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PREVALENCE OF LIPID PEROXIDATION AND HEPATIC PROFILE IN HEPATITIS C PATIENTS RECEIVING SOFOSBUVIR AND RIBAVIRIN FROM LAHORE-PAKISTAN

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Abstract:

Background: *Hepatitis C Virus is a member of flaviviridae family in hepacivirus genome. HCV effect liver that develop in CHC contagion in maximum patients. About 160 million individuals are infected by HCV contagion that leads Long-lasting hepatitis C, liver cirrhosis, hepatocellular carcinoma. Direct damaged hepatic cells by HCV cause CHC and Chronic Hepatitis C leads lipid accumulation in liver.* ***Objective:*** *To evaluate the lipid peroxidation and Hepatic profile in hepatitis C patients receiving ribavirin and sofosbuvir.* ***Methodology:*** *Venous blood samples (5.0 ml) of 50 diagnosed Hepatitis C patients receiving sofosbuvir and ribavirin and 50 Blood samples (5.0 ml) of Healthy individuals were taken in clotted gel vials. Blood serum was further tested for estimation of Malondialdehyde (MDA), Nitric Oxide (NO), Advanced Glycation End products (AGE's), Serum Liver Functions tests (LFTs), Lipid Profile, Urea and Creatinine by using spectrophotometer.* ***Results:*** *Serum MDA level significantly elevate (8.60) in diseased individuals receiving sofosbuvir and ribavirin than control (1.3) and NO level significantly increase in patients (35.21) as compare to healthy individuals (6.41). Serum hepatic profile status reveals that value of ALT significantly rises in disease persons (44.56) that control individuals (36.22). Serum AST level increase significantly in diseased individuals (40.33) as compare to healthy individuals (29.81). All parameters showed significant statistically ($P = 0.000 < 0.05$).* ***Conclusions:*** *Due to high lipid peroxidation, serum MDA level and NO level elevated remarkably in Hepatitis C patients that is highly significant. AGE's level and Creatinine level decreased in Hepatitis C patients. ALT, AST, ALP, Total Bilirubin, Urea, Cholesterols and Triglyceride level increased in HCV patients that may leads to the progression of disease.*

Key Words: *HCV, Ribavirin, Sofosbuvir, ALT, AST, MDA*

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INTRODUCTION:

Hepatitis C disease is liver tenderness affected via HCV that stimulate stellate cells. Deposition of collagen occur due to activation of stellate cells that leads fibrosis progression within the liver (1). Around 160 million persons have HCV effect that leads crucial liver cancer, chronic liver infection and late phase of liver damaging (fibrosis). Total patients experiencing continual long-lasting hepatitis C effects and HCC suspected towards further intensification in subsequently 25 years (2). Main reason for Acute and CHC is HCV. Almost 20% of acute hepatitis C incidents in all over the world, initiated via Hepatitis C Virus. Clinically acute hepatitis C bears resemblance to further acute viral related hepatitis forms along the beginning of illness, vomiting, and right upper quadrant soreness monitored through darker urine and yellowish pigmentations on skin and eyes. Phase between HCV contagion revelation and first indications appearance is 8 weeks (assortment, 3-20 weeks) (3, 4). Though, HCV viral markers become apparent, a long time before the oncoming of symptoms. In 1-2 weeks revelation, HCV RNA becomes identifiable contained by serum and levels of HCV RNA rises by 10^6 to10^8 genomes/mL (5, 6).About every 3rd acute HCV infected patients evolve jaundice or else signs til now, but other remaining patients evolve hepatitis without jaundice and the hepatitis is not severe enough to present readily symptoms. If it clearly visible clinically, disease usually proceed for 2-12 weeks. Hepatitis C virus exceptionally leads fulminant hepatitis (7). Aminotransferase levels become normal and in few weeks of symptoms inception, HCV RNA becomes unidentifiable in acute self-limited disease. Unluckily, acute, self-limited disease is not supreme mode of acute HCV infection. ALT levels habitually persist raised and HCV RNA perseveres but symptoms of acute hepatitis C disappear in most patients (8, 9).

In fact, the most discriminating characteristic of hepatitis C is a tendency towards chronicity that happen in almost 80% patients with severe HCV contagion. Features are not clear, initiating long-lasting hepatitis C. The envelope gene of virus have propensity to mutate fastly and hepatitis C virus have quasi species nature that may be key factors, due to antigenic epitopes transmutations to which some neutralizing body formed, virus constantly remain safe from immune identification (10, 11). Hepatitis C Virus interact receptors existing on exterior of cell, incorporates within cell. Single-stranded, positive-sense RNA genome releases in cell cytoplasm by cellular and viral membranes coalition that actuating via low pH of endocytic compartment. In hepatitis C virus life cycle, single-stranded, positive-sense RNA genome perform many tasks such as firstly, for viral protein translation it acts as mRNA secondly, for RNA replication it acts as template and thirdly, it involves in nascent genome packaging into new HCV.

Typically, few symptoms appear in chronic hepatitis C patients that generally wide, irregular, and temperate. In critical disease condition anorexia nervosa, vomiting, right upper quadrant soreness, darker urine, and pruritus happened, but most common symptoms exist fatigue. Chronic hepatitis C patients also have lipid accumulation in liver. Excessive lipid accumulation in the cytoplasm of hepatocyte called Hepatic steatosis that leads cirrhosis (12). Reactive oxygen species are short-lived molecules that attack polyunsaturated fatty acid, leads lipid peroxidation with cellular outcomes and reactive oxygen species exert local effects (13). Lipid Peroxidation produces MDA and HNE molecules, these molecules move from provenance to intracellular and extracellular spaces raise oxidative stress. The main causes of HCV infection are blood transfusion, nosocomial transmission, injected drug use such as blood-blood contact, sharing syringes and needles or it may be caused by further parenteral revelation like needle twig damages, body modification (14). Sofosbuvir is a uridine analog prodrug that used along ribavirin and pegylated interferon. Sofosbuvir along ribavirin and pegylated interferon used as treatment of HCV genotypes one, four, five and six contagions. And sofosbuvir along ribavirin also used as treatment for HCV genotype 2 and 3 contagions. Ribavirin along with pegylated interferon, typical therapy used to treat HCV contagion (15). To persuade sustained virological response, oral ribavirin monotherapy is not operative (16). Peg-interferon alfa 2a and peg-interferon alfa 2b, these two interferon formations approved as HCV therapy. 25-39% SVR proportions aimed for pegylated interferon monotherapy and 54-60 % aimed for pegylated interferon plus ribavirin (17). To improve therapy efficacy, care, and permissibility used to cure chronic HCV contagion, other interferons like consent interferon, albinterferon alfa 2b and ribavirin substitutes like taribavirin established (18).

AIMS AND OBJECTIVES

Objective of present study was to determine lipid peroxidation and Hepatic profile in Hepatitis C patients receiving ribavirin and sofosbuvir.

MATERIALS AND METHODS:

PLACE OF WORK

Whole experimental work was performed in the Biochemistry Lab, School of Biochemistry and

Medical Lab Technology, Faculty of Allied Health Sciences, after the approval of Ethical and Research Committee, Minhaj University Lahore.

STUDY DESIGN

Whole study was divided into two groups i.e. 1st group A consist of Diseased Persons and 2nd group B Consist of Healthy individuals.

Sr. No	Group	Sample Size (n)
A	Diseased Persons (Hepatitis C receiving sofosbuvir and ribavirin)	50
B	Control / Healthy	50

BLOOD/DATA COLLECTION

Venous blood samples (5.0 ml) of 50 Hepatitis C patients receiving sofosbuvir and ribavirin and 50 Blood samples (5.0 ml) of Healthy individuals were taken in clotted gel vial from Hepatology department, Gulab Devi Chest hospital Lahore. Blood was further tested for estimation of Malondialdehyde (MDA), Nitric Oxide (NO), Advanced Glycation End products (AGE's,) Serum Liver Functions tests (LFTs), Lipid Profile, Serum urea and Creatinine.

BLOOD/SAMPLE ANALYSIS

Samples were collected into EDTA tubes or gel clotted vials. Samples were centrifuged at 4000 rpm for 10 minutes and serum was separated.

RESULTS:

Table 1	**Comparison of Anti-Oxidant Parameters Between Control and HCV Patients Receiving Sofosbuvir and Ribavirin.**

Parameters	**Control (n=50) Mean ± S.D**	**Disease(n=50) Mean ± S.D**	**P-Value (P≤0.05)**
AGE's (mU/ml)	**15.21± 2.09**	**4.33±0.13**	**0.000**
MDA (µmol/ml)	**1.3± 0.21**	**8.60±1.74**	**0.000**
NO (µM)	**6.41±1.39**	**35.21±1.30**	**0.000**
MDA Normal Range = 1.0-3.0 µmol/mL,			

Data interpretation in Table 1 and Figure 1 represents clear cut picture of different variables of antioxidative status distressed by HCV patients receiving sofosbuvir and ribavirin. Serum AGE's level decline *(4.33±0.13)* than control individuals *(15.21± 2.09)* and statistics expose that it is highly significant *(P=0.000<0.05).* Serum antioxidative status expose in HCV patients that MDA level elevate curiously *(8.60±1.74)* as compare to control individuals *(1.3± 0.21)* that reveals high lipid peroxidation rates in HCV patients and statistics exposes that it is highly significant *(P=0.000<0.05).* Serum NO level raised *(35.21±1.30)* as compare to control individuals *(6.41±1.39)* and it is highly significant statistically *(P=0.000<0.05).*

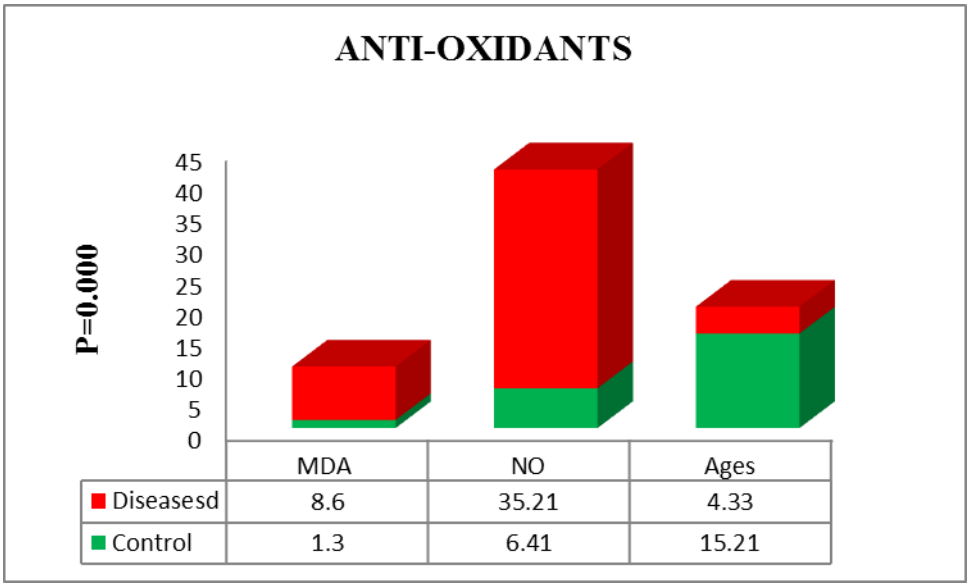

Figure 1: Graphical Representation of Antioxidants status between Hepatitis C and Control Persons

Table 2 Comparison of Serum LFT,s Parameters Between Control and HCV Patients Receiving Sofosbuvir and Ribavirin

Parameters	Control (n=50) Mean ± S.D	Disease (n=50) Mean ± S.D	P-Value (P≤0.05)
ALT (U/L)	36.22± 1.92	44.56±3.64	0.000
AST (U/L)	29.81± 4.33	40.33±1.82	0.000
ALP (U/L)	79.73± 5.96	192.06±13.81	0.000
Tot. Bili (mg/dL)	0.71± 0.02	2.73±0.92	0.000
Normal Ranges: total bilirubin=0.2-0.8ml\dL, ALT=7-55U\L,AST=8-48U\L			

Data analyses in above table 2 and Figure 2 indicate that different variables of hepatic profile status distressed by HCV patients receiving sofosbuvir and ribavirin. Serum hepatic profile status expose in HCV patients that ALT level elevate curiously *(44.56±3.64)* as compare to control individuals *(36.22± 1.92)* and statistics exposes that it is highly significant *(P=0.000<0.05)*. Serum AST level was also raised *(40.33±1.82)* than control individuals *(29.81± 4.33)* and statistics expose that it is highly significant *(P=0.000<0.05)*. Serum ALP level elevated remarkably in diseased persons *(192.06±13.81)* as compare to control individuals *(79.73± 5.96)* and it is highly significant statistically *(P=0.000<0.05)*. Serum total bilirubin level also rise *(2.73±0.92)* as compare to control *(0.71± 0.02)* and it is highly significant statistically *(P=0.000<0.05)*.

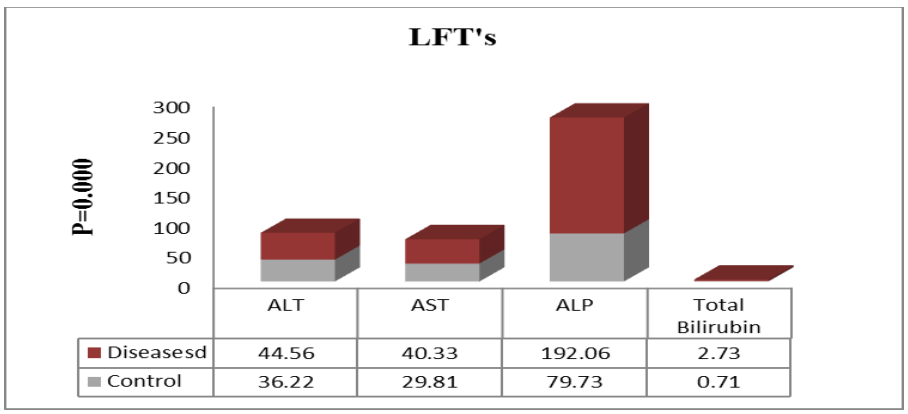

Figure 2: Graphical Representation of LFT's status between Hepatitis C and Control Persons

Table 3	Comparison of Serum RFT,s Parameters Between Control and HCV Patients Receiving Sofosbuvir And Ribavirin.		
Parameters	**Control (n=50) Mean ± S.D**	**Disease (n=50) Mean ± S.D**	**P-Value (P≤0.05)**
Creatinine (mg/dL)	**0.82 ± 0.02**	**0.71±0.11**	**0.000**
UREA (mg/dL)	**19.55± 2.11**	**29.00±3.69**	**0.000**
Normal Ranges: Urea=7-20mg\dL, Createnine=0.6-1.2mg\dl			

Data valuation in above table 3 and Figure 3 implies clear cut representation of different variables of renal profile status distressed by HCV patients receiving sofosbuvir and ribavirin. Serum renal profile status exposes in HCV patients that CREATININE level reduce *(0.71±0.11)* as compare to control individuals *(0.82 ± 0.02)* and statistics exposes that it is highly significant *(P=0.000<0.05).* Serum UREA level raise *(29.00±3.69)* than control individuals *(19.55± 2.11)* and statistics expose that it is highly significant *(P=0.000<0.05).*

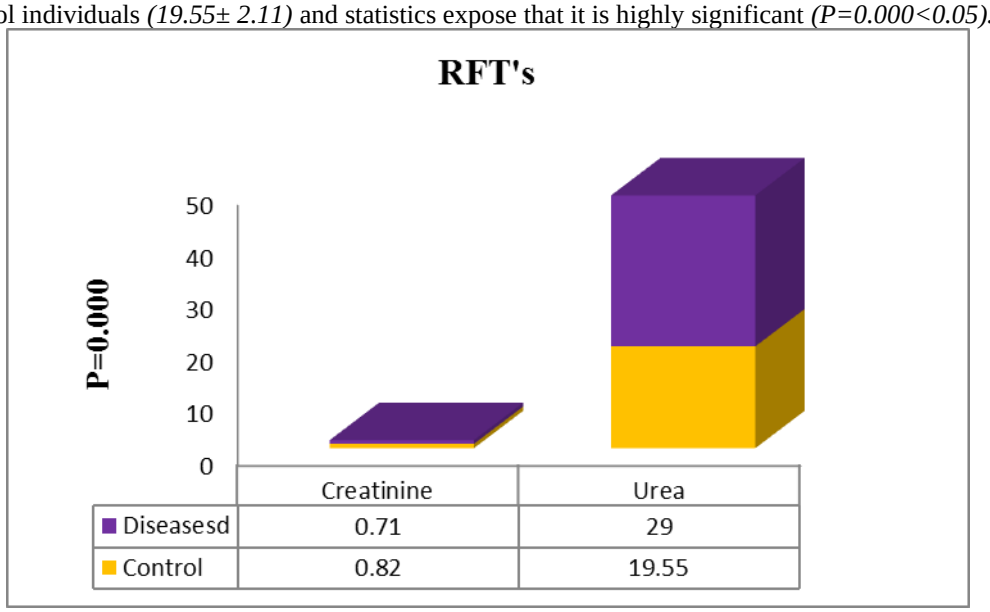

Figure 3: Graphical Representation of RFT's between Hepatitis C and Control Persons

Table 4	Comparison of Lipid Profile Parameters Between Control and HCV Patients Receiving Sofosbuvir and Ribavirin

Parameters	**Control(n=50) Mean ± S.D**	**Disease(n=50) Mean ± S.D**	**P-Value (P≤0.05)**
Cholesterol (mg/dL)	**154.21±4.39**	**172.86±7.88**	**0.000**
Triglycerides (mg/dL)	**113.23±6.49**	**140.76±6.65**	**0.000**
Normal Ranges: Tg = <150 mg\dl, Cholesterol = <200 mg\dl			

Data evaluation in above Table 4 and Figure 4 shows different variables of lipid profile status distressed by HCV patients receiving sofosbuvir and ribavirin. Serum lipid profile status revelations in HCV patients that Cholesterol level rise *(172.86±7.88)* than control individuals *(154.21±4.39)* and it is highly significant statistically *(P=0.000<0.05).* Serum Triglycerides level increase *(140.76±6.65)* than control individuals *(113.23±6.49)* and statistics expose that it is highly significant *(P=0.000<0.05).*

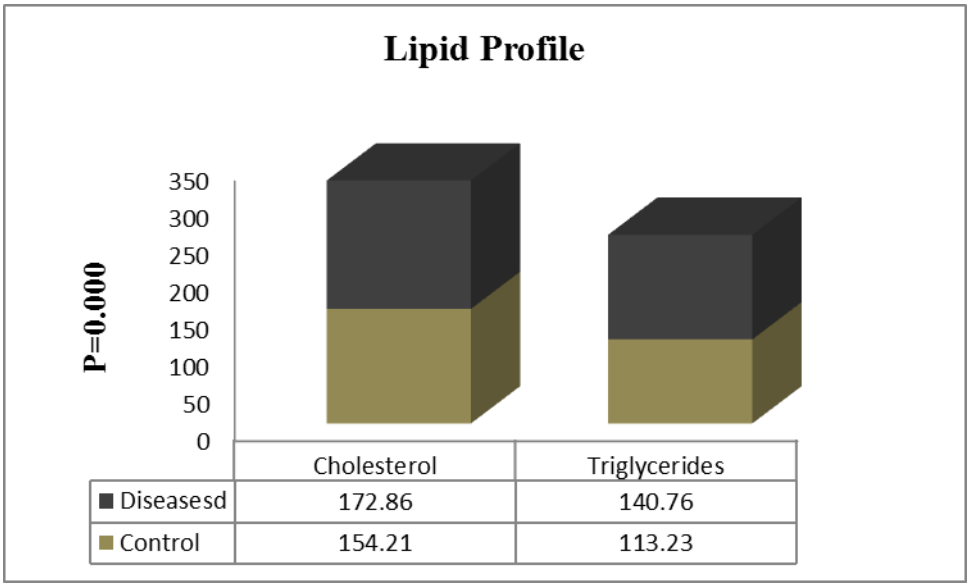

Figure 4: Graphical Representation of Lipid profile between Hepatitis C and Control Persons

Table 5	**Pearson's Correlations of Different Variables Estimated in HCV Patients Receiving Sofosbuvir and Ribavirin.**

Parameters	**Correlation (r)**	**P-Value**
NO Vs AST	**0.428***	**0.018**
NO Vs Urea	**0.369***	**0.045**
Total Bilirubin Vs ALP	**-0.469****	**0.009**
AST Vs ALT	**0.729****	**0.000**
AST Vs ALP	**0.424***	**0.020**
ALP Vs Triglycerides	**0.362***	**0.049**

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

DISCUSSION:

Hepatitis C virus contagion affects liver that develop into chronic HCV infection in more patients. Eventually it causes hepatic cirrhosis, hepatic failure and hepatoma that liable for maximum deaths every year (19). Lipid peroxidation, a mechanism leading by free radical that involve in acute and chronic Hepatitis C contagion progression. Lipid peroxidation form aldehyde products for example MDA and HNE that bind with several molecules and damaging them and cause harmful effect of this mechanism. The previous studies describe Instabilities in ROS created in overabundance and features inhibiting their harmful influences happened that leads oxidative stress. GSH-Px provides protection to cell from lipid peroxidation reactions by decaying hydrogen peroxide and it also alters lipid peroxide into innocuous molecules. Through reduced glutathione oxidation, GSH-Px eradicates H_2O_2. GSR and NADPH alter oxidized glutathione into reduced glutathione over again. Oxidative stress identified nearly in every medical and investigational chronic liver diseases situation. De Maria *et al.* exposed that MDA level increased in liver and blood which is the consequence of polyunsaturated fatty acid peroxidation (20). Viral burden and raised serum alanine aminotransferase (ALT) levels might ensure clinical applicability in persons with chronic hepatitis C.

Leakage of ALT into blood from liver occurs by injured parenchymal liver cells that cause increased alanine aminotransferase values in bloodstream. Partial HCV infected patients, not undergo through treatment have normal or

negligibly increased serum ALT levels. ALT level in serum expressively and unconventionally associated along periportal necrosis, viral burden and period of HCV contagion. ALT level in serum, as degree of biochemical hepatitis action, elevate expressively along periportal necrosis. There is negative and significant correlation among viral burden and mean ALT values. Ito *et al* demonstrated that HCV diseased persons having continuously standard ALT values also possess significantly raised mean viral load (21). Alanine Transaminase, Aspartate Transaminase and Alanine Phosphatase are highly significant (P<0.001) along HCV RNA viral burden. Alanine Transaminase, Aspartate Transaminase and Alanine Phosphatase formed by liver, have advantages in salts and amino acids production, which forms proteins. HCV leads hepatic tenderness; elevated viral burden cause increased liver inflammation. Raised ALT levels commonly display injured liver or tender liver. Liver enzymes Level have a tendency of elevation and reduction at consistent intervals in HCV infected patients. AST levels utilized to display liver tenderness and injury including combination along other tests.

Elevated Alanine Phosphatase from liver display obstructed bile ducts initiated through liver disease. Bilirubin association along HCV RNA viral burden not significant, bilirubin is not effective biochemical HCV indicator (19). Zechini B *et al* verified HCV RNA associate along aminotransferase levels and statistically it proved significant. They also demonstrated statistically significant ALT association with histological parameters and Serum Aspartate level. ALT level, particularly AST, might associate with hepatic injury (22). Osman *et al.* demonstrated that HCV contagion increase total nitric oxide (NO) level comprising nitrites and nitrates. Toll like receptors of innate immunity predict HCV RNA and create IFN-1a and IFN-1b.IFN-1a activate nitric oxide synthase that indicated in liver cells and macrophages. Serum nitrate and nitrite levels are greater in individuals with high hepatitis C viral load as compare to healthy individuals and hepatitis c contagion without viral load. In CHC, processes causing liver damage are not understood well. Zaki *et al.* evaluated that nitrate including NO2 *(P=0.000),* NO3 *(P=0.000)* are highly significant and ALT *(P<0.005)* significant in hepatitis C patients (23). The process in which long-lasting HCV contagion disturbs lipid metabolism is poorly understood. Virus assembly inside the cell utilized metabolic pathways convoluted in lipid metabolism. Persistently, patients with HCV-infection habitually possess low plasma betalipoprotein, triglyceride and cholesterol values. More likely, lipid accumulation induced by virus seems to be direct cytopathic lesion persuaded specially however not absolutely through HCV genotype 3a contagion. An uninterrupted HCV character maintained through vanishing of these deformities when contagion treated via antiviral therapy and through their recurrence after HCV contagion of giver liver in HCV-affected transplant individuals (24).

In this study we examine the correlation of various clinical findings such as NO, AST, Urea, ALP, ALT and Bilirubin in HCV infected patients receiving sofosbuvir and ribavirin. The results in Table 5 reveal that there is a strong negative or inverse correlation between **Total Bilirubin** and **ALP** *(r = -0.469**)* and statistically it is significant *(P = 0.000<0.01)*. High bilirubin level commonly related with secondary liver cancer and tumors on or in the liver cause HCC and liver cirrhosis via active or non-active HCV.

Results shows a direct relationship between **NO** and **AST** *(r = 0.428*)* and it is significant statistically *(P=0.01<0.05)*.It shows that the when NO level elevate, the level of AST also elevates.

There is a positive or direct association present between **NO** and **Urea** *(r = 0.369*)*, through statistical analysis it proved significant *(P = 0.04<0.05)*. As NO level increases as the level of Urea also increases.

There is a positive or direct connection present between **AST** and **ALT** *(r = 0.729**)*, through statistical analysis it proved significant *(P = 0.000<0.01)*. As AST level increases as the level of ALT also increases.

A positive or direct interaction exist among **AST** and **ALP** *(r = 0.424*)* and it is statistically significant *(P = 0.02<0.05)*. It shows that the when AST level elevate, the level of ALP also elevates.

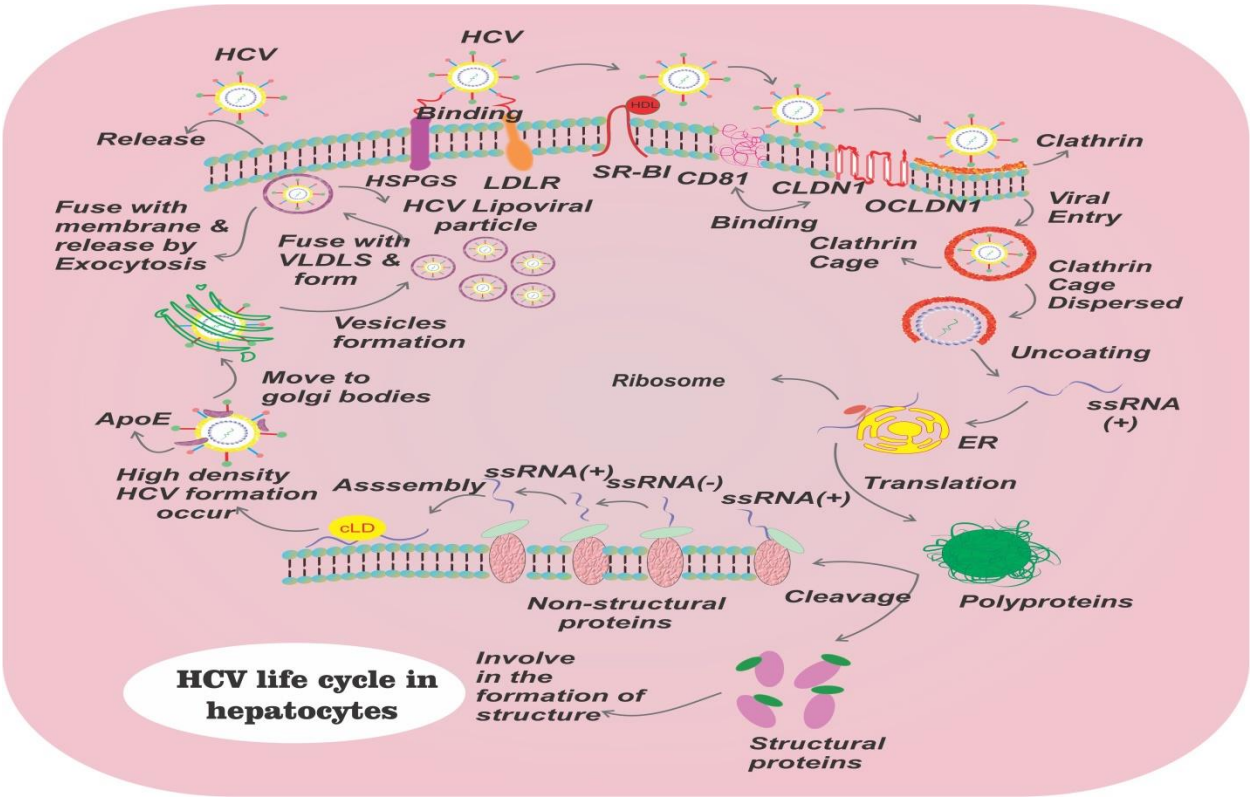

Figure 5: HCV Life cycle in Hepatocytes

CONCLUSION:

Oxidative stress is important component that involve in the progression of Hepatitis C disease. In present study, MDA, NO, AGE's, ALT, AST, ALP, Total Bilirubin, Creatinine, Urea, Triglycerides and Cholesterol parameters were studied. Present study concluded that deep associations exist between oxidative stress, hepatic, renal and lipid profile parameters in HCV patients taking sofosbuvir and ribavirin. Due to high lipid peroxidation, serum MDA level and NO level elevated remarkably in Hepatitis C patients that is highly significant. AGE's level and Creatinine level decreased in Hepatitis C patients. ALT, AST, ALP, Total Bilirubin, Urea, Cholesterols and Triglyceride level increased in HCV patients that may leads disease progression.

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