



# TGF- $\beta$ 1 gene – 509C>T promoter polymorphism modulates TGF- $\beta$ 1 levels in hepatitis E patients



Sanjay B. Rathod, Anuradha S. Tripathy \*

Hepatitis Group, National Institute of Virology, Pune, India

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## ABSTRACT

Elevated levels of transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) and its positive correlation with Foxp3 expression in hepatitis E patients have indicated involvement of TGF- $\beta$ 1 in hepatitis E pathogenesis. The current study determined polymorphisms in TGF- $\beta$ 1 gene, plasma TGF- $\beta$ 1 levels and T effector (Teff) cell proliferation and explored their association in a case control study. Polymorphisms in three selected sites (–509C>T, +869T>C and +915G>C) of TGF- $\beta$ 1 gene by PCR & restriction fragment length polymorphism methods, plasma TGF- $\beta$ 1 quantitation by ELISA and Teff (CD4+CD25–) cell proliferation by CFSE method were carried out in 277 hepatitis E patients (HE) with self-limiting infection and 233 ethnically matched healthy controls (HCs) from western India. Frequency of CT genotype of –509C>T site was significantly higher in hepatitis E patients compared to healthy controls ( $p = 0.017$ ; OR 1.53, 95% CI 1.07–2.17). Plasma TGF- $\beta$ 1 levels were significantly higher in HE compared to HCs. TGF- $\beta$ 1 level of patient group having CT genotype of –509C>T site was significantly higher compared to those having CC or TT genotypes. Teff cell proliferation was negatively correlated with plasma TGF- $\beta$ 1 levels in HE patients ( $r = -0.568$ ;  $p = 0.014$ ). Influence of TGF- $\beta$ 1 promoter (–509C>T) polymorphism on plasma TGF- $\beta$ 1 levels and inverse correlation of Teff cell proliferation with plasma TGF- $\beta$ 1 levels in self-limiting hepatitis E patients suggest key role of TGF- $\beta$ 1 in augmentation of reported T regulatory cell mediated pathogenesis in hepatitis E.

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## 1. Introduction

Hepatitis E, an epidemic as well as sporadic disease, prevalent in the developing countries is mostly characterized by a self-limiting course and a severe course, i.e. fulminant hepatitis with high mortality, in pregnant women (Taneja et al., 2009; Navaneethan et al., 2008). Pathogenesis of self-limiting hepatitis E is partially explored, while that of fulminant hepatitis is poorly understood (Saravanabala et al., 2008; Jilani et al., 2007; Kar et al., 2008). Since, a large number of HEV-infected pregnant women also clear the infection, it is important to understand the factors determining differential outcomes of HEV infection. Understanding the crucial role of host factors, in the rapid progression to fulminant hepatitis and in recovery from infection are important for (a) development of novel immune-based treatment strategies and for (b) identification of prognostic markers. Cytokines/chemokines in relation to their

immunological role and pathological events have been studied broadly in different viral hepatitis (Sheron et al., 1991; Smith, 1996; Turner et al., 1997). Plasma TGF- $\beta$ 1, a key cytokine, is reported to be significantly elevated in acute hepatitis E patients and in recovered individuals from hepatitis E infection. Restoration of T effector cell suppression by blocking TGF- $\beta$ 1 (unpublished data), further suggests TGF- $\beta$ 1 to be one of the mediators behind the self-limiting course of hepatitis E infection.

TGF- $\beta$ 1, a cytokine produced by both immune and non-immune cells, exhibits a broad range of functions including tissue repair and immune response (Smith, 1996). Polymorphisms in cytokine genes affect gene transcription and cause inter-individual variations in cytokine production (Turner et al., 1997).

The TGF- $\beta$ 1 gene is located on chromosome 19q13 (Lawrence, 1996; Clark and Coker, 1998). Seven genetic polymorphisms of TGF- $\beta$ 1 have been identified; 3 in the upstream region at –988, –800 and –509; 1 in a non-translated region at +72; 2 in the signal peptide sequence of exon one at codon 10 (+869C>T) and 25 (+915G>C); and 1 in the protein coding region at codon 629 (Awad et al., 1998). It is proposed that the TGF- $\beta$ 1 production is under genetic control (Grainger et al., 1999; Shah et al., 2006). TGF- $\beta$ 1 gene polymorphisms at codon 10 and 25 regulate the TGF- $\beta$ 1 production in vivo and in vitro (Blobe et al., 2000; Lueddecking et al., 2000; Meng et al., 2005). The –509C>T is located

**Abbreviations:** HEV, hepatitis E virus; ALT, alanine transaminase; PBMCs, peripheral blood mononuclear cells; HCV, hepatitis C virus; HBV, hepatitis B virus; TGF- $\beta$ 1, transforming growth factor beta 1; RFLP, restriction fragment length polymorphism; Teff, T effector cells; Treg, T regulatory cells; HE, hepatitis E patients; HCs, healthy controls; OR, odds ratio; CI, confidence interval.

\* Corresponding author at: Hepatitis Group, National Institute of Virology, 130/1, Sus Road, Pashan, Pune, Maharashtra 411021, India.

E-mail address: [anuradhastripathy@hotmail.com](mailto:anuradhastripathy@hotmail.com) (A.S. Tripathy).

within an YY1 consensus binding site and –509T allele has been associated with increased TGF- $\beta$ 1 plasma level (Shah et al., 2006; Lueddecking et al., 2000) and reduced T cell proliferation (Meng et al., 2005).

Our group has previously shown polymorphism in TNF- $\alpha$  to be associated with susceptibility and polymorphism in IFN- $\gamma$  to be associated with the clinical outcome of hepatitis E infection (Mishra and Arankalle, 2011). In a similar line, Devi et al. have shown association of TNF- $\alpha$  –308 AA, IFN- $\gamma$  +874 AA, TGF- $\beta$ 1 codon 10 +869 TT & codon 25 GG genotypes with preterm delivery, TNF- $\alpha$  –308 GG & IL-6 –174 CC genotypes with low birth weight, IL-6 –174 CC genotype with fetal loss and IL-6 –174 CC & TGF- $\beta$ 1 codon 10 +869 TC genotypes with small-for-dates in HEV infected pregnant women compared to uninfected controls (Devi et al., 2014).

In the absence of data on the association of specific mutations in the TGF- $\beta$ 1 gene with a given pattern of secretion of the same, in the current study, we have estimated plasma TGF- $\beta$ 1 levels, genotyped the SNPs in the TGF- $\beta$ 1 gene, measured T effector (Teff) cell proliferation and have evaluated their correlation in a Western Indian populations of hepatitis E patients and matched healthy controls.

## 2. Subjects & methods

### 2.1. Ethics statement

All procedures performed in current study involving human participants were in accordance with the ethical standards of the Institutional Ethical Committee for Research on Humans.

### 2.2. Informed consent

Written informed consents were taken from all individual participants included in the study.

### 2.3. Study population

A total of 498 study subjects including 277 hepatitis E patients (168 males and 109 females) (mean age  $\pm$  standard deviation;  $29.8 \pm 12.7$ ) and sex, age and ethnicity matched healthy controls, 221 (126 males and 95 females) (mean age  $\pm$  standard deviation;  $31.4 \pm 9.74$ ) were enrolled in the current study. The patients as well as controls enrolled were from Western India.

### 2.4. Serology

Hepatitis E diagnosis was based on the presence of IgM antibodies to HEV (IgM-anti-HEV) by ELISA (Arankalle et al., 2007). The control group consisted of age and sex-matched apparently healthy individuals. All the study subjects were screened for IgG and IgM-anti-HEV antibodies, IgM-anti-HAV antibodies, HBsAg, IgM-anti-HBc, anti-HCV and anti-HIV antibodies (ELISA, Abbott, USA). The patients were negative for the serological markers for HAV, HBV, HCV and HIV, while the controls were negative for these markers as well as for IgM and IgG anti-HEV antibodies. The acute patients were confirmed to be positive for serum anti-HEV IgM antibody and had elevated ALT levels. The recovered individuals having a recent history of acute hepatitis E had normalized ALT levels, positive for anti-HEV IgG antibody and were positive/negative for anti-HEV IgM antibody. Together, the patient group is referred as HE. Exclusion criteria for study group included infection with viral co-infections, past history of pre-existing liver disease and any other associated diseases.

### 2.5. Genotyping of TGF- $\beta$ 1 polymorphism

#### 2.5.1. DNA extraction

Genomic DNA was extracted from blood/PBMCs using QIAamp DNA Blood Mini extraction kit (Qiagen, Germany). DNA was quantified by

spectrophotometer (ND-1000, Nanodrop Technologies) and the quality was assessed by running on an agarose gel.

#### 2.5.2. Genetic analysis

Genotyping of the 3 SNPs in the TGF- $\beta$ 1 gene (–509C>T, +869T>C and +915G>C) reported to be associated with alteration in plasma TGF- $\beta$ 1 levels (Fig. 1a) was performed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (Romani et al., 2011).

#### 2.5.3. PCR amplification

The PCR conditions for +869T>C and +915G>C SNPs were as follows: 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 58.4 °C for 30 s, and 72 °C for 30 s, with an extension at 72 °C for 10 min. The PCR conditions for the –509C>T SNP were as follows: 95 °C for 5 min, 30 cycles of 95 °C for 30 s, 62 °C for 45 s, and 72 °C for 30 s, with an extension at 72 °C for 10 min.

For genotyping of –509C>T SNP, the PCR product was digested using the Eco81I restriction enzyme (RE) (New England Biolabs, USA). For genotyping of +869T>C and +915G>C SNPs, a pair of indigenously designed primers that amplified both polymorphic sites were used. This PCR product was used as a template for two RFLP reactions with MspA1I for +869T>C and BglI for +915G>C (New England Biolabs, USA). The primer sequences, RE, digestion sites and fragments for the detection of each SNPs are depicted in Table 1. Amplification of 100 ng (5  $\mu$ L) of template DNA for each of the 2 PCR reactions was performed using a total volume of 25  $\mu$ L containing final concentration of forward and reverse primers 10 pmol (2.5  $\mu$ L each), 1.25 U of Taq polymerase (2.5  $\mu$ L) (Applied Biosystems, USA), 200  $\mu$ M of each dNTP (2.5  $\mu$ L) (Fermentas, Latvia), 37 mM of MgCl<sub>2</sub> (2.5  $\mu$ L), dimethyl sulfoxide (1.25  $\mu$ L) (Sigma Aldrich, Germany), and distilled water (6.25  $\mu$ L).

#### 2.5.4. Restriction enzyme digestion

10  $\mu$ L of the PCR amplification product for studied SNPs +869T>C, +915G>C and –509C>T were digested with MspA1I, BglI and Eco81I RE respectively in a 20  $\mu$ L volume mixture containing 2  $\mu$ L CutSmart Buffer for MspA1I, BglI and 3.1 buffer for Eco81I and 1  $\mu$ L RE and 7  $\mu$ L Milli Q water. The reaction mixture was incubated at 37 °C for 45 min. The RFLP products for +915G>C and –509C>T SNPs were run on a 2.5% agarose gel electrophoresis at 100 mV for 1 h in 100 mL TAE buffer, and RFLP products for +869T>C were separated and run by using 16% polyacrylamide gel electrophoresis at 80 mV for 5 h in 100 mL TBE buffer. The size of the restriction fragments for three RFLP reactions products were determined using 100 bp DNA ladder (Invitrogen, USA).

### 2.6. Direct DNA sequencing

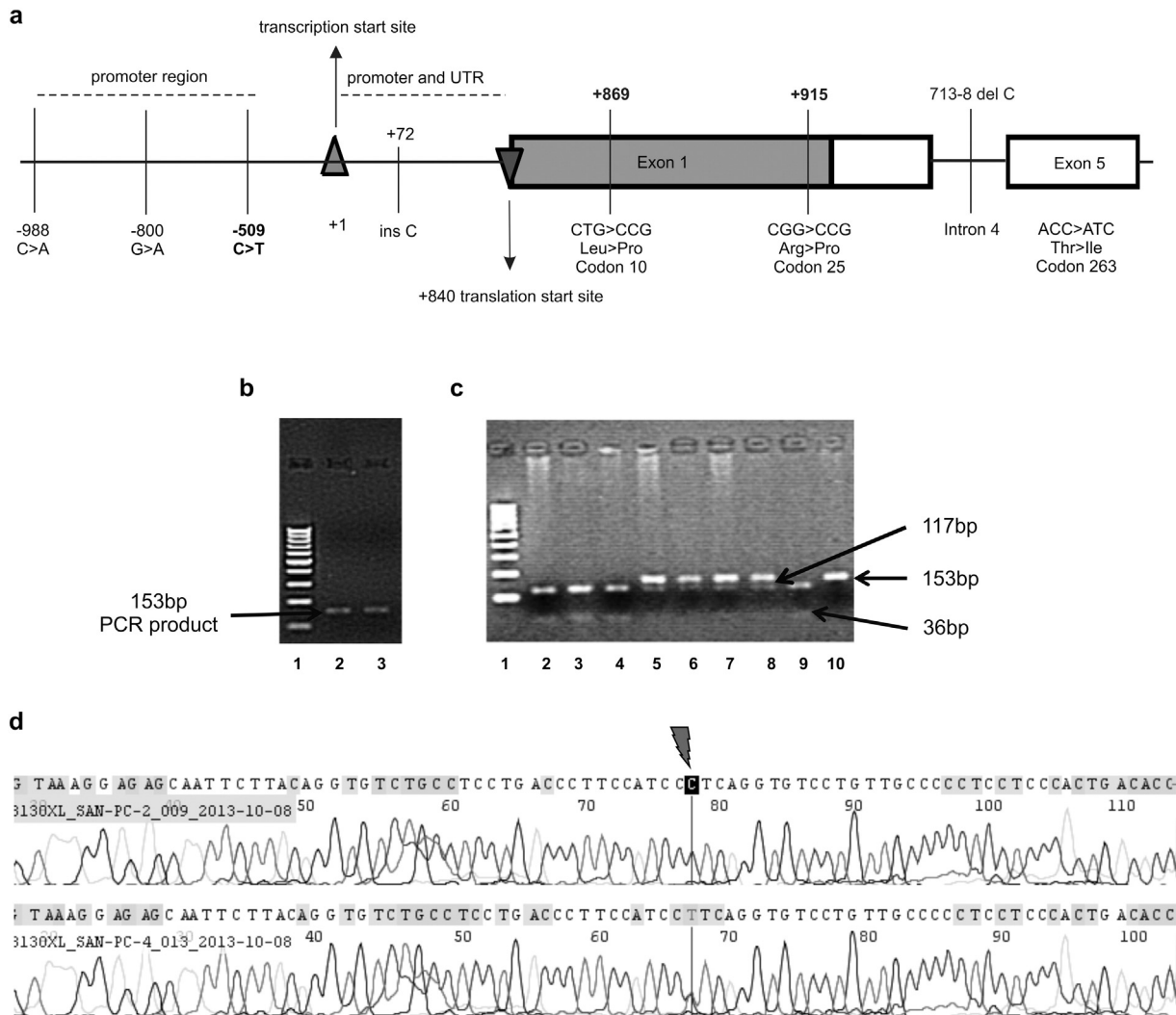
To confirm the RFLP genotyping results, 20% of the samples were genotyped to match the product sequence with the established TGF- $\beta$ 1 sequence. The PCR product was purified using MinElute PCR Purification Kit and both the strands were sequenced using Big Dye Terminator cycle sequencing Ready Reaction Kit (ver. 3.1; Life Technologies, USA) and automatic sequencer (ABI 3130 XL, USA) (Fig. 1b–d).

### 2.7. TGF- $\beta$ 1 quantitation

Using standard ELISA kits (R&D Systems, USA) and following the manufacturer's instructions, TGF- $\beta$ 1 plasma levels were estimated in duplicate from HE patients ( $n = 95$ ) and HCs ( $n = 69$ ).

### 2.8. Teff cell proliferation assay

Isolation of Teff cells and CFSE (carboxyfluorescein succinimidyl ester) (Invitrogen, USA) based proliferation was carried out in 18 acute hepatitis E patients as previously described by Rathod et al. (2014).



**Fig. 1.** Polymorphism sites, PCR/RFLP product and sequence of  $-509C>T$  polymorphism in the TGF- $\beta$ 1 gene. (a) Analyzed polymorphism sites are indicated in bold letters. +1 – first major transcription start point; +840 – translation start point; the shaded region of exon 1 encodes the signal peptide sequence (codons 1 to 29). Allelic substitutions were found 20 at positions  $-988C>A$ ,  $-800G>A$ ,  $-509C>T$ ,  $+869T>C$ , and  $+915G>C$ . Allelic insertions were found at positions +72C and 263C>T. (b) Representative PCR product (153 bp) of the TGF- $\beta$ 1 gene ( $-509C>T$  polymorphism). (c) EcoRII cut PCR product and representative RFLP pattern of  $-509C>T$  shown on 2.5% agarose gel. Lane 1: 100 bp ladder. Lanes 2–4 & 9 (CC homozygote; two bands: 117 bp, 36 bp), lanes 5–8 (CT heterozygotes; three bands: 153 bp, 117 bp, and 36 bp) and lane 10 (TT homozygote; single band: 153 bp). (d) Sequence of  $-509C>T$  site.

### 2.9. Calculation of gene frequencies and statistical analysis

Allele and genotype frequencies were calculated by direct counting. Chi square test was used for comparison of allele and genotype frequencies between different study groups. *p* values, odds ratio (OR) with 95% confidence limits (CI) were calculated using MedCalc software. Statistically significance between groups was further assessed using ANOVA with Bonferroni post-hoc corrections. Statistical analyses were performed with SPSS 20 software (SPSS Inc., USA).

### 3. Results

#### 3.1. Position $-509C>T$

Seventy three (26.35%) of HE and 68 (29.18%) of HCs were homozygote CC, 153 (55.23%) of HE and 104 (44.63%) of HCs were heterozygote CT, whereas homozygote TT was found in 51 (18.41%) of HE and 49 (21.03%) of HCs at this position. The frequency of only CT genotype was significantly higher in HE group

**Table 1**  
RFLP conditions for studied genotypes.

Polymorphism site in TGF $\beta$ <sub>1</sub> gene	Restriction enzyme	Primer sequences	Restriction site	Genotype	Restricted fragments, base pairs size
$-509C>T$ (promoter)	Eco811	F: 5-CAGTAAATGTATGGGGTCCGAG-3 R: 5-GGTGTCAGTGGGAGGAGGG-3	5'..CC▼TNAGG..3' 3'..GGANT▲CC..5'	TT CT CC	153 153, 117, 36 117, 36
+869C>T (codon 10)	MspA11	F: 5-GTTATTTCCTGGGATACTGAGAC-3 R: 5-GACCTCCTTGGCGTAGTAGTCG-3	5'..CMG▼CKG..3' 3'..GKC▲GMC..5'	CC CT TT	12, 40, 67, 108, 230 12, 40, 67, 108, 230, 242 40, 67, 108, 242
+915G>C (codon 25)	BglI	As codon 10	5'..GCCNNNN▼NGGC..3' 3'..CGGN▲NNNNCCG..5'	GG GC CC	212, 252, 60 212, 252, 312 212, 312

compared to the HC group ( $p = 0.017$ ; OR 1.53; 95% CI 1.07–2.17) (Table 2).

### 3.2. Position +869T>C

Eighty three (29.96%) of HE, and 67 (28.75%) of HCs were homozygote CC, 142 (51.26%) of HE and 111 (47.63%) of HCs were heterozygote CT and homozygote TT was found in 52 (18.77%) of HE and 43 (18.45%) of HCs at this position. No significant difference in the genotype distribution and allele frequency was found (Table 2).

### 3.3. Position +915G>C

Eighty seven (31.40%) of HE and 72 (30.90%) of HCs were homozygote GG, 145 (52.34%) of HE and 110 (47.21%) of HCs were heterozygote GC and homozygote CC was found in 45 (16.24%) of HE and 39 (16.73%) of HCs at this position. No significant difference in the genotype distribution and allele frequency was found (Table 2).

### 3.4. Plasma TGF- $\beta$ 1 levels

Irrespective of the genotype, plasma TGF- $\beta$ 1 level in the HE group was significantly higher than HCs ( $42.74 \pm 22.57$  pg/mL vs.  $11.74 \pm 8.10$  pg/mL;  $p = 0.0001$ ) (Fig. 2a). At  $-509C>T$  site, in HE group plasma TGF- $\beta$ 1 level was significantly higher in CT genotype compared to homozygous CC ( $p = 0.0003$ ) or TT ( $p = 0.013$ ) genotypes, while the same was comparable in HCs (Fig. 2b). At  $+869T>C$  and  $+915G>C$  SNPs no significant difference was observed among the genotypes with respect to plasma TGF- $\beta$ 1 level (Fig. 2c–d).

### 3.5. Spearman correlation analysis

In a set of 18 acute hepatitis E patients, concentration of plasma TGF- $\beta$ 1 was correlated with their Teff cell proliferation. A negative association between plasma TGF- $\beta$ 1 level and Teff cell proliferation was observed ( $r = -0.568$ ,  $p = 0.014$ ) (Fig. 2e).

## 4. Discussion

Participation of TGF- $\beta$ 1 with Treg cells to mediate immunosuppression, its function in maintaining peripheral tolerance and in restoration

of suppressive activity of Treg cells are established phenomena (Nakamura et al., 2004; Bommireddy and Doetschman, 2007). Increased protein level of TGF- $\beta$ 1 in hepatitis E patients, elevation of TGF- $\beta$ 1 on both unstimulated and HEV antigen stimulated PBMCs of acute hepatitis E patients and recovered individuals at both phenotypic and gene expression levels have suggested TGF- $\beta$ 1 to be a pivotal cytokine in the pathogenesis of hepatitis E (Rathod and Tripathy, 2014; Rathod et al., 2014; Gewaltig et al., 2002).

An association of SNPs in the TGF- $\beta$ 1 gene  $-509C>T$  with rate of progression of HCV-induced fibrosis has been reported in a study from Germany (Gewaltig et al., 2002). In an Italian study, Falletti et al. have shown CT and TT genotypes of TGF- $\beta$ 1  $-509C>T$  to be higher in cirrhotic group than control group indicating its detrimental association (Falletti et al., 2008). Egyptian study by Hanafy and Abdo reporting different genotype and allele distributions of TGF- $\beta$ 1  $-509C>T$  site in chronic HCV patients and controls and similar scenario again on Taiwanese HCV patients by Dai et al. further established involvement of this genotype in hepatitis C (Hanafy and Abdo, 2011; Dai et al., 2008). In a different tune, Kimura et al. have demonstrated  $-509C$  allele to be associated with a higher clearance rate of HCV as well as with lower promoter activity (Kimura et al., 2006). However, in the contest of hepatitis B and Iranian patients, absence of association between TGF- $\beta$ 1  $-509C>T$  and  $+915G>C$  polymorphisms with chronic hepatitis B indicated that these changes do not play a significant role in increasing the risk of chronic infection in Iranian patients (Razavi et al., 2014; Romani et al., 2011). Our finding of TGF- $\beta$ 1 polymorphism at position  $-509$  with increase in  $C>T$  genotype in the patient group suggests its protective role, since all the patients recovered.

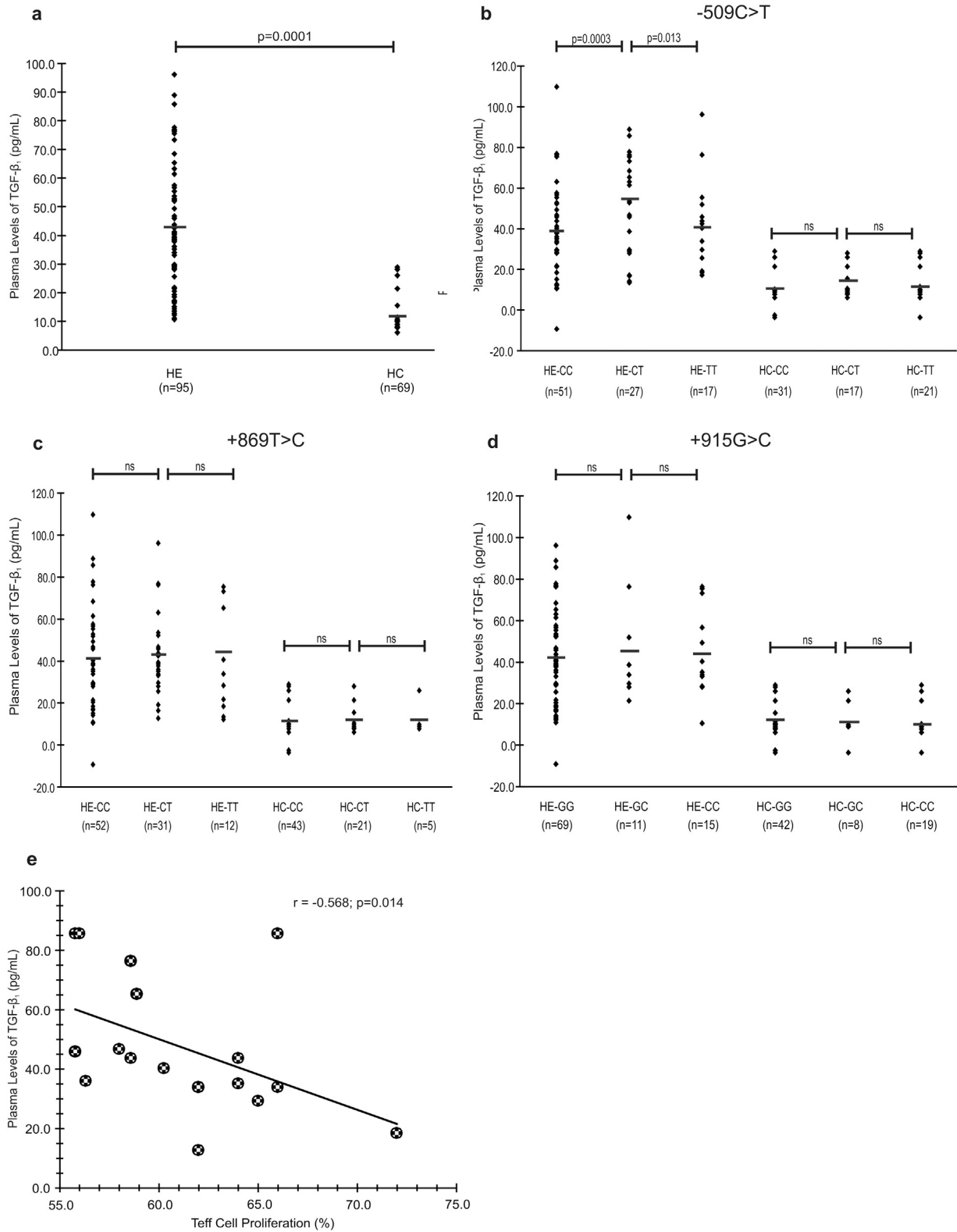
Changes in the production of TGF- $\beta$ 1 have been linked to numerous disease states. Elevated levels of circulating TGF- $\beta$ 1 in acute viral hepatitis and in its fulminant form have been demonstrated (Nakamura et al., 2000; Koulentaki et al., 2002). TGF- $\beta$ 1 is a central regulator in chronic liver disease contributing to all stages of disease progression from initial liver injury through inflammation and fibrosis to cirrhosis and HCC (Bissell, 2001). Serum levels of TGF- $\beta$ 1 are elevated in fibrosis and in patients with chronic HCV infection, viral core protein up-regulates TGF- $\beta$ 1 transcription exacerbating liver fibrosis progression (Falletti et al., 2008; Kopp et al., 1996; Taniguchi et al., 2004). Miwa et al. detected increased levels of plasma TGF- $\beta$ 1 in patients with fulminant viral hepatitis B (Miwa et al., 1997). Plasma TGF- $\beta$ 1 has been suggested as a useful serologic marker in the diagnosis of small HCCs (Song et al., 2002). Higher plasma TGF- $\beta$ 1 of the current hepatitis E patients needs further evaluation.

Elevated plasma TGF- $\beta$ 1 levels associated with the  $-509C>T$  polymorphism have been suggested to suppress immune activation and higher risk of allergy (Kim et al., 2007). Polymorphisms of TGF- $\beta$ 1 gene at  $-509C>T$  and  $869T>C$  are not related to plasma TGF- $\beta$ 1 levels and are not associated with increased susceptibility to development of chronic obstructive pulmonary disease from cigarette smoking in Hong Kong Chinese population (Mak et al., 2009). Report that  $-509C>T$  polymorphism is significantly associated with a high plasma concentration of TGF- $\beta$ 1 (Grainger et al., 1999) goes in parallel with our observation of higher TGF- $\beta$ 1 plasma level in  $-509C>T$  site of HE patients with a CT genotype compared to patients with either CC or TT genotypes.

Genotype distribution and allele frequencies at position  $+869T>C$  and  $+915G>C$  SNPs of the current study were not significantly different in the patient and control groups. However in HEV infected pregnant women, TGF- $\beta$ 1 cytokine polymorphism at  $+869TT$  &  $+915GG$  genotypes are reported to be associated with preterm delivery and TGF- $\beta$ 1  $+869TC$  genotype with small-for-dates compared to not infected with HEV and controls (Devi et al., 2014). Since, the same group has previously shown higher TGF- $\beta$ 1 levels in HEV infected pregnant women, carrying out polymorphism at  $-509C>T$  site might have yielded significant results (Kumar et al., 2014).

**Table 2**  
Genotype distribution and allele frequencies of studied TGF- $\beta$ 1 polymorphisms.

TGF- $\beta$ 1 genotype and alleles	HE (n = 277) %	HCs (n = 221) %	OR (95% CI)	p-Value
<i>Genotype: -509 (C&gt;T)</i>				
CC	73 (26.35)	68 (29.18)	0.80 (0.54–1.19)	0.27
CT	153 (55.23)	104 (44.63)	1.53 (1.07–2.17)	0.017
TT	51 (18.41)	49 (21.03)	0.79 (0.55–1.22)	0.29
<i>Allele</i>				
C	299 (61.78)	240 (59.26)	1.11 (0.84–1.45)	0.44
T	185 (38.22)	168 (40.72)	0.91 (0.68–1.17)	0.44
<i>Genotype: +869 (T&gt;C)</i>				
CC	83 (29.96)	67 (28.75)	0.98 (0.66–1.44)	0.93
CT	142 (51.26)	111 (47.63)	1.04 (0.73–1.44)	0.81
TT	52 (18.77)	43 (18.45)	0.95 (0.61–1.49)	0.84
<i>Allele</i>				
C	308 (61.72)	245 (60.79)	1.02 (0.78–1.33)	0.86
T	191 (38.28)	158 (39.21)	0.97 (0.74–1.27)	0.86
<i>Genotype: +915 (G&gt;C)</i>				
GG	87 (31.40)	72 (30.90)	0.94 (0.64–1.38)	0.78
GC	145 (52.34)	110 (47.21)	1.12 (0.78–1.60)	0.51
CC	45 (16.24)	39 (16.73)	0.90 (0.56–1.41)	0.67
<i>Allele</i>				
C	90 (22.56)	78 (23.49)	0.91 (0.65–1.29)	0.63
G	319 (79.95)	254 (76.51)	1.08 (0.77–1.53)	0.63



**Fig. 2.** Plasma TGF- $\beta_1$  levels stratified by TGF- $\beta_1$  SNP genotypes in HE and HCs. (a) Plasma TGF- $\beta_1$  levels in HE patients and HCs. (b) Plasma level of TGF- $\beta_1$  was significantly more in heterozygous CT individuals compared to homozygous CC or TT individuals of only HE patient group at position -509C>T. (c) No significant differences between homozygous or heterozygous genotypes at position +869T>C in HE patients and HCs. (d) No significant differences between homozygous or heterozygous genotypes at position +915G>C in HE patients and HCs. (e) Negative correlation between plasma TGF- $\beta_1$  levels with CD4 + CD25 – Teff cell proliferation in acute hepatitis E patients. Each dot represents an individual data point and the horizontal lines represent the mean. Data are representative of mean  $\pm$  SD.

Suppressive activity of the Treg cells towards Teff cells in hepatitis E patients could be attributed to TGF- $\beta_1$ , known to promote human peripheral Treg cells development (Nakamura et al., 2004). Negative

correlation of plasma TGF- $\beta_1$  levels with Teff cell proliferation in the acute patients of the current study confirms the suppressing effect of TGF- $\beta_1$  and goes in parallel with previously reported (Meng et al., 2005).

## 5. Conclusion

We identified (a) higher frequency of C to T base change at  $-509C>T$  site of TGF- $\beta$ 1 gene (b) higher plasma TGF- $\beta$ 1 levels in patients having CT genotype of  $-509C>T$  site and (c) negative correlation between Teff cell proliferation and plasma TGF- $\beta$ 1 levels indicating the importance of the interaction of three factors in self-limiting HEV infection. Future studies with severe fulminant form of hepatitis E patients will be needed to ratify the results of the present study.

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## Conflict of interest

No conflict of interest existed.

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