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Vitamin C supplementation ameliorates liver function profile and antiviral treatment response in Hepatitis C patients

Iffat Nayila[1,2], Muhammad Shahid Javed[3], Muhammad Asaf Khan[4], Ghulam Hussain[5], Syed Ali Raza Naqvi[6], Safdar Ali[1], Zahra Jabeen[7], Saira Shahnawaz[1,8], Rabia Akram[5], Tehreem Iman[5], Muhammad Zahoor-Ul Hassan Dogar[1*] and Shoaib Ahmad Malik[1*]

[1]Department of Biochemistry, Sargodha Medical College, University of Sargodha, Sargodha, Pakistan
[2]Department of Biotechnology, The University of Lahore, Sargodha Campus, Sargodha, Pakistan
[3]Department of Physiology, Sargodha Medical College, University of Sargodha, Sargodha, Pakistan
[4]Institute of Plant Breeding and Biotechnology, MNS University of Agriculture, Multan, Pakistan
[5]Neurochemicalbiology and Genetics Laboratory, Department of Physiology, Government College University, Faisalabad, Pakistan
[6]Department of Chemistry, Government College University, Faisalabad, Pakistan
[7]Department of Biochemistry, Government College University, Faisalabad, Pakistan
[8]Department of Biochemistry, Niazi Medical and Dental College, Sargodha, Pakistan

Abstract: An imbalance between oxidative stress and antioxidative defence mediates a variety of diseases pathogenesis. The present study aims to assess the possible outcome of supplementation of oral vitamin-C (VC), an antioxidant, in Viral Hepatitis C (HCV) treatment as an adjuvant therapy. 200 HCV-patients were selected, 100 were given Vitamin-C (1000 mg/day) along with anti HCV treatment (sofosbuvir plus daclatasvir) while the other 100 took only anti-HCV treatment for 4weeks. The serum ascorbic acid (Vitamin-C) levels and functions of the liver were tested before and after the VC supplementation. HCV patients with relatively low serum ascorbic acid showed significant improvement after the intake of vitamin C. After 4 weeks of treatment, AST, ALP, albumin, and total, direct and indirect bilirubin were improved significantly in the VC group; whereas only ALT and indirect bilirubin were improved in both groups when associated with the control subjects. Comparing the two treatment groups at 4weeks; more effective and significant improvement was observed in ALT ($p<0.01$), AST ($p<0.001$), direct ($p<0.01$) and indirect bilirubin ($p<0.001$), total proteins ($p<0.001$) and albumin ($p<0.05$) in patients with VC supplementation on anti-viral treatment compared to only anti-viral treatment group. Thus, VC supplementation improves the antiviral therapy outcome by bestowing a beneficial effect in minimizing liver damage in HCV cases.

Keywords: Vitamin C, antioxidants, viral hepatitis C, liver function tests.

INTRODUCTION

Hepatitis C, instigated by the hepatitis C virus (HCV), is an infection that targets hepatocytes eventually leading to inflammation, injury, and decease of liver cells (Tong *et al*., 1995; Comanescu, 2015). HCV infection is a health issue globally and a viral pandemic with probable 170 million individuals suffering from HCV worldwide, a leading reason for chronic disease of the liver. It is estimated that 70-85% of individuals with HCV develop a chronic infection and 30% of those show progressive liver disease with other complications, for example, cirrhosis and hepatocellular carcinoma (HCC), HCC being the leading cause of death worldwide (Armstrong *et al*., 2006). The low- and middle-income countries are more vulnerable and prone to get this disease, as it is found more prevalent among people of poor socioeconomic status. In Pakistan, this disease is spreading at an alarming rate, as about 5-8% population is infected with HCV. Pakistan is among the world's second-highest prevalent countries of hepatitis C infection, which is second only to Egypt (Suthar and Harries, 2015). According to available data, people above 10-11 million in the country are infected with Hepatitis C (Umer and Iqbal, 2016; World Health Organization, 2018) and of these, 8% are diagnosed with hepatocellular carcinoma (Ghias and Pervaiz, 2009).

HCV infection is treated with antiviral medications that clear the virus from the body. Direct-acting antivirals (DAAs) are the potent HCV treatment class, that is capable of clearing the infection of HCV and can substantively lessen the HCV viral load and thereby further transmission in chronic cases. Initially, Sofosbuvir plus Ribavirin was a recommended therapy, especially for patients with hepatitis C genotype 2 and 3 but nowadays, sofosbuvir plus daclatasvir has been proven more efficacious for genotypes 1,2,3,4,5 or 6 of HCV even with compensated cirrhosis (European Association for the Study of the Liver, 2017). The antivirals eradicate viral load effectively, but numerous ancillary supplements are being used in practice to treat HCV efficiently and to minimize the side effects of antivirals (Santana et el., 2021). In this scenario, searching for new and adjuvant

*Corresponding author: e-mail: drsamalik@gmail.com; postdoc233@yahoo.com

therapies can be quite helpful.

Vitamin C (Ascorbic Acid) is a well-known water-soluble vitamin with antioxidant properties. Vitamin C (VC) is a potent antioxidant in human and animal cells and body fluids with the ability of scavenging free radicals and participating as a reducing agent in several enzymatic reactions. Several important roles of VC in metabolism have been established as it effectively scavenges reactive oxygen *species* (*ROS*) which are produced during metabolic pathways (Buettner, 1993). VC is protective against toxic free radicals as it limits lipid peroxidation (Ray *et al*., 2007), also has a key role in the homeostasis maintenance of both circulating and hepatic lipids (Ipsen *et al*., 2014). VC is associated with the healing of tissues and enhance immuno-competence through regulating functional enzymes (Bashandy *et al*., 2018). Interestingly, VC, in various studies, show protective effect following a liver injury (Ozdil *et al*., 2004, Su *et al*., 2014, Ipsen *et al*., 2014). Although, VC exerts hepato-protection in immunological and chemical-induced liver injuries, but VC cannot be synthesized by humans and a some other species, and it must be acquired by dietary means (Djurašević *et al*., 2008). Intriguingly, VC levels in non-treated Hepatitis C patients were shown to be negatively linked to the aminotransferases levels, proposing that VC could be another marker of hepatitis C severity in chronic HCV patients (Souza dos Santos *et al*., 2008). A recent study gave the descriptive analysis of the existing data on the use of several antioxidant molecules, including vitamin C, in the treatment of liver diseases both in vivo and in vitro, and concluded that antioxidants may be useful depending upon the stage of infection (Lozano-Sepúlveda *et al*., 2019).

Given the foregoing, we surmised that VC supplementation, as an adjuvant drug, along with antiviral therapies can improve the ascorbic acid level in HCV patients and that could help in clearing the viron more rapidly and effectively. Here, we have demonstrated that, whether VC supplementation along with antiviral drugs (Sofosbuvir plus Daclatasvir) in hepatitis C patients could help in normalizing abnormal levels of various liver function parameters in the tested period of four weeks.

MATERIALS AND METHODS

Collection of samples

In this study, a total 200 number of subjects (both male and female) were selected by convenient purposive sampling. Hepatitis C was confirmed with polymerase chain reaction (PCR) with detectable HCV RNA in serum. Inclusion criteria included patients with age from 25 to 65 years with confirmed HCV diagnosis, raised ALT values (>80IU/L), and importantly the patients on anti-viral sofosbuvir plus daclatasvir for HCV treatment. The patients who gave history or presence of any liver disease, including all types of viral hepatitis, fatty liver disease, decompensated disease of the liver such as liver cirrhosis, or any coexistent diseases causing liver pathology such as diabetes, were excluded from the study. The patients who gave a history of Peg-interferon treatment or recent (within 2 weeks period) use of hepato-tonic agents such as silymarin, garlic oil were also excluded from the study.

Experimental design

The study design is a parallel observational study. Two separate groups were made for the recruited patients. Group A: Hepatitis C positive patients receiving their anti HCV treatment (sofosbuvir plus daclatasvir), who were not supplemented with Ascorbic Acid/Vitamin C. Group B: HCV positive patients receiving Vitamin C (VC) supplementation of 1000mg per day along with the intake of their regular anti-viral medications (Sofosbuvir plus Daclatasvir) for 04-weeks. Values before the start of the treatment (at 0 weeks) are called pre-treatment values whereas the results at 04 weeks are labeled as post-treatment. The study was approved by the Board of Advanced Studies and Research, University of Sargodha, Pakistan. Ethical approval of the study was taken from the Institutional Ethics Research Board (ERB) of the Faculty of Medical & Health Sciences, University of Sargodha, Pakistan.

Serum Ascorbic Acid analysis

The determination of ascorbic acid in plasma was done through Roe and Keuther's method, using 2,4-dinitrophenyl hydrazine, as described by Nino and Shah (1986) (Dogar *et al.,* 2010). Briefly, the ascorbic acid was converted to dehydroascorbic acid by using a cupric sulphate solution. Then the dehydroascorbic acid is coupled with 2,4- dinitrophenyl hydrazine by using thiourea as a mild reducing agent. Sulphuric acid is used to convert dinitrophenyl hydrazine, a red coloured compound that is assayed calorimetrically.

Biochemical Tests

All biochemical tests were performed by using standard methods described by the manufacturer. These biochemical tests included direct bilirubin (DB), total bilirubin (TB), serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), serum proteins (total, albumin, and globulin). All materials and quality controls were provided by Roche (Pakistan), Merck (France), and Ecoline (Germany).

STATISTICAL ANALYSIS

Descriptive indices (mean, standard deviation, and standard error) were analysed in this study. The 'paired t-test was employed to assess the comparability among the means of two groups whereas the 'independent sample t-test' was taken for the independent variables' evaluation. P-values<0.05 was considered statistically significant. All of the statistical analyses were done by using SPSS 23 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Influence of Vitamin C supplementation on Ascorbic Acid levels in serum

The levels of vitamin C (Ascorbic Acid) were determined at the start and after the 04 weeks of study. In the group with no supplementation of VC (Group A), the levels of ascorbic acid were 0.73±0.21 (Mean ± SD) mg/dL and 0.71±0.20 (Mean ± SD) mg/dL before the start and after the end of the study respectively. The values of ascorbic acid in the group having VC supplementation (Group B) were 0.72±0.22 (Mean ± SD) mg/dl before and 1.22±0.30 (Mean ± SD) mg/dL after the intake of VC for 4 weeks. Statistically substantial improvement in the ascorbic acid levels (p-value<0.001) in serum was seen post-treatment in Group Bas shown in fig. 1.

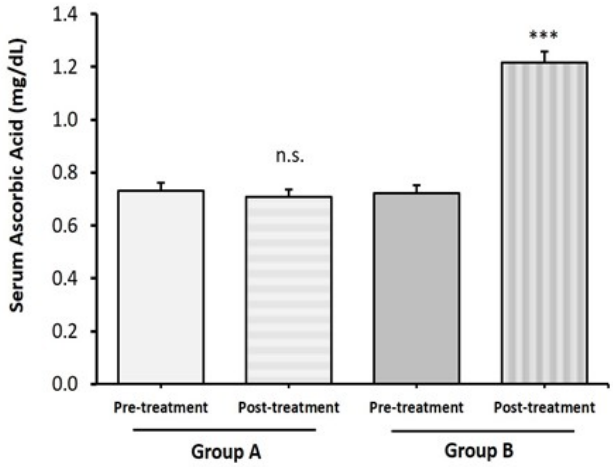

Fig. 1: Ascorbic Acid levels in serum. The VC (Ascorbic Acid) levels at the start and after the 04 weeks of study in HCV patients on Antiviral treatment with or without ascorbic acid supplementation. Significance: ***p<0.001, **p<0.01, *P<0.05 and p>0.05 non-significant (n.s.).

Effect of Vitamin C supplementation on the levels of liver enzymes

In the group, without VC supplementation (Group A), the pre-treatment value of ALT was 178±7 (Mean ± SEM) IU/L, and post-treatment it was observed 151±5 (Mean ± SEM) IU/L. The antiviral treatment only lowered the ALT levels significantly after 04 weeks of treatment (p-value<0.001). Similarly, in the other group with VC supplementation (Group B), elevated ALT levels in HCV patients with a pre-treatment value of 177±10 (Mean ± SD) IU/L was significantly decreased to 130±7 (Mean ± SD) IU/L post-treatment. This better trend towards the normalization of ALT is more pronounced (p-value<0.01) in Group Bas compared to the patients who haven't taken VC supplements (Group A) on comparing the post-treatment values of both groups (fig. 2A). Similarly, AST was measured in both groups with similar results as shown in fig. 2B. In group A, the AST was 111±6 IU/L at the start while post-treatment was 107±6 (Mean ± SD) IU/L. Inpatients with VC supplementation for 04 weeks (group B), AST was 108±7(Mean ± SD) IU/L before and 80±5 (Mean ± SD) IU/L after the intake of supplementation. The AST levels show a better trend towards its normalization (p-value<0.001) as compared to the patients who haven't taken VC supplements on comparing the post-treatment values of both groups (fig. 2B). The ALP level in group A was 188±8 IU/L before and 181±6 (Mean ± SD) IU/L post-treatment (p-value =0.31). The ALP level in group B was significantly improved with 189±8 (Mean ± SD) IU/L at the start and 175±5 (Mean ± SD) IU/L (with p-value=0.02) after 04 weeks of antiviral therapy with VC supplementation, but the post-treatment comparison of both groups was non-significant (p-value=0.57) (as shown in fig. 2C).

Influence of Vitamin C Supplementation on Bilirubin levels

The values of Total bilirubin (fig. 3A), direct (fig. 3B), and indirect bilirubin (fig. 3C) were deranged in the Hepatitis C patients. It was observed that the deranged values of the Total Bilirubin (p-value<0.001), Direct (p-value<0.001) and Indirect (p-value<0.001). Bilirubin significantly improved towards better levels in the group with VC supplementation (Group B) on comparing pre-treatment and post-treatment values. Whereas only the Indirect Bilirubin level (p-value=0.02) was improved in the patient without VC intake (Group A) when the results were compared between pre-treatment and post-treatment. Moreover, comparing the post-treatment values of both groups, VC supplementation is found to improve the Serum Direct Bilirubin (p-value<0.001) and Indirect Bilirubin (p-value<0.01) whereas it has no effect on Total Bilirubin (p-value=0.52), as shown in fig. 3.

Vitamin C Supplementation Effect on Serum Total Proteins, Albumin & Globulin Levels

Serum Total Proteins (fig. 4A), Albumin (fig. 4B) & Globulin (fig. 4C) levels were assessed in both groups before the start (pre-treatment) and after the four weeks of treatment (post-treatment). The serum Albumin level (p-value<0.001) was significantly enhanced in the group with VC supplementation (Group B), whereas serum Globulin and Total Proteins were changed insignificantly in both groups when the pre- and post-treatment values were compared, as shown in fig. 4. It is found that the pre-treatment serum total Protein level in group A (without VC supplementation) was 6.26±0.12 (Mean ± SEM) g/dL, and it was 5.96±0.09 (Mean ± SEM) mg/dL (p-value=0.08) at post-treatment. Whereas in group B, the values were 6.31±0.12 g/dL before supplementation and it improved to 6.48±0.09 g/dL (p-value=0.17) after 04-weeks of supplementation (post-treatment). Interestingly, total Protein levels improved significantly on comparing post-treatment values of both groups (p<0.001). Serum

Albumin level in group A (without VC supplementation) was 3.52±0.04 (Mean ± SEM) g/dL at the start and 3.47±0.03 (Mean ± SEM) g/dL after 04 weeks (non-significant change), whereas in group B was 3.36±0.05 (Mean ± SEM) g/dL before supplementation and post-treatment it improved significantly to 3.70±0.08 (Mean ± SEM) g/dL (p-value<0.01). Similar to total Protein levels, serum Albumin levels were improved significantly on comparing post-treatment values of both groups (p<0.05) (fig. 4B).

Serum Globulin level in the group A (without VC supplementation) was 1.67±0.08 g/dL at start and 1.73±0.09g/dL (p-value=0.27) after 4 weeks, whereas in group B it was 1.64±0.08 g/dL before and 1.71±0.07 g/dL (p-value=0.17) after 04 weeks of supplementation, both groups gave non-significant results. Moreover, evaluating the results at 04 weeks of treatments, VC supplementation has no effect on serum Globulin (p-value=0.85), as shown in fig. 4C.

DISCUSSION

In the study, we hypothesized that Vitamin C (VC) supplementation could minimise the oxidative stress accompanying the abnormal functions of the liver in HCV patients, and its use as an adjuvant drug or in diet (along with antivirals) is beneficial in restoring the antioxidant defence system, which may lower elevated markers of liver inflammation in HCV patients. The anti-oxidative agents help in improving liver functions due to reduction in oxidative stress, signifying that the antioxidants can protect liver damage and leads to a decrease in the elevated liver function markers induced by hepatotoxicity (Prabu *et al*., 2011). Here, we demonstrated that Ascorbic Acid, as an adjuvant agent, can be beneficial in Hepatitis C when used with HCV treatment (Sofosbuvir plus Daclatasvir) to normalize aberrantly deranged liver function parameters and thereby supportive in curing the disease more effectively.

Vitamin C is a powerful water-soluble antioxidant and naturally occurring vitamin, that employs potent pharmacological properties like anti-aging, anti-inflammatory, and antioxidant activities (Djurašević *et al*., 2008). Vitamin C (VC) has been shown to inhibit hepatotoxicity induced by different drugs, some chemical agents, organophosphate insecticides, and heavy metals. VC was being reported to normalize the levels of serum aminotransferase (such as alanine aminotransferase, aspartate aminotransferase, gamma-glutamyl transferase), alkaline phosphatase, serum bilirubin, lactate dehydrogenase and other biological parameters in intoxicated experimental animals. Interestingly, the animal experimental data support that the VC deficiency results in the augmented hepatic oxidative stress, along with the elevation of lipids levels both in the liver and plasma, leading to inflammation and fibrosis (Ipsen *et al*., 2014, Ergul *et al*., 2010, Ozturk *et al*., 2009). Conversely, VC treatment in animal models of liver diseases has been demonstrated to decrease hepatic markers of oxidative stress as shown by normalized liver function tests in drug-induced hepatitis; such as methotrexate (MTX) (Akbulut *et al*., 2014), and isoniazid- (INH) induced hepatitis (Ergul *et al*., 2010). In another comparable study done on rats, VC supplementation turns out to minimize the liver damage caused by CCL_4 poisoning as depicted by the normalization of the deranged liver enzymes' levels such as aminotransferases (ALT, AST) and ALP (Ozturk *et al*., 2009). In humans, VC has been shown to be beneficial in diseases like non-alcoholic steatohepatitis (NASH), fatty liver disease, and other forms of hepatitis. The hepatoprotective property of VC is credited to its antioxidant effects. VC levels are shown negatively linked with the levels of AST in humans, signifying the role of VC as an additional marker of hepatitis C severity (Souza dos Santos *et al*., 2008).

In this study, we have validated that the level of VC (ascorbic acid) was lower than normal in HCV patients, and the serum ascorbic acid levels were significantly restored by VC supplementation. VC supplementation markedly improved specific indicators of the liver function in HCV affected patients that are treated with antiviral therapy along with VC supplementation as compared to HCV patients treated only with antiviral therapy (without VC supplementation), as shown in fig. 2 for serum ALT, AST, and ALP enzymatic levels; fig. 3 for improved serum Bilirubin (Total, Direct and Indirect) levels. This is due to the reason that VC augments antioxidant capacity, diminishes cellular inflammation, and thereby enhances liver functionality and performance (Ipsen *et al*., 2014) and thus the evaluated improved parameters of LFTs in this study are may be due to the protective effect of ascorbic acid supplementation. In two independent studies, ascorbic acid alone or in combination with vitamin E decreased some abnormally elevated liver-related biochemical parameters in individuals having hepatotoxicity (Ji *et al*., 2014) and in patients suffering from NASH (Harrison *et al*., 2003). We found that the serum Total Proteins and Albumin levels (fig. 4) were improved while comparing the effectiveness of treatment in both groups post-treatment. VC efficiently conserves cellular integrity and thereby reserved the ALT and AST activity in the oxidative-mediated hepatotoxicity animal model (Bashandy *et al*., 2018). It is also reported that dietary VC significantly regress the endogenous protein levels that are associated with an oxidative insult to cells in liver disorders (Ipsen *et al*., 2014), and in line with that we have found that VC supplements restored the synthetic functions of liver in HCV patients showing that serum Albumin levels and Direct Bilirubin were improved significantly.

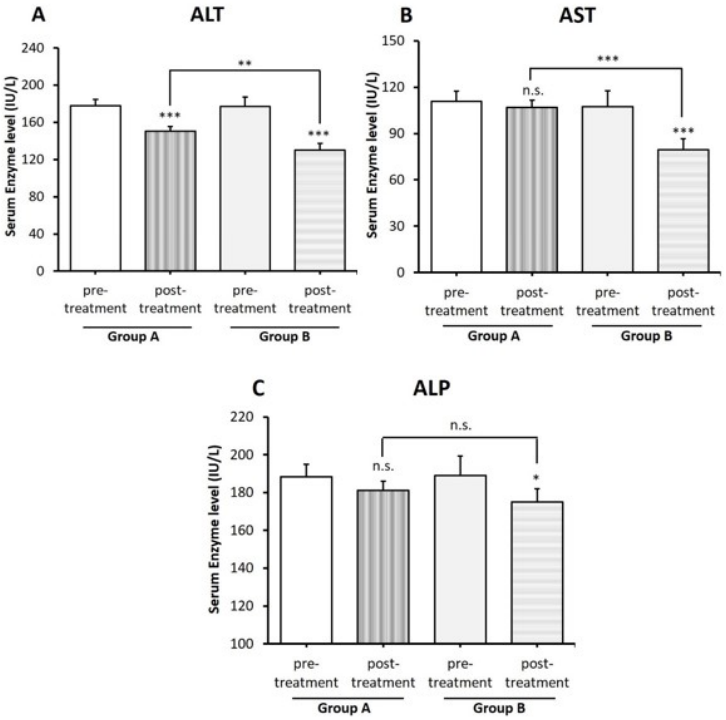

Fig. 2: Levels of Liver enzymes. The pre-treatment and post-treatment levels of ALT (2A) AST (2B) and ALP (2C) estimated in the serums of HCV patients on Antiviral with (Group A) or without (Group B) Ascorbic Acid supplementation. Data are shown as Mean ± SEM. Significance: ***p<0.001, **p<0.01, *P<0.05 and p>0.05 non-significant (n.s.). The normal range of ALT is 4 to 36 IU/L, AST is 8 to 33 IU/L and ALP is 44 to 147 IU/L (Pincus *et al*, 2017).

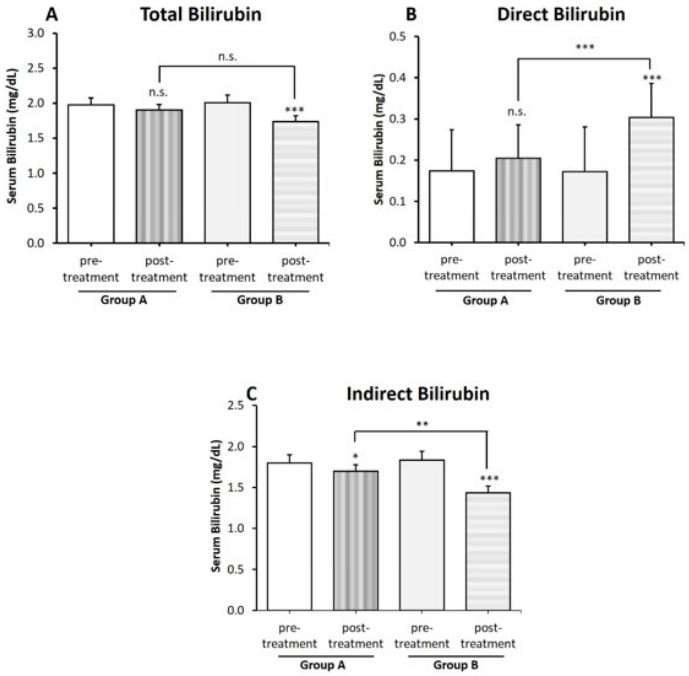

Fig. 3: Serum Bilirubin levels. The pre-treatment and post-treatment levels of serum Total Bilirubin(3A), Direct Bilirubin (3B), and Indirect Bilirubin (3C) estimated in HCV patients on Antiviral with (Group A) or without (Group B) Ascorbic Acid supplementation. Data are shown as Mean ± SEM. Significance: ***p<0.001, **p<0.01, *P<0.05 and p>0.05 non-significant (n.s.). The regular range of Total Bilirubin is 0.1 to 1.2mg/dL and Direct Bilirubin is less than 0.3mg/dL (Pincus *et al*, 2017).

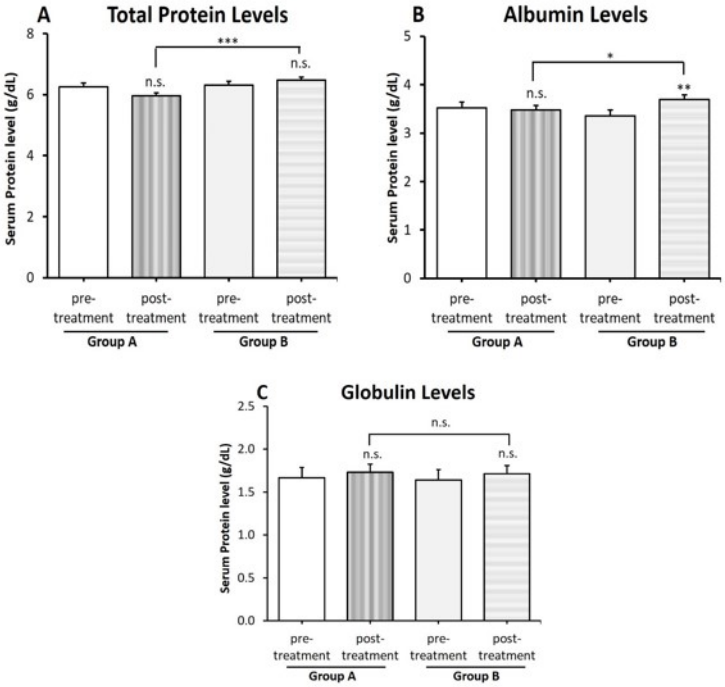

Fig. 4: Serum Proteins levels. The pre-treatment and post-treatment levels of serum Total Proteins (4A) serum Albumin (4B) and serum Globulin (4C) estimated in HCV patients on Antiviral with (Group A) or without (Group B) Ascorbic Acid supplementation. Data are shown as Mean ± SEM. Significance: ***p<0.001, **p<0.01, *P<0.05 and p>0.05 non-significant (n.s.).

Pakistan and other South Asian countries have a high proportion of individuals with reduced levels of ascorbic acid, which might explain the higher rates of different metabolic diseases in South Asians (Khan *et al*., 2006; Iqbal *et al*., 2006). Fascinatingly, VC seems to be essential for maintaining good health as it correlates with the improved markers of metabolic health. The adults with a greater level of VC showed lower weight, waist circumference, and BMI, including HbA1c, insulin, and triglycerides (Pearson *et al*., 2017). Therefore, VC supplementation in developing countries can eventually reduce the overall health care cost for the patients.

The current study has assessed the role of VC intake with Hepatitis C on a considerably good sample size with the exclusion of a substantial number of potential perplexing factors and it is the first study examining the aforementioned role of VC in HCV patients in the Pakistani population. The comparison of the plasma ascorbic acid levels is done in both groups of HCV patients understudy in the local population, however, the comparison of plasma Ascorbic Acid levels between normal subjects and HCV patients is needed to elaborate the causal relationship.

CONCLUSION

Conclusively, improved serum Vitamin-C (VC) levels with vitamin C supplementation in HCV patients could help in normalizing the abnormal level of various liver function parameters more quickly and effectively. Therefore, VC supplementation may be considered as an inexpensive, safe, and potent adjuvant therapy along with antiviral drugs (Sofosbuvir plus Daclatasvir) in HCV patients to get better antiviral effects.

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