when the Hb level dropped below 10 g/dl. in three patients, this finding was observed in many other trials (Derbala, *et., al* 2003, Sherif A *et., al* 2004, Hassan F MD *et., al* 2004, Motaz, Stephens J. *et., al* 2004 and Dusheiko G; *et., al* 2008.

Conclusion: The combination of Pegylated INFα-2a and Ribavirin is an effective and safe treatment for chronic HCV genotype 4 non cirrhotic patients. aGST and PCHE is a very stable and specific serum markers of hepatocellular damage similar to conventional liver biochemistry in the evaluation of chronic HCV. In addition, aGST and PCHE measurement act as a predictive index of response to IFN therapy. While the poor response in patients with cirrhosis needs further studies and new strategies to improve anti viral response in this group. The low pre-treatment viral load, young age and absence of cirrhosis are good predictors of responsiveness to treatment.

REFERENCES

Zekry, A.R., A.A. Bahanssy and A.S. Ramadan, 2001. hepatitis c virus genotyping versus serotyping in Egyptian patients, Infection, Jan-Feb; 2a(1): 24-26.

Attia, M.A., 1998. prevalence of H.BV. HCV in Egypt and Africa, Antivir ther, 3 supplement, 3: 1-9.

Mc Hutchinson, J.G., S.G. Gordon and E.I.N.F. Schiff, 1998. α-2a alone or in combination with ribavirin as antiviral treatment of chronic hepatitis C, NEJM, 339: 1485-92.

Poynard, T., P. Marcellin and Leess, 1998. randomized trial of INF α-2b plus ribavirin for 48 weeks or 24 weeks versus INF α-2b plus placebo for 48 weeks for treatment of chronic HCV hepatitis., Lancet, 532: 1426-32.

Nelson, D.R., H.L. Lim, D. Oliver, K. Qian, G.L Davis and J.Y. Lau, 1995. α-Glutathione S-transferase as a marker of hepatocellular damage in chronic hepatitis C virus inection.Amer J of Clin Pathol, 104: 193-198.

Abdel Rahman H., 1990. Evaluation of the diagnostic value of the serum cholinesterase activity in chronic liver disease. MD Thesis; Internal medicine, Faculty of Medicine, Cairo University.

Mannervik, B., Y.C. Awasthi and P.G. Board, 1992. Nomenclature for human glutathione-S transferase (letter) Biochemical journal, 282: 305-306.

Hayes, P.C., I.A. Bouchier and G.J. Beckett, 1991. Glutathione S-transferase in humans in health and disease. Gut:, 32: 813-313.

Mulder, T.P., W.H. Peters, D.A. Court and J.B. Jansen, 1996. Sandwich ELISA for glutathione-S-transferase alpha1-1: Plasma concentration in contros and in patients with gastrointestinal disorders: Clin Chem., 42:3: 416-419.

Manns, M.P., J.G. Mc Hutchinson and S.C. Gordon, 2001. INF α-2a plus ribavirin compared with INF α-2b plus ribavirin for initial treatment of chronic hepatitis, a randomized trial, Lancet, 358: 958-65.

El-Zayadi, A., O. Slim, S. Haddad, P. Simmonds, H. Hamdy, H.M. Badran and S. Shawky, 1999. Combination treatment of interferon alpha-2b and ribavirin in comparison to interferon monotherapy in treatment of chronic hepatitis C genotype 4 patients. Ital J Gastroenterol Hepatol, 31: 472-475.

Krawitt, E.L., S.R. Gordon, N.D. Grace, T. Ashikaga, M.A. Ray, M. Palmer, J.C. Yarze and S. Moskowitz, 2006. A study of low dose peginterferon alpha-2b with ribavirin for the initial treatment of chronic hepatitis C. Am J Gastroenterol. 101(6): 1268-73 (ISSN: 0002-9270).

Dusheiko, G.D. K.R. Nelson and Reddy, 2008. Ribavirin considerations in treatment optimization. Antivir Ther., 13 Suppl 1: 23-30 (ISSN: 1359-6535).

Hanouneh, I.A., C. Miller, F. Aucejo, R. Lopez, M.K. Quinn and N.N. Zein, 2008. Recurrent hepatitis C after liver transplantation: on-treatment prediction of response to peginterferon/ribavirin therapy. Liver Transpl., 14(1): 53-8.

Frank, C., M. Mohamed and G. Strickland, 2000. The role of parenteral anti shistosomal therapy in the spread of hepatitis C in egypt, Lancet, 11(35): 887-891.

Nelson D.R., 1995. aglutathione S-transferase as a marker of hepatocellular damage in chronic hepatitias C virus infection**.** Am. J. Clin. Pathol., 104: 193-198.

Rees, J.W., 1995. Validity of an enzyme immunoessay for the detection of a glutathione S-transferase in human serum. Ann Clin. Biochem., 32: 575-583.

Manal Diab, Nagla El Sherif, Mona Mousa, Hanem Ahmed, Nihal El Assaly, Maged El Ghannam, Naema

El Ashry and Maha Akl, 2001. Autoimmunity in Chronic Liver Disease: Clinical , Serological, and Immunohistochemical Correlations. Egyptian journal od medical microbiology, April, 10(1): 227 -237.

Czaja, A.J, M.P Manns, J.G. McFarlane, *et al.,* 2000. Autoimmune Hepatitis: The investigational and clinical challenges. Hepatol, 31(5): 1194-1200.

Hassan, M., Hayfa Askar and Gamila EL-Kalidy, 2001. The incidence of genotype 4 among HCV chronic hepatitis C patients in Egypt. Journal of Clinical Gastrology, august, 33(2): 123.

Diago, M., S. Hadziyannis and H. Bodenhehmer, 2002. optimized virologic response in genotype 4 chronic HCV patients treated by PEG INF α-2a and ribavirin. Hepatology, 36(4): 2 of 2.

Martinot-Pegnoux, M., Marcellin P. and M. Pouteau, 1995. Pretreatment serum hepatitis C virus RNA levels and hepatitis C virus genotype are the main and independent prognostic factors of sustained response to interferon alfatherapy in chronic hepatitis C. Hepatology, 22: 1050-6.

Propert, D.N. and C.J. Brackenridge, 1976. The relation of sex, age, smoking status, birth rank, and parental ages to pseudocholinesterase activity and phenotypes in a sample of Australian Caucasian adults. Hum Genet., 32: 181-8.

Abdel Fattah A., 1997. aGST as a new marker for liver damage in chronic hepatitis C virus (HCV). M Sc Thesis in clinical and chemical pathology. Faculty of Medicine, Ain Shams University.

Mostafa I, Hassan Mona, Nessim Iris G., Fakhry S. and Y. Abdel Rahman, 2000. Alpha-Glutathione S transferase as a predictive Index of Response to Interferon Therapy in HCV Related Chronic Hepatitis. Kasr Aini Medical Journal, 6(1): 149-162.

Thakeb, F., Mahmoud Omar, M. Mostafa and Y. Sherif Randomized, 2003. controlled trial of Peginterferon alfa- 2a plus Ribavirin for chronic hepatitis C virus genotype 4 among Egyptian patients. oral presentation at the 54th Annual Meeting of the AASLD Boston, Massachusetts, October 24, 2003.

Sherif, A. Fattah and Y EL Shazly *et al.,* 2004. End of Treatment Response to Peginterferon Alfa-2a (40KD) (PEGASYS®) and Ribavirin: a prospective, multicenter, open label trial in Egyptian patients with chronic hepatitis C genotype 4, EASL Berlin.

Hassan, F.M.D., M.D. Haifa Askar, A.l. Jameel and M.D. Khaldi, *et al.* 2004. INF α-2b plus ribavirin for the treatment of hepatitis C genotype 4, American Journal of Gastroenterology., 1733-1737.

Motaz Derbala, Alia Amer, Abdulbari Bener *et al.* 2003. pegylated INF α-2b –ribavirin combination in Egyptian patients with genotype 4 chronic hepatitis C, the Egyptian Journal of Gastroenterology, Vol 8, No 1 Suppl. January 2003 71-82.

Stephens, J., M.D. Hadzianess, M.D. Hoel Sette Jr, R.M.D. Timothy *et al.*, 2004. Peg interferonμ2a and ribavirin combination therapy in chronic hepatitis C. Annals of internal medicine, No 346:357

Hayashi, J., Y. Kishihara, K. Ueno, K. Yamaji, Y. Kawakami, N. Furusyo, Y. Sawayama and S. Kashiwagi, 1998. Age-related response to interferon alfa treatment in women vs men with chronic hepatitis C virus infection. Arch Intern Med 1998. Jan 26: 158(2): 177-81.