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Research Article

# Serum Vitamin D and ferritin levels for predicting Early virological Response in Chronic Hepatitis C Egyptian Patients

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## Abstract

**Background:** Vitamin D has been shown to play an important immunomodulatory and anti-inflammatory role. Vitamin D deficiency has been associated with evolution of Chronic Hepatitis C (CHC) infection. Elevated serum ferritin level reflects a systemic inflammatory state as well as increased iron storage, both contribute to unfavorable outcome of CHC treatment. **Aim:** to study serum 25(OH) vitamin D [25(OH)D] and ferritin levels in Chronic Hepatitis C Egyptian patients achieving early virological response during Pegylated Interferon and Ribavirin combination therapy. **Patients and methods:** 30 Egyptian patients with CHC were included in the study, and treated with pegylated interferon α2a and ribavirin. PCR for HCV (RNA) was done for all the patients to detect early virological response (EVR) and non-EVR group. Serum 25(OH)D and ferritin levels were measured for all patients. **Results:** EVR group showed significantly lower Serum ferritin levels and higher 25(OH)D at week 0, 4 and 12 of treatment compared to non-EVR group with cut off value of week 0s.ferritin ≤ 219 ng/ml, week 0 25(OH)D ≥ 15 ng/ml and week 4 ≥ 20 ng/ml in predicting EVR. At week 4 the predictive power of serum 25(OH)D in EVR group was found significantly higher than ferritin (AUCs = 0.9 versus 0.687, p = 0.05). **Conclusion:** determination of serum 25(OH) vitamin D level at week 4 of treatment provides satisfactory diagnostic accuracy in predicting EVR.

**Keywords:** 25 (OH) vitamin D, ferritin, Chronic hepatitis C, Early virological response.

## Introduction

HCV is a mounting global health challenge, causing a significant proportion of chronic liver disease around the world (1). Chronic hepatitis C (CHC) infection has a global prevalence of 2%-3%. Approximately 170 million people are thought to be currently infected (approximately 3% of the world's population), and an additional 3-4 million are infected each year (2). The Egyptian Demographic Health Survey (EDHS), a cross sectional survey including hepatitis C virus (HCV) biomarkers, was conducted in 2008 on a large nationally representative sample. It estimated HCV prevalence among the 15–59 years age group to be 14.7%. Accordingly, Egypt has the highest HCV prevalence in the world (3).

HCV interferes with the host's iron metabolism, and hepatic iron measures were correlated with the grade and stage, as well as with the treatment outcome, of CHC (4). Infection with HCV leads to iron accumulation in the liver and increased serum ferritin levels, which can be, at least partially, explained by down-regulation of hepcidin, a key regulator of iron homeostasis (5) However, serum ferritin is also frequently elevated in inflammatory conditions. Excess iron in the liver promotes liver inflammation, oxidative stress, and mitochondrial dysfunction (6).Vitamin D has immunomodulatory properties, exerts an anti-hepatitis C virus (HCV) effect in vitro and improves response to interferon-based therapy in patients with chronic hepatitis C (CHC). Low serum levels of 25(OH) vitamin D [25(OH)D] are frequently found in CHC patients and seem to be related to more advanced stages of liver fibrosis (7). The association between vitamin D and infectious disorders has been suggested to be linked to its ability to modulate both innate and adaptive immune responses (8).

Different parameters had been determined for prediction of treatment outcome in CHC patients receiving dual combination therapy. Results concerning vitamin D and ferritin levels are conflicting, so this study was conducted to clarify the role of serum 25(OH)D and ferritin levels in CHC Egyptian patients achieving EVR during Pegylated Interferon and Ribavirin combination therapy.

## Patients and Methods

### Study design

The present study was conducted on 30 treatment-naïve patients (male: 20 and female: 10) with chronic HCV attending the outpatients clinics ofInternal Medicine, Hepatology and Gastroenterology Department in Ain Shams University and military hospitals, during the period from May 2014 to August 2014 who received IFN-based dual combination therapy[pegylated INF alpha 2a(180 ug/wk) and weight based ribavirin].

According to the value of quantitative assay of HCV by PCR at week 12 patients were classified into:
EVR group: undetectable serum HCV RNA by PCR at week 12.
Non-EVR group: failure to achieve reduction in serum HCV RNA of at least 2 logs compared to baseline.

All patients were selected according to the following

### Inclusion criteria

Patients aged from 30 to 60 years, Both male and female sex. HCV infection diagnosis based on the presence of anti-HCV antibody (4th generation ELISA) and detectable HCV RNA by polymerase chain reaction (PCR).Chronic Hepatitis C patients (persistent elevation of ALT more than 6 months).Patients with hemoglobin >12 g/dl (in females), >13gm/dl (in males), platelets>100000/mm 3 and total leucocytic count > 4000/mm 3.Patients with body mass index (BMI) <30, Compensated liver disease (child A score).

### Exclusion criteria

patients excluded from treatment with interferon therapy: (Decompensated liver disease, Psychological disorders, Thyroid dysfunction, Autoimmune disease); Liver disease other than Hepatitis C (Hepatitis B (HBsAg +ve, HBcAb IgG +ve), Autoimmune hepatitis, Alcoholic liver disease, Wilson disease, Hepatocellular carcinoma, Hemochromatosis); Patients with conditions or disease that may affect serum iron level (Patients receiving oral or parenteral iron therapy, Hemolytic anemias, Iron depletion therapy, Hemoglobinopathies); Concomitant use of any drug affecting vitamin D concentration and or induce liver disease, diabetic patients discontinue treatment due to side effects.

A written informed consent was obtained from all patients enrolled in the study and approval was taken from the medical ethical committee of Ain Shams University Hospitals.

All patients were subjected to Full history taking and complete clinical examination, Laboratory investigations [CBC, Viral markers (HBsAg, HCV Ab), Quantitative PCR for HCV RNA (baseline, and 12 weeks), Measurement of serum ferritin and vitamin D level (baseline, at 4 and 12 weeks), Liver function tests( ALT, AST, S.Alb, Total and D.bil, ALK ph, PT and INR), Renal function tests (Serum creatinine, Blood urea ,Serum sodium and potassium), Thyroid function test (TSH), Autoimmune markers (ANA, AMA, ASMA)], Abdominal ultrasonography. Fundus examination.

Follow up of cases at week 4 & week12) by Clinical examination and Laboratory investigation (CBC, AST, ALT, T.Bil., D.Bil).

### Quantitative PCR for HCV RNA (Gene Proof Company)

Technology: Real-time PCR.
Gene Target: Conservative section of 5´UTR sequence.
Specificity: HCV genotype 1 – 6.
Sensitivity (LOD): Reaches 18.354 IU/ml with the probability of 95%.
Accuracy of measurement : Within the range of 106 - 104 IU/ml the establishment accuracy is 0.5 log.
Linear range of measurement: 1010 - 18.354 IU/ml
Extraction/Inhibition Controls Included control of PCR Inhibition and Quality of Extraction (ISEX version)
Sample Material: serum, plasma
Reporting Units: IU/ml (1 IU/ml = 3,2 copies/ml)
Kit Storage: -85 °C to -10 °C

### Serum ferritin

(GENWAY BIOTECH INC Company).
Sample type: Serum

### Principle of the test:

The Ferritin Quantitative Test is based on a solid phase enzyme-linked immunosorbent assay (ELISA)..
Storage and Stability: Store at 2 to 8°C.
With reference range: 12-250 ng/ml.

### Vit. D level

(CALBIOTIC Company).

### Principle:

The Calbiotech (25-OH) Vitamin D kit is a solid phase enzyme-linked immunoassay (ELISA).

### Storage and Stability:

Product should be stored at 2-8 °C. Product is stable for 24 months from the date of manufacturing.
With reference range: 20-100 ng/ml

### Drugs used in this study

Pegylated Interferon alpha 2a (180 ug), SC injection once / week. Ribavirin; weight based ribavirin, 15mg/Kg daily (0.8-1.4 g/day oral capsules) i.e. the number of capsules administrated daily varied according to body weight.

### Statistical analysis

The collected data was revised, coded, tabulated and introduced to a PC using Statistical package for Social Science (SPSS 15.0.1 for windows; SPSS Inc, Chicago, IL, 2001). Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

### Descriptive statistics

Mean, Standard deviation (± SD) and range for parametric numerical data, Frequency and percentage of non-numerical data.

### Analytical statistics

Student T Test was used to assess the statistical significance of the difference between two study group means. Correlation analysis: To assess the strength of association between two quantitative variables. The correlation coefficient defines the strength and direction of the linear relationship between two variables. Paired t-test was used to assess the statistical significance of the difference between two means measured twice for the same study group.

**Logistic regression:** used in the prediction of an outcome (response) based on a set of independent variables. The ROC Curve (receiver operating characteristic) was constructed to obtain the most sensitive and specific cutoff for each technique. To evaluate the most discriminating markers between the compared groups, AUC can also be calculated.

Diagnostic validty test include:

The diagnostic sensitivity: it is the percentage of diseased cases truly diagnosed among total diseased cases. The diagnostic specificity: it is the percentage of non-diseased truly excluded by the test among total non-diseased cases. The predictive value for a +ve test : it is the percentage of cases truly diagnosed among total positive cases. The predictive value for –ve test : it is the percentage of cases truly negative among total negative cases.

P- value (propability test): level of significance
P>0.05: Non significant (NS).
P≤0.05: Significant (S).
P≤0.01: Highly significant (HS).

## Results

The present study was conducted on 30 treatment-naïve patients with chronic HCV attending the outpatient's clinic of the Internal Medicine, Hepatology and Gastroenterology Department in Ain Shams University and military hospitals during the period from May 2014 to August 2014.

### Demographic characteristics of patients enrolled in the study:

There were 20 males (66.7%) and 10 females (33.3%). Their age ranged from 32 to 59 years with mean age (47.57±7.72), and their BMI ranged from 21.6 to 30 with mean (26.63±2.38).

Serum 25(OH) vitamin D, ferritin, liver enzymes and PCR at different weeks of treatment among studied patients (n=30): the results obtained were tabulated (table 1).

**Table (1): Description of laboratory data of the studied patients (n=30) at different weeks of treatment**

| | Week 0 (Mean±SD) | Week 4 (mean±SD) | Week 12 (mean±SD) |
|---|---|---|---|
| Hb(mg/dl) | 14.03±1.65 | 12.42±1.72 | 10.83±1.42 |
| ALT(u/L) | 67.13±44.27 | 56.7±24.53 | 62.37±23.23 |
| AST(u/L) | 57±41.91 | 60.93±25.82 | 59.67±21.75 |
| T. Bil(mg/dl) | 0.79±0.34 | 1.27±0.32 | 1.42±0.41 |
| D. Bil(mg/dl) | 0.29±0.22 | 0.67±0.34 | 0.72±0.35 |
| TSH(µU/L) | 1.62±0.8 | - | 3.51±1.99 |
| Ferritin(ng/ml) | 249.18±202.08 | 297.31±213.98 | 306.85±210.2 |
| 25(OH)D (ng/ml) | 25.35±28.24 | 22.43±26.65 | 19.35±24.62 |
| PCR(Iu/ml) | 2380433.3±3038277.7 | - | 490390±1284265.5 |

Hb=haemoglobin, ALT=Alanine aminotransferase, AST=Aspartate aminotransferase, T.Bil=Total Bilirubin, D.Bil =Direct Bilirubin, TSH=Thyroid stimulating hormone PCR=Polymerase chain reaction, 25(OH)D= 25 hydroxyvitaminD

***Comparison between EVR and Non-EVR groups as regarding 25(OH)D, ferritin level, liver enzymes and PCR:***

**At week 0:** Mean 25(OH) vitamin D was (48.50±27.24) in EVR group compared to (13.78±21.03) in non-EVR group, with high statistical significance (P value=0.001), Mean serum ferritin was (136.36±80.18) in EVR group compared to (305.59±221.9) in non-EVR group, with high statistical significance (P value=0.005), and Mean PCR was (1869100±3107583) in EVR group compared to (2636100±3051033) in non-EVR group with no statistical significance.

**At week 4:** Mean (25-OH) vitamin D was (43.40±26.21) in EVR group compared to (11.95±20.28) in non-EVR group, with high statistical significance (P value=0.001), Mean serum ferritin was (191.9±101.39) in EVR group compared to (350.02±237.16) in non-EVR group, with statistical significance (P value=0.017).

**At week 12:** Mean (25-OH) vitamin D was (34.05±23.98) in EVR group compared to (12.01±21.96) in non-EVR group, with high statistical significance (P value=0.001), Mean serum ferritin was (208.75±112.35) in EVR group compared to (355.90±232.08) in non-EVR group, with statistical significance (P value=0.027). (Table 2).

***Multivariate analysis for logestic regression*** was done. It revealed that the most independent factors associated with EVR was BMI (p=0.021), and baseline (week0) vit D (p= 0.045) **Table 3.**

***Correlation between serum ferritin and 25(OH)Dat different weeks of treatment with different parameters in EVR group*** shows a significant linear correlation between baseline serum ferritin and BMI(r=0.425, p=0.019).There was a positive linear correlation between baseline Vit D and Total bilirubin with R=0.371, R=0.04 respectively. Also vit D at week 12 correlated inversely with PCR (r= -0.545)p=0.013 (table 4)

***ROC curve analysis*** was used to find the diagnostic performance of baseline(week0), week 4 and 12 of serum 25(OH)D and serum ferritin for discriminating EVR from non-EVR. The cut-off values of serum 25(OH)D and s.ferritin in different weeks of treatment was shown in table 5.

**Table 2: Comparison between EVR and non-EVR at different weeks of treatment as regard different variables**

| Variable | Non EVR | | EVR | | P |
|---|---|---|---|---|---|
| | **Mean** | **±SD** | **Mean** | **±SD** | |
| Age | 48.3 | 7.13 | 46.1 | 9.01 | 0.757 |
| BMI | 27.55 | 2.11 | 24.78 | 1.8 | **0.001**** |
| Hb: week 0 | 13.96 | 1.8 | 14.18 | 1.39 | 0.912 |
| week 4 | 12.29 | 1.84 | 12.7 | 1.51 | 0.596 |
| week 12 | 10.58 | 1.6 | 11.32 | .82 | 0.158 |
| ALT: week 0 | 65.8 | 45.13 | 69.8 | 44.75 | 0.613 |
| week 4 | 55.9 | 24.86 | 58.3 | 25.1 | 0.808 |
| week 12 | 66.7 | 24.51 | 53.7 | 18.6 | 0.152 |
| AST week 0 | 55.2 | 43.53 | 60.6 | 40.49 | 0.645 |
| week 4 | 61.55 | 27.54 | 59.7 | 23.34 | 0.725 |
| week 12 | 65.05 | 22.3 | 48.9 | 16.74 | 0.053 |
| T.Bil week 0 | 0.78 | 0.36 | 0.81 | 0.31 | 0.739 |
| week 4 | 1.28 | 0.35 | 1.26 | 0.27 | 0.982 |
| week 12 | 1.5 | 0.45 | 1.27 | 0.28 | 0.144 |
| D.Bil: week 0 | 0.27 | 0.22 | 0.34 | 0.22 | 0.311 |
| week 4 | 0.67 | 0.35 | 0.66 | 0.35 | 0.930 |
| week 12 | 0.77 | 0.38 | 0.62 | 0.25 | 0.271 |
| TSH: week 0 | 1.65 | 0.83 | 1.56 | 0.76 | 0.947 |
| week 12 | 3.91 | 1.92 | 2.73 | 1.98 | 0.068 |
| Ferritin: week 0 | 305.59 | 221.9 | 136.36 | 80.18 | **0.005**** |
| week 4 | 350.02 | 237.16 | 191.9 | 101.39 | **0.017*** |
| week 12 | 355.9 | 232.08 | 208.75 | 112.35 | **0.027*** |
| Vit D week 0 | 13.78 | 21.03 | 48.5 | 27.24 | **0.001**** |
| week 4 | 11.95 | 20.28 | 43.4 | 26.21 | **0.001**** |
| week 12 | 12.01 | 21.96 | 34.05 | 23.98 | **0.001**** |
| PCR week 0 | 2636100 | 3051033 | 1869100 | 3107583 | 0.225 |
| week 12 | 3208945 | 668700 | 0.00 | 0.00 | ---- |

* Statistically significant ** statistically highly significant

**Table 3: Regression analysis of different variables to study independent factor associated with EVR**

| | Adjusted OR | P | Sig |
|---|---|---|---|
| Age | 78.345 | 0.995 | NS |
| Males | 0.000 | 0.999 | NS |
| BMI | 0.279 | 0.021 | S |
| At week 0: Vit D | 2.477 | 0.045 | S |
| S.ferritin | 1.027 | 1 | NS |
| At week 4: Vit D | 0.000 | 0.997 | NS |
| S.ferritin | 1.059 | 1 | NS |
| At week12: Vit D | 0.024 | 0.999 | NS |
| S.ferritin | 0.859 | 0.999 | NS |
| Alt | 3.046 | 0.998 | NS |
| Ast | 0.452 | 1 | NS |

OR = Odds Ratio

**Table 4: Correlation between serum ferritin and 25(OH)D at different weeks of treatment with different parameters in EVR group:**

| Parameter | Test time(in weeks) | Serum ferritin | | 25(OH)D | |
|---|---|---|---|---|---|
| | | R | P | R | P |
| **Age** | Week 0 | 0.111 | 0.560 | 0.043 | 0.823 |
| | 4 | 0.061 | 0.750 | 0.093 | 0.624 |
| | 12 | 0.058 | 0.760 | 0.113 | 0.553 |
| **BMI** | Week 0 | 0.425 | **0.019*** | -0.166 | 0.382 |
| | 4 | 0.271 | 0.148 | -0.166 | 0.381 |
| | 12 | 0.345 | 0.062 | -0.207 | 0.273 |
| **Hb** | Week 0 | -0.110 | 0.564 | 0.252 | 0.179 |
| | 4 | 0.066 | 0.730 | 0.281 | 0.133 |
| | 12 | 0.039 | 0.839 | 0.470 | 0.009 |
| **ALT** | Week 0 | 0.320 | 0.084 | 0.360 | 0.050 |
| | 4 | 0.256 | 0.172 | 0.197 | 0.296 |
| | 12 | 0.351 | 0.057 | -0.205 | 0.276 |
| **AST** | Week 0 | 0.281 | 0.132 | 0.297 | 0.111 |
| | 4 | 0.217 | 0.250 | 0.104 | 0.585 |
| | 12 | 0.270 | 0.149 | -0.208 | 0.271 |
| **T.bil** | Week 0 | 0.138 | 0.468 | 0.371 | **0.044*** |
| | 4 | -0.042 | 0.826 | -0.115 | 0.544 |
| | 12 | 0.085 | 0.655 | 0.012 | 0.949 |
| **D.bil** | Week 0 | -0.033 | 0.863 | 0.322 | 0.083 |
| | 4 | 0.048 | 0.800 | -0.075 | 0.692 |
| | 12 | 0.001 | 0.995 | -0.042 | 0.824 |
| **TSH** | Week 0 | 0.001 | 0.995 | -0.184 | 0.331 |
| | 12 | 0.081 | 0.672 | -0.274 | 0.142 |
| **PCR** | Week 0 | -0.082 | 0.665 | -0.033 | 0.864 |
| | 12 | 0.226 | 0.339 | -0.545 | **0.013*** |

* Statistically significant

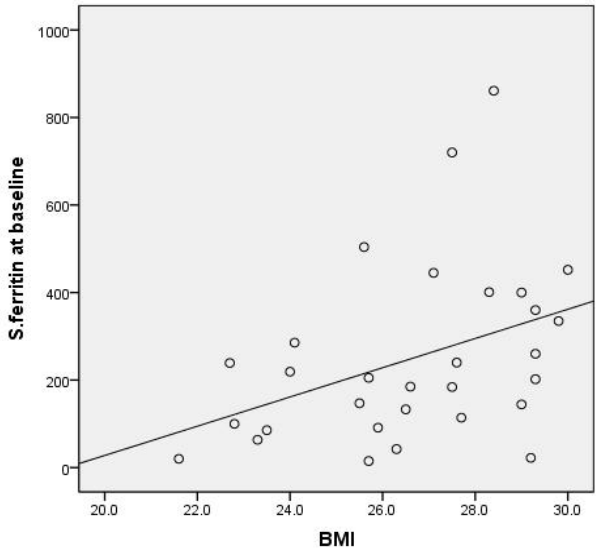


**Figure (1): Spearman's linear correlation between baseline serum ferritin and BMI in EVR group.**

**Table (5): receiver operating characteristic (ROC) curve for serum 25(OH) D and serum ferritin in different weeks of treatment in prediction of EVR**

| | | Cutoff value (ng/ml) | AUC(CI) | Sensitivity | Specificity | PPV | NPV | P(Sig) |
|---|---|---|---|---|---|---|---|---|
| Serum ferritin | Week 0 | ≤219 | 0.75(0.559 to 0.889) | 90% | 60% | 52.9% | 92.3% | **0.005 (HS)** |
| | Week 4 | ≤320 | 0.687(0.493 to 0.843) | 100% | 45% | 47.6% | 100% | 0.053(NS) |
| | Week12 | ≤310 | 0.690(0.496 to 0.845) | 90% | 50% | 47.4% | 90.9% | **0.05 (S)** |
| 25(OH)D | Week 0 | ≥15 | 0.907(0.744 to 0.98) | 90% | 85% | 75% | 94.4% | **0.001(HS)** |
| | Week 4 | ≥20 | 0.90(0.73to 0.97) | 80% | 95% | 88.9% | 90.5% | **0.001(HS)** |
| | Week12 | ≥8.61 | 0.892(0.72 to 0.97) | 100% | 70% | 62.5% | 100% | **0.001(HS)** |

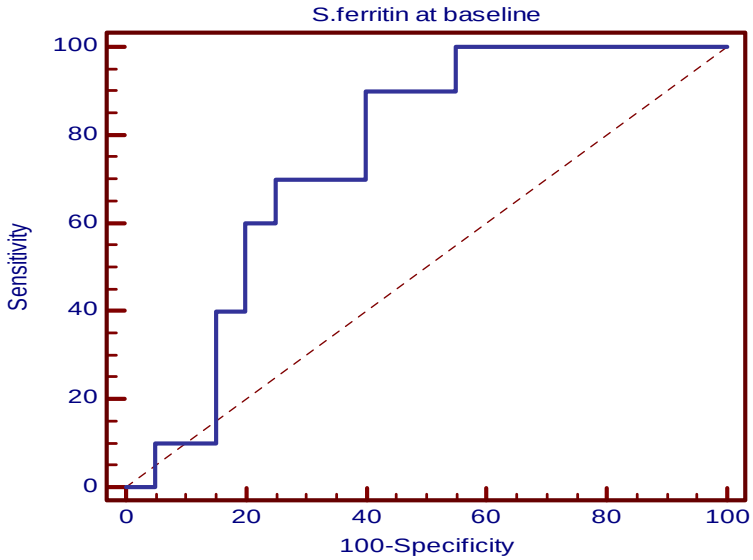


**Figure (2): ROC curve of serum ferritin at week 0 for predicting EVR**

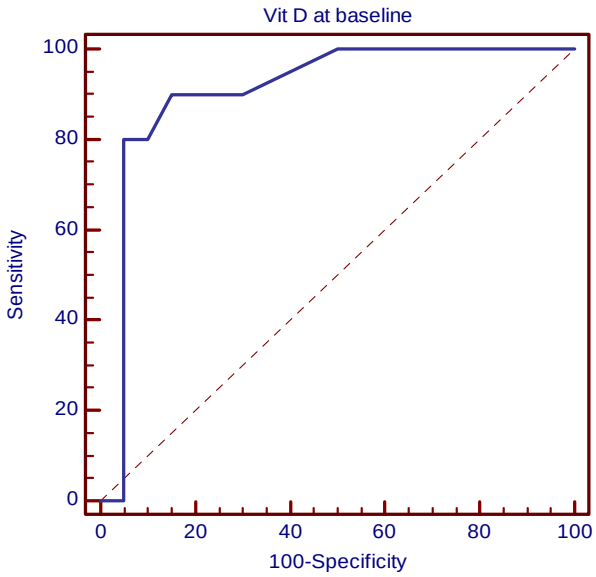


**Figure (3): ROC curve of 25(OH)D at week 0 for predicting EVR**

***Comparison of serum 25(OH)D with s.ferritin in different weeks of treatment*** to evaluate the performance measures of 25 (OH)D level in predicting EVR, AUCs of 25(OH)D and serum ferritin were compared(Fig 4-6). At week 0; 25(OH)D AUC was 0.908 (Standard error =0.0673, 95% CI= 0.77 to 1)compared to that of s.ferritin AUC=0.75 (Standard error0.0901, 95% CI=0.573 to 0.927) p=0.13.

At week 4; 25(OH)D AUC was 0.9 (Standard error =0.0698, 95% CI= 0.76 to 1)compared to that of s.ferritin AUC=0.687 (Standard error0.099, 95% CI=0.493 to 0.882) p=0.05. At week 12; 25(OH)D AUC was 0.89 (Standard error =0.0611, 95% CI= 0.773 to 1) compared to that of s.ferritin AUC=0.69 (Standard error0.097, 95% CI=0.499 to 0.881) p=0.109.

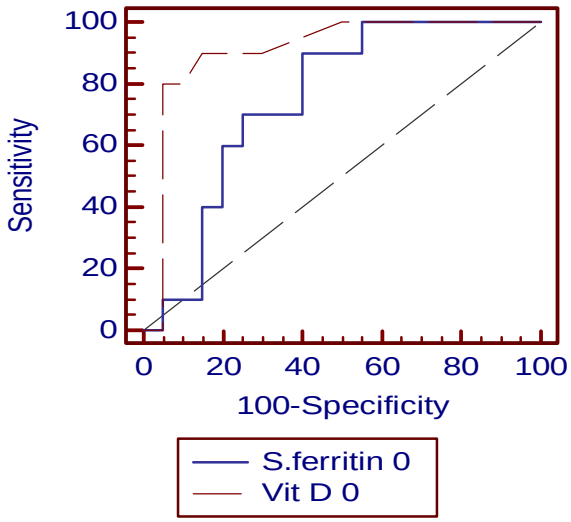


**Fig (4): Comparison of AUC of ROC analysis of week 0 serum ferritin and 25(OH)D for prediction of EVR**

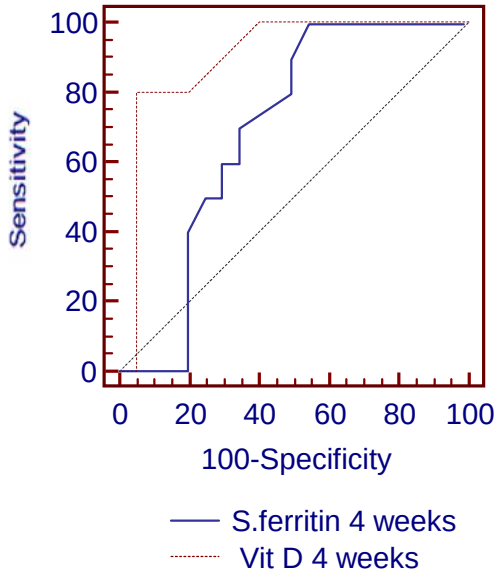


**Figure (5): Comparison of AUC of ROC analysis of week 4 serum ferritin and 25(OH)D for prediction of EVR**

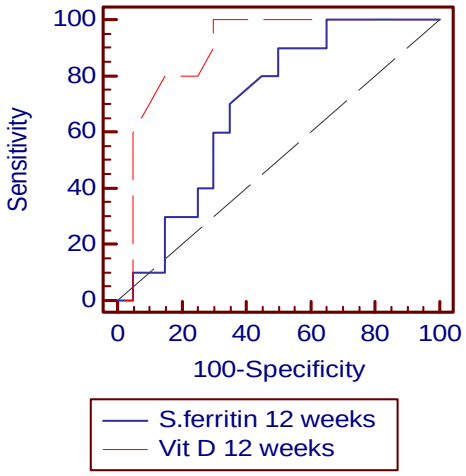


**Figure (6): Comparison of AUC of ROC analysis of week 12 serum ferritin and 25(OH)Dfor prediction of EVR**

## Discussion

In the present study, we found that the level of week 0 serum ferritin was lower in the EVR group than the non EVR group (136.36 ng/ml VS 305.59ng/ml) respectively, (p=0.005). This indicates that lower week 0 serum ferritin has been associated with better early virological response, these results were agreed with **Barut et al., (9)** who enrolled 97 CHC patients 64 patients achieved sustained virological response (SVR), while 33 didn't achieve SVR. He found that patients who achieved SVR had lower baseline ferritin levels, and it was observed that serum ferritin levels increased dramatically in both the SVR and non-SVR groups after starting therapy, remained high until the end of the treatment period, and returned to baseline levels after completion of treatment.

On comparing level of serum ferritin at week 4 for EVR group was (191.90±101.39) and non-EVR group was (350.02±237.16), with P value (0.017).this agrees with previous findings of **Barut et al (9)** who concluded thatan increase in serum ferritin at week 4 was an independent factor predicting SVR, and that 1 fold increase in serum ferritin at week 4 resulted in a 24% increase in the probability of SVR.

We found that serum ferritin was (208.75±112.35) for EVR group compared to (355.90±232.08) for non-EVR group, with statistical significance (P value=0.027), this goes in agreement with **Lange et al., (10)** who enrolled a study on 876 CHC patients, found elevated serum ferritin was among the strongest pretreatment predictors of failure to achieve SVR (P < 0.0001, odds ratio [OR] favoring SVR ¼ 0.45), And he demonstrated that serum ferritin was strongly and independently associated with a failure to achieve HCV eradication by IFN based therapy.

**Yada et al., (11)** who enrolled 175 patients in their study and examined difference in serum ferritin levels before and during therapy between patients who achieved SVR and non-SVR. Evaluate that there was no significant statistical difference in serum ferritin level between both groups.

Moreover, **Distante et al., (12)** stated that an increased serum ferritin level during the antiviral therapy corresponds with a favorable virological response. This was explained by mentioning activation of macrophage ceels by interferon, which leads to production of ferritin from macrophages.

In our study we found that the cutoff value for pretreatment serum ferritin was (<219ng/ml) for discriminating early virological response with sensitivity 90%, specificity 60%, positive predictive value 52.9%, negative predictive value 92.3%, and P value (0.005)with high statistical significance. And at week 4 of treatment the cutoff value was (<320ng/ml), P value (0.053) with no statistical significance and at week 12 of treatment the cutoff value was (<310ng/ml), P value (0.05) with statistical significance.

Concerning the relevance of (25-OH) vitamin D in the clinical setting have clearly shown that true deficiency, whether mild (<20ng/ml) or severe (<10 ng/ml), is important, whereas simple variations of(25-OH) vitamin D serum levels above the limit of normality seem to have a negligible biological effect. Thus, the choice to analyze vitamin D as a categorical variable appears to be appropriate **(13)**.

In our study we found that the level of week 0 (25-OH) Vitamin D was higher in the EVR group than the non EVR group (48.50ng/ml VS 13.78ng/ml) respectively, (p=0.001). This indicates that higher week 0 (25-OH) vitamin D level had been associated with better early virological response; these results were agreed with **Petta et al (14)**who enrolled 197 CHC patients, he stated that (25-OH) vitamin D levels were found to influence the achievement of viral clearance after antiviral therapy in patients with chronic HCV infection. In particular, there was a highly significant association between progressively lower baseline serum (25-OH) vitamin D levels and the rates of viral clearance. This outcome was evident in all HCV genotypes.

Another study done by **Bitetto et al., (15)** who enrolled 89 CHC patients and found that (25-OH) vitamin D status at the time of starting antiviral therapy was found to be associated with the achievement of SVR following treatment. The association was strictly related to the degree of (25-OH) vitamin D deficiency: patients with severe vitamin D deficiency (<10ng/ml) almost never achieved SVR, whilst those with near normal or normal vitamin D (>20 ng/ ml) prior to starting antiviral treatment obtained a SVR rate in about half the cases.

**Petta*et al*., (14)** have shown a low serum (25-OH) vitamin D level to be related to severe fibrosis and low responsiveness to interferon-based therapy in chronic hepatitis C.

**Arteh , et al., (16)** have shown that adding vitamin D(25-OH) to conventional Peg/RBV therapy for naïve patients with chronic HCV infection significantly improves SVR.

On comparing (25-OH) vitamin D level at week 4 was (43.40±26.21) in EVR group compared to (11.95±20.28) in non-EVR group, with high statistical significance (P value=0.001), and at week 12(25-OH) vitamin D was (34.05±23.98) in EVR group compared to (12.01±21.96) in non-EVR group, with high statistical significance (P value=0.001).

In our study we found the cutoff value for week 0 (25-OH) vitamin D was (>15 ng/ml) for discriminating early virological response with sensitivity 90%, specificity 85%, positive predictive value 75%,negative predictive value 94.4%, and P value (0.001) highly significant, the cutoff value for week 4 (25-OH) vitamin D was (>20 ng/ml) for discriminating early virological response with sensitivity 80%, specificity 95%, positive predictive value 88.9%, negative predictive value 90.5%, and P value (0.001) highly significant, and the cutoff value for week 12 (25-OH) vitamin D was (>8.61 ng/ml) for discriminating early virological response with sensitivity 100%, specificity 70%, positive predictive

value 62.5%,negative predictive value 100%, and P value (0.001) highly significant.

Regarding correlation between (serum ferritin levels and ALT and AST levels) and (serum 25-OH vitamin D levels and ALT and AST levels) it was found that there was no statistically significant correlation, these results were agreed with **Ferrara et al (17) and Petta et al., (14)** respectively who found no significant correlation between ALT and serum ferritin at week 12 and 24 of therapy each time point. And no significant correlation between ALT and AST and (25-OH) vitamin D levels at week 4 and 12 of therapy.

We have found that BMI was lower in the EVR group than the non EVR group (24.78 VS 27.55 kg/m$^2$) respectively, (p=0.001). BMI was significantly correlated with baseline serum ferritin (r= 0.425, p=0.019). On the other hand **Barut et al., (9)** who mentioned that there is no association was found between BMI and either baseline serum ferritin or serum ferritin rise during treatment.

The predictive power of serum (25-OH) vitamin D and serum ferritin levels at week 4 was assessed by comparing AUC of ROC curve analysis which show that AUC of (25-OH) vitamin D was significantly higher than that of serum ferritin , AUC of (25-OH) vitamin D was (0.900) versus serum ferritin (0.687) with P value (0.05).

## Conclusion and Recommendation:

The pretreatment serum ferritin was lower in patients achieving EVRwith a cut off value <219 ng/ml, positive predictive value 52.9% and sensitivity 90%. The pretreatment vitamin D was higher in patients achieving EVR, with cut-off value >15 ng/ml, positive predictive value 75% and sensitivity 90%.The early virological response was achived in patients who have an increase in serum ferritin during dual combination therapy than those who didn't. the impact of 25(OH) vitamin D supplementation on EVR in CHC patients receiving dual combination therapy is to be verified .Further studies to be done to assay serum 25(OH) vitamin D level in prediction of SVR 12 week in triple combination therapy (Sofosbuvir, pegulated interferon and ribavirin).

Therefore we recommend that serum ferritin and vitamin D levels should be included in the biochemical workup of CHC patients before and during therapy, particularly in situations where resources for an appropriate virological workup are limited, as they are cheap markers for the prediction of treatment outcome in CHC patients receiving interferon-based therapy.

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