Can zinc levels predict response to pegylated-interferon and ribavirin therapy in hepatitis c genotype 4 infected Egyptian patients ?

Amal A. Mohamed[1], Maggie M. Abbassi[2], Waleed A. Hamed[3], Mohamed A. EzzEl-Arab[4], Ahmed M. Aref[5]

(1) Biochemistry Department, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt ; (2) Clinical Pharmacy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt ; (3) Tropical Medicine Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt ; (4) Internal Medicine Department, National Hepatology and Tropical Medicine Research Institute, Cairo, Egypt ; (5) Biotechnology Department, Faculty of Dentistry, MSA University, Cairo, Egypt.

Abstract

Background and Aims : **Zinc has been found to be low in chronic hepatitis patients. Its level was correlated with response to Interferon/ribavirin therapy in patients infected with hepatitis C genotype 1. In Egypt, inexpensive predictors to treatment response in Hepatitis C genotype 4 infected patients are desperately needed. We aim to explore if pretreatment zinc serum levels correlate with response to pegylated- interferon and ribavirin therapy in Egyptian patients.**

Methods : **This is an observational prospective study where 57 treatment naïve hepatitis C genotype 4 infected patients that were Hepatitis B and Human Immunodeficiency virus negative were recruited in a hospital setting. The study was performed from October 2010 till June 2012. Patients had Liver biopsy and basic biochemical profiles were performed pretreatment for all patients. Treatment consisted of 48 weeks of pegylated-interferon-alpha2a and ribavirin therapy. Blood samples were withdrawn from 21 healthy subjects to compare zinc levels and other biochemical markers. Patients were followed up to 72 weeks.**

Results : **Pretreatment serum zinc levels were significantly lower in hepatitis C infected patients compared to healthy volunteers ($p < 0.05$). Moreover, zinc levels correlated to sustained virological response in treated patients ($p = 0.00$).**

Conclusion : **Serum zinc levels can be used as an inexpensive predictor to effective Pegylated-interferon/ribavirin therapy in Egyptian patients infected with Hepatitic C genotype 4.** (Acta gastroenterol. belg., **2014**, 77, **217-223**)

Key words : hepatitis C, Genotype 4, Zinc, and Egypt Introduction.

Zinc levels have been shown to be low in patients with chronic liver disease due to impaired trace element metabolism (1-3). Zinc affects a myriad of biological processes including the activity of around 300 enzymes *in vivo* (4), the metabolism of nucleic acids and protein and the suppression of inflammation (5). It also acts as a signaling molecule in immune cells, suggestive of a beneficial therapeutic role of zinc supplementation in several viral infections (6). Not only has zinc been shown to inhibit the replication of several viruses *in vitro* ; including human immunodeficiency virus, rhinovirus, herpes simplex virus, and respiratory syncytial virus and hepatitis C virus (HCV) (7-10) ; but was also found to stimulate human interferon (11-13). Zinc has an important role in growth and in combating childhood conditions such as stunted growth, anorexia and morbidity from cough, diarrhea, fever, and vomiting (14). Deficiency in zinc results in several signs and symptoms including weight loss, emotional disturbance, dermatitis, alopecia, impaired taste acuity, night blindness, poor appetite, delayed wound healing, and elevated blood ammonia levels (15) ; suggesting a contribution to the side effects of interferon/ribavirin (IFN/ribavirin) therapy (16).

In non-alcoholic liver cirrhosis ; zinc deficiency was attributed to poor zinc absorption (17,18).

Albeit, zinc dietary intake was found to be significantly higher in HCV patients (genotypes 1, 2, 3 and 4) who responded to IFN therapy than in non-responders (19).

Pre-treatment serum zinc level was correlated with the response to IFN in HCV patients of genotype 1b (20), IFN-α 2b in HBV infected children (21) as well as to IFN-α and lamivudine (22), signifying a possible role for zinc levels as a good predictor to therapeutic outcomes in treatment of viral hepatitis. Nevertheless, whether zinc supplementation can enhance response to therapy and reduces its side effects in HCV infected patients genotype1b (16,23-26) has yet to be confirmed. Copper metabolism is also impaired in chronic hepatitis patients, leading to higher levels than normal (1,2). It is not clear whether copper could have a role as an indicator to treatment response in chronic viral hepatitis.

Almost 15% of the Egyptian population is infected with HCV, 90% of which have genotype 4 (27), with higher prevalence among persons residing in rural versus urban areas (12% versus 7%) (28) constituting a huge burden on the Egyptian economy. We aim to find predictors to response to Peg-IFN/ribavirin, standard therapy for HCV in Egypt (29).

Most of Phase 2 and 3 clinical trials for treatment of HCV are carried out in North American and Western European centers, where HCV genotypes 1, 2 and 3 account for > 95% of the patient infections. As a result information about predictors, and even sustained virological response (SVR) rates, in genotype 4, 5 and 6 patients are relatively limited (30). Patients infected with HCV genotype 4 have intermediate response to pegylated-interferon plus ribavirin (Peg-IFN/ribavirin) in contrast to genotype 2 or 3 who have better SVR rates (up to 80%) and genotype 1 having the worst (45%) (31,32).

Correspondence to : Maggie M. Abbassi, Assistant Professor, Clinical Pharmacy Department, Faculty of Pharmacy, Cairo University, Kasr elaini street 11562, Cairo, Egypt. E-mail: maggieabbassi@gmail.com

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Considering that different HCV genotypes have different response rates to treatment, it has yet to be determined whether HCV genotype 4 patients response to treatment is affected by serum zinc level.

The goal of this study was to investigate the role of serum zinc level as a predictor of therapeutic response in HCV genotype 4 infected patients.

Methods

Study Subjects

This study was conducted on 57 naive Egyptian patients with chronic HCV infection. Adult subjects aged between 18-60 years, who were diagnosed with chronic HCV infection, genotype 4 were enrolled in the study. Subjects were excluded if co-infected with human immuno-deficiency virus (HIV) infection or hepatitis B virus (HBV), had evidence for other liver diseases, had any underlying renal, cardiovascular, gastrointestinal, autoimmune disease, or malignant diseases. Active intravenous drug users, any patient taking vitamin supplements or any vitamin/mineral supplements were also excluded.

The control group consisted of 21 healthy subjects, free of HCV and HBV, of matched age, gender and body mass index (BMI) to the test group.

Study Setting and Design

This is a prospective, observational study conducted between October 2010 and June 2012, in the department of Tropical Medicine, Ain Shams University Hospitals, Cairo, Egypt. All recruited HCV genotype 4 patients were treated with subcutaneous (Sc) administration of PEG-IFN-alpha2a (Pegasys, Roche, Basel, Switzerland) at a dose of 180 μg/week plus oral ribavirin at a dose of 1000-1200 mg/day according to body weight (1000 mg/day for a body weight of < 75 kg, 1200 mg/day for a body weight of > 75 kg) for 48 weeks (33).

The study was performed in accordance with the principles of the Declaration of Helsinki and its appendices (34) and approved by local Institutional Review Board and Ethics Committee, with written informed consent obtained from all cases. All the recruited patients and control group were subjected to full clinical assessment prior to drug administration and at 72 weeks. Screening included also age, sex, body weight, height, and BMI as well. Liver biopsy at inclusion was available for all patients. Hepatic inflammation and fibrosis were graded according to Modified Knodell's (Ishak) fibrotic Score (35,36). Clinical and anthropometric data were collected at the time of liver biopsy.

Baseline Characteristics

A 12-hour overnight fasting blood sample was drawn at the time of biopsy to determine serum levels of alanine aminotransferase (ALT) (IU/mL), aspartate aminotransferase (AST) (IU/mL), blood glucose concentration (mg/dL), total bilirubin (mg/dL), direct bilirubin (mg/dL), albumin (g/dL), alpha-fetoprotein (AFP) (ng/dL), prothrombin time (PT) %, thyroid stimulating hormone (TSH) (mIU/L), creatinine (mg/dL), and hemoglobin (Hb) (g/L), zinc (µg/dL), copper (µg/dL), white blood cells (WBCs) (cells/mL) and platelets (cells/mL). The analysis of serum zinc and copper were performed using Quimica Clinica Aplicada S.A Kit, Amposta, Spain.

Assessment of Efficacy of PEG-IFN

The efficacy of the PEG-IFN was assessed based on the sustained virological response (SVR) adopting viral titer (Real Time PCR System-*Applied Biosystem*) using a detection limit of 50 IU (100 copies) × 10^3/L. The viral titer was obtained at the time of biopsy from all the screened patients at baseline just before injection of PEG-IFN, at 24 weeks, 48 weeks (end-of-treatment, EOT) and 72 weeks (Sustained virological response, SVR). For this assessment, 5 mL blood samples were withdrawn for the entire screened subjects and frozen at -80ºC for further investigations.

Genotyping was performed by INNO-LIA, HCV (Innogenetics, USA). Side effects due to treatment were only noted if their severity warranted a change in the dose.

Statistical Analysis

Pairwise comparisons were performed using Mann-Whitney, Wilcoxon-Signed-Rank, or Pearson's Chi square tests as needed at a level of significance of 0.05. Correlations were tested using Spearman's correlation. Simple logistic regression was used to test whether any factors correlated with SVR.

Results

Baseline Characteristics

A total of 57 patients HCV positive and HBV negative were recruited. Patient characteristics are summarized in Table I. Twenty one normal subjects were matched for age, gender and BMI with those diagnosed with HCV. Patients' age ranged from 20 to 54 years, 30% with normal BMI (< 25). Viral load at baseline was less than 100,000 copies for 51% of the patients. Thirty-three patients (58%) showed sustained virological response (SVR) defined as negative PCR at 72 weeks from initiation of treatment (or 24 weeks after finishing treatment). ALT, AST, AFP and direct bilirubin were significantly higher in patients than in control subjects ($p < 0.005$). On the other hand, blood glucose levels, WBC's, zinc and copper were significantly lower ($p < 0.005$).

Zinc Levels

Zinc levels were compared pretreatment in HCV infected patients to control subjects. The baseline serum

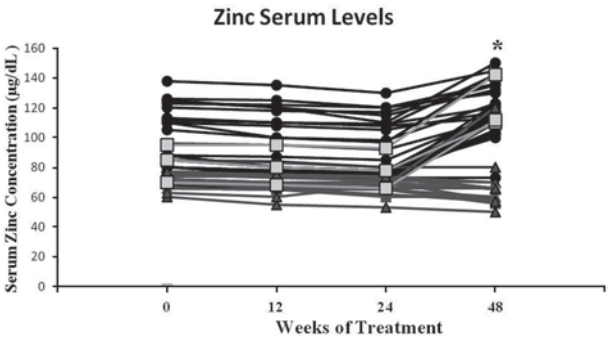

Fig. 1. — Zinc serum levels (μg/dL) in hepatitis C patients at baseline (0 weeks), 12 weeks and 48 weeks of treatment, ● patients who responded to treatment, ▲ patients who did not respond to treatment, □ patients who relapsed at 72 weeks. * Wilcoxon-signed rank test comparing zinc serum levels in responders at 48 weeks to baseline ($p < 0.05$).

zinc (85 ± 19 µg/dL) was found to be significantly lower than control groups (106 ± 17, $p = 0.000$). Serum Zinc was also significantly higher in HCV patients at the end of treatment (48 weeks) compared to baseline. Comparing responders to Peg-IFN/ribavirin therapy to non-responders, serum zinc was found to be significantly higher in responders (Fig. 1).

Response to Therapy

Initial viral load is summarized in Table I. At 24 and 48 weeks (EOT), 36 out of 57 (63.2%) patients responded to therapy (had negative PCR for HCV RNA), however at 72 weeks three patients relapsed and the percentage response dropped to 58%. No severe side effects were observed to necessitate dose adjustment during the study period.

Correlation of Zinc Levels to Baseline Characteristics

Spearman's correlation was used to test whether baseline zinc serum correlated with markers of liver disease (Table II). The correlation was found to be statistically significant for albumin, Hb, viral load at baseline, platelet count and copper ($p < 0.05$).

Factors Associated with Sustained Virological Response

Responders to therapy were compared to non-responders for baseline characteristics using Wilcoxon signed rank test (Table III). Only ALT, viral load at baseline, serum zinc and serum copper were found to be significantly different among groups. Using simple logistic regression to test whether any of the biochemical tests were predictive of SVR, only zinc levels were significantly predictive ($p = 0.045$). Neither BMI nor baseline

Table I. — **Baseline characteristics of 57 patients infected with HCV genotype 4 and 21 healthy control subjects data represented as mean and standard deviation (SD)**

Parameter	Baseline Parameters HCV Patients (N=57)	Control (N= 21)	P*
Gender Distribution (male %)	37(65%)	12 (57%)	NS
Age (years) (range, SD)	40.2 ± 7.9	36.9 ± 9.8	NS
Viral Load			
Number (%) of Patients with Low Load (< 10^5)	27 (47)		
Number (%) of Patients with Moderate (10^5-10^6)	12 (21)		
Number (%) of Patients with High (> 10^6)	18 (32)		
Aspartate Aminotransferase (IU/mL)	61 ± 24	33 ± 10	0
Alanine Aminotransferase (IU/mL)	59 ± 22	32 ± 8	0
Total Bilirubin (mg/dL)	1.0 ± 0.4	0.8 ± 0.2	NS
Direct Bilirubin (mg/dL)	0.25 ± 0.17	0.2 ± 0.1	0.002
Albumin (g/dL)	3.9 ± 0.3	3.8 ± 0.2	NS
Blood Glucose Level (mg/dL)	98 ± 17	107 ± 17	0.023
Alpha-fetoprotein (ng/dL)	12.9 ± 12.2	5.9 ± 2.0	0
Prothrombin Time %	94 ± 6	96 ± 5	NS
Thyroid Stimulating Hormone (mIU/L)	3.6 ± 0.8	2.9 ± 1.2	0.008
Creatinine (mg/dL)	0.96 ± 0.16	1.0 ± 0.2	NS
Hemoglobin (g/L)	11.9 ± 1.8	11.9 ± 1.8	NS
BMI (kg/m^2)	27 ± 9	24 ± 6	NS
Zinc (µg/dL)	85 ± 19	106 ± 17	0
Copper (µg/dL)	71 ± 9	108 ± 15	0
WBC's × 10^3 (cells/mL)	8.1 ± 1.4	6 ± 1.9	0
Platelets × 10^3 (cells/mL)	288 ± 73	251 ± 63	0.030

* Level of significance $p < 0.05$: Mann-Whitney U test, NS : not significant.

Table II. — **Correlation of zinc serum levels with the biochemical profile of 57 patients before Peg-interferon/ribavirin treatment**

Parameter	Correlation Coefficient (Spearman)	P*
Age (Years)	0.131	0.333
Aspartate Aminotransferase (IU/mL)	0.060	0.655
Alanine Aminotransferase (IU/mL)	0.242	0.069
Total Bilirubin (mg/dL)	0.010	0.943
Direct Bilirubin (mg/dL)	0.053	0.694
Albumin (g/dL)	0.308	0.020
Blood Glucose Level (mg/dL)	-0.015	0.910
Alpha-fetoprotein (ng/dL)	0.100	0.460
Hemoglobin (g/L)	0.267	0.045
Viral Load at Baseline	0.280	0.035
Thyroid Stimulating Hormone (mIU/L)	0.129	0.340
Creatinine (mg/dL)	0.049	0.717
Prothrombin Time (%)	-0.055	0.684
BMI (kg/m^2)	0.164	0.221
Copper (µg/dL)	0.698	0.000
WBCs (cells/mL)	-0.060	0.657
Platelets (cells/mL)	-0.263	0.048

* Level of significance $p < 0.05$.

Table III. — **Baseline clinical and demographic characteristics of the studied population (N = 57). Patients' Characteristics before treatment are divided according to response at SVR. Categorical variables are presented as number of patients (percentage) and continuous variables are presented as medians (range)**

Parameter	Responders (N = 33)	Non-Responders (N = 24)	P*
Male gender	21 (64%)	16 (67%)	NS**
Age (years)	41 (20-54)	37.5 (28-52)	NS
Aspartate Aminotransferase (IU/mL)	60 (36-160)	52 (22-100)	NS
Alanine minotransferase (IU/mL)	60 (35-140)	50 (34-87)	0.018
Total Bilirubin (mg/dL)	1 (0.5-2)	0.9 (0.6-2.5)	NS
Direct Bilirubin (mg/dL)	0.2 (0.1-0.8)	0.2 (0.1-0.9)	NS
Albumin (g/dL)	4 (3-4.5)	3.9 (3.4-5)	NS
Blood Glucose Level (mg/dL)	100 (70-150)	90 (80-153)	NS
Alpha-fetoprotein (ng/dL)	12 (3-91)	10 (3-20)	NS
Prothrombin Time %	96 (80-100)	95.5 (80-100)	NS
Thyroid Stimulating Hormone (mIU/L)	3.8 (2-5)	3.6 (2-6)	NS
Creatinine (mg/dL)	1 (0.6-1.3)	0.9 (0.8-1.5)	NS
Hemoglobin (g/L)	12 (9-15)	12 (9-14)	NS
BMI (kg/m^2)	29 (18-45)	20 (18-45)	NS
Viral Load at Baseline x 10^3	800 (1-74 × 10^6)	5 (0.3-31 × 10^6)	0.021
Fibrosis *** (1-2)	8 (24%)	2 (8%)	NS
Fibrosis Score (3-6)	25 (76%)	22 (92%)	NS
HAI *** (4-7)	30 (91%)	21 (87.5%)	NS
HAI (8-9)	3 (9%)	3 (12.5%)	NS
Zinc (µg/dL)	85 (68-138)	71.5 (60-95)	0.00
Copper (µg/dL)	70 (55-90)	67.5 (56-99)	0.042
WBCs × 10^3 (cells/mL)	8.5 (6-10)	8.8 (4.5-10)	NS
Platelets × 10^3 (cells/mL)	258 (165-420)	300 (190-450)	NS

* Level of significance $p < 0.05$; Pearson Chi-square test for categorical variables and Mann-Whitney test for continuous variables.
** NS : Not significant.
*** Fibrosis score and HIA (The Modified Histological Activity Index) are graded according to Modified Knodell's (Ishak) fibrotic Score (24,25).

viral load were found to be significant factors for predicting SVR in this set of patients.

Discussion

This is the first study to correlate zinc serum levels with response to Peg-IFN/ribavirin therapy in Egyptian patients infected with HCV genotype 4. Zinc was proven to be an inhibitor of HCV replication *in vitro* (10), in addition to its involvement *in vivo* in immune function, its anti-inflammatory and anti-oxidative effects, through inducing metallothionein (37-39), and in the activity of cytotoxic T lymphocytes. These effects could counter the pathological changes in hepatic tissue associated with chronic hepatic disease. Zinc was also proven to induce the production of antiviral IFN-α and IFN-γ *in vitro* (12,13), to potentiate the antiviral action of human IFN-a tenfold (11) and to mediate the dimerization of IFN molecules required for the activation of the IFN receptors (40).

In this study, overall sustained response was found to be 58% in agreement with other studies in Egyptian patients who showed SVR rates between 48% and 69% (27). Zinc levels were found to be initially higher in patients who responded to therapy versus non-responders at 72 weeks. However, zinc levels were overall lower in HCV infected patients compared to healthy subjects. Earlier studies confirmed the decreased zinc level in HCV genotype 1b infected patients and HBV infected patients (20-22) and its correlation with response along the course of treatment, albeit this was not the case in our study. Zinc levels were only significantly higher at 48 weeks for responders but decreased from baseline to 24 weeks (Fig. 1).

Zinc levels were found to be correlated with albumin, platelets and initial viral load in accordance to a previous report correlating zinc levels to the biochemical profiles of HCV infected patients mainly genotype 1a (41). However, zinc level was not correlated to ALT and AST levels even though their levels were previously demonstrated to decrease with zinc supplementation (42). Correlation with baseline viral load was positive rather than negative which would have been expected from the fact that zinc is poorly absorbed in chronic liver disease, and that zinc levels declined in accordance with the severity of the disease (17,18). This could be explained by the fact that the viral titer does not essentially reflect the degree of liver damage (43). In a study where zinc was administered to HCV positive patients without antiviral treatment (42) HCV RNA titer did not change after 6 months of treatment. In addition, in this cohort of patients, the viral load at baseline was also unexpectedly significantly higher in patients with sustained virological response in contrast to earlier studies that either correlated SVR with lower baseline viral load (44,45) or where there was no such correlation (30) .

Zinc was not correlated with neither HAI nor Fibrosis score in contrast to what was previously demonstrated (22). Copper levels were lower in HCV infected patients than control subjects and in non-responders compared to responders in contrast to previous research (1,46).

Although higher zinc dietary intake was correlated with improved response to interferon (19), studies investigating the role of oral zinc supplementation on response to IFN or IFN/ribavirin therapy failed to give a conclusive evidence of such an effect (16,23-26,42,47). Failure to demonstrate effect of zinc on clearance of HCV in some cases could be due to the small subset of patients examined and low zinc dose and was in some cases accompanied only by decrease in side effects of treatment. It was also clear from the above studies that the effect of zinc was more pronounced with IFN treatment than with combined IFN/ribavirin.

It should be noted that the above studies were performed in Asian populations. Earlier research demonstrated that there was a significant ethnic difference in response to IFN/ribavirin therapy, African Americans and Hispanics were poor responders while Asians were better responders (48). It is not clear whether Egyptians would respond favorably to zinc supplementation as revealed by a subset of the above reports.

In Egypt, there is a huge health and economic burden due to HCV infection. Around 15 % of Egyptians are infected and with healthcare cost of treatment per adults around $3000, this could mean that most patients in Egypt (about 10-12 million infected individuals, annual income per capita for 2011 according to the World Bank : $ 2,781), could not afford therapy. A treatment and care program was established in Egypt for HCV treatment with an estimated cost for the Egyptian government of $80 million annually, representing only 40% of total costs. The remaining 60% is paid by insurance companies and patients (29).

It would be cost-saving for the government and for private patients if research could help establish predictive markers of response to therapy in order to identify patients who could best benefit from therapy.

It has been pointed out that serum zinc levels influences biochemical profiles in HCV patients (41). In addition, zinc and other indices of free radical-mediated damage can offer a tool for monitoring the degree of liver damage, the response to antiviral therapies and to the design of new therapeutic strategies (38,49). Even though there are genetic markers that has been highly associated with response to therapy, most notably IL28B and ITPA (50), genetic testing is still highly expensive.

Zinc levels were found to be low in rural population in Egypt in 1966 (51). In 2004, a national survey was conducted to determine bone mineral density among Egyptians showing that 8.5 percent of adolescents and 5.6 percent of adults had low serum zinc levels, equally among genders and geographical locations (52). A Food And Agriculture Organization of The United Nations (FAO) report (52) has concluded that zinc intake in Egyptians among other micronutrients is considered insufficient.

We previously demonstrated the role of vitamin D as a predictor for response to IFN/ribavirin treatment (44). If more than one marker could be involved, therapy outcome could be predicted more reliably with a possibility that supplementation with vitamins and minerals along with IFN/Ribavirin therapy could enhance overall response in patients.

Indeed, low cost markers to determine response to therapy and the degree of liver disease are essential in a low middle income country as Egypt where HCV is an epidemic, constituting a huge economic burden. This study being performed in a single center with a rather limited number of patients, limit its generalization to Egyptian population. Further studies need to be performed in different areas of Egypt to explore both the effect of pretreatment zinc levels and of the supplementation of zinc on response to Peg-interferon and ribavirin therapy.

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