rs2230201 polymorphism may dictate complement C3 levels and response to treatment in chronic hepatitis C patients

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SUMMARY. The basis of response of chronic hepatitis C (CHC) patients to treatment is still unclear, and there may be many other factors which influence treatment outcome other than the existing ones. The serum concentration of C3 closely reflects the total complement activity, and individuals affected by C3 deficiency suffer from recurrent pyogenic infections. This study aims to find out relationship between levels of C3 in serum and its functional SNPs with response to treatment. The study included 132 CHC patients of which 48 received Pegylated IFN+Ribavirin and 81 controls. C3 levels and its three known functional SNP's genotyped by ELISA and SSP PCR, respectively. C3 Level of the healthy group was significantly higher (88.5 ± 19 mg/dL) when compared to CHC group (56 ± 18 mg/dL; P < 0.001). Thirty-three of 36 responders were rs2230201 CC genotype carriers, whereas 9 of 12 nonresponders were non-CC genotype. The 'C' allele of rs2230201 was found to be associated with increased serum C3 levels when compared to other genotypes in healthy group, whereas CT genotype was associated with lowered serum C3 in CHC group. A serum C3 value of <53 mg/dL was predictive of SVR with sensitivity 63.89% and specificity 66.67%. The study supports the observation that rs2230201 'C' allele is associated with increase of serum C3 levels when compared to 'T' allele which may confer advantage in attaining SVR when present in homozygous condition. The study suggests that patients with serum C3 value <53 mg/dL and non-CC genotypes may not respond to treatment.

Keywords: complement system component 3, chronic hepatitis C, treatment response, serum S3 levels.

INTRODUCTION

The hepatitis C virus (HCV) is a major public health problem affecting an estimated 180 million people worldwide and leads to chronic liver disease [1]. HCV is the leading cause of liver transplantation and death. The introduction of effective therapy for the prevention of such life-threatening infection is the ultimate goal.

The complement system is a set of 30 soluble and membrane bound proteins that help clear pathogens. The complement system links the innate and adaptive immune response by various mechanisms which include regulating antibody effector mechanism, T-cell function modulation [2]. It leads to cellular response like apoptosis, opsonization, amplification of inflammatory reaction, etc [3]. C3 is the third component of complement system and known to play a protective role in viral infection [4,5]. It has been observed that the serum C3 levels are depleted in case of HCV-infected cirrhotic patient. Humoral component of the immune system's defence against infectious agents, the alternative pathway of complement system, is based on C3 cleavage fragment C3b and £B bound on activator surface and so forms another essential mechanism whereby the immune system tries to contain an infection for which an antibody response has not been produced or developed. Homozygous C3 deficiency results in recurrent pyogenic infections such as septicaemia, pneumonia and meningitis, and the absence of C3 is frequently lethal [6,7]. The human C3 locus contains a single gene and is located on chromosome 19 [8]. C3 cleavage by C3 convertase gives rise to two fragments C3a and C3b. The factor C3b activates the C5 convertase which in turn activates the complement cascade [9]. C3b facilitates phagocytosis of foreign particles by macrophages [10]. Studies have also shown C3 deficiency resulting in diminished liver regeneration, accompanied by transient or fatal liver failure after partial hepatectomy [11]. Few of the C3 polymorphisms have been seen to be associated with susceptibility to various diseases like sys-
temic lupus erythematosus (SLE) and age-related macular degeneration (ARMD) [12,13] and also to increased susceptibility to infection. The infections have included pneumonia, bacteremia, meningitis and osteomyelitis caused by encapsulated pyogenic bacteria. Various viruses have made C3 there target as C3 is a common denominator in the activation of all three pathways of the complement system. This study aims at finding the role of C3 expression levels and its functional SNP's role in hepatitis C patients and its correlation with treatment.

MATERIALS AND METHODS

Patient material

The study included 132 chronic hepatitis C patients diagnosed as per American Association for Study of Liver Disease (AASLD) practice guidelines [14], attending the medical wards and outpatient department of Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi, India, from January 2012 to August 2013. The control group comprises of blood samples from voluntary donors (as per ethical ground) who did not have any evidence of hepatobiliary disease. Blood samples were collected from subjects and stored at −70 °C for HCV genotyping, viral load, quantification of serum C3 levels and C3 SNP analysis. The study protocol conforms to the ethical guidelines of the Institutional Ethical Committee.

Of the total number of patients included in the study, 48 patients were self-assigned to receive peginterferon alpha-2b of 1.5 μg/ks/week dosed according to body weight. Ribavirin was given at 800 mg for genotype 3 and 4. Ribavirin dosage for genotype 1 was 1000 mg for ≤75 kg and 1200 mg for ≥75 kg. Treatment was carried out for 24 weeks for genotype 3 and 4 and 48 weeks for genotype 1 patients. Control groups comprised of 81 healthy blood donors who did not have any history of liver disorders. These patients were evaluated for previous history of arthritis or hypocomplementemia. The infections have included pneumonia, bacteraemia, meningitis and osteomyelitis caused by encapsulated pyogenic bacteria. Various viruses have made C3 there target as C3 is a common denominator in the activation of all three pathways of the complement system. This study aims at finding the role of C3 expression levels and its functional SNP's role in hepatitis C patients and its correlation with treatment.

Clinical SVR and liver histology

SVR was defined when serum HCV RNA was undetectable at the end of therapy and at 24-week follow-up after the treatment. Nonresponders were those patients in whom the viral RNA re-appeared after 6 months of cessation of treatment or when viral load decreases 2 logs after 12-week treatment, or when viral RNA was detectable at the end of treatment. Liver biopsy samples were collected from patients after getting their consent. Histological activity index (HAI) was assessed by pathologists who had no knowledge of the clinical or viral load state. Histological activity index was quantified ranging from 0 to 18 and fibrosis from 0 to 5 (0: no fibrosis, 1: mild fibrosis, 2: moderate fibrosis, 3: severe fibrosis, 4+: cirrhosis).

RNA quantification and genotyping

HCV RNA was extracted using the TRI® reagent (Sigma, MO, USA) from plasma, and HCV RG quantitative real-time PCR kit (Genome Diagnostics Pvt. Ltd, HP, India) was used to quantify the HCV RNA. The genotyping was performed by type-specific primers in a multiplex PCR system as described by Kazuaki Chayama [15].

Quantification of serum C3 levels

C3 levels in serum were determined utilizing commercially available kit (Abnova, Taiwan, China). The C3 levels were determined at the beginning of the therapy as well as at the end of the therapy.

Genotyping of C3 polymorphism (rs3745567), (rs7951), (rs2230201)

DNA was purified from peripheral blood mononuclear cells (PBMCs) using QI Amp DNA Maxi Kit (Qiagen, Hilden, Germany). The SNP's that were included in the study are based on previous literatures which suggest their ability to alter C3 expression level as well as their

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established association with diseases. Three candidate SNP’s were selected and tested which are rs3745567 [16], rs7951 [13], rs2230201 [13]. Genotyping of these C3 polymorphisms was carried out by allele-specific PCR method. The polymorphism rs2230201 and rs7951 also had established association in disease conditions like ARMD and SLE. The primers were designed using online tool RexPrimer2 [17] and have been mentioned in Table 1. Reaction condition that was used for amplifying rs2230201, rs7951 and rs3745567 polymorphism consisted of 1x PCR buffer SB (XT-Taq, Merck, Mumbai, India), 0.2 mM of each dNTP, 0.3 μM of each primers, 1.75 mM of total Mg2+ concentration, 0.7U of XT-5 Taq(Merck). The genomic DNA was used at 50 ng, PCR volume was 20 μL, and reaction was carried out on TPersonal Thermocycler (Biometra GmbH, Goettingen, Germany). The cycling parameter consisted of 95 °C for 5 min; 14 cycles at 94 °C for 30 s, 62 °C for 30 s, 72 °C for 30 s; 19 cycles at 94 °C for 30 s, 59 °C for 30 s, 72 °C for 30 s and final extension step of 72 °C for 5 min. The products were amplicons of size 178 bp, 207 bp, 251 bp, respectively, and visualized in 3% agarose gel.

**Statistical analysis**

The statistical significance of the association between allele and disease states and response to treatment was determined by chi-square (χ²) test. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated for each polymorphism using epi info online tool [18]. ANOVA and Student’s t-test were used to find association within a group of measurable variables. Mann–Whitney U-test was carried out for samples which were not normally distributed using MedCalc version 12.7 (MedCalc Software, Ostend, Belgium). Logistic regression was performed to ascertain factors that would affect treatment response. To obtain area under curve (AUC), Youden’s index, specificity, positive predictive value and negative predictive values were determined using MedCalc version 12.7. P-values ≤ 0.05 were considered statistically significant.

**RESULTS**

Of the 132 CHC patients whose mean age ± SD was 35.11 ± 11.40 years included, 81 were males (61.36%) and 51 were females (38.63%). Of 81 healthy controls included in the study, 48 were males (59.26%) and 33 were females (40.74%) with mean age ± SD of (37.34 ± 15.2). The demographic and clinical profiles of the patients are mentioned in Table 2.

**Serum C3 levels and relation to polymorphism rs3745567, rs2230201 and rs7951**

Henceforth, C3 levels indicate the baseline serum C3 levels unless explicitly mentioned otherwise. Baseline C3 component quantification was carried out to find whether any statistical difference was present within the healthy group and CHC group. C3 level of the healthy group was 88.5 ± 15 mg/dL, whereas the C3 level of the chronic hepatitis C group was 56 ± 18 mg/dL (P < 0.0001, t = 13.6047), indicating that in HCV infection, there is lowering of complement system C3 components. To find out whether any of the mentioned polymorphisms were involved in lowering of the C3 levels, we had compared the allele genotypes of each polymorphism with the C3 levels in both healthy and chronic hepatitis C patients. No significant association was observed between the genotypes of rs7951, rs3745567 and the levels of C3 in healthy controls and CHC group (Table 3). However, significant association was found between genotypes of rs2230201 and C3 expression levels in case of chronic hepatitis C group and healthy group (Table 3). In the healthy group, the mean ± SD C3 levels of CC (n = 54), CT (n = 24) and TT (n = 3) were 96 ± 12 mg/dL, 89.5 ± 15 mg/dL, 80 ± 10 mg/dL, respectively, which was significantly varied (P = 0.034, F = 3.5). It was observed that ‘C’ allele was associated with increase in C3 levels when compared to the ‘T’ allele (P = 0.038, F = 3.5). It was also observed that in CHC group patients with CT (n = 42) allele, the mean ± SD of C3 levels was 48 ± 14 mg/dL, that of CC (n = 68) allele patients was 61 ± 15 mg/dL and that of TT allele carrier (n = 2) was 59 ± 10.4 mg/dL. This showed that there was significant (P < 0.00003, F = 11.2) variation of C3 level in patients carrying CT, TT and CC genotype (Table 3). It was observed that significant reduction in C3 level was observed in CT
Table 2: Demographic and clinical parameters of chronic hepatitis C patients. The above chart indicates that mean age at entry, HCA RNA during treatment initiation and fibrosis scores to be the independent predictors of treatment response.

<table>
<thead>
<tr>
<th>Treatment group (n = 48)</th>
<th>1 responder (n = 36)</th>
<th>2 nonresponder (n = 12)</th>
<th>P-value (1 vs 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at entry (Mean ± SD) years</td>
<td>35.11 ± 11.40</td>
<td>28 ± 9.37</td>
<td>40 ± 11.13</td>
</tr>
<tr>
<td>Male/female</td>
<td>1:8.1(81/51)</td>
<td>1:1(21/15)</td>
<td>1:1(4/4)</td>
</tr>
<tr>
<td>AST (mean ± SD) IU/mL</td>
<td>112.37 ± 76.92</td>
<td>121.36 ± 83.86</td>
<td>108.5 ± 45.67</td>
</tr>
<tr>
<td>ALT (mean ± SD) IU/mL</td>
<td>151.3 ± 131.1272</td>
<td>80.36 ± 47.17</td>
<td>92.75 ± 36.38</td>
</tr>
<tr>
<td>HCV RNA (IU/mL)</td>
<td>6435894 ± 2255662</td>
<td>159489 [1872-7581431]</td>
<td>483124 [3368-4588097]</td>
</tr>
<tr>
<td>Genotype distribution</td>
<td>GT 1: 39 (29.55%)</td>
<td>GT1:12(33.34%)</td>
<td>GT1:3(25%)</td>
</tr>
<tr>
<td></td>
<td>GT 3: 79 (59.84%)</td>
<td>GT3:20(55.55%)</td>
<td>GT3:9(75%)</td>
</tr>
<tr>
<td></td>
<td>GT 4: 14 (10.61%)</td>
<td>GT4:4(11.11%)</td>
<td>GT4:0(0%)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>2.16 ± 1.71</td>
<td>1.5 ± 1.69</td>
<td>4.25 ± 1.25</td>
</tr>
<tr>
<td>HAI</td>
<td>4.2 ± 2.3</td>
<td>4 ± 3.07</td>
<td>4.34 ± 0.57</td>
</tr>
</tbody>
</table>

*Indicates significant values P-value.
**Indicates highly significant values (P-value < 0.0001).
†Indicates that Mann–Whitney U-test carried out to compare parameters as data are not normally distributed.

Table 3: Levels of baseline complement component C3 of the genotypes in the polymorphism in both healthy and chronic hepatitis C. The above chart indicates that there is significant variance between the various genotypes of rs2230201 in healthy as well as in the CHC group. No variation was observed in the genotypes of rs3745567 and rs951 in both healthy controls and CHC groups.

<table>
<thead>
<tr>
<th>CC (Mean ± SD) mg/dL (n)</th>
<th>CT (Mean ± SD) mg/dL (n)</th>
<th>TT (Mean ± SD) mg/dL (n)</th>
<th>P-value, F-stat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3745567</td>
<td>90.25 ± 14 mg/dL (69)</td>
<td>87 ± 13 mg/dL (10)</td>
<td>88.25 ± 10 mg/dL (2)</td>
</tr>
<tr>
<td>rs23203021</td>
<td>96 ± 12.8 mg/dL (54)</td>
<td>89.5 ± 15 mg/dL (24)</td>
<td>80 ± 10 mg/dL (3)</td>
</tr>
<tr>
<td>rs7951</td>
<td>91.3 ± 15 mg/dL (67)</td>
<td>87.2 ± 20 mg/dL (9)</td>
<td>87 ± 12 mg/dL (5)</td>
</tr>
<tr>
<td>Chronic hepatitis C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs3745567</td>
<td>57.5 ± 15 mg/dL (108)</td>
<td>52.5 ± 14 mg/dL (22)</td>
<td>58 ± 16 mg/dL (2)</td>
</tr>
<tr>
<td>rs23203021</td>
<td>61 ± 15 mg/dL (88)</td>
<td>48 ± 14 mg/dL (42)</td>
<td>59 ± 10.4 mg/dL (2)</td>
</tr>
<tr>
<td>rs7951</td>
<td>57.2 ± 17 mg/dL (112)</td>
<td>54.8 ± 10 mg/dL (10)</td>
<td>56 ± 12 mg/dL (10)</td>
</tr>
</tbody>
</table>

*Indicates significant values P-value.
**Indicates highly significant values (P-value < 0.0001).

...genotype carriers when compared to both CC allele and TT allele in CHC group.

Relation of polymorphism to treatment response

The rs3745567 and rs23203021 were in complete agreement with the Hardy–Weinberg equilibrium, but rs7951 was not. When rs3745567, rs23203021 and rs7951 polymorphism study was carried in CHC patients and controls, there was no significant association observed (Table 4). But significant association was observed when the comparison was made between the responder and nonresponder group carrying rs23203021. Of the total number of 48 patients undergoing treatment, 36 (75%) were responders and 12 (25%) were nonresponders. It was observed that patients carrying rs23203021 heterozygous CT genotype had an increased risk of not attaining sustained virological response after being administered conventional Interferon + Ribavirin (P < 0.0001). It was observed that of 12 CT allele carrying patients who received treatment, only 3 (25%) patients responded and other 9 (75%) patients did not, whereas of 36 CC allele carrying patients, only 3 (8.3%) were nonresponders, whereas the rest 33 (91.7%) were responders (Table 4).

Cut-off values of serum C3 levels from ROC analysis of other predictor of treatment outcomes

A logistic regression was performed to ascertain the effect of rs23203021 polymorphism, HCV RNA load and C3 levels...
Table 4 Frequency of rs3745567, rs2230201 & rs7951 frequency in healthy, chronic hepatitis C group and the group with Interferon+Ribavirin drug

<table>
<thead>
<tr>
<th>Allele</th>
<th>Healthy (81)</th>
<th>Responder (36)</th>
<th>Nonresponder (12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>2 (2.4%)</td>
<td>1 (2.78%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>CT</td>
<td>10 (12.34%)</td>
<td>4 (11.11%)</td>
<td>2 (16.67%)</td>
</tr>
<tr>
<td>CC</td>
<td>69 (85.18%)</td>
<td>31 (86.11%)</td>
<td>10 (83.33%)</td>
</tr>
<tr>
<td>P-value (a vs b)</td>
<td>0.323</td>
<td>0.75</td>
<td>0.516</td>
</tr>
<tr>
<td>P-value (c vs d)</td>
<td>0.08</td>
<td>0.427</td>
<td>0.272</td>
</tr>
</tbody>
</table>

*Indicates significant values \( P\text{-value} \)
**Indicates highly significant values \( P\text{-value} < 0.0001 \)

Table 4 Frequency of rs3745567, rs2230201 & rs7951 frequency in healthy, chronic hepatitis C group and the group with Interferon+Ribavirin drug on the likelihood that patients respond to treatment. The model explained 78.7% (Nagelkerke \( R^2 \)) of the variance in response to treatment and correctly classified 89.6% of cases and was statistically significant (\( P < 0.001 \)). CT allele and C3 levels at the initiation of the treatment were found to be associated with treatment outcome (Table 5). To find out the baseline C3 levels that could be used as a predictive marker, ROC curve was utilized to find the area under curve (AUC) for C3 levels, and viral RNA levels. The comparison indicated that AUC for viral RNA during initiation of treatment was 0.669 and that of C3 levels was 0.833 (Fig. 1). The calculated cut-off value of serum C3, indicating a sustained virological response to therapy, was a value >53 mg/dL. The area under the curve was 0.833 (CI = 0.698–0.925) with a sensitivity of 86.11% and specificity of 75% (Fig. 1).

Specific serum C3 level and clinical liver parameters comparison

The levels of C3 were compared with enzymology, liver histological parameters and virus genotypes in the 132 chronic hepatitis C patients. No significant correlations were found between specific C3 activity and alanine aminotransferase, HCV genotype, histological activity index. However, it was found that the mean age ± SD of responder group was 28 ± 9.37 and was significantly lower than that of nonresponder group which was 40 ± 11.13 (\( P = 0.0006, df = 46 \)). It was also found that in the responder group viral RNA levels, before treatment initiation, were lower than that of nonresponder group (\( P = 0.05, df = 1 \)). The fibrosis score ± SD of the nonresponder group (4.25 ± 1.25) was significantly higher than the responder group (1.5 ± 1.69) (\( P < 0.0001 \)) (Table 2).

DISCUSSION

Chronic HCV infection involves in a complex interaction of virus with host innate and adaptive immunity. It has been seen that the acute hepatitis is less placable to therapy, approximately 80% HCV infections progress to chronic stage. Several host factors including age at the time of infection, coinfection with the hepatitis B virus or human immunodeficiency virus and gender are known to affect disease courses in HCV infection [19–22]. Research studies have provided with evidence that supports involvement of host genetics in immune responses, which may predict the treatment response [23–25]. HCV evades the host immune surveillance by incorporating a myriad of mechanisms which start from triggering production of viral inhibitory enzymes and negative signal regulatory proteins that block signal transduction pathways of human innate immune system. In addition, HCV employs multiple escaping strategies including viral genetic variability, baseline viral load and high viral turnover to protect HCV from host humoral
Table 5 Logistic regression of factors to find predictors of treatment response

<table>
<thead>
<tr>
<th>Variables in the equation</th>
<th>B</th>
<th>Df</th>
<th>Sig.</th>
<th>Exp(B)</th>
<th>95% C.I. for EXP(B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>viral_load</td>
<td>0.000</td>
<td>1</td>
<td>0.319</td>
<td>1.000</td>
<td>1.000</td>
</tr>
<tr>
<td>C3Levels</td>
<td>0.319</td>
<td>1</td>
<td>0.035*</td>
<td>1.376</td>
<td>1.022 1.853</td>
</tr>
<tr>
<td>CT(1)</td>
<td>6.079</td>
<td>1</td>
<td>0.014*</td>
<td>436.519</td>
<td>3.361 56693.712</td>
</tr>
<tr>
<td>Constant</td>
<td>-19.236</td>
<td>1</td>
<td>0.028</td>
<td>0.000</td>
<td></td>
</tr>
</tbody>
</table>

CT(1) is the group which has CT genotype of rs2230201.

*Indicates significant P-value (P < 0.05).

Fig. 1 Receiver operating characteristics curve between baseline C3 level and viral load. Here in the image C3 levels seem to occupy greater area than the Basal viral RNA levels which indicate C3 levels to be a better predictor than viral RNA. A cut-off value of >53 mg/dL was observed to be involved with positive treatment response. The cutoff for viral RNA was 342 184 IU/mL which was in agreement with current standards of 400 000 IU/mL.

and cellular immunity. This is the first study that attempts to co-relate the C3 levels and its SNP that influences its expression in chronic hepatitis C infection and also its relation to treatment. In this study, we have observed that there is a significant reduction in the complement C3 levels in chronic hepatitis C patients and that this reduction may be because of polymorphism that have previously been seen to be involved in other pathogenic conditions. Our data are in corroboration with Majumdar et al. [26] who found that the C3 levels were lowered in chronic hepatitis C infected patients. The study confirms the assertion of complement system component C3 involvement in chronic hepatitis C infection performed in population size that is larger compared with Majumdar et al. However, Dunestre-Ferd et al. [27] were not able to find any significant difference in the level of C3 component within the chronic hepatitis C patients. The study is also in accordance with study by Teisberg and Gjone where they found that chronic active hepatitis patients have reduced C3 and C4 levels [28]. This study may reveal an insight that complement system component C3 can be an indicator of immunological activity.

The study also indicates a putative role of rs2230201 polymorphism in alteration of C3 levels as well as response to treatment with conventional Interferon + Ribavirin. This polymorphism has been seen to be associated with susceptibility to diseases like SLE by Miyagawa et al. [12]. Their study indicated that the C3 SNP rs2230201 'C' allele and rs7951 'T' allele were significantly associated with SLE. Their study also indicated that the mean serum C3 level of carriers of the rs7951 'C' allele was significantly lower than that of noncarriers of the 'C' allele in SLE patients. Another study carried out by Yang et al. [16] also indicated that rs3745567 was involved in significant lower production of C3 levels. However, in their study, they were not able to find any significant relation between the C3 levels and rs2230201. The study, however, indicates that rs2230201 'C' allele was associated with significant increase of C3 levels in healthy controls. We also found that controls carrying single 'C' allele of the rs2230201 had significantly higher levels of serum C3 than did healthy controls with no rs2230201 'C' allele, as shown in Table 3. We predicted rs2230201, rs7951, rs3745567 effect on transcription and translation, by online prediction tools, which include using TFSsearch [29] for transcriptional regulation, Ensembl-NS [30] for protein coding alteration, Human Splice Finder [31] for splicing regulation and sulfinator [32] for post-translational modification. It was predicted that both rs2230201 and rs7951 SNP had splicing regulatory effect and that they did not possess adroitness to regulate protein coding or post-translational modification, whereas rs3745567 had transcriptional regulatory effect. Rs2230201 polymorphism is included in a region of exonic splice enhancer (ESE). When this polymorphism was fed in predictor software Splice Finder, it was observed that 'C' allele resulted in breaking of ESE sequence, whereas 'T' allele incorporation showed formation of new ESE site. Although above-mentioned effect was tested on prediction software, the
The effect of the polymorphism in this context needs to be further evaluated.

In this study, we also compared serum C3 levels with baseline RNA levels to find out whether C3 level is a better predictor of treatment response by utilizing receiver operating characteristics (ROC) curve. Our study revealed that even though the AUC for serum C3 and baseline RNA were not significantly different statistically, C3 occupied more area suggesting that serum C3 levels incorporation as a treatment response factor (Fig. 1). This was even further proved by binary logistic regression test whereby it was found that C3 levels and CT genotype were associated with treatment response (Table 5). Our study indicates patients with C3 serum level >53 mg/dL will attain SVR, whereas those with value <53 mg/dL will not be able to clear HCV RNA on treatment (Fig. 1). AUC analysis revealed the cut-off value of base HCV RNA that was the predictor of treatment was 342184 IU/mL (sensitivity 63.89% and specificity 66.67%) which is in close accordance with the study by Jules Levin where they found a value of 400 000 IU/mL as the cut-off for viral load in HCV [33]. C3 levels, both baseline and at the end of the treatment, did not correlate with the viral load. However, it was observed that there was significant difference in the C3 levels at the end of the treatment between the responder and nonresponder group.

Our study was not able to find any correlation between the levels of C3 and alleles of rs7951 and rs3745567. However, our study shows C3C patients carrying two copies of the rs2230201 'CC' allele, however, had higher levels of serum C3 (Table 3) than the CT genotype carriers. This was also observed in case of healthy controls where 'CC' genotype carriers had significant lowered C3 level than those carrying 'CT' and 'TT' genotype. The vast difference between the levels of C3 between CT genotype and non-CT genotype of rs2230201 between the chronic hepatitis C may be because of hepatitis C virus core protein capability in inhibiting C3 promoter expression as suggested by Mavumdur et al. [26]. However, the added effect of increased C3 levels by the 'C' allele cannot be undermined. The above study suggests, as for C3 deficiency, decreased levels of C3 may be associated with HCV persistence and numerous, to an extent confounding, factors may be involved in alteration of C3 concentrations. This condition that is why becomes very important specifically in case of patients receiving treatment. As our study did not include patients with TT genotype undergoing treatment, the response of TT allele to treatment and its correlation was not evaluated. However, this indicates that the 'C' allele may have a protective role which is substantially effective only in homozygous condition. We have observed that in patients receiving treatment, 'CC' genotype can be a positive predicting factor for the outcome of interferon and ribavirin double therapy.

CONCLUSION

rs2230201 'CC' genotype (i.e. 2 copies of the C allele for the rs2230201 single nucleotide polymorphism) is associated with increased likelihood of attainment of SVR or viral clearance when compared to 'CT' genotype or 'TT' genotype. A serum C3 level of <53 mg/dL is the cut-off value which may indicate attainment of SVR. The increase of serum C3 level seems to be correlated with the 'C' allele when compared to the 'T' allele. The study suggests that rs2230201 and serum C3 level could be included as one of the predictors of treatment response in CHC.

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