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Expression Profiling of the Tripartite Motif Family Genes in Chronic Hepatitis C Patients

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Cite This: *ACS Omega* 2023, 8, 25370−25377

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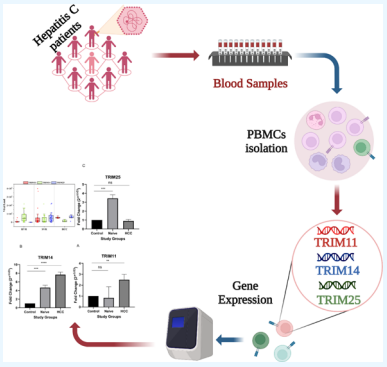
ABSTRACT: Hepatitis C virus (HCV) is one of the most prevalent pathogens which causes significant morbidity and mortality in 2% of the world's population. Several interferon-stimulated genes (ISGs) are involved in HCV clearance by interacting with the viral proteins. Among these ISGs, the tripartite motif (TRIM) family genes are elevated during HCV infection. This study aims to evaluate the expression of three TRIM family genes in chronic hepatitis C patients, distributed among different groups, including TRIM11, TRIM14, and TRIM25. A total of 242 participants were recruited in this study, including 182 infected patients, 37 naïve individuals, and 23 control individuals. Out of 182 infected patients, 100 achieved sustained virologic response (SVR), 61 achieved rapid virologic response (RVR), and 21 patients developed hepatocellular carcinoma (HCC), showing no response to the given treatments. Our results indicate highest expression levels of TRIM mRNA transcripts in the RVR group with the highest increase of 7.5 folds in TRIM25, 6.68 folds in TRIM14, followed by the data from patients of the SVR group. The elevation was also evident in other groups, i.e., SVR and HCC, in different patterns among all the three TRIM genes. In addition to elevation in expression levels, a linear correlation is observed between the TRIM mRNAs and viral loads of HCV. These results showed the potential role of TRIM family genes in HCV restriction.



1. INTRODUCTION

Hepatitis C virus (HCV) is the leading cause of liver-associated mortality and morbidity worldwide. Approximately 71 million people are infected globally with HCV, while most of the individuals are unaware of their status of infection. The prevalence of HCV is heterogeneously distributed within different regions and countries, with the overall global prevalence being 1.8%.[1] This is a significant increase from 2017, with the global prevalence estimated at 1.0%.[2] In Pakistan, the HCV prevalence rate is 4.8%, and around 1 in 20 individuals is infected with HCV.[3−5] Apart from the regions, HCV distribution and prevalence ratio are also affected by age, gender, race, and immune response; however, the accurate occurrence cannot be calculated because of the low progression rates of HCV.[6] The World Health Organization (WHO) has advocated that HCV could be eliminated globally by 2030 by recommending new strategies for injection safety, blood safety, harm reduction, and HCV treatment. However, according to the current statistics, this target is unlikely to be achieved.[7]

HCV is primarily transmitted through percutaneous blood exposure, illegal drug injections, use of non-sterile needles, sexual contact, mother-to-infant transmission, surgical procedures, and blood transfusion from infectious donors.[8,9] Current therapy with direct-acting antivirals (DAAs) offers >90% cure rates. However, attaining sustained virologic response (SVR) does not immediately reverse HCV-related liver complications like fibrosis and cirrhosis.[10] Also, considerable relapse rates are found in HCV patients after DAA therapy. As of 2017, DAA therapy relapse rates were reported to be 37.5% in Pakistani population.[11,12] However, literature shows a direct correlation between HCV relapse and subsequent deaths even after receiving a second therapy dose. The mortality rate after relapse can go as high as 41% in HCV patients.[13]

Our research provides empirical evidence that suggests the role of TRIM family genes in lowering the viral titer of HCV. In this study, the expression of three different TRIM family genes was studied to understand their antiviral properties on a molecular level against HCV.

TRIM14 interferes with the viral replication by regulating innate immune response[14] and acts directly against the NS5A protein of HCV.[15] NS5A is one of the non-structural proteins encoded by HCV and consists of three main domains (D1, D2,

Received: April 24, 2023
Accepted: June 1, 2023
Published: July 7, 2023

and D3) separated by two linkers. D1 is dimeric in nature and contains an RNA-binding domain at the interface of the dimer and helps anchor HCV RNA on intracellular membranes by forming a protective replication chamber. D2 promotes NS5A dimerization and induces resistance against HCV immune response by suppressing IFN response,[16] and D3 helps assemble viral particles.[17] Immune response activation requires the detection of PAMPs (pathogen-associated molecular patterns) with the help of cellular PRRs (pattern recognition receptors), which trigger the cellular signaling cascade that induces the pro-inflammatory cytokines and type-1 interferon.[18] RLR (retinoic acid-inducible gene I RIG-I-like receptor), specifically RIG-1, recognizes the viral RNA, which, upon binding, undergoes a conformational change to be recruited to MAVS (mitochondrial antiviral-signaling), which in turn activates two kinase complexes, TBK1 (TANK binding kinase 1) and IKK (IkappaB kinase), with the help of downstream molecules. TBK1 causes the phosphorylation of IFN regulatory factor 3/7 (IRF3/7), which is then translocated to the nucleus and starts the transcription of interferons, whereas IKK, which comprises NEMO (NF-κB essential modulator), releases NF-κB that stimulates pro-inflammatory gene expression.[19,20]

Here, TRIM14 affects the immune response by facilitating RLR-mediated activation of NF-κB and IRF3 (interferon regulatory factor 3) by localizing to mitochondria and acts as a mediator of MAVS. It is suggested that the 365th amino acid in TRIM-14 is essentially required for its interaction with NEMO, which leads to viral replication inhibition by the enhancement of type 1 IFN and NF-κB production.[21] Type 1 interferons are the primary line of defense against viral infection which induce interferon-stimulated genes (ISGs) that restrict the antiviral activity of different viruses through several mechanisms; however, only few ISGs have been found with anti-HCV activity. Out of these ISGs, TRIM14 has been identified as the potent inhibitor of HCV replication and infection.[15] TRIM14 is a RINGless member of TRIM family proteins, and its structural analyses showed the presence of B-box, coiled-coil portion, and PRYSPRY C-terminal domain (Figure 1). TRIM14 interacts with NS5A/NS5B and contaminate NS5A (an RNA-dependent RNA polymerase) in HCV. The degradation occurs dose dependently and is based on the interaction between NS5A domain 1 and the SPRY domain of TRIM14 that ultimately causes the degradation and inhibits the replication.[15,22] Apart from HCV, TRIM14 also plays important antiviral roles against multiple viruses, including hepatitis B virus,[23] herpes simplex virus-1, vesicular stomatitis virus,[24] and human immunodeficiency virus.[25]

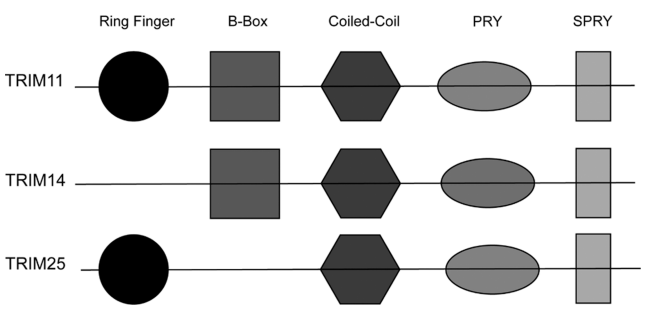


Figure 1. Structural organization of TRIM proteins. Schematic representation of domain organization in TRIM11, TRIM14, and TRIM25. The scheme shows the N-terminal tripartite motif and its composing domains (RING domain, B-box domain, and coiled-coil region) and the variable C-terminal domains. TRIM14 lacks a ring domain, whereas TRIM25 lacks a B-box compared to TRIM11.

TRIM11 is located on human chromosome 11 and has a PRY and SPRY domain, in addition to the RBCC domain (Figure 1).[26] The significance of TRIM11 was first indicated in the nervous system's activity, such as in Alzheimer's disease, expression of dopamine β-hydroxylase, Pax6-dependent neurogenesis, and survival of neuronal cells.[27] TRIM11 displays an oncogenic mechanism regulated by the epidermal growth factor receptor signaling pathway.[28] TRIM11 works as an oncogene in lung cancer by facilitating cell proliferation, migration, and invasion.[29] Chen and colleagues discovered that TRIM11 overexpression is strongly associated with the development of HCC and poor patient survival but can act as a potential therapeutic target suggesting the antiviral properties of TRIM11.[30] TRIM11 is also involved in HIV restriction by accelerating premature viral uncoating and inhibiting reverse transcription.[31]

TRIM25 contains a RING domain, a C-terminal SPRY domain, and a CC dimerization domain (Figure 1). TRIM25 is crucial in different cellular processes, such as cancer and adaptive and innate immunity.[32] The best role of TRIM25 is through K63-linked polyubiquitination, which regulates RIG-I signaling.[33] No direct interaction is reported that shows the functioning of TRIM25 against HCV. However, multiple studies show antiviral properties of TRIM25 through the RIG-1 pathway.[34−36] TRIM25 exhibits antiviral properties against infectious bursal disease virus (IBDV)[37] and Sindbis virus.[38,39]

In this study, we have examined the antiviral properties of three TRIM family mRNAs including TRIM11, TRIM14, and TRIM25 against HCV by observing the expression pattern of TRIM transcripts concerning the HCV viral loads. Our main objective is the comparison of mRNA transcript levels of all the three TRIM mRNAs in infected vs naïve individuals and healthy controls. Understanding the molecular mechanisms of TRIM mRNAs and unraveling their antiviral roles might lead us toward identifying potential drug targets in combating HCV. Although current medications against HCV, including DAAs and interferon therapy, give promising results, the high relapse rates imply the need for new therapeutics as an alternative option, as reported in our previous study.[40] Another aim is to find an association of TRIM protein levels in viral clearance as indicated by different groups, including SVR and RVR (rapid virologic response).

2. MATERIALS AND METHODS

2.1. Sample and Data Collection. A total of 242 individuals were recruited for the present study and divided into different groups, including the following:

1. Infected individuals (182)—infected with different genotypes of HCV, identified from various areas of Pakistan and following the different regimens of medications.
2. Naïve individuals (37)—HCV-positive individuals in their pre-treatment phase.
3. Control group (23)—healthy individuals with no other associated co-morbidities.

The study has been approved by the institutional review board, and informed written consent was obtained from all the participants included in the study. A unique sample identifier has been allocated to each sample for data analysis and future

https://doi.org/10.1021/acsomega.3c02800
ACS Omega 2023, 8, 25370−25377

correspondence. Individuals were contacted at various time points during the study and course of treatment to track their regimen's outcome.

Approximately 5 mL of blood was collected in EDTA-anticoagulant vials, labeled, and stored at −20 °C, and peripheral blood mononuclear cells (PBMCs) were isolated, and RNA was extracted from collected samples. The infected individuals were examined repeatedly to assess the viral loads at 6, 12, and 24 weeks.

2.2. Isolation of PBMCs. 5 mL of heparinized/defibrinated blood (stored in heparin vacutainers) was layered on top of 10 mL of histopaque −1077 (Sigma-Aldrich) in a 15 mL falcon tube with the help of a micropipette. The tubes were then centrifuged at 3000 rpm for 30 min at 4 °C. The whitish buffy coat at the interphase between cell debris and histopaque was aspirated and transferred to a fresh tube. 10 mL of 1× phosphate-buffered saline (PBS) was added into the fresh tubes containing PBMCs and again centrifuged at 3000 rpm for 10 min. PBS is used to wash and resuspend the pellet. PBMCs were stored at 4 °C until further analysis.

2.3. RNA Extraction. 300 μL of the liquid sample (homogenized PBMCs) was added to 1 mL of TRIZOL (Invitrogen, Carlsbad, CA, USA). The mixture was mixed well with the help of a vortex and incubated at room temperature for 5 min 300 μL of chloroform was added to the tube-containing Trizol and PBMCs and incubated for 3 min at room temperature. After that, centrifugation was carried out at 12,000*g* for 15 min, resulting in three distinct phases: upper aqueous phase, interphase, and lower phase. The upper aqueous phase was carefully pipetted into a new tube, whereas the other two layers were discarded. 500 μL of isopropanol was added, mixed by gentle inversion, incubated at room temperature, and centrifuged at 12,000*g* for 10 min. The supernatant was discarded, and the pellet was washed with 1 mL of 75% ethanol and centrifuged at 7500*g*. The supernatant was again discarded, and the pellet was air-dried for 5−10 min. The pellet was suspended in DEPC-treated water, and RNA purity and quantity were measured by NanoDrop (ND-100 UV/Vis, USA).[41]

2.4. cDNA Synthesis. A total of 2 μg of RNA extracted from each sample is reverse-transcribed into cDNA using M-MLV reverse transcriptase (Thermo Fisher Scientific, Waltham, MA, USA). The reaction mixture containing 5× FS buffer (Thermo Fisher Scientific), 0.1 M DTT (Invitrogen), M-MLV, nuclease-free water (Fermentas), and RNase inhibitor (Thermo Fisher Scientific) were added in the PCR tubes in specified quantities.

In each tube, 9 μL of the RNA sample, 2 μL of dNTPS, and 1 μL of the reverse primer of TRIM14 were added. The primer sequences for TRIM11, TRIM14, and TRIM25, as well as for GAPDH as a control, are shown in Table 1. GAPDH cDNA synthesis and subsequent quantification were performed and used as a positive control for normalization. Each tube was short-spanned and incubated in a thermocycler for 1 h at 42 °C and 5 min at 85 °C. The cDNA quantification was carried out by a Nano-Drop (ND-100 UV/vis, USA).

2.5. Detection of TRIM cDNA by Real-Time PCR. The synthesized cDNA was subjected to the amplification of TRIM11, TRIM14, and TRIM25. The cyclic conditions of real-time PCR (Rotor-Gene Q; Qiagen) were; 95 °C for 3 min. Then, 35 cycles of PCR were performed using the following protocol: 94 °C for the 30 s, 59 °C for 30 s, 72 °C for 30 s, followed by final extension at 72 °C for 10 min. qRT-PCR of the target and housekeeping gene was performed with the 2× Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific). The expression level of TRIM14 was normalized to that of the housekeeping gene GAPDH using the Livak method, also known as Ct ($2^{-\Delta\Delta ct}$), which calculates fold change in the gene expression of samples after real-time PCR.[24] All the samples were assessed in triplicates.

Table 1. Primer Sequences for Gene Amplification Used in the Current Study

genes	primer	sequence
TRIM11	forward	5′-ATGGCCGCCCCCGACCTGTC-3′
	reverse	5′-TCACTGGGGAGCCAGGGTGT-3′
TRIM14	forward	5′-TGAAGGGGAAATTCACTGAACTC-3′
	reverse	5′-AGCCTCTGGACAGGATCGG-3′
TRIM25	forward	5′-AAAGCCACCAGCTCACATCCGA-3′
	reverse	5′-GCGGTGTTGTAGTCCAGGATGA-3′
GAPDH	forward	5′-CGGATTTGGTCGTATTGGG-3′
	reverse	5′-CTCGCTCCTGGAAGATGG-3′

2.6. Statistical Analysis. Relative gene expression of TRIM cDNAs was analyzed by comparing mean group values using one-way ANOVA. Baseline analyses and graphical representations were performed by GraphPad Prism (version 8.4.3; San Diego, CA). A *p*-value of less than 0.05 was considered significant in all statistical analyses. Graphical representations were also generated with GraphPad Prism software.[42]

3. RESULTS

The current study includes 182 infected individuals, out of which 100 attained SVR, 61 individuals achieved RVR, and 21 individuals progressed toward hepatocellular carcinoma (HCC). Study participants are assigned into different groups based on the therapy outcome they showed following the medication regimen. Individuals assigned to the SVR group showed undeletable levels of HCV in the serum after 24 weeks of therapy, and the RVR group showed undetectable levels of HCV in the serum after only 4−6 weeks of therapy. The medication regimen followed by study participants, along with the treatment outcome and associated genotypes, is shown in Table 2.

3.1. Demographic Features of Studied Participants. Clinical and demographic features of different studied groups are presented in Table 3. A total of 182 HCV-infected patients were recruited in the current study, along with 23 healthy controls and 37 naïve individuals. Detailed patient interviews were conducted, and complete blood count (CBC) and liver function tests were carefully examined in order to assess different parameters and results are presented as mean values. Out of all the study groups, HCC patients and naïve individuals showed a significant elevation in various parameters as assessed by *t*-test. All the other groups showed insignificant variations from the control values in all parameters, except ALP.

3.2. Relative Expression Analysis of TRIM mRNA. The mRNA transcript levels of TRIM proteins were significantly higher in HCV-infected patients as compared to the healthy controls, which was taken as 1. Out of all the study groups, the maximum increase was observed in patients that achieved RVR, after 12 weeks of therapy (determined by quantitative real-time PCR), where highest elevation is seen in TRIM25 (7.5-folds), followed by TRIM14 (6.68-folds) and TRIM11

https://doi.org/10.1021/acsomega.3c02800
ACS Omega 2023, 8, 25370−25377

Table 2. Treatment Regimen for HCV Patients along with Treatment Outcome[a]

regimen	duration	genotype	treatment response
SOF	16/24 weeks	3	SVR (n = 23)
			RVR (n = 18)
			NR/HCC (n = 8)
SOF/DCV	12/24 weeks	3, 1	SVR (n = 36)
			RVR (n = 17)
			NR/HCC (n = 6)
SOF/RBV	12/24 weeks	3, 1	SVR (n = 17)
			RVR (n = 7)
SOF/RBV/DCV	12/24 weeks	3, 1	SVR (n = 24)
			RVR (n = 19)
			NR/HCC (n = 2)

[a]A total of 182 infected individuals received different treatment regimens. The table shows the division of all test individuals depending on the treatment regimen and observed outcomes. Abbreviations: SOF: sofosbuvir, RBV: ribavirin, DCV: daclatasvir, SVR: sustained virologic response, RVR: rapid virologic response, NR: non-responders.

(1.2-folds). The elevated levels of TRIM transcripts are also evident in patients who achieved SVR; after 24 weeks of therapy, a similar pattern of RVR is followed by the SVR group, where TRIM25 shows the highest elevation (7.1-folds), followed by TRIM14 (3.98-folds) and TRIM11 (2.2 folds). In both groups, the increase in transcript levels is significantly high, as calculated by one-way ANOVA (Figure 2). The levels of significance are indicated in Figure 2.

Next, the levels of TRIM transcripts were determined in naive patients who did not receive any medication and those who progressed toward HCC to see the behavior of TRIM proteins under various conditions. In most cases, the patients, usually non-responders (NR) to the provided treatment, ultimately progressed toward HCC. The highest increase was observed in TRIM14 (Figure 3A), with significantly higher values in both naïve (4.3-folds) and HCC (7.6-folds) patients, which reflects an association of TRIM14 with untreated viral infections. A similar pattern can be seen in the case of TRIM11, with a significant increase in HCC patients (2.5-fold). However, in naïve patients, the increase in TRIM11 mRNA transcript is non-significant (Figure 3B). Last, the analyses of TRIM25 levels revealed an utterly opposite pattern as that of TRIM11, with a significant increase in naïve

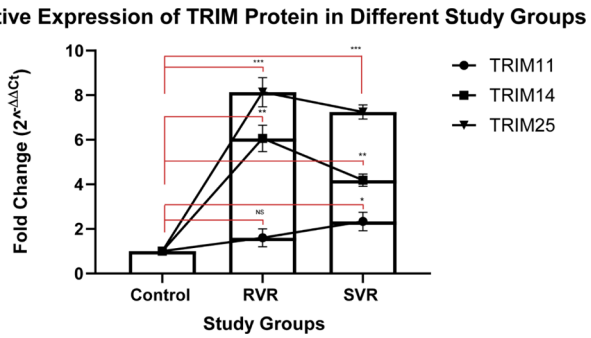


Figure 2. Relative expression of TRIM protein (fold change) is shown in three different study groups, i.e., SVR (n = 100), RVR (n = 61), and control (n = 23), whose expression is taken as 1. Error bars represent the standard error of the mean, level of significance was calculated by ordinary one-way ANOVA. * Shows the level of significance. Ns means non-significant.

individuals (3.4-folds) but a non-significant difference in HCC patients (Figure 3C), showing the varied behavior of different TRIM proteins under different stages of infection.

3.3. TRIM mRNA Transcripts Showed a Linear Correlation with Viral Loads of HCV. The expression analyses of all the three TRIM proteins showed a linear increase in mRNA transcripts as compared to the viral loads. In all the patients (divided into different study groups), the relative expression of TRIM mRNA transcripts was detected at different time points throughout the study. With few exceptions at specific points, all TRIMs under study are directly proportional to the increase in viral loads as the patients advance in infection (Figure 4). The highest increase can be seen in TRIM14, with an overall elevation of ∼13-fold following a viral infection. TRIM11 and TRIM25 also showed a significant linear increase in the antiviral activity.

3.4. Comparison of Viral Load among Different Study Groups. Next, the viral loads were compared among different study groups to see the relevancy of treatment outcome. The results showed varying amounts of viral loads in RVR, SVR, and HCC. Figure 5 shows that TRIM14 followed an opposite pattern compared to TRIM11 and TRIM25 where the viral load is highest in the RVR group, followed by SVR and HCC. These results contrast the other two TRIM proteins, where the viral load is significantly lesser in RVR while increasing progressively in SVR and HCC patients.

Table 3. Clinical and Demographic Parameters of HCV Patients Are Given among Different Groups[a]

groups	ALT (IU/L)[b]	AST (IU/L)[c]	ALP (IU/L)[d]	TLC (/mL)[e]	platelet count (/μL)[f]	HB (g/dL)[g]	BLB (mg/dL)[h]	viral load	age
naive	73.8	77.9	244	8374	281749.5	M = 14.5	0.73	9787596	47
						F = 13.8			
SVR	54.9	49.2	233	7199	213718.9	M = 14.1	0.67	3406197	43
						F = 12.7			
RVR	46.8	44.1	218	7177	248544.4	M = 13.9	0.67	7954063	45
						F = 12.8			
HCC	62.9	72.5	222	7845	195237.5	M = 12.9	0.69	2860853	53
						F = 10.3			
healthy controls	54.3	52.5	153	94700	380636.5	M = 15.8	0.75	1664809	42
						F = 12.2			

[a]The table shows the mean values of different assessed parameters. [b]ALT = alanine transaminase (normal range = 7−55 U/L). [c]AST = aspartate transaminase (normal range = 8−48 U/L). [d]ALP = alkaline phosphatase (normal range = 44−147 U/L). [e]TLC = total leukocyte count (normal range = 4000−11,000/μL). [f]Platelet count − (normal range = 150,000−450,000/μL). [g]HB = hemoglobin (normal range = M (male), 13.5−17.5 g/dL. F (female), 12.0−15.5 g/dL). [h]BLB = bilirubin (normal range = 0.1−1.2 mg/dL).

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ACS Omega 2023, 8, 25370−25377

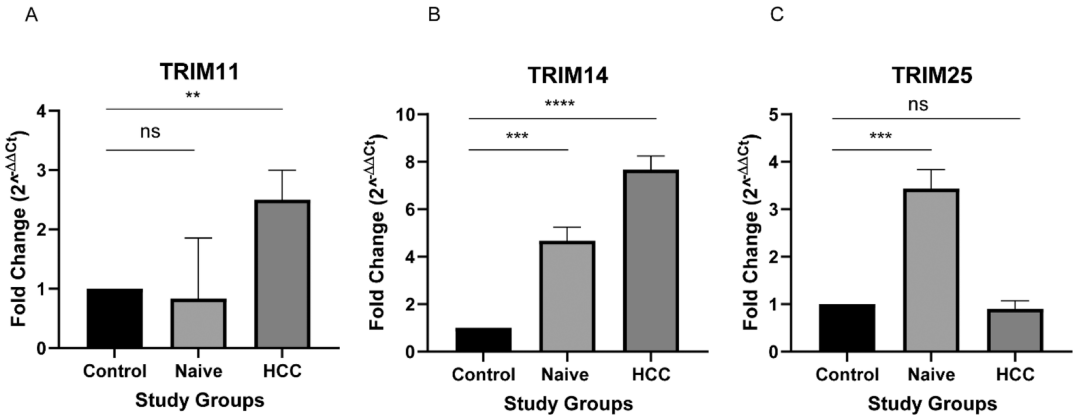

Figure 3. Expression analysis of TRIM proteins in naïve vs HCC patients. Analysis of mRNA transcript levels in naïve (n = 37) vs HCC (n = 21) patients as compared to the control group (n = 23) for (A) TRIM11, (B) TRIM14, and (C) TRIM25. Ordinary one-way ANOVA determined the level of significance. * indicates significant p-values, and "ns" indicates non-significant changes.

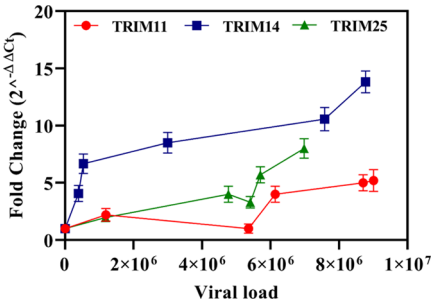

Figure 4. Correlation of TRIM proteins expression levels is shown with viral loads. The expression was analyzed at different time points and documented along with the viral loads at respective time points. The highest elevation is observed in TRIM14, followed by TRIM25. Each dot point represents an average value from all the patients recruited during the study (n = 182).

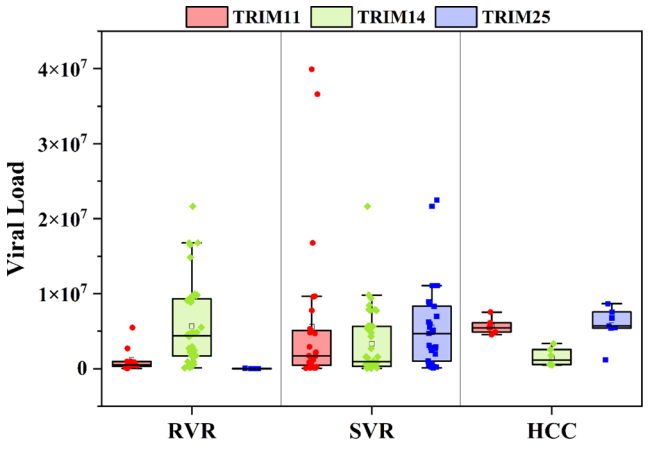

Figure 5. Comparative analysis of viral loads in different study groups. The viral loads have been detected and documented in RVR (n = 61), SVR (n = 100), and HCC (n = 21) for all the three of the TRIM mRNA transcripts. The RVR group showed highest viral loads and highest levels of TRIM14 mRNA expression as opposed to the other groups.

4. DISCUSSION

HCV is considered as a significant target for clinical and research areas due to its persistent infection and lethal consequences. Annually, several studies are reported, which focus on new dimensions to cure HCV and eradicate it globally. In the Pakistani population, HCV is spreading rapidly and is a significant health concern.[43] Interferon therapy was majorly used to treat HCV until 2011, but due to the high relapse rates and other side effects, interferon therapy has been replaced with DAAs.[44]

This study focuses on TRIM mRNA expression variations observed in four study groups, including SVR, RVR, naïve individuals, and HCC patients. The comparison of mRNA transcript levels in these groups showed high variation in comparison to one another as well as to healthy controls.

The results clearly indicate that significantly high expression levels of TRIM transcripts are associated with different study groups entailing patients on different medication regimes. One of the aims is to find the association of TRIM mRNA levels in sustained virologic responders (SVR) and rapid virologic responders (RVR) in order to understand the level of involvement of each TRIM mRNA in achieving rapid clearance (as can be seen in the RVR group).

TRIM11 showed slightly elevated levels in both RVR and SVR. However, the elevation is limited and insignificant, indicating the minimal role in viral clearance in both the study groups and naïve individuals. On the other hand, TRIM11 showed significantly elevated levels in HCC individuals that reflects an association with the progression of HCC. These results are in agreement with a previously reported study showing the oncogenic effect of TRIM11 in HCC.[45]

TRIM14 showed significantly elevated levels in all the study groups as compared to the healthy controls. Elevated levels of TRIM14 in RVR compared to SVR may indicate an association with rapid viral clearance, hinting toward the active participation of TRIM14 in combating the virus in less time and decreasing its expression while progressing toward HCC. This is also evident by the direct correlation of TRIM14 mRNA transcripts with viral loads. Similarly, naïve individuals also showed a significant increase in TRIM14 transcripts, indicating its antiviral role even in untreated individuals. Similar results are also observed in multiple previous studies, e.g., an in vitro study suggested the high levels of TRIM14 in cell lines under HCV infection and the potent inhibition of infection, which supports our study and opens new avenues and future perspectives for the use of TRIM14 as a potential biotherapeutic. The overall results showed that TRIM14 is a potential ISG that could limit the infection caused by HCV by

https://doi.org/10.1021/acsomega.3c02800
ACS Omega 2023, 8, 25370−25377

inhibiting NS5A.[15] The antiviral properties of TRIM14 are not only limited to HCV, but TRIM14 can also inhibit multiple viral infections, e.g., influenza A virus[46] and hepatitis B virus.[23] In the case of TRIM25, the results are similar to TRIM14, as the elevated expression can be seen in the RVR group associated with protective effect and rapid clearance against HCV. However, unlike both TRIM11 and TRIM14, TRIM25 does not show elevated levels in HCC patients, indicating its absence from the process of HCC progression. TRIM25 also exhibits antiviral effect against other numerous viruses.[24,36,47]

The data obtained from this study could potentially be used for diagnostic purpose by analyzing negative and positive correlations. Out of the three TRIM protein studies herein, TRIM14 could serve as a most potent biomarker as it shows direct relation with viral load and inverse relation in HCC progression. Apart from the TRIM proteins discussed in this study, many other TRIM proteins are involved in viral clearance. For instance, higher levels of TRIM22 transcript have been observed in HCV infection, while TRIM27 also positively regulates viral mRNA.[48]

5. CONCLUSIONS

The present study showed significant variation in TRIM mRNA expression among four groups, including RVR, SVR, HCC, and healthy controls. The expression of TRIM mRNA in rapid virologic responders is substantially higher as compared to other groups, indicating the antiviral properties of TRIM proteins. Of the three proteins studied herein, TRIM11 does not impart antiviral properties against HCV; instead, it is involved in HCC progression. TRIM14, on the other hand, imparts its role against HCV but is also involved in HCC progression incidentally. Last, TRIM25 imparts its antiviral role, and its significantly lower levels in HCC patients make it the most suited antiviral protein. The results indicate TRIM proteins as potential biotherapeutics owing to their unique antiviral properties and as biomarkers in HCC progression.

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A.A., A.K., and A.A.-H. contributed to conceptualization; R.K. and S.B. contributed to methodology; S.R. and S.A.H. contributed to software; M.I. and M.W. contributed to formal analysis; M.H.A. contributed to investigation; A.A., A.K., and A.A.-H. contributed to resources; J.U. contributed to data curation; F.A. and A.A. contributed to writing—original draft preparation; S.A.H., M.W., and A.K. contributed to writing—review and editing; M.H.A. contributed to visualization; A.A., A.K., and A.A.-H. contributed to supervision; and J.U. and A.A.-H. contributed to funding acquisition.

Funding

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through the Large Groups Project under grant number (RGP. 2/100/44).

Notes

The authors declare no competing financial interest.

The study was approved by the Institutional Ethics Committee of Hazara University Mansehra, 21120, Pakistan (HU/ORIC/2022/1091), for studies involving humans.

Informed consent was obtained from all subjects involved in the study.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Khalid University for funding this work through the Large Groups Project under grant number RGP. 2/100/44.

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