Studying the Kynurenine, Some Hematological and Biochemical Changes on Egyptian Patients with Chronic Hepatitis C During and After Treatment with Sofosbuvir

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Abstract
Background: Hepatitis C virus (HCV) is a serious health problem where its prevalence is very high in Egypt. Development of new therapeutics Sofosbuvir, a recently approved nucleotide analog, is a highly potent inhibitor of the NS5B polymerase in the HCV, and has shown high efficacy in combination with several other drugs with and without pegylated interferon.

Objectives: This work aimed to investigate the effect of the treatment with the antiviral Sofosbuvir on serum Kynurenin, some hematological and biochemical changes in patients with HCV during and after treatment.

Subjects and methods: The study was conducted on 180 individuals classified as follows: 47 healthy normal adult served as control group and 133 chronically infected HCV adult Egyptian patients divided into 2 groups according to the type of treatment, group II patients who received dual combination therapy of Sofosbuvir (SOF) and Ribavirin (RBV) for 24 weeks, and group III patients who received a triple combination therapy of SOF, pegylated interferon (PEG-IFN) Alfa, and RBV for 12 weeks. Fasting blood samples pre-, during and after treatment were collected.

Results: All reported data were compared to control group. The obtained data showed that there were no significant differences between all studied groups regarding gender and age. The data showed a significant increase (p >0.01 or 0.001) in serum aspartate aminotransferase (AST) & alanine aminotransferase (ALT) activities, total & direct bilirubin and kynurenine (KYN), white blood cells (WBCs) and prothrombin time in Gr. IIA & Gr III A as compared to control group, whereas there were significant decrease (p >0.05) in blood platelets count (PLTs) and serum albumin, urea & creatinine in Gr. IIA & Gr III A. During treatment in Gr. II B & Gr. III B, serum ALT, AST activities, direct bilirubin, and PLT & PT were improved, yet still showing significant increase (p>0.05), while the increase serum KYN was non-significant p >0.05 from control in Gr. II B and Gr. II C. Serum albumin, creatinine & urea and WBCs were returned to their normal levels after treatment(Gr .II C & Gr. III C), while hemoglobin (Hb) & red blood cells (RBCs) showed significant decrease(p >0.05 ) after treatment (Gr .II C& Gr. III C).The other tested biochemical parameters were returned to their normal levels except total bilirubin that showed significant increase(p >0.05) in Gr. II C & Gr. III C and serum ALT & KYN that showed significant increase (p >0.05) in Gr III C. While, Hb & RBCs and PLT showed (p >0.01) , significant decrease. Both WBCs & HCT showed normal values in Gr. II B & Gr. II C, whereas those in Gr. III C showed significant decrease. No significant changes in fasting blood sugar (FBS) in Gr. II & Gr. III (Pre, during and after treatment).

Conclusion: It can be concluded that the dual therapy of Sofosbuvir for 24 weeks caused improvement of most of the tested parameters of HCV patient. Thus it has little metabolic harm.

Keywords: Hepatitis C, Kynurenin, Sofosbuvir, pegylated interferon, Ribavirin.

Introduction
Hepatitis C is a contagious disease that primarily affects the liver, causing severe damage as the disease progresses. It is caused by the Hepatitis C virus (HCV), a small enveloped RNA virus [1,2]. Approximately 3.7 million people between 15 and 59 years of age had chronic hepatitis C [3]. Hepatitis C viral infection is endemic in Egypt with the highest prevalence rate in the world [4] and up to 85% of HCV infections persist for life; leading to chronic hepatitis [5,6]. The most common HCV RNA genotype in Egypt is genotype 4, representing 92.5% of all HCV cases [7].

In Egypt, the standardized treatment for chronic hepatitis C virus (HCV) infection was combination of pegylated interferon (PEG-IFN) and ribavirin (RBV), that attains viral clearance in 40% of the patients while 60% fail this therapy, which does not attack the virus directly but stimulates the immune system and activated it to become more efficient to eliminate the virus [8,9]. HCV treatment with sofosbuvir was launched in Egypt by Ministry of Health and the Egyptian National HCV Control Program. The drug sofosbuvir acts directly on the virus life cycle to prevent proliferation of the virus, this is by attacking enzymes necessary to manufacture the parts of the virus by preventing it from entering the liver cell or blocking the necessary proteins required for the formation of the virus [10]. The drug also showed a high efficacy in combination with several other drugs, with and without PEG-IFN, against HCV.

Kynurenine is an endothelial-derived relaxing factor and neuroactive molecule [11]. It is a small molecule generated by the oxidation opening of the indole ring of tryptophan. Tryptophan has been considered as the main amino acid for proliferation.
and activation of T- cells, in particular various cells as macrophages and multiple malignant cells [12]. The biological activity of kynurenine and its metabolites (kynurenines) is well recognized [13]. Kynurenine has been shown to act as an immune regulatory molecule that mediates immunosuppressive effects in the tissue microenvironment [14], and its metabolism and disposition are reviewed with particular emphasis on the hepatic Kynurenine pathway (HKP) in health and disease. Over 95% dietary TRP is metabolized in the HKP [15]. Evidence suggests that increased kynurenine production may precipitate depressive symptoms associated with interferon treatment for hepatitis C [16].

**Objectives of the study**

Therefore, the present study was suggested to test the impact of sofosbuvir treatment on some hematological and biochemical parameter of treated patients.

**Materials and methods**

This study was conducted on 180 individuals classified as follows: 47 healthy adult (30 males and 17 females) with age range of 20-70 years served as normal control group (Gr. INC). One hundred and thirty three adult Egyptian patients (92 males and 41 females) with age range of 20 to 70 years diagnosed with chronic HCV infection (with positive HCV-PCR results), recruited from the outpatient clinics of Health Insurance Hospital, in Benha, Egypt. Starting from December 1st 2015 till December 1st 2016 to serve as Gr.II & Gr.III according to the type of combination therapy, depending on pretreatment history, or contraindications according to the approved therapy recommendations: Group II (Gr. II A, Gr. II B&Gr. II C as pre-, during and after treatment) respectively including 74 patients (55 males & 19 females) who received dual combination therapy of sofosbuvir (SOF) and Ribavirin (RBV) for 24 weeks, and Gr. III (Gr. III A,Gr. III B and Gr. III C as pre-, during and after treatment respectively including 59 patients (37 males & 22 females) who received a triple combination therapy of SOF, pegylated interferon (PEG-IFN) Alfa, and RBV for 12 weeks. At the end of treatment course HCV-PCR blood samples were collected for hematological and biochemical analysis.

All patients received sofosbuvir (Sovaldi, Gilead Sciences, Inc.) at a dose of 400 mg daily, pegylated INF alpha 2a 180 µgonce per week and oral ribavirin guided by weight of patients. Those less than 75 kg received 1000 mg daily and those more than 75 kg received a 1200 mg daily for 12 weeks.

**Exclusion criteria:**

The patients were clinically examined to exclude those suffering from previous history of cardiac diseases, abnormal clinical or electrocardiographic (ECG) findings, severe psychiatric disorders, diabetes mellitus, hypertension; pregnancy or advanced renal impairment.

**Blood samples:**

Fasting venous blood samples (5 ml) were drawn once from control individuals and HCV patients: pre, during (at week 12 and 6 for Gr. II and Gr. III, respectively) and after treatment (after 24 and 12 weeks for Gr. II and Gr. III, respectively). Each sample was distributed in three vacutainer tubes, the first tube contained sodium citrate where the plasma was separated and used for coagulation assays, the second tube contained ethylene diamine tetra acid (EDTA) was used for complete blood counts (CBC), while the third tube contains no anticoagulant was allowed to stand for 30 minutes at room temperature then centrifuged at 3000 r.p.m. for 5 minutes. Serum was immediately separated and stored at −20°C until biochemical analysis.

**Hematological analysis:**

The prothrombin time (PT) was determined by STA®-Neoplastine ®CIPlus (PT) according to [17]. Complete blood counts (CBC) and Hb content were measured by using a cell counterSysmex KX-21 N (Sysmex America, Inc., Mundelein. Illinois. USA).

**Biochemical analysis:**

Liver function tests which include serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) activities, total and direct bilirubin (T. & D bilirubin) and albumin concentrations. Kidney functions tests (urea, creatinine) and fasting blood sugar (FBS) were measured by using the DiaSys Diagnostic Systems for In Vitro Diagnostic using the method developed by [18]. Serum kynurenine (KYN) was determined by sandwich Enzyme linked Immunosorbent Assay (ELIZA) by the use of commercial kit from Bluegene Biotic Com., China according to the instructions supplied by the manufacturer, where the micro titer plate wells were coated by a polyclonal antibody specific to KYN which capture the KYN of the samples. Conjugate was added to each well to sandwich the KYN immobilized on the plate. After incubation, and washing the wells to remove all unbounded components, substrate was added exhibiting change in colour. The enzyme – substrate reaction was terminated by the addition of a sulphoric acid solution and the colour change was measured spectrophotometrically at a wavelength of 450 nm. The concentration of KYN in the samples is determined by comparing the absorbance of the samples to that of the standard curve [19].

**Statistical analysis:**

All data was entered and analyzed on SPSS (Statistical Package for Social Science) program for statistical analysis (version 21). Various assay conditions were evaluated by using an analysis of variance (ANOVA) test was carried out to compare healthy controls with each of the HCV patient groups. All data are presented as mean± Standard error (SE). P-values >0.05 were considered
statistically not significant, P-values <0.05; <0.01 and <0.001 were significant.

Results and discussion

Worldwide, more than one million people die each year from hepatitis C virus (HCV) related diseases, and over 300 million people are chronically infected with hepatitis B or C [20]. Sofosbuvir, an NS5B polymerase inhibitor, has a pan-genotypic anti-HCV activity and emerged as an important component of currently available anti-HCV regimens for the treatment of genotypes 1, 2, 3 and 4 of HCV virus infections.

The age of our studied patients ranged from 20 to >60 years with a male predominance where male: female (M: F) ratio was 2.24:1. It has been reported that Hepatitis C virus infection is a disease that disproportionately affects men more than women [21].

All reported data were compared to their respective controls (Gr.INC). The data indicated that there were no significant differences were obtained between the studied groups regarding age and gender.

Hematological data (table 1) showed significant increase in white blood cells (WBCs) count except for groups (Gr. II B and C) and prothrombin time showed significant decrease (p >0.05) and in blood platelets count (PLTs) of pretreatment groups compared to control group. During treatment in Gr. II B & Gr. III B PLT & PT were significantly decrease and increased, respectively (p >0.05), while WBCs returned to their normal levels in group II but still significantly decrease in groupIII compared with control one. Hemoglobin (Hb) & red blood cells (RBCs) showed significant decrease (p >0.05) compared to control one. Post treated individuals (Gr. II C& Gr. III C) show significant decrease in Hb & RBCs at (p >=0.01) and in PLT at (p >0.05) in both subgroups respectively while WBCs & HCT in Gr. III C were moderately decreased as compared to control groups.

Result concerning hematological parameters indicated improvement of WBCs during and after treatment than reported at the initial diagnosis in Gr. II B & C, while in Gr. III B and C showed significant decrease in white blood cells. It was found that certain antiviral medications used to treat HCV may cause decreased WBCs which is due to Interferon suppresses bone marrow production of leukocytes, which leads to a drop in the white blood count or neutropenia [22]. These results were in agreement with [23] who showed that the most common side effect of combination therapy with Peg-IFNα and Ribavirin was severe neutropenia. Also [24] found that when sovaldi is administered with ribavirin or peg interferon alfa/ribavirin may cause low blood cell count. On the other hand the data were on the contrary of [25] who showed that no neutropenia or any serious adverse events are associated with sofosbuvir treatment. [27] studied also the efficacy and safety of sofosbuvir plus ribavirin for treatment of patients with genotype 4 hepatitis C virus. The results showed that during treatment WBCs and blood platelets were not affected by the treatment.

The results showed significant decline of hemoglobin content in Gr. II B & Gr. III B and highly significant decline in Gr. II C & Gr. III C (p <0.05 & p >0.01 respectively). Anemia is a major complication of antiviral therapy in chronic hepatitis C and is one of the major adverse events in patients treated with Peg IFN/RBV due to the hemolytic effect of ribavirin and on rare occasions, the bone marrow depression induced by interferon [27, 28]. It has been reported that interferon has inhibitory effects on bone marrow, and reduced levels of blood cells, including the count of RBCs; moreover, Ribavirin can cause RBCs lysis and enhances the inhibitory effect of interferon [29]. Therefore, the combination of these two drugs has synergistic effects and decrease hemoglobin concentration [30, 31].

Our results are in agreement with the study by [26] and also [32] who reported that the sofosbuvir-containing treatment led to significant decrease in hemoglobin. Thus; it appears that the ribavirin component may be responsible for the side effect of anemia [33]. Similarly, a study by [34] showed that there was significant decline during different follow-up periods as regard hemoglobin with significant anemia in about 76% of patients at the end of his study. In addition [35] showed Blood Platelets count (PLTs) showed significant decrease (p <0.05) in both groups (Gr II A&B) & (Gr. III A,&B),and showed highly significant decrease in Gr II C & Gr. III C (p <0.01) . These results are in line with those reported by [36] who reported that severe thrombocytopenia (A low platelet count) occurred in 3 (1.7%) patients with dual therapy and 4(6%) with triple therapy. In addition the present study was in agreement with [37, 38].

On the contrary, the present study is in disagreement with [39] that treated 60 chronic hepatitis C patients of Egyptian ancestry with sofosbuvir and ribavirin for 12 week. or 24 week. The authors reported increases in platelets during treatment.

The data of prothrombin time (PT) in Gr. II A &Gr. III A showed highly significant increase at (p> 0.01) in PT values and this indicates that there was improvement during treatment. A significant increase (p >0.05) in Gr. II B and there was reduced in PT values in treatment in Gr. II C. These results are consistent with previous reports [32, 40]. The revealed significant improvements in PT. Prothrombin are one of several proteins produced by the liver that play an important role in blood clotting [41].

Both Gr. II A& Gr. III A (tables 2,3,4) showed significant increase in serum AST & ALT activities, D. bilirubin, KYN (p < 0.01) and T. bilirubin (p < 0.001), and significant decreases (p < 0.05) in serum
albumin, urea and creatinine. During treatment in Gr. II B & Gr. III B liver function test were improved, yet still showing significant increase (p < 0.05), and while serum KYN was improved only in Gr. II B. Serum Alb., creatinine & urea returned to their normal levels. At the end of the treatment in Gr. II C & Gr. III C all biochemical parameters were returned to their normal levels except T. Bil which showed significant increase (p >0.05) in both groups. Serum ALT and KYN showed significant increase (p >0.05) only in Gr. III C as compared to control group. Biochemical data (table 4) showed no significant changes in FBS in Gr. II & Gr. III compared to control group.

The biochemical results indicated a significant decrease in serum albumin in Gr. II A & Gr. III A (p<0.01) . This decrease is consistent with the results of [42, 43]. Albumin is the major form of protein present in the blood synthesized by the liver, and its low concentration is a marker of liver damage [44]. A decrease in serum levels of albumin, may suggest decreased hepatic production due to decreased liver function following hepatocellular disease [45]. This decrease of serum albumin was returned to its normal levels in Gr. II B & C and Gr. III B & C, which is consistent with the results of [43]. They found that treatment with both pegylated interferon-alpha and ribavirin to The data revealed a significant increase in serum AST&ALT activities in Gr. II A &Gr. III A (p > 0.01) as compared to the control group. These results are in accordance with [48, 42]. Also, [49] demonstrated that, the levels of serum ALT ,AST, and ALP were significantly higher in positive HCV-infected patients compared with negative HCV patients. The infection with HCV leads to attack of the liver causing liver tissue damage and altered membrane permeability which considered one of the main causes of elevated liver enzymes [50] .Therefore elevation of these enzymes in the blood is used as an indirect marker of liver inflammation or injury [51]. The results of ALT and AST showingsignificant increase during treatment in Gr. II B & Gr. III B and returned about their normal levels after treatment in Gr. II C only , while ALT is still showing significant increase in Gr. III C. These results are in accordance to [52] who observed an ALT elevation during sofosbuvir plus pegylated interferon and ribavirin therapy for chronic HCV Egyptian patients). The results were also in accordance to [43] who found that post treatment with interferon and Ribavirin to patient with hepatitis C. The activates of plasma ALT and AST significantly reduced.

In the present study the results showed significant increase in levels of serum totaland direct bilirubin (T&D Bil) in Gr. II A & Gr. III A (p > 0.001 & 0.01 respectively). These results are in similar to the results of [53]. Bilirubin is the catabolic product of hemoglobin produced within the reticulo endothelial system, released in unconjugated form which enters into the liver, converted to conjugated forms bilirubin mono and diglucuronides by the enzyme UDP-glucuronyl transferase [54]. During treatment this increase was still showing significant increase in Gr. II B &Gr. III B and only D-Bil returned to the normal level of the control after treatment in Gr. II C &Gr. III C .The result are consistent with the results of [46,55].

A significant decrease in the level of serum urea and creatinine was noticed in Gr. II A& Gr. III A (p> 0.05). These results were in agreement with those of [46,56] who found a significant reduction (p > 0.05) in urea and creatinine levels in HCV patients as compare to normal individuals. Decreased kidney function is a common complication found in patients with HCV. Creatinine is a product of the metabolism of creatinine, which is produced in the liver and stored in muscle to be used as a source of energy once phosphorylated and it is removed from the blood by the kidney [57]. Patients with chronic liver disease have a significantly lower baseline serum creatinine concentration than the general population [58]. While urea is a nutritional pointer connected to protein intake and it is formed in the liver and carried by the blood to the kidneys for excretion. Urea synthesis is reduced in liver disease, and the reduction in the maximal capacity for urea production in patients with hepatitis is due to a decreased activity all five urea-cycle enzymes [59].

The decreases noticed in serum urea and creatinine was significant during and after treatment in Gr. II B &C, and non-significant in Gr. III B &C for creatinine while urea showed significant increases. This finding is agreed with the result of [60], that demonstrated the creatinine level became normal after therapy with interferon the results also agreed with the finding of [61] who showed antiviral therapy based on interferon alfa can significantly increase urea in patients with chronic hepatitis C . In addition, our results are in agreement with a review of the [32] who showed that patients experienced increase in serum urea nitrogen to levels above the upper limit of normal. Therefore patients with hepatitis C infection who are administered a sofosbuvir-containing regimen with or without ribavirin require close monitoring of renal function [33]. On the other hand our results were did not agreed with result of a recent study by [53] who found that the antiviral therapy consisting of IFNα-2b combined with RBV for 48 weeks decreased the serum creatinine level and improved both the estimated glomerular filtration rate.

In the largest, so far, study, concentration of serum kynurenine in Gr. II A& Gr. III A were significantly higher than in those healthy controls. Acute chronic liver failure is characterized by systemic
Conclusion:
During and after treatment serum KYN showed non-significant changes in Gr. II B and C, while it showed significant increase in Gr. III B and Gr. III C. Pegylated IFN-α is a pro-inflammatory cytokine and has strong anti-viral and anti-proliferation characteristics [65]. It was found that IFN-based immunotherapy significantly increased the activity of IDO [66].

It was found that the dual sofosbuvir therapy given for 24 weeks in genotype4 infected Egyptian patients better than triple therapy given for 12 weeks, where in patients who treated with dual therapy all biochemical parameters were returned about its normal levels at the end of treatment. Also, sofosbuvir-based combination therapy showed an improvement in liver function tests in patients of group II and group III. Although, anemia and thrombocytopenia were a common side effects occurred after treatment. Increased serum KYN levels have been documented in individuals with chronic HCV infection.

References
[22] J.P. Ong, and Z.M. Younossi. Managing the hematologic side effects of antiviral therapy for chronic hepatitis C: anemia,


that Ribavirin) genotype 4 Hepatitis C viral strain therapy Sharafeldin. study the effect of hepatic triple Hepatitis C Infected Egyptian Patients in the Northeast Provin. The


### Table (1): Hematological data in in male and female control, HCV patient treated with Sofosbuvir and Ribavirin combination (Gr. II),andSofosbuvir, pegylated - interferon and Ribavirin combination (Gr. III) at different ages.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=47)</th>
<th>Gr. II</th>
<th>Gr. III</th>
<th>HCV Patients (n=133)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(n =30 males &amp; 17 females)</td>
<td>(n =74) (55 males &amp; 19 females)</td>
<td>(n =59) (37 males &amp; 22 females)</td>
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<tr>
<td>Parameter</td>
<td>Age</td>
<td>WBCs (x10^9/L)</td>
<td>Hb (g/dl)</td>
<td>WBCs (x10^9/L)</td>
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<td>Hb</td>
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<td></td>
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<td>(mean±S.E.)</td>
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<td>WBCs</td>
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<td>RBCs</td>
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<td>HCT (%)</td>
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<tr>
<td>PLT (x10^3/L)</td>
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<td>PT (sec)</td>
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</table>

Data are expressed as: mean ± standard error. (ns)Non significant difference, (*P<0.05, (**P<0.001 & (***)P<0.0001 indicate significant difference.Compared to the healthy normal control group.

Abbreviations: M: males; F:females;WBCs: White blood cells; Hb: Hemoglobin concentration; RBCs: Red blood cells; HCT: Hematocrit ; PLTs: platelets count; PT: prothrombin time.

a)number of male control 30(10(20-40);10(41-60);10(61). b) number o female control 17(6(20-40);5(41-60);6(61). c) number male of Gr. II=5(20-40);22(41-60);18(61). d) number female of Gr. II=9(20-40);7(41-60);5(61). e) number male of Gr. III=37(20-40);17(41-60);7(61). f) number female of Gr. III=22(9(20-40);6(41-60);7(61).
Table (2): Liver function data in male and female control, HCV patient treated with Sofosbuvir and Ribavirin combination (Gr. II), and Sofosbuvir,pegylated interferon and Ribavirin combination (Gr. III) at different ages.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normal (n=47)</th>
<th>Control (30 males &amp; 17 females)</th>
<th>HCV Patient (n=133)</th>
<th>Gr.II Sofosbuvir-Ribavirin (n=74)</th>
<th>Gr.III Sofosbuvir-Ribavirin Plus Pegylated Interferon (n=59)</th>
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<tr>
<td></td>
<td>Gr.I INC</td>
<td>Gr. II A</td>
<td>Gr. II B</td>
<td>Gr. II C</td>
<td>Gr. III A</td>
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<td>ALB</td>
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<td>(g/dl)</td>
<td>41-60</td>
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<td>&gt;60</td>
<td>4.2±0.22</td>
<td>2.7±0.18</td>
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<tr>
<td>ALT</td>
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<td>24.4±0.26</td>
<td>19.0±4.33</td>
<td>24.4±0.26</td>
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<tr>
<td>(U/L)</td>
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<td>75.12±8.47</td>
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<td>AST</td>
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<td>(U/L)</td>
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<td>T-BIL</td>
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<td>1.2±0.04</td>
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<tr>
<td>(mg/dl)</td>
<td>41-60</td>
<td>0.5±0.04</td>
<td>1.2±0.04</td>
<td>0.5±0.04</td>
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<td>&gt;60</td>
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<tr>
<td>(mg/dl)</td>
<td>41-60</td>
<td>0.1±0.05</td>
<td>0.5±0.55</td>
<td>0.1±0.05</td>
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<tr>
<td></td>
<td>&gt;60</td>
<td>0.1±0.05</td>
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</tr>
</tbody>
</table>

Data are expressed as: mean ± standard error. (ns)Non significant difference. (*) P<0.05. (**) P<0.001 & (***) P<0.0001 indicate significant difference. Compared to the healthy normal control group.

Abbreviations: M: males; F: females; ALB: Albumin; AST: Aspartate Aminotransferase; ALT: Alanine aminotransferase; T- BIL: Total Bilirubin; D- BIL: Direct Bilirubin

Table (3): kidney function data in male and female HCV patient treated with Sofosbuvir and Ribavirin combination (Gr. II), Sofosbuvir, pegylated - interferon and Ribavirin combination (Gr. III) at different ages.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Age</th>
<th>Normal (n = 47)</th>
<th>HCV Patient (n = 133)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control (30) (males &amp; 17 females)</td>
<td>Gr. II (n = 74) (55 males &amp; 19 females)</td>
</tr>
<tr>
<td>creat (g/dl)</td>
<td></td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>20-40</td>
<td></td>
<td>0.79±0.02</td>
<td>0.73±0.07</td>
</tr>
<tr>
<td>&gt;60</td>
<td></td>
<td>0.76±0.05</td>
<td>0.71±0.04</td>
</tr>
<tr>
<td>Urea (g/dl)</td>
<td></td>
<td>20-40</td>
<td>33.48±0.29</td>
</tr>
<tr>
<td>41-60</td>
<td></td>
<td>30.14±0.15</td>
<td>28.34±0.67</td>
</tr>
<tr>
<td>&gt;60</td>
<td></td>
<td>28.84±0.87</td>
<td>30.12±0.80</td>
</tr>
</tbody>
</table>

Data are expressed as: mean ± standard error. (ns) Non significant difference, (*) P<0.05, (**) P<0.001 & (***) P<0.0001 indicate significant difference. Compared to the healthy normal control group.

Abbreviations: M: males; F: females; Creat: Creatinine; Urea: Blood urea nitrogen.

a) number male of control 30=10 (20-40); 10 (41-60); 10 (>61).

b) number female of control 17=6 (20-40); 5 (41-60); 6 (>61).

c) number male of Gr. II 55=15 (20-40); 22 (41-60); 18 (>61).

d) number female of Gr. II 19=7 (20-40); 7 (41-60); 5 (>61).

e) number male of Gr. III 37=13 (20-40); 17 (41-60); 7 (>61).

f) number female of Gr. III 22=9 (20-40); 6 (41-60); 7 (>61).
Table (4): Biochemical data in male and female HCV patient treated with Sofosbuvir and Ribavirin combination (Gr.II), Sofosbuvir, pegylated - interferon and Ribavirin combination (Gr. III) at different ages.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Age</th>
<th>Gr.I INC</th>
<th>Gr. II</th>
<th>Gr. II B</th>
<th>Gr. II C</th>
<th>Gr. III A</th>
<th>Gr. III B</th>
<th>Gr. III C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>FBG (mg/dl)</td>
<td>20-40</td>
<td>95.24±25.1</td>
<td>92.195±18.1</td>
<td>112.13±9.23</td>
<td>109.25±9.12</td>
<td>99.39±6.84</td>
<td>90.1±6.45</td>
<td>91.3±6.13</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>104.9±12.65</td>
<td>98.89±6.93</td>
<td>110.63±5.2</td>
<td>108.45±5.5</td>
<td>107.65±5</td>
<td>107.4±6.2</td>
<td>107.4±6.2</td>
</tr>
<tr>
<td>Kyn (ng/ml)</td>
<td>20-40</td>
<td>331.64±24.05</td>
<td>325.43±18.88</td>
<td>472.60±16.65</td>
<td>466.42±12.45</td>
<td>359.41±15.04</td>
<td>358.22±11.75</td>
<td>352.53±13.31</td>
</tr>
<tr>
<td></td>
<td>41-60</td>
<td>350.58±20.88</td>
<td>339.47±14.65</td>
<td>491.52±18.15</td>
<td>480.43±13.82</td>
<td>373.51±12.35</td>
<td>374.52±14.33</td>
<td>374.52±14.33</td>
</tr>
<tr>
<td></td>
<td>&gt;60</td>
<td>365.67±12.65</td>
<td>347.33±11.11</td>
<td>506.56±15.05</td>
<td>488.33±14.12</td>
<td>381.36±17.64</td>
<td>381.36±17.64</td>
<td>381.36±17.64</td>
</tr>
</tbody>
</table>

Data are expressed as: mean ± standard error. (ns) Non significant difference , (*) P<0.05, (**) P<0.001 & (***) P<0.0001 indicate significant difference. Compared to the healthy normal control group.

Abbreviations: M: males; F: females; FBG: Fasting blood glucose; KYN: kynurenine.

a) number male of control 30=10(20-40);10(41-60);10(>61).

b) number female of control 17=6(20-40);5(41-60);6(>61).

c) number male of Gr. II 55=15(20-40);22(41-60);18(>61).

d) number female of Gr. II 19=7(20-40);7(41-60);5(>61).

e) number male of Gr. III 37=13(20-40);17(41-60);7(>61).

f) number female of Gr. III 22=9(20-40);6(41-60);7(>61).