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Analysis of IFN-γ +874 T/A Gene Polymorphism and Serum IFN-γ Levels in Chronic Hepatitis B and Liver Cirrhosis Patients

(Analisis Polimorfisme Gen IFN-γ +874 T/A dan Tahap IFN-γ Serum pada Pesakit Hepatitis B Kronik dan Sirosis Hati)

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ABSTRACT

Interferon gamma (IFN-γ) plays a central role in antiviral immunity and immune-mediated liver injury in chronic hepatitis B virus (HBV) infection. Genetic polymorphism affecting IFN-γ production may influence disease susceptibility and progression. This study investigated the association between the IFN-γ +874 T/A polymorphism, serum IFN-γ levels, and clinical outcomes in patients with chronic hepatitis B (CHB) and liver cirrhosis (LC). A total of 125 patients were enrolled, including 88 with CHB and 37 with LC. IFN-γ +874 T/A polymorphism was analysed using PCR–RFLP, and serum IFN-γ levels were measured. Genotype and allele distributions were compared between groups, and genotype-specific differences in serum IFN-γ were evaluated. The AA genotype and A allele were the most prevalent in both CHB and LC groups, with no significant differences in genotype or allele distributions. In contrast, serum IFN-γ level were significantly higher in patients with LC compared with those with CHB (p=0.018). Stratified analysis showed no significant differences in IFN-γ levels among genotypes within each group; however, LC patients carrying the AA genotypes had significantly higher IFN-γ levels than AA carriers in the CHB group (p=0.047). In conclusion, elevated serum IFN-γ levels are associated with advanced liver disease in chronic HBV infection, whereas IFN-γ +874 T/A polymorphism alone does not independently predict progression from CHB to LC. These finding underscore the importance of disease-stage-dependent immune dysregulation HBV-related liver disease.

Keywords: Chronic hepatitis B; IFN-γ +874 T/A; liver cirrhosis; serum IFN-γ

ABSTRAK

Interferon gama (IFN-γ) memainkan peranan utama dalam imuniti antivirus dan kecederaan hepar yang dimediasi oleh sistem imun dalam jangkitan virus hepatitis B kronik (HBV). Polimorfisme genetik yang mempengaruhi penghasilan IFN-γ mungkin mempengaruhi kerentanan terhadap penyakit dan perkembangan penyakit. Kajian ini bertujuan untuk menilai hubungan antara polimorfisme IFN-γ +874 T/A, paras IFN-γ serum dan hasil klinikal dalam kalangan pesakit hepatitis B kronik (CHB) dan sirosis hati (LC). Seramai 125 pesakit telah direkrut, terdiri daripada 88 pesakit CHB dan 37 pesakit LC. Polimorfisme IFN-γ +874 T/A dianalisis menggunakan kaedah PCR-RFLP, manakala paras IFN-γ serum diukur. Taburan genotip dan alel dibandingkan antara kumpulan dan perbezaan paras IFN-γ serum mengikut genotip turut dinilai. Genotip AA dan alel A merupakan yang paling kerap ditemui dalam kedua-dua kumpulan CHB dan LC tanpa perbezaan signifikan dalam taburan genotip atau alel. Sebaliknya, paras IFN-γ serum adalah lebih tinggi secara signifikan dalam kalangan pesakit LC berbanding CHB (p=0.018). Analisis berstrata menunjukkan tiada perbezaan signifikan paras IFN-γ antara genotip dalam setiap kumpulan. Namun, pesakit LC yang membawa genotip AA mempunyai paras IFN-γ yang lebih tinggi signifikan berbanding pembawa genotip AA dalam kumpulan CHB (p=0.047). Kesimpulannya, peningkatan paras IFN-γ serum berkaitan dengan penyakit hepar yang lebih lanjut dalam jangkitan HBV kronik, manakala polimorfime IFN-γ +874 T/A secara bersendirian tidak meramalkan perkembangan penyakit hati daripada CHB kepada sirosis hati. Penemuan ini menekankan kepentingan disregulasi imun yang bergantung pada peringkat penyakit dalam penyakit hepar berkaitan HBV.

Kata kunci: Hepatitis B kronik; IFN-γ +874 T/A; IFN-γ serum; sirosis hati

INTRODUCTION

Chronic hepatitis B virus (HBV) infection remains a major global health problem, affecting over 250 million individuals and causing approximately 900,000 deaths annually due to complications such as liver cirrhosis (LC) and hepatocellular carcinoma (HCC) (WHO 2024a). The clinical manifestations of HBV infection are highly heterogenous, ranging from asymptomatic carriage and acute self-limiting hepatitis to chronic hepatitis, fulminant hepatic failure, and progression to LC and HCC (Ribeiro et al. 2020). This variability in clinical outcomes arises from complex interactions among viral characteristics, environmental factors, and host genetic determinants (Zhang et al. 2019).

Among host-related factor, genetic polymorphisms, particularly in cytokine genes, have been implicated in the persistence of HBV, disease progression, and the development of complication such as liver cirrhosis. Several cytokine gene variants have been associated with virological parameters (HBV DNA levels), and non-invasive markers of liver injury, suggesting a significant role for genetically regulated cytokine responses in modulating HBV disease course (Adu et al. 2024). Intrahepatic cytokine and chemokine activity plays a central role in HBV immunopathogenesis, where chronic antigen exposure contributes to immune exhaustion and the development of a pro-fibrotic inflammatory microenvironment (Zhong et al. 2021). At the population level, meta-analytic evidence indicates that specific cytokine polymorphisms, including IFN-γ rs2430561, are associated with cirrhosis risk in certain contexts, highlighting the heterogeneous and ethnicity-dependent contributions of cytokine genetic variation to HBV progression (Zheng et al. 2023).

Among the various cytokine genes that have been investigated, the IFN-γ +874 T/A polymorphism has frequently been associated with differences in immune responses to HBV infection. Polymorphisms in the regulatory region and intronic region of the IFN-γ gene, particularly the +874 T/A variant, may influence IFN-γ expression in patients with acute infection and chronic carriers, thereby increasing the risk of progression to hepatitis B (Heidari, Moudi & Mahmoudzadeh-Sagheb 2020). The IFN-γ +874 T/A polymorphism (rs2430561) is located at a binding site for the transcription factor NF-κB and is known to affect IFN-γ expression, with the A allele or the AA genotype linked to lower cytokine production and the T allele associated with higher expression, potentially influencing the strength of antiviral immune responses (Hillaire, Lawrence & Lagrange 2023). Meta-analysis and haplotype studies suggest that IFN-γ +874 T/A variant, alone or in combination with other linked polymorphisms, is associated with increased susceptibility to chronic hepatitis B and liver cirrhosis, likely through its influence on IFN-γ expression and consequent modulation of fibrotic disease progression (Sun et al. 2015a).

Despite the importance of this polymorphism, data on the distribution and clinical implications of IFN-γ +874 T/A in the Indonesian population, particularly in West Nusa Tenggara (NTB), remain very limited. Moreover, similar studies from Southeast Asia are still scarce, particularly those integrating IFN-γ +874 T/A polymorphism with circulating IFN-γ levels across the spectrum of chronic hepatitis B and HBV-related cirrhosis. Given that genetic variation can differ across populations, understanding its role in HBV-related liver disease in this region is crucial. Therefore, this study aimed to investigate the association between the IFN-γ +874 T/A gene polymorphism and IFN-γ cytokine levels, and to assess its relationship with liver cirrhosis among patients with chronic hepatitis B.

MATERIALS AND METHODS

STUDY DESIGN AND EVALUATED POPULATION

A cross-sectional study was performed with the inclusion of consecutive patients with chronic hepatitis B. This study was conducted at the outpatient clinics of the Department of Internal Medicine, General Hospital of West Nusa Tenggara Province (Indonesia) from July 2024 to January 2025. Participant were adults aged ≥ 18 years with chronic hepatitis B who provided written informed consent to participate. Only patient who were conscious and clinically stable, without an emergency condition at the time of enrolment, were included. Patients diagnosed with hepatitis C virus and HIV were excluded from the study. The study protocol was approved by The Research Ethics Committee of General Hospital of West Nusa Tenggara Province with ethical clearance number 00.9.1/KEP/2024 in March 2024. Patients were classified into two groups based on the internist's diagnosis: chronic hepatitis B without liver cirrhosis (CHB) and chronic hepatitis B with liver cirrhosis (LC). CHB was defined as persistence HBsAg ≥ 6 months without clinical, laboratory, or imaging findings suggestive of LC. LC was diagnosed based on clinical, laboratory, imaging, and/or endoscopic evidence of cirrhosis or portal hypertension, in accordance with established guidelines (EASL 2017; Perhimpunan Peneliti Hati Indonesia 2021; WHO 2024b).

GENOTYPING OF IFN-γ +874T/A (rs2430561)

Host DNA was extracted from whole blood samples using *QIAamp® Blood Mini Kit* (Cat.51104, Qiagen, Inc., Germany) in accordance with the manufacture's-instructions. PCR was carried out on host genome using PCR MyTaq HS Red Mix (cat. No. 25047; Bioline, USA). The IFN-γ +874 A/T polymorphism was amplified using the following primers (Table 1). The PCR reaction mixture had a total volume of 20 µL, consisting of : 12.5 µL MyTaq HS™ Red Mix, 2.5 µL nuclease-free water (NFW), 1 µL of each primer, 3 µL DNA template. The PCR process was carried out under the following thermal cycling conditions: (1) initial denaturation at 94 °C for 5 min; (2) followed by 35 cycles of : denaturation at 94 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 30 s and final extension

at 72 °C for 5 min. The PCR product (176 bp) was digested with HinfI enzyme at 37 °C for 2 h. Restriction fragments (176 bp for allele A; 148 bp and 28 bp for allele T) were separated using 2 % agarose gel electrophoresis, stained with ethidium bromide (Redsafe™ Nucleic Acid Staining Solution, iNtRON), and run at 100V for 30 min. SNPs were identified via agarose gel electrophoresis analysis based on the presence of an SNP at position +874 on IFN-**γ** intron, a 176 bp band (A allele) is visible, while 148 and 28 bp fragments are identified for the corresponding wild type (T allele). The procedures were conducted at the Institute of Tropical Disease, Surabaya, Indonesia. To confirm the RFLP results, sequencing was performed on several representative samples.

SERUM IFN-γ MEASUREMENT

Peripheral venous blood (3 mL) was collected from the subject. Serum was isolated and stored at -80 ℃ for cytokine detection. Circulating levels of cytokine IFN-**γ** was measured using the ELISA method. HS Human IFN-**γ** (Interferon Gamma) (Cat No. E-HSEL-H0007, Elabscience®, United States). The wavelength at which absorbance may be detected was 450 nm. The procedures were conducted in the Central Laboratory Installation, Clinical Pathology Unit, Dr. Soetomo Hospital (Surabaya, Indonesia) according to the manufacturer's instructions.

STATISTICAL ANALYSIS

All collected data were analyzed using IBM Statistics version 26. Descriptive data presented as mean ± standard deviation (SD) and n (percentage). Data distribution normality was assessed using The Shapiro-Wilk test. Chi-square, Mann-Whitney U, and Kruskal-Wallis tests were applied to determine statistical significance depending on variable type. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

CHARACTERISTICS OF STUDY PARTICIPANTS

A total of 125 patients participated in this study and were classified into 88 CHB patients (70.4%) and 37 LC patients (29.6%). The study population consisted of 70 males (56 %) and 55 females (44 %). Although sex distribution did not differ significantly between groups (p=0.091). The LC cohort showed a clear trend toward male predominance (67.6% vs 51% in CHB). Notably, patients with LC were significantly older than those with CHB (50.65±10.88 vs 41.69 ±13.03 years; p<0.001), indicating that advanced disease status in this cohort clustered with older age, which may reflect cumulative infection duration and progressive liver injury (Table 2).

The significantly higher age observed in the LC groups is biologically plausible, as increasing age is consistently associated with a higher risk of fibrosis progression and cirrhosis among individuals with chronic hepatitis B, reflecting prolonged exposure to necro inflammatory processes and cumulative hepatic remodelling (Le et al. 2021). Although the male predominance in LC group was not statistically significant in the present cohort, this may relate to sample size and population heterogeneity. Notably, while male sex is frequently associated with worse HBV-related outcomes, this protective sex effect is not absolute; large registry data indicate that in older age groups (> 50 years), the female advantage in slower progression diminished, with a more rapid increase in cirrhosis prevalence among elderly women (Brown, Goulder & Matthews 2022). Globally, males remain at higher risk for chronic HBV infection and its complications, including a 2.5 fold increased risk of cirrhosis, a 3 to 6 fold increased risk of HCC, and a 1.8-fold increased risk of mortality. Mechanistically, elevated androgen activity in the context HBV infection enhanced viral transcription, creating a positive feedback loop that promotes viral replication and increases oncogenic potential (Cooper et al. 2023).

GENOTYPE AND ALLELE FREQUENCY DISTRIBUTIONS OF IFN-**γ** +874 T/A POLYMORPHISMS

The IFN-**γ** +874 T/A polymorphisms was identified using PCR-RFLP analysis. Figure 1 illustrate distinct banding patterns corresponding to AA, AT, and TT genotypes were observed following Hinfl digestion and were further confirmed by sequencing analysis (Figure 2).

The distribution of IFN-γ +874 T/A genotype and alleles in patients with chronic hepatitis B and liver cirrhosis is presented in Table 3. In addition, the overall distribution of IFN-γ +874 T/A genotypes showed a similar pattern between groups, with AA remaining the predominant genotype in both the CHB (45.5 %) and LC (54.1 %), followed by the AT and TT genotypes. Although AA appeared higher in LC and AT lower in LC compared with CHB, this differences did not reach statistical

TABLE 1. Primer sequence and method used for investigating the IFN-**γ** +874 T/A gene polymorphisms (Zambon et al. 2005)

Polymorphism	Method	Primer	Enzyme	Alleles
IFN-**γ** +874 T/A	RFLP	Forward: 5- GAT TTT ATT CTT ACA ACA CAA AAT CAA GAC-3’	Hinfl	A: 176 bp
		Reverse: 5- GCA AAG CCA CCC CAC TAT AA-3’		T: 148 bp, 28 bp

TABLE 2. Demographic data of study participant

Parameter	Total population	CHB	LC	p-value
Sex[a]				0.091
Male	70 (56 %)	45 (51.1 %)	25 (67.6 %)	
Female	55 (44 %)	43 (48.9 %)	12 (32.4 %)	
Age[b]	44.34±13.05	41.69 ±13.03	50.65±10.88	0.000*

[a]Chi-square test, the value is presented in n (%). [b]Independent T-test, the value is presented in mean ± SD. *p value <0.05 is significant

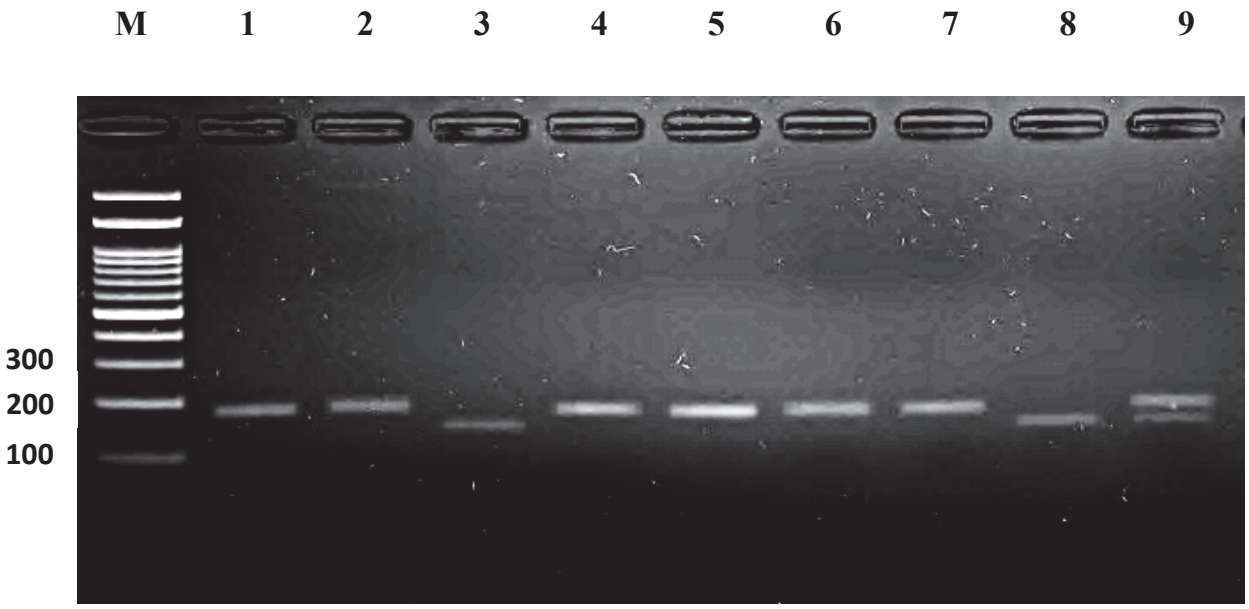

FIGURE 1. The electrophoresis pattern of IFN-**γ** +874 T/A gene polymorphisms. The digestion pattern of Hinfl restriction enzyme on 2 % agarose gel at +874 SNP. M, 100 bp DNA ladder with representative bands at 100, 200, 300 bp; genotype AA (176 bp) : lanes 1, 2, 4, 5, 6 and 7; genotype AT (176 and 148 bp) : lane 9; genotype TT (148 bp and 28 bp (not visible)): lanes 3 and 8

significance (p = 0.36). Similarly, allele frequencies were comparable between CHB and LC (p=0.76), with the A allele consistently more frequent than the T allele in both clinical groups.

ASSOCIATION BETWEEN IFN-**γ** +874 T/A POLYMORPHISMS AND HBV-RELATED DISEASES

In this study, the AA genotypes was the most frequently observed in both CHB group (45.5%) and LC groups (54.1%), and the A allele was more prevalent than the T allele in both groups, with no significant differences in IFN-γ +874 T/A genotype or allele distributions between CHB and LC patients (Table 3). These findings suggest that the IFN-γ +874 T/A polymorphism may not represent an independent genetic determinant of disease progression from chronic hepatitis B to liver cirrhosis in our cohort. This result is consistent with several previous studies reporting heterogeneous or null association between IFN-γ +874 T/A and HBV-related liver disease progression, suggesting that this SNP alone may be insufficient to explain the transition from CHB to cirrhosis despite the established role of interferon gamma in antiviral immunity. Differences in disease severity may instead be influenced by the regulation of cytokine expression or other immunological factors (Sun et al. 2015a). With regard to chronic hepatitis B itself, a study reported no association between the IFN-γ+874A/T polymorphisms and susceptibility to chronic HBV infection, supporting the concept that IFN-γ gene polymorphism are not reliable predictors of HBV susceptibility in The Egyptian population (Dondeti et al. 2022).

In contrast, other studies have demonstrated significant associations, showing that the T allele commonly linked to higher IFN-γ production, may influence HBV susceptibility or clinical outcomes in certain populations, underscoring that the direction and magnitude of genetic effects are population- and study design–dependent (Hillaire, Lawrence & Lagrange 2023; Li 2020). Polymorphisms in IFN-γ have been reported more frequently in Asian

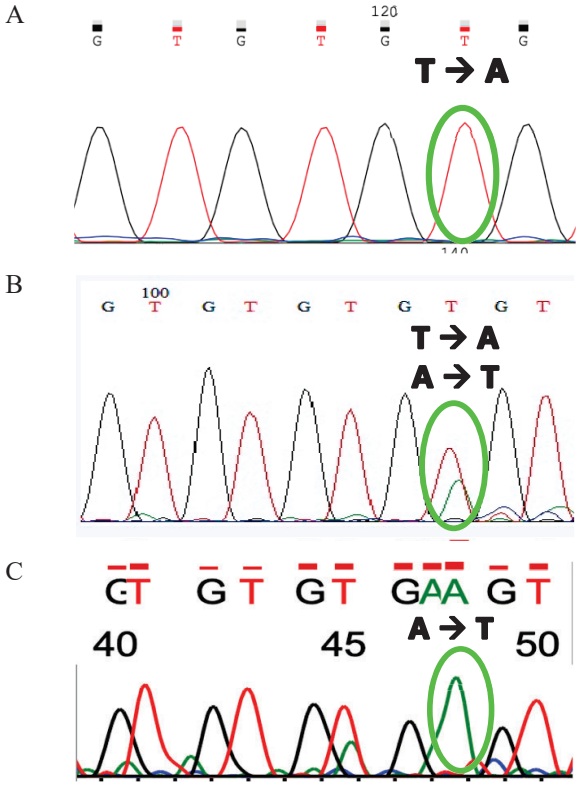

FIGURE 2. Reverse sequencing result in IFN-γ +874 T/A gene polymorphisms. Target-site nucleotides were identified based on their complementary pairing. (A) AA genotype; (B) AT genotype; (C) TT genotype

TABLE 3. Distribution of IFN-γ alleles and genotypes in chronic hepatitis B and liver cirrhosis patients

Genotype	CHB (88)	LC (37)	p value
AA	40 (45.5 %)	20 (54.1 %)	0.36
AT	33 (37.5 %)	9 (24.3 %)	
TT	15 (17.0 %)	8 (21.6 %)	
A	113 (64.2 %)	49 (66.2 %)	0.76
T	63 (35.8 %)	25 (33.8 %)	

Chi-square test, the value is presented n (%). A p-value <0.05 is significant

population, where the A allele more common than in other ethnic groups. A meta-analysis including 13 studies reported that individuals with the +874TT and +874TA genotypes had a reduced risk of HBV infection, particularly in East Asian populations; however, the literature remains inconsistent, as other studies reported a higher frequency of the +874AA and +874TA genotypes among individuals with chronic infection (Li 2020). The IFN-γ +874T/A polymorphism may be associated with the chronic HBV infection, according to genetic model AA vs. AT and TT (Al Kadi & Monem 2017). Two studies conducted in Iranian populations consistently reported a significant association between IFN-γ +874T/A polymorphism and susceptibility to chronic HBV infection. In both studies, the +874A allele was more frequent among chronically infected patients, whereas the T allele was associated with reduce susceptibility and higher like hood of viral clearance, suggesting a protective role of the T allele against chronic HBV persistence (Heidari, Moudi & Mahmoudzadeh-Sagheb 2020; Naghizadeh et al. 2017). This population-specific implication may be partly explained by differences in genetic background and host-virus interaction. HBV genotype distribution may also contribute to these differences, as genotype D is predominant in Iran and

HBV genotypes are known to influence disease burden, prognosis, and antiviral responses (Haghshenas, Arabi & Mousavi 2014). Consistent with the role of IFN-γ in modulating immune-mediated liver injury, a Brazilian study reports that the IFN-γ +874T/A polymorphism was significantly associated with severe liver fibrosis in chronic HBV patient (OR=1.85;p=0.029), while no association was observed with susceptibility to HBV infection (Ferreira et al. 2015). Although studies from Southeast Asia specifically evaluating IFN-γ +874T/A polymorphism in chronic hepatitis B and HBV-related liver cirrhosis are scarce, this polymorphism has been investigated in other Southeast Asian population and disease context, including tuberculosis in Indonesia and Malaysia, periodontitis, schizophrenia, and cervical cancer in Indonesia (Damanik, Effendy & Husada 2020; Daulay et al. 2021; Kiteswara, Sari & Pratamawati 2024; Lubis, Salim & Hafy 2023; Maulani et al. 2022; Nik Zuraina et al. 2025; Panigoro et al. 2012). This finding indicates that IFN-γ +874T/A has been explored as an immunogenetic marker in Southeast Asia.

Mechanistically, the IFN-γ +874T/A polymorphism may influence susceptibility to hepatitis virus–related diseases. IFN-γ production and downstream immune signalling. The IFN-γ +874TT genotype, associated with higher IFN-γ expression, may enhanced host antiviral immunity, whereas the AA and TA genotypes are associated with lower IFN-γ production, potentially increasing susceptibility to hepatitis virus infection. IFN-γ exert its biological effects trough binding to its specific cell-surface receptor (IFN-γR), and activating downstream signalling such as JAK–STAT pathways, which are critical for antiviral defence and immune regulation. Alteration in IFN-γ signalling may influence not only viral control but also inflammatory cascade associated with fibrogenesis and carcinogenesis. In addition, because the IFN-γ +874T/A locus is located within a nuclear factor kappa B (NF-κB) binding site, disruption of NF-κB dependent transcriptional regulation may impair inflammatory balance, promotes oxidative stress, and contribute to hepatocellular injury and progressive liver fibrosis under condition of persistent viral inflammation (Ben Selma et al. 2021).

Moreover, the same polymorphism may exert different implications at different disease stages progression, whereby strong IFN-γ signalling may be beneficial for viral control early in infection, while sustained immune activation overtime may contribute to chronic inflammation and tissue damage over. This dual role of inflammatory pathways is well recognized in chronic viral hepatitis and could partly explain divergent associations across studies and outcomes. Accordingly, the IFN-γ +874T/A polymorphism could influence both susceptibility to chronic infection and the severity of downstream liver disease by regulating IFN-γ production and related inflammatory signalling. Supporting this concept, a systematic review reported that carriers of the IFN-γ +874 T allele had a higher risk of developing cirrhosis compared with carriers of the A allele, suggesting that genotype-associated differences in immune signalling may modulate the trajectory of chronic liver injury toward cirrhosis (Xiao et al. 2023). Interferon-gamma (IFN-γ) is a central proinflammatory cytokine involved in immune dysfunction in chronic liver diseases. In chronic hepatitis B, IFN-γ produced by T cells, NK cells, and NKT cells contributes to viral control by activating cellular immunity. However, its persistent expression promotes sustained inflammation, hepatic injury, and progression to fibrosis. In liver cirrhosis, IFN-γ is systemically elevated together with TNF-α and IL-6, driving hepatocellular injury, hepatic stellate cell activation, and excessive extracellular matrix deposition. This stages is characterized by cirrhosis-associated immune dysfunction, in which FN-γ loses its antiviral effectiveness and instead exacerbates tissue damage and fibrotic remodelling (Pratim Das & Medhi 2023).

Beyond its role as an inflammatory biomarker, IFN-γ also represents a relevant therapeutic target and functional readout in emerging immunotherapeutic strategies for chronic hepatitis B. Therapeutic vaccines and toll-like receptor (TLR) agonists aim to restore antiviral immunity by enhancing HBV-specific T cell and NK cell responses, as reflected by increased production of IFN-γ together with TNF and IL-2. Thus, modulation of IFN-γ may serve not only as an indicator of immune activation but also as a surrogate marker of immunotherapy efficacy in re-establishing functional antiviral T cell responses (Watson, Mulay & Gill 2025). An updated meta-analysis evaluating the association between IFN-γ +874 T/A polymorphism and susceptibility of chronic hepatitis B with liver cirrhosis demonstrated that the allelic model (T vs A), dominant model (TT+TA vs AA) and heterozygote model (TT vs AA) were associated with a significantly decreased risk of CHB with liver cirrhosis. In contrast, the recessive model (TT vs TA+AA) was associated with an increased risk of CHB, and no significant difference was observed in the homozygous comparison (TT vs TA) (Febriawan 2022).

ASSOCIATION OF IFN-γ +874T/A POLYMORPHISM AND SERUM IFN-γ LEVELS IN CHRONIC HEPATITIS B AND LIVER CIRRHOSIS

Serum IFN-γ levels differed significantly between clinical groups (Table 4; Figure 3(A)). Patients with LC showed higher median IFN-γ levels (2.2 pg/mL; range : 0.09-43.14)) than patients with CHB (1.32 pg/mL; 0.02-712.35), and this difference was statistically significant (p=0.018), suggesting that systemic IFN-γ elevation is associated with the progression of liver disease from CHB to LC.

Interferon γ (IFN-γ) is the sole type II interferon and is predominantly produced by activated immune cells rather than hepatocytes; therefore, its systemic elevation primary reflects immune cells activation rather than direct hepatic synthesis (Lei et al. 2024). This provides a biological rationale for interpreting higher circulating IFN-γ in cirrhosis as a marker of stage-dependent immune

activation, which may occur even when IFN-γ +874 T/A genotype distributions remain comparable between CHB and LC group.

When IFN-γ levels were stratified by IFN-γ +874T/A genotypes within each clinical group (Table 5; Figure 3(B)-3(C)), no significant differences were detected among AA, AT, and TT genotypes in CHB (p=0.234) or in LC (p=0.619). However, genotype-stratified comparison between disease stages (Table 6) demonstrated a significant increase of IFN-γ in LC compared with CHB within the AA genotype subgroup, (3.38 vs. 1.16 pg/mL, p =0.047), whereas no significant differences were observed for AT (p=0.592) and TT (p=0.519).

Recent immunological profiling studies suggest that higher IFN-γ levels in advanced chronic liver disease may reflect persistent immune activation rather than effective antiviral immunity. In chronic HBV, prolonged antigen exposure can drive T-cell disfunction or exhaustion, where inflammatory mediators remain elevated but antiviral effector function is impaired (Li et al. 2022). This may help explain why IFN-γ increases in cirrhosis without necessarily indicating improved viral control.

Elevated interferon gamma (IFN-γ) in the LC group may reflect the inflammatory component of cirrhosis-associated immune dysfunction (CAID), a dynamic state characterized by concurrent systemic inflammation and immunodeficiency. In cirrhosis, increased gut permeability and altered microbiota promotes bacterial translocation, which can amplify systemic inflammatory signalling and cytokine release. Moreover, cirrhotic patients exhibit adaptive immune abnormalities, including dysfunctional activated CD8+ T-cell subsets associated with susceptibility to infection, supporting the notion that cytokine elevation in advanced disease may reflect immune dysregulation rather than effective antiviral control (Irvine et al. 2019; Liaskou & Hirschfield 2019). The gut-liver axis provides an additional mechanistic framework for these findings, as increased permeability and microbial translocation can sustain systemic inflammatory signalling and IFN-γ-associated immune activation (Tan et al. 2018). This support the interpretation that elevated IFN-γ in cirrhosis may arise from chronic inflammatory stimulation driven by microbial products, potentially overriding genotype dependent regulation of cytokine expression.

An increase in IFN-γ levels in patients with liver cirrhosis can be understood within the broader framework of systemic inflammation and immune dysfunction associated with advanced liver disease. In cirrhosis, prolonged chronic inflammation contributes to hepatocellular injury and fibrosis progression, and several proinflammatory cytokines, including TNF-α, IL-1, IL-6, and IFN-γ have been implicated in hepatocyte necrosis and fibrogenesis. Therefore, elevated IFN-γ levels observed in patients with cirrhosis are biologically plausible and may reflect the inflammatory milieu characteristic of advanced liver disease (Pratim Das & Medhi 2023). Recent clinical reviews further emphasizes that interferon signalling may become increasingly maladaptive with disease progression; while IFN-γ contributes to antiviral defence early in infection, sustained activation of interferon-related pathways in advanced disease can promote ongoing tissue injury, stellate cell activation, and fibrotic progression, supporting a pathogenic interpretation of IFN-γ elevation in cirrhosis (Rodríguez-Negrete et al. 2024).

Furthermore, cirrhosis is increasingly recognized as a condition characterized by CAID, in which systemic inflammation coexists with impaired immune competence. As liver disease progresses from compensated cirrhosis to decompensated cirrhosis and acute-on-chronic liver failure (ACLF), both inflammatory burden and immune dysregulation intensify, potentially explaining why inflammatory mediators, including IFN-related pathways, are more pronounced in advanced disease stages (Hasa, Hartmann & Schnabl 2022). Consistent with this concept, persistent systemic inflammatory activation in cirrhosis may occur alongside immune paralysis and defective antimicrobial responses. This dual state has important implications for interpreting cytokine measurements:

TABLE 4. Comparison of serum IFN-**γ** level in chronic hepatitis B and liver cirrhosis patients

	CHB (88)	LC (37)	p value
IFN-**γ** (pg/mL)	1.32 (0.02-712.35)	2.2 (0.09-43.14)	0.018*

Mann-Whitney U test, the value is presented in median (min-max). A p-value <0.05 is significant

TABLE 5. Comparison of serum IFN-**γ** level of different genotypes in chronic hepatitis B and liver cirrhosis patients

Genotype	CHB (88)	p value	LC (37)	p value
AA	1.16 (0.02-23.66)	0.234	3.38 (0.18-43.14)	0.619
AT	1.34 (0.15-49.45)		1.38 (0.09-14.11)	
TT	1.65 (0.23-712.35)		3.83 (0.17-18.51)	

Kruskal-Wallis test, the value is presented in median (min-max). A p-value <0.05 is significant

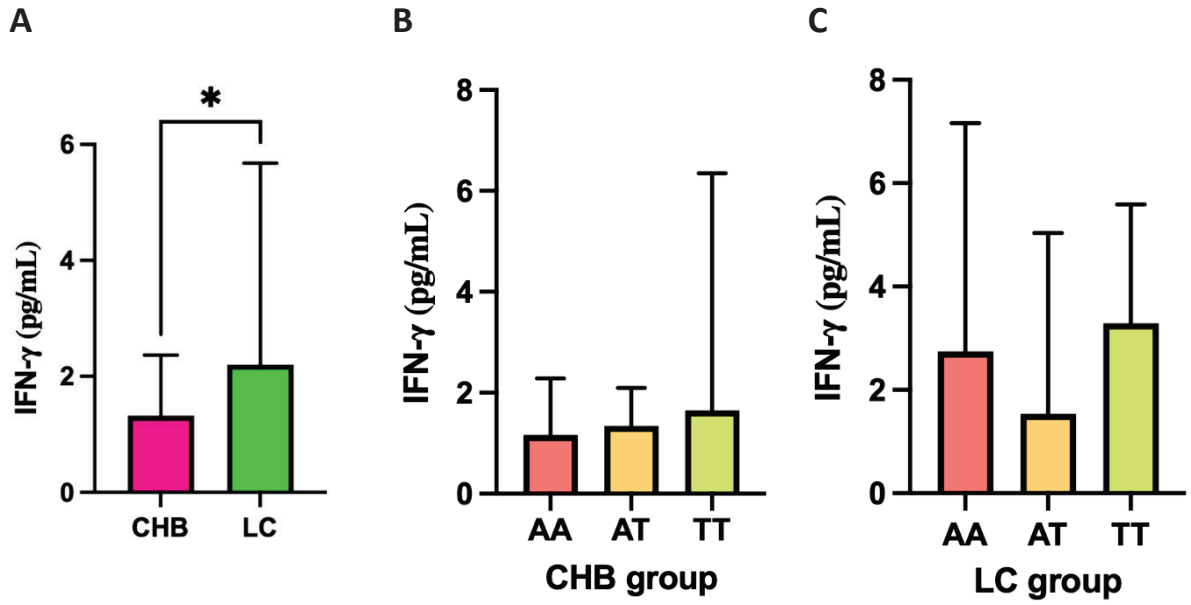

FIGURE 3. Serum IFN-**γ** level between (A) chronic hepatitis B and liver cirrhosis, and among carriers of the different genotypes from (B) Chronic hepatitis B and (C) Liver cirrhosis groups. The data represent median with interquartile range. Statistical analysis is defined as: *, $p<0.05$

TABLE 6. Comparison of IFN-γ levels between CHB and LC patients according to IFN-γ +874T/A genotypes

Genotype	CHB (88)	LC (37)	p value
AA	1.16 (0.02-23.66)	3.38 (0.18-43.14)	0.047*
AT	1.34 (0.15-49.45)	1.38 (0.09-14.11)	0.592
TT	1.65 (0.23-712.35)	3.83 (0.17-18.51)	0.519

elevated IFN-γ levels in cirrhosis do not necessarily indicate enhanced antiviral immunity, but may instead represent chronic immune activation that occurs alongside functional immune impairment (Irvine et al. 2019). Systemic inflammation in cirrhosis involves complex immune-metabolic and multi organ interactions, in which IFN-γ participates alongside other inflammatory mediators (Lu et al. 2024). In this context, elevated IFN-γ may correlate more closely with immune imbalance and disease severity than with viral burden alone, reinforcing its relevance as a marker of advanced liver disease rather than a direct surrogate of antiviral effectiveness.

From a genetic and molecular perspective, IFN-γ gene is a key pro-inflammatory cytokine involved in antiviral immunity against HBV, and host genetic factors substantially influence its expression and function (Hillaire, Lawrence & Lagrange 2023). Host genetic factors are known to substantially influence cytokine function and production. This effect is largely attributable to genetic polymorphisms affecting cytokine coding region or regulatory element, including promoter regions and the untranslated region, which modulate transcriptional activity and cytokine production (Ben Selma et al. 2021). Among these, the +874T/A (rs2430561) has been the most extensively studied, particularly in Asian population, due to its functional relevance in regulating IFN-γ expression (Sun et al. 2015b). This polymorphism is located in the first intron within a nuclear factor kappa B (NF-κB) binding site, and variation at this locus may influence transcriptional regulation and IFN-γ production (Naghizadeh et al. 2018). Functionally, the +874T allele is associated with higher IFN-γ production ('high-producer' phenotype), whereas the A allele particularly the AA genotype is associated with lower IFN-γ production ('low-producer' phenotype). However, the present finding indicate that in advanced disease stages such as cirrhosis, inflammatory and immunological alterations related to disease severity may override genotype dependent regulation of IFN-γ expression. This may explain why elevated IFN-γ levels were observed in LC despite the predominance of the AA genotype, and why previous studies have reported heterogeneous association between IFN-γ +874T/A and HBV susceptibility or disease progression .

This study integrates the analysis of IFN-γ +874T/A genetic polymorphism with serum IFN-γ levels, providing a combined immunogenetic and functional perspective on chronic HBV infection. The inclusion of both chronic hepatitis B and liver cirrhosis patients enables disease stage-specific comparisons. Several limitations should also be acknowledged. The cross-sectional design limits the evaluation of temporal changes in IFN-γ expression and their direct relationship with disease progression. In addition, serum IFN-γ was assessed at a single time point, which may not fully capture dynamic immune responses over the course of chronic HBV infection. Given the dynamic nature of chronic HBV infection, future longitudinal studies with serial IFN-γ measurements at baseline and periodic follow up intervals, such as every 3-6 months, may better clarify immune dynamics during HBV-related liver disease (Chen et al. 2023).

CONCLUSIONS

In conclusion, this study demonstrated that the distribution of IFN-γ +874 T/A genotypes and alleles did not differ between patients with chronic hepatitis B and with liver cirrhosis, suggesting that this polymorphism alone may not represent an independent determinant of disease progression. In contrast, serum IFN-γ levels were significantly elevated in cirrhotic patients, indicating an association between increased FN-γ levels expression and advanced liver disease. The observed genotyped-specific increase in IFN-γ levels among AA carriers with cirrhosis highlights the importance of disease-stage-dependent immune dysregulation, in which inflammatory processes may override genotype-dependent regulation of cytokine production. Collectively, these finding support a role for IFN-γ as a marker of inflammatory and fibrotic progression in chronic HBV infection, while underscoring the complex and context-dependent contribution of IFN-γ +874 polymorphism. Further longitudinal and functional studies are warranted to clarify the interplay between host genetics, cytokine regulation, and disease progression from chronic hepatitis B to cirrhosis.

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