Impact of insulin resistance and hyperferritenemia on Serum interferon gamma and early virological response to interferon therapy among Egyptian HCV patients

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Abstract: Introduction: Resistance to interferon therapy in HCV patients represents a major health problem. The aim of the study was to assess the effect of insulin resistance (IR) and hyperferritenemia on the antiviral and immunomodulatory activities of interferon alpha (IFNα) therapy in Egyptian patients with hepatitis C virus **(HCV) Design and Methods:** The current study is a prospective, observational case- control study, a total of 165 patients with HCV recruited from Internal Medicine and Tropical Departements- Mansoura University Hospital, Egypt were enrolled in the study. According to their response to IFN α therapy were classified into responders and non responders. Serum HCV m RNA, glucose, insulin, ferritin and interferon gamma (IFNγ) were measured. (IR) was estimated by the homeostasis model of assessment for insulin resistance (HOMA-IR). **Results:** Non responder patients revealed elevated levels of serum ferritin and HOMA index accompanied by elevated HCV m RNA and IFN γ levels as compared to responders patients ***(p<0.0001).*** **Conclusions:** The findings of the present study showed that IR and hyperferritenemia interfere with the antiviral and immnomodulatory effects of IFN α therapy as assessed by serum HCV m RNA and IFN γ levels respectively.

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Introduction:

Hepatitis C virus (HCV) represents a major health problem worldwide. HCV patients are at risk of progressive liver disease which favors the generation of long term complications such as cirrhosis, end stage liver disease and hepatocellular carcinoma **(Spengler and nattermann,2007)**

Interferons (IFNS) are key players in the innate immune response to virus infections **(shoder et al., 2004).**Type I interferons (IFN α, IFN β) are secreted by almost all virus infected cells and in much higher amounts by specialized T cells **(Trincheri,2010)**.The type II IFN subtype is presented by a single gene product, interferon gamma (IFNγ), an inflammatory cytokine secreted primarily by activated T cells and (NK) cells and recognized for its antiviral and immunomodulatory effects **(Horras et al.,2010)**

Treatment of HCV using combination of pegylated interferon (PEG-IFNα) plus ribavirin fails in about 50% of patients and is physically and economically demanding **(Asselh et al., 2010).** Thus, it is highly important to understand the mechanisms of non response and to identify factors that can help to predict the chance of each patient to respond to treatment **(Lecube et al. 2006).**Different factors are associated with non response to IFN α therapy including viral factors and host factors such as insulin resistance **(Alberti et al., 2005)** and iron overload **(Shan et al.,2005; Fargion et al., 2002).**

On this basis the aim of the present study was to verify the impact insulin resistance and hyperferritenemia on modulation the antiviral and immunomodulatory effects of IFN α therapy assessed by serum HCV m RNA and IFNγ levels respectively.

Subjects and methods:

I. Study design

The current study is a prospective, observational case- control study, conducted between January 2011 and January 2013 at Internal Medicine and Tropical Departements- Mansoura University Hospital, Egypt.Selected patients were assigned to pegylated interferon alpha-2a in a fixed dose of 180μg weekly by subcutaneous injections. All patients received ribavirin daily in an adjusted dose according to

body weight; patients < 75 Kg were given 1000 mg and those> 75 Kg were given 1200 mg. the safety was assessed by clinical by clinical evaluation and laboratory tests at week 1, 2, 4 and monthly thereafter during treatment.

Viral load, HOMA-IR, serum INF-γ, ferritin, fasting and postprandial glucose levels were assed by liver enzyme activities,, and fasting serum insulin levels were also measured at base line and 12 weeks after study entry. Patients were considered as having early virologic response (EVR) if they had HCV m RNA below the limit of detection or at least more than 2 log reduction after 12 weeks of the combination therapy. Subjects who failed to attain a decline in their HCV m RNA levels of >2log, as compared with baseline, at week 12 of combination therapy were considered non responders. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated with the formula defined by Matthews **(Matthews et al., 1985)** as follows: HOMA-IR= [fasting serum insulin (μIU/mL)×fasting serum glucose (mg/dL)×0.055⁄22.5]. Insulin resistance was defined as HOMA-IR index>2.114.

II. Inclusion criteria:

Eligible patients were ≥ 18 years of age who had chronic hepatitis C (CHC), genotype 4, based on the presence of anti HCV and detectable serum HCV m RNA for 6 months or more in combination with a liver biopsy findings compatible with chronic viral hepatitis **(Bedossa and poynard, 1996)** In the preceding 12 months. All patients were treatment naïve.

III. Exclusion criteria:

Patients were excluded if they suffered from diabetes mellitus diagnosed according to the American diabetic association (ADA) classification criteria (**American diabetes association, 2010).** decompensated cirrhosis, other causes of liver disease, concomitant HBV, HIV, and schistosoma mansoni co-infection, autoimmune diseases. Patients with a history of alcohol intake, a serious concomitant medical conditions, and those with evidence of malignant neoplastic disease before the start of antiviral therapy were also excluded from the study. Pregnant females, females unwilling to practice contraception, non genotype 4 and age < 18 years were not eligible for the current study.

The study was approved by the ethical committee of Mansoura School of Medicine, and informed consent was obtained from every study participants.

IV-Biochemical laboratory measurements:

An overnight 10 m L fasting blood samples was taken from every patients.Serum was separated by centrifugation for quantitative measurement of HCV mRNA, alanine aminotransferase activity(ALT), aspartate aminotransferase activity(AST) activities, serum glucose, insulin, IFN-γ and ferritin levels.

1-Quantitative determination of HCV mRNA

HCV mRNA was detected by quantitative polymerase chain reaction (PCR) using (Robogene HCV quantitation kits, Germany) **(Rolfe et al., 2005)**

***2-Determination of serum glucose*:**Serum glucose was measured according to the method of **Trinder, 1969)** using kits provided by (Spinreact,SPAIN).

3- Determination of serum AST and ALT activities:

Serum ALT and AST activities were measured according to the method of **(Herny et al., 1960)** using kits provided by (Human, Germany).

4-Dermination of serum insulin levels:

Serum insulin was assayed by solid phase enzyme –linked immunosorbent assay" ELISA technique" using a commercially available kits from (immnospec UK.) The assay system utilizes one antibody for solid phase (micotiter wells) immobilization and another antiinsulin antibody-enzyme (horseradish peroxidase) conjugate solution. **(McCann and Kirkish, 1985)**The sensitivity of this assay was found to be 2 μIU/ mL. The coefficient of variation of this method is <2%.

5-Dermination of serum IFN γlevel:

Serum levels of interferon-gamma (IFN-γ) were detected by Enzyme immunoassay of IFN-γ which is a sandwich type assay with two steps. The first step leads to capture of IFN-γ by monoclonal anti-IFN-γ antibody bound to the wells of a microstate plate. In the second step, a second monoclonal anti-IFN-γ antibody, which is biotinylated antibody, binds to the solid phase antibody antigen complex and in turn binds the conjugate. After incubation the wells are washed and the binding of streptavidin peroxides via biotin is followed by the addition of chromogenic substrate of the peroxides. The intensity of the coloration produced is proportional to the IFN-γ concentration in the sample or standard. The kits were supplied by Beckman Coulter Company. according to the method of (Engvall and Perlman, **1976).**

6- Determination of serum ferritin level

Serum ferritin was determined by chemiluminescence assay using Elecsys immunoassay analyzers **(Lotz et al., 1997)**

Statistical analysis:

Data entry and statistical analysis were done using SPSS software

package version (17). Data were expressed as means±SD and

frequencies. Comparisons between groups were conducted using independent t-test, and X2 test when applicable. Pearson's correlation analysis was used for estimating correlation between variables. Probability levels< 0.05were considered significant. Linear regression analysis was performed to investigate the independent association of the study parameters with posttreatment HCV m RNA as a dependent variable.

Resuls:

1-Clinical and laboratorial characteristics of the studied population at the initial of study (Table1)

The mean age of studied patients was (46.1± 7.1), the female patients represent 61.8% of the studied population while, male patients constitute 83.2% of all participants. Patients with insulin resistance account for 61.2% of all patients **(Table 1).**The mean value of HCV m RNA was 1,292,605± 741,355 while, The mean values of ALT and AST activities were 89.8± 36.5 IU/ml and 67.1± 207 IU/m L respectively **(Table 1**).With regard to fasting serum glucose and insulin levels, their mean values were 14.5± 11.8 mg/dl and 14.5± 11.8mg/dl respectively. **(Table1).**Concerning homeostasis model of assessment of insulin resistance (HOMA), the mean value of HOMA index was 4± 3.6 in all studied patients. **(Table1).** With respect to serum ferritin and IFN γ levels, their mean values were 219.7± 28 and 325± 64.6 respectively.

2- Biochemical laboratory parameters in the studied population(Table2)

Viral burden as defined by serum HCV m RNA levels, AST and ALT activities, serum ferritin and IFN γlevels showed significant increase among non responders pre and post IFN α treatment as compared to responders **(P<0.0001) (Table 2).** With respect to serum fasting insulin and HOMA index, significant increase were detected in non responders as compared to responders both pretreatment and post treatment with IFNα. ***(p<0.0001)*** **(Table2).**

3-Correlation between posttreatment HCV m RNA and basal and posttreatment laboratory parameters

-A strong positive correlation (*p<0.0001*) was observed between post treatment HCV m RNA levels and pretreatment HCV m RNA levels (r=0.3), Posttreatment HCV m RNA levels were positively correlated with ALT activities(r=0.4), fasting serum glucose (r=0.7), serum ferritin (r=0.3) and serum IFNγ levels (r=0.4) in all patients enrolled in the study **(Table 3).** Both pretreatment insulin levels and HOMA Index showed strong positive correlation with post treatment HCV m RNA levels in all studied patients. (r= 0.7 *p<0.0001*) **(Table3).**post treatment HCV m RNA levels showed Strong positive correlation.(*p<0.0001*) with post treatment AST &ALT activities (r=0.9), Serum fasting glucose (r=0.7), insulin (r=0.8), ferritin and IFN γ levels (r=0.9) **(Table3)**.Post treatment HOMA index showed a strong positive correlation with post treatment HCV m RNA in all patients (r=0.8,p<0.0001)**(Table3)**

4-Correlation of serum ferritin levels with basal and posttreatment laboratory parameters

A significant positive correlation was detected between pretreatment serum ferritin levels and both pretreatment serum fasting glucose and insulin levels (r= 0.3, *p<0.0001*) **(Table: 4).** Pretreatment serum ferritin levels is positively correlated with posttreatment fasting serum glucose r=0.2,p=0.004), fasting insulin (r=0.25, p=0.001), IFN γ levels, HCV m RNA levels and ALT &AST activities *(r=0.3, p<0.0001)* **(Table 4)**. Post treatment serum ferritin levels showed strong positive correlation with post treatment HCV m RNA levels, ALT &AST activities (r=0.9, p<0.0001) HOMA index, fasting serum glucose, insulin levels (r= 0.9,p<0.0001) and serum IFN γ levels (r=0.9, p<0.0001) **(Table4)** Moreover, Post treatment ferritin levels showed strong positive correlation with pretreatment serum fasting glucose (r=0.5), insulin levels (r=0.7), ferritin levels (r=0.4), IFN γ levels (r=0.4) and ALT activities (r=0.3,p<0.0001).

5-Correlation of serum interferon gamma levels with basal and posttreatment laboratory parameters

Pretreatment serum IFNγ levels correlated positively with pretreatment HCV m RNA *(r=0.3,p<0.0001),* pretreatment ALT activity (r=0.2,p=0.003) and pretreatment fasting glucose levels (r=0.3,p=0.001). Pretreatment ferritin levels were positively correlated with post treatment serum IFNγ levels (p<0.0001**) (Table5)**. Post treatment IFNγ levels correlated positively with pretreatment each of HCV m RNA levels (r=0.22,p=0.02), ALT activity (r=0.3,p<0.0001), fasting serum glucose (r=0.5,p<0.0001), fasting insulin and HOMA index (r=0.4,p<0.0001) **(Table5).**Both Pre and post treatment serum IFNγ levels showed significant positive correlation with post treatment HCV m RNA levels, ALT &AST activities, insulin levels, HOMA index fasting serum glucose) and serum ferritin levels (, p<0.0001), **(Table5)**

6-Correlation of HOMA index with basal and posttreatment laboratory parameters

Pretreatment and post treatment HOMA index were positively correlated with pretreatment fasting insulin, fasting blood glucose, ferritin, IFNγ (p<0.0001) levels and ALT activities (r=0.3,p<0.0001) **(Table 6).**Both Pre treatment and post treatment HOMA index were positively correlated with post treatment fasting insulin, fasting

serum glucose, ferritin, IFNγ levels and ALT&AST activities, ($p<0.0001$) **(Table 6)**.

Discussion:

HCV has been shown to be an etiologic agent responsible for chronic liver disease with eventual progression to cirrhosis in 20% of patients **(Asselh et al., 2010)**.The present study was designed to investigate the impact of insulin resistance and hyperferritenemia on the immunomodulatory and antiviral activities of IFN α therapy.The elevated HOMA index as a marker of insulin resistance, among studied non responders patients had negative sequences as mirrored by elevated viral load, increased transaminases activities and elevated IFN γ levels. These effects are partly attributed to the effect of Hyperinsulinemia in the setting of insulin resistance in both inhibiting the response to IFN-α therapy and promoting disease progression *via* different mechanisms. One possible mechanism is the reduction of PI3K/AKT signaling leading to subsequent inhibition of HCV replication suppressor such as p21 activated kinase 1 **(Ishida et al., 2007). Moreover,** IR may play a pivotal role in progression of fibrosis through stimulating the proliferation of hepatic stellate cells (HSCS)and hence promoting collagen I synthesis **(Svegliati et al.,1999).** In addition, insulin signaling through IRS/PI3K cascade enhancing matrix metalloproteinase (MMP) activity that have been implicated in hepatic fibrosis **(Hemman et al.,2007).** Concerning the impact of IR on IFN α signaling, it was reported that there is reciprocal interference between IFN α and insulin signaling in liver cells as insulin mediated IRS-1 serine phosphorylation could be responsible for the interference with the non classical IFN signaling pathway resulting in lower transcription of interferon stimulated genes (ISGs**) (Franceschini et al.,2011).**

On the other hand HCV structural, non structural proteins, inflammation and immune system each on its own may play a role in exacerbation of IR **(Paraviz et al.,2011).**It was proposed that HCV core protein stimulated increased levels of the molecule suppressor of cytokine signaling (SOCS3), leading to ubiquitination and proteasomal degradation of insulin receptor substrate 1 (IRS-1) and insulin receptor substrate -2 (IRS2**) (Kawaguchi et al.,2004).** In addition, HCV core protein also increases the expression of tumor necrosis factor α (TNF α) which is required for the phosphorylation of serine residues of IRS-1 eventually leading to the downregulation of insulin signaling **(Pal et al.,2010).** Protein phosphatase 2 A (PP2A) is another molecule that is upregulated by HCV non structural protein (NS5A) and **may** play a role in HCV induced insulin resistance by dephosphorylating and thus inactivating AKT **(Bernsmeier et al., 2008**). Moreover, IFN γ mediates the loss of insulin stimulated glucose uptake in human adipocytes accompanied by downregulation of the, insulin receptor substrate 1(IRS-1) **(McGillicuddy et al., 2009)**

With respect to serum ferritin levels, The present study demonstrated a strong positive correlation between serum ferritin levels and elevated HCV m RNA levels accompanied by increased transaminases activities**.**This was in agreement with the study conducted by **(Theurl et al., 2004).**These effects are attributable to the role of iron in modulating the course of HCV infection by at least three mechanisms, First, iron is known to interact directly with cell-mediated immune pathways through weakening IFNγ activity and Th1 –mediated effector mechanisms and inducing the expression of anti-inflammatory cytokines by Th2 cells, which is an unfavorable condition for fighting infectious disease including HCV **(Weiss, 2002)** Second, Iron may catalyze the formation of highly toxic hydroxyl radicals by the Fenton reaction, leading to progressive liver fibrosis **(Pietrangelo, 2003)** Third, iron has a direct effect on HCV translation via induction of (eukaryotic initiation factor 3 (e IF3) expression, an indispensable factor for effective initiation of HCV translation **(Weiss, 2002)** In addition, iron is a profibrogenic factor, acting as an activator of both hepatic stellate cells and kupffer cells **(Pietrangelo, 2003).** Moreover, a further attenuating effect on response to IFN α therapy may be mediated by the cross talk between iron and glucose metabolism as insulin is known to stimulate ferritin synthesis and facilitates iron uptake by the cell through the translocation of transferrin receptors from the intracellular compartment to the cell surface **(Shan et al.,2005).** Conversely, iron influences glucose metabolism as iron is a potent pro oxidant that increases the cell oxidative stress, causing inhibition of insulin internalization and actions, results in hyperinsulinemia and insulin resistance **(Farigon et al., 2002).**

Concerning (IFNγ), as a mediator of the immunomodulatory role of exogenous IFN α therapy **(Asselh et al., 2010**). The elevation in serum IFNγ levels among studied non responder patients can be attributed to several factors. First, the increased production of IFN γ by activated T-lymphocytes, such as NK cells which either reside in the liver or recruited to the liver in response to inflammation and injury **(Horras et al., 2011).**Second, IFNγ production was increased in lymphocytes exposed to heavy chain ferritin (H-ferritin**) (Weiss, 2002)** Third, The role of insulin resistance in modulating a shift to a Th1 – cytokine profile dominated by the production of IFN γ **(Horras et al., 2011)**.On the other hand the decreased

IFN γ levels among responders who do not experience insulin resistance or hyperferritenemia may partly attributed to the effect of exogenous high levels of type I interferons -mediated inhibition of IL-12 production by antigen-presenting cells leading to secondary inhibition of IFNγ **(Horras et al., 2011).** Besides its role as a predictor of the immunomodulatory effect of exogenous IFN α therapy, IFNγ may play an important role in disease exacerbation as detected by The increased transaminases activity in patients with elevated serum IFNγ. These effects are attributed to the role of IFNγ in exacerbation of liver damage during viral hepatitis **(Horras et al., 2011).** IFN γ induces the hepatic expression of IFN γ inducible genes such as chemokines CXCL9 and CXCL 10 and enhances the recruitment of antigen non specific mononuclear and polymorphnuclear cells to the liver where they accumulate in necroinflammatory foci **(Horras et al., 2011).** Moreover, IFN γ is a potent stimulus for macrophage activation, and activated macrophages produce an abundance of cytokines such as TNFα which may impact hepatocyte function. **(Horras et al., 2011).** On the other hand IFN γ plays a pivotal role in viral clearance through several mechanisms, including direct inhibition of viral replication, increasing antigen processing, transport and major histocomptability expression in virus infected cells to facilitate viral clearance **(Horras et al., 2011)**.The attenuated viral clearance effect of IFNγ despite its elevated serum levels among non responders may be ascribed to the role of both hyperinsulinemia and hyperferritenemia in interfering with its signaling pathway as it was stated that elevated serum ferritin levels result in an impaired response to stimulation by the Th1- cytokine IFN γ **(Pietrangelo, 2003)**.On the other hand hyperinsulinemia in the setting of insulin resistance is known to induce suppressor of cytokine signaling 3 (SOCS3) which inhibits IFNγ signaling**(Franceschini et al., 2011)**

In summary, an activated T-cell response was present in HCV patients and subsequently may be regulated by IFN-α therapy. Modulation of T-cell function may be one mechanism whereby IFN-α therapy results in decreased viral load. Insulin resistance and hyperferritenemia may interfere with this immunoregulatory effect of IFN-α.. By further understanding of both the immune response in HCV patients and pleiotropic immunomodulatory properties of IFN α, future treatment strategies can be designed for HCV infection to overcome different inhibitory signals and allow more rational exploitation of IFN-α therapy.

Results

Table (1): Demographic and laboratorial characteristics of the studied population at the initial of study

Parameters	**Total population (n=165)**
Age (years)	46.1± 7.1
Gender M F	102 (61.8%) 63 (38.2%)
Insulin resistance	101 (61.2%)
HCV m RNA	1,292,605± 741,355
ALT**(IU/m L)**	89.8±36.5
AST**(IU/m L)**	67.1±20.7
FBG **(mg/ dl)**	106.8±10.4
Fasting insulin **(µI u/m L)**	14.5±11.8
HOMA-IR	4± 3.6
ferritin **(ng/ml)**	219.7±28
INF-γ **(pg/mL)**	325±64.6

(IFN γ): Interferon gamma, (ALT), alanine aminotransferase
(AST) Aspartate aminotransferase
(HOMAI): Homeostasis model of assessment of insulin resistance
FBG: fasting blood glucose

Table (2): comparison of serum HCV m RNA, insulin, fasting glucose, ferritin and interferon gamma (IFN γ) levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities and homeostasis model of assessment (HOMAI) in responders and non responders HCV patients pretreatment and 12-weeks after treatment with interferon alpha (IFNα),

PARAMETERS	Pre-IFNα TREATMENT		12 week post treatment with IFNα	
	Responder (n=132) (M/F=62.1%)	Non-responder (n=33) (M/F=60.6%)	Responder (n=132) (M/F=62.1%)	Non-responder (n=33) (M/F=60.6%)
HCV m RNA	1,265,546± 666,420	1,400,842±991,737*	18,799± 16,808	1,529,879±760,264**
ALT (IU/m L)	84.2±32.8	111.9±42.2*	18± 4.5	174.6± 41**
AST (IU/mL)	67±19.9	67.3±23.7*	17.3± 5.2	183.1± 36.4**
Fasting blood glucose (mg /dl)	104.1±9.7	117.3±5*	96.8± 6.3	117.5±2.5**
Serum Fasting insulin (µI u/m L)	9.8±5.5	33±12.4*	3.1±1.4	36.1± 12.2**
HOMA –IR	2.6± 1.6	9.6± 3.9*	0.8±0.3	10.4± 3.6**
Serum ferritin (ng/ml)	215.7±27.2	335.4±26*	215.8±27	235.4± 26**
Serum IFNγ(pg/mL)	312.7±60.6	374.5±56.6*	191± 8.9	432.4±78.5**

*** Significant of non responders versus responder pretreatment IFN α treatment (p<o.ooo1)**
**** Significant of non responders versus responder 12 weeks post treatment with IFN α treatment (p<o.ooo1)**
n: number of patients M/F: Male to Female ratio
(IFN γ): Interferon gamma, (ALT), alanine aminotransferase
(AST) Aspartate aminotransferase
(HOMAI): Homeostasis model of assessment of insulin resistance

Table (3): Pearson's correlation between post treatment HCV m RNA and pretreatment (basal) and post treatment levels of serum insulin, fasting glucose, ferritin interferon gamma (IFN γ) levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST)and homeostasis model of assessment (HOMAI) in the total studied group of patients (n=165).).

	posttreatment HCV m RNA	
	R	P
Age	0.1	Ns
HCV mRNA	**0.3***	**<0.0001**
Pretreatment ALT**(IU/mL)**	**0.4**	**<0.0001**
Pretreatment AST(**IU/mL**)	0.001	Ns
Pretreatment FBG **(mg /dl)**	**0.4***	**<0.0001**
Pretreatment fasting insulin **(µI u/m L)**	**0.7***	**<0.0001**
Pretreatment HOMA-IR	**0.7***	**<0.0001**
Pretreatment ferritin (**ng/ ml)**	**0.3***	**<0.0001**
Pretreatment INF-γ**((pg/mL)**	**0.4***	**<0.0001**
post treatment ALT (IU/Ml)	**0.9***	**<0.0001**
post treatment AST IU/mL	**0.9***	**<0.0001**
post treatment FBG (mg /dl)	**0.7***	**<0.0001**
post treatment fasting insulin µI u/m L	**0.8***	**<0.0001**
post treatment HOMA-IR	**0.8***	**<0.0001**
post treatment ferritin(ng/ m L)	**0.9***	**<0.0001**
Post treatment INF-γ (pg/mL)	**0.9***	**<0.0001**

(IFN γ): Interferon gamma, (ALT), alanine aminotransferase
(AST) Aspartate aminotransferase FBG: Fasting blood glucose
(HOMAI): Homeostasis model of assessment of insulin resistance
*** significant positive correlation with HCV m RNA (P<0.0001)**

Table (4): Pearson's correlation between both pre & **post treatment** ferritin levels **and pre- and post treatment levels of HCV m RNA, serum insulin, fasting glucose, ferritin, interferon gamma (IFN γ) levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST) activities and homeostasis model of assessment (HOMAI) in the total studied group of patients (n=165)**

	pretreatment ferritin		**posttreatment ferritin**	
	R	P	R	P
Age	-0.1	Ns	0.03	Ns
pretreatment HCV m RNA	-0.1	Ns	0.1	Ns
pretreatment ALT **(IU/mL)**	-0.03	Ns	**0.3****	**<0.0001**
pretreatment AST **(IU/mL)**	0.01	Ns	-0.004	Ns
Pretreatment FBG (mg/dL)	**0.3***	**0.001**	**0.5****	**<0.0001**
Pretreatment fasting insulin (µI u/m L)	**0.3***	**<0.0001**	**0.7****	**<0.0001**
Pretreatment ferritin(ng/ m L)	-	-	**0.3****	**<0.0001**
Pretreatment IFN γ **(pg/mL)**	-0.01	Ns	**0.4****	**<0.0001**
Post treatment HCV m RNA	**0.3***	**<0.0001**	**0.85**	**<0.0001**
Post treatment ALT (IU/mL)	**0.3***	**<0.0001**	**0.9**	**<0.0001**
Post treatment AST (IU/mL)	**0.3***	**<0.0001**	**0.9**	**<0.0001**
Post treatment FBG (mg/dl)	**0.2**	**0.004**	**0.8**	**<0.0001**
Post treatment fasting insulin (µI u/m L)	**0.25***	**0.001**	**0.9**	**<0.0001**
Post treatment HOMA-IR	**0.3***	**0.001**	**0.9**	**<0.0001**
posttreatmentferritin (ng/ m L)	**0.3***	**<0.0001**	-	-
posttreatmentIFN γ(pg/mL)	**0.3***	**<0.0001**	**0.9**	**<0.0001**

(IFN γ): Interferon gamma, (ALT), alanine aminotransferase
(AST) Aspartate aminotransferase FBG: Fasting blood glucose
(HOMAI): Homeostasis model of assessment of insulin resistance
*** significant positive correlation with pretreatment serum ferritin (P<0.0001)**
**** significant positive correlation with post treatment serum ferritin (P<0.0001)**

Table 5: Pearson's correlation between both pre & **post treatment** interferon gammsa (IFN γ) levels **and pre- and post treatment levels of HCV m RNA, serum insulin, fasting glucose, ferritin, interferon gamma (IFN γ) levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST)and homeostasis model of assessment (HOMAI) in the total studied group of patients (n=165).)**

	pretreatment IFN γ		**posttreatment IFN γ**	
	R	P	R	P
Age/ years	**0.1**	**Ns**	**0.03**	**Ns**
Pretreatment HCV m RNA	**0.3**	**<0.0001***	**0.2**	**0.02**
Pretreatment ALT**(IU/mL)**	**0.2**	**0.003***	**0.3**	**<0.0001****
pretreatment AST**(IU/mL)**	**-0.2**	**Ns**	**-0.02**	**Ns**
pretreatment FBG(mg/dl)	**0.3**	**0.001**	**0.5**	**<0.0001****
Pretreatment fasting insulin **(µI u/m L)**	**0.4**	**<0.0001***	**0.4**	**<0.0001****
Pretreatment HOMA-IR	**0.4**	**<0.0001***	**0.4**	**<0.0001****
pretreatment ferritin **(ng/ m L)**	**-0.01**	**Ns**	**0.3**	**<0.0001****
Post treatment HCV m RNA	**0.4**	**<0.0001***	**0.9**	**<0.0001****
Post treatment ALT **IU/mL**	**0.35**	**<0.0001***	**0.9**	**<0.0001****
Post treatment AST **IU/mL**	**0.4**	**<0.0001***	**0.9**	**<0.0001****
posttreatment FBG mg/dl	**0.24**	**0.002***	**0.8**	**<0.0001**
Post treatment fasting insulin **µI u/m L**	**0.4**	**0.0001***	**0.4**	**<0.0001****
Post treatment HOMA-IR	**0.4**	**0.0001***	**0.4**	**<0.0001****
Post treatment ferritin ng/mL	**0.3**	**<0.0001***	**0.9**	**<0.0001****
Post treatment IFN γ **(pg/mL)**	0.4	<0.0001	-	-

(IFN γ): Interferon gamma, (ALT), alanine aminotransferase
(AST) Aspartate aminotransferase FBG:fasting blood glucose
(HOMAI): Homeostasis model of assessment of insulin resistance
*** significant positive correlation with pretreatment serum IFN γ(P<0.0001)**
**** significant positive correlation with post treatment serum IFNγ (P<0.0001**

Table 6:Pearson's correlation between both pre & **post treatment** HOMA index **and pre-and post treatment levels of HCV m RNA, serum insulin, fasting glucose, ferritin, interferon gamma (IFN γ) levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST)and homeostasis model of assessment (HOMAI) in the total studied group of patients (n=165).**

	Pretreatment HOMA-IR		**Post-treatment HOMA-IR**	
	r	P	r	P
Age (years)	0.04	Ns	0.1	Ns
Pretreatment HCV m RNA	0.01	Ns	0.1	ns
Pretreatment ALT**(IU/mL)**	**0.3**	**<0.0001***	**0.3**	**<0.0001****
Pretreatment AST(**IU/mL**)	-0.04	Ns	-0.002	ns
pretreatment FBG (mg/dl)	**0.7**	**<0.0001***	**0.5**	**<0.0001****
pretreatment fasting insulin **(µI u/m L)**	**0.997**	**<0.0001***	**0.5**	**<0.0001****
pretreatment IFN γ (pg/mL)	**0.4**	**<0.0001***	**0.4**	**<0.0001****
pretreatment ferritin(ng/mL)	**0.3**	**<0.0001***	**0.3**	**0.001****
posttreatment HCV m RNA	**0.7**	**<0.0001***	**0.8**	**<0.0001****
posttreatmentALT IU/mL	**0.8**	**<0.0001***	**0.9**	**<0.0001****
posttreatment AST IU/mL	**0.8**	**<0.0001***	**0.9**	**<0.0001****
posttreatment FBG mg/dL	**0.7**	**0.002***	**0.8**	**<0.0001****
posttreatmentfasting insulin µI u/m L	**0.7**	**0.0001***	**0.995**	**<0.0001****
posttreatment HOMA-IR	**0.4**	**0.0001***	**-**	**-**
posttreatment ferritin (ng/mL	**0.7**	**<0.0001***	**0.9**	**<0.0001****
Posttreatment IFN γ(pg/m L)	**0.8**	**<0.0001***	**0.9**	**<0.0001****

(IFN γ): Interferon gamma, (ALT), alanine aminotransferase
(AST) Aspartate aminotransferase FBG: fasting blood glucose
(HOMAI): Homeostasis model of assessment of insulin resistance
*** significant positive correlation with pretreatment HOMA index(P<0.0001)**
**** significant positive correlation with post treatment HOMA index (P<0.0001**

Multiple linear regression analysis

Step wise multiple linear regression analysis selected pretreatment levels of HCV m RNA, HOMA-IR and ferritin as best independent predictors for post treatment HCV m RNA levels (R^2= 0.6, P< 0.0001). **(Figure 1, 2,3)**

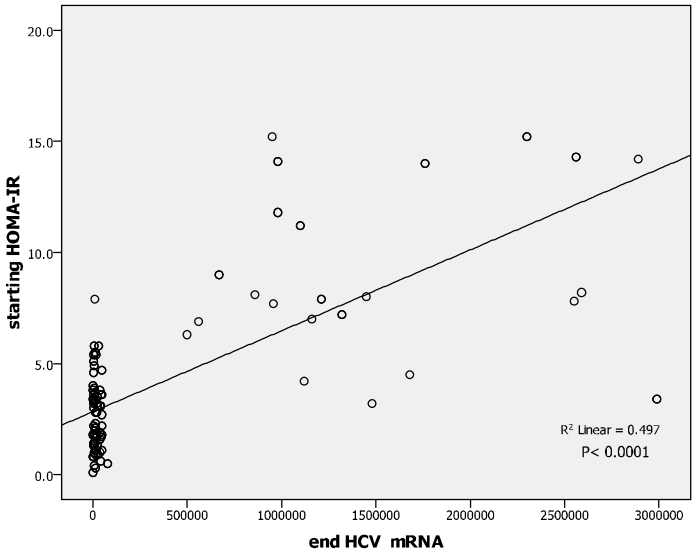

Figure (1) linear regression analysis between post treatment HCV m RNA levels and pretreatment HOMA-IR

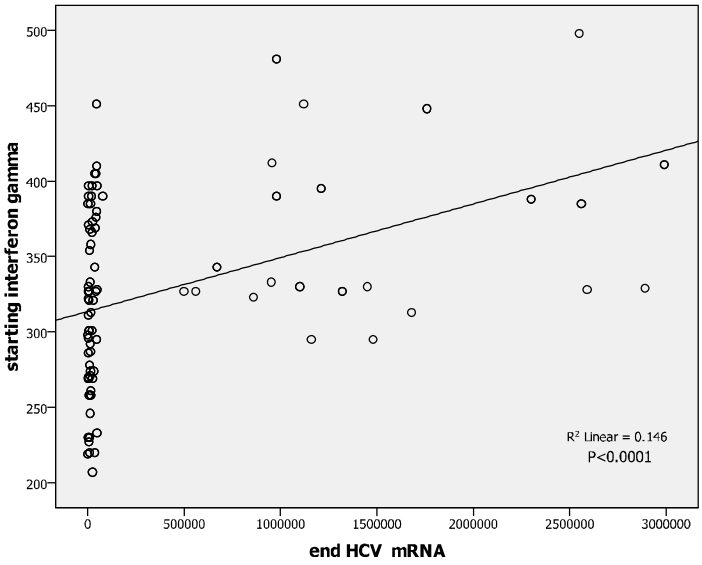

Figure (2) linear regression between post treatment HCV m RNA and pretreatment serum INF-γlevels

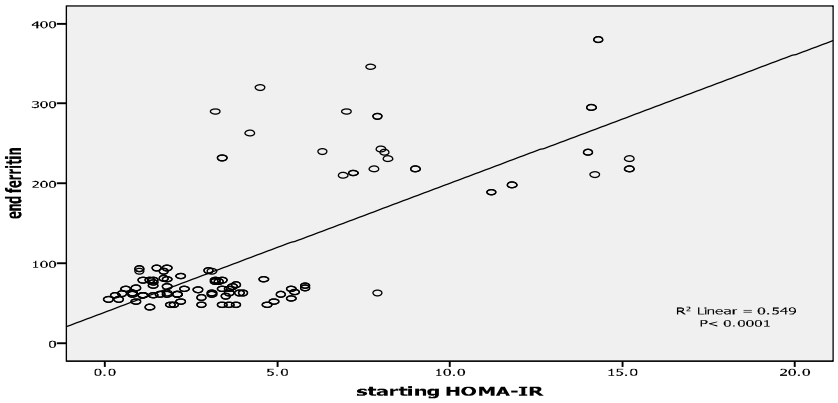

Figure (3): pretreatment HOMA-IR and pretreatment fasting insulin are best independent predictors for posttreatment serum ferritin.

Running title: Resistance to interferon therapy in Egyptian HCV patients

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