Evaluation of Interlukin 10 Gene Promotor Polymorphism in Children with Acute Hepatitis A Induced Liver Failure

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ABSTRACT

Background: Pediatric Acute liver failure (ALF) is a rare but rapidly and frequently mortal condition. A large number of inflammatory markers have been implicated in causing liver injury. The aim of the present study was to assess IL10 level and polymorphism in children with acute viral hepatitis (AVH) and ALF. Patients and methods: This study included 48 children and carried out at the hepatology Unit, Pediatric Department Zagazig University Children Hospital in co-operation with the scientific and Medical Research Center of the Faculty of Medicine, Zagazig University and the pediatric Department of the National Liver Institute. The studied patients were divided equally into the fulminant hepatitis group and the control group (acute viral hepatitis). Results: There is statistically significant difference between studied groups regarding platelet count, hemoglobin and WBCs, ALT, AST, bilirubin, INR. The best cutoff of serum Il-10 in prediction of FHF is ≥39.5 pg/ml with area under curve 0.998, sensitivity 95.8%, specificity 95.8%, positive predictive value 95.8%, negative predictive value 95.8%, overall accuracy 95.8%.There is statistically significant difference between studied groups regarding IL-10 genotype polymorphism at A-G transition concerning genotype and alleles. C allele significantly increase risk of FHF by 14.44 folds denoting that AA (wild type) genotype protects against the development of fulminant hepatitis.C allele significantly increase risk of FHF by 4.71 folds denoting that TT genotype significantly protects against the development of fulminant liver failure. Conclusion: Regulatory polymorphism in the IL-10 gene promoter has a possible and significant association with the severity and the outcome in patients with AVH and ALF.

Keywords: Liver Failure; Interlukin 10; Promotor Polymorphism; Acute Hepatitis

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INTRODUCTION

Acute liver failure (ALF) is a rare but rapidly and frequently mortal condition, the estimated frequency of acute liver failure in all age groups in the United States is approximately 17 cases per 100,000 populations per year, but the frequency in children is unknown. ALF accounts for approximately 10 percent of pediatric liver transplants performed in the United States annually (1).

Pediatric ALF definition as (a) evidence of liver dysfunction within 8 weeks of onset of symptoms (b) uncorrectable (6-8 hours after administration of one dose of parenteral vitamin K) coagulopathy with International Normalized Ratio (INR) >1.5 in patients with hepatic encephalopathy, or INR> 2.0 in patients without encephalopathy and (c) no evidence of chronic liver disease either at presentation or in the past (2).

Globally, 1.4 million cases of hepatitis A virus (HAV) occur annually with a majority of the cases concentrated in the less developed countries where the risk factors facilitate transmission, of which near 1% will develop fulminant liver failure (3).

Interleukin 10 (IL10) is an important antiinflammatory cytokine that regulates immune cells as B, T, NK cells and thus involved in pathophysiology of ALF (4). The promoter region polymorphism of IL10 gene determine the variability in IL10 production. Since sequence variation in the IL10 gene promotor may alter IL10 expression, polymorphism in IL10 promotor region may impact liver damage and disease progression to ALF (5).

Therefore, this study aimed to assess IL10 level and polymorphism in children with acute viral hepatitis (AVH) and ALF.

PATIENTS AND METHODS

This study was carried out at the hepatology Unit, Pediatric Department Zagazig University Children Hospital in co-operation with the scientific and Medical Research Center of the Faculty of Medicine, Zagazig University and the pediatric Department of the National Liver Institute and was held over 21 months period between 2019 and 2022. The studied 48 children divided into 2 groups:

(i) The fulminant hepatitis group:

This group included 24 children aged 1 to 18 years diagnosed as fulminant liver failure according to the following criteria:

(a) Evidence of liver dysfunction within 8 weeks of onset of symptoms

(b) Uncorrectable (6-8 hours after administration of one dose of parenteral vitamin K) coagulopathy with International Normalized Ratio (INR) >1.5 in patients with hepatic encephalopathy, or INR> 2.0 in patients without encephalopathy .

(c) No evidence of chronic liver disease either at presentation or in the past.

This group is further subdivided into survivors and non survivors

Exclusion criteria:

Acute viral liver failure coincident with drug induced liver injury, sepsis, inherited liver diseases and mpaired kidney Functions

(ii) The control group (acute viral hepatitis):

Twenty four children, 12 males and 12 females with a mean age of 8.07 ± 3.17, who were diagnosed as acute hepatitis A virus in the outpatient clinic based on elevated liver enzymes and positive hepatitis A virus IgM.

Ethical Consideration:

An approval of the study was obtained from Zagazig University Academic and Ethical Committee. Written informed consent of all the participants was obtained. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

All patients were subjected to full history taking, complete physical examination and routine investigations including :

a. Complete Blood Count (CBC).

b. Liver Function Tests (Albumin,ALT,AST, GGT, ALK, Total and direct bilirubin)

c. PT, PTT, INR.

d. HAV IgM, HCV Ab, HBs Ag, anti-HBc IgM.

- **Estimation of Interlukin-10:**

IL10 level by ELISA and IL10 polymorphism by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) at IL-10-1082 G/A, IL-10 –819 C/T, and for IL-10–592C/A.

- **Identification of Interlukin 10 gene promotor polymorphism:**

The identification of the gene polymorphism was carried out using polymerase Chain Reaction-Restriction Fragment Length Polymorphism technique (PCR-RFLP).

Genomic DNA extraction was carried out from whole blood using the commercially available Innu PREP Blood DNA Mini Kit (Analytik Jena AG, Jena, Germany).

Statistical analysis:

Data analyzed using Microsoft Excel software then imported into Statistical Package for the Social Sciences (SPSS version 20.0) software. According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean ± SD. Differences between quantitative independent multiple by ANOVA. P value was set at <0.05 for significant results &<0.001 for high significant result.

RESULTS

The current study showed a statistically non-significant difference between studied groups regarding gender or age **(Table 1).**

There is statistically significant difference between studied groups regarding platelet count, hemoglobin and WBCs (significantly higher in viral hepatitis group), ALT, AST, bilirubin, INR (significantly higher in fulminant liver cell failure) **(Table 2).**

According to cause of mortality, 50 % of our cases died of cerebral edema, whereas 25% were died due to sepsis **(Figure 1).**

There was a significant positive correlation between INR and the poor outcome of fulminant hepatitis **(Figure 2).**

The best cutoff of serum Il-10 in prediction of FHF is ≥39.5 pg/ml with area under curve 0.998, sensitivity 95.8%, specificity 95.8%, positive predictive value 95.8%, negative predictive value 95.8%, overall accuracy 95.8% (p<0.001) **(Table 3; Figure 3).**

There is statistically significant difference between studied groups regarding IL-10 genotype polymorphism at A-G transition concerning genotype and alleles. A allele significantly increase risk of FHF by 4.96 folds denoting that GG (wild type) genotype is protective against the development of fulminant hepatitis **(Table 4).**

there is statistically significant relation between IL-10 genotype polymorphism in the fulminant hepatitis group at A-G transition and outcome. AG genotypes (heterozygous type) significantly protect against mortality (COR=0), whereas 100 % percent of the non survivors have AA genotype (mutant type). **(table 5)**

There is statistically significant difference between studied groups regarding IL-10 genotype polymorphism at A-C transition concerning genotype and alleles. C allele significantly increase risk of FHF by 14.44 folds denoting that AA (wild type) genotype protects against the development of fulminant hepatitis **(Table6)**.

there is statistically significant relation between IL-10 genotype polymorphism at A-C transition and outcome. CC (mutant type) genotypes indefinitely increases odds of mortality among patients suffering fulminant hepatitis**.(table 7)**

There is statistically significant difference between studied groups regarding IL-10 genotype polymorphism at T-C transition concerning genotype and alleles. C allele significantly increase risk of FHF by 4.71 folds denoting that TT genotype significantly protects against the development of fulminant liver failure **(Table8)**.

there is statistically significant relation between IL-10 genotype polymorphism at T-C transition and outcome. TC, and TT genotypes significantly protect against mortality (COR=0) **(table 9).**

Table (1): Comparison between studied groups regarding demographic data:

Parameter	Viral hepatitis group N=24(%)	Fulminant liver cell failure group N=24(%)	χ^2	p
Gender: Female Male	12 (50%) 12 (50%)	12 (50%) 12 (50%)	0	>0.999
	Mean ± SD	Mean ± SD	t	p
Age (year)	7.07 ± 3.17	6.29 ± 3.21	0.846	0.402

t independent sample t test χ^2chi square test

Table (2): Comparison between studied groups as regarding laboratory data:

	Viral hepatitis group	Fulminant liver cell failure group	T	P
	Mean ± SD	Mean ± SD		
Hemoglobin (g/dl)	9.69 ± 1.07	8.43 ± 1.06	4.075	<0.001**
WBCs ($10^3/mm^3$)	5.89 ± 1.49	3.18 ± 0.45	8.534	<0.001**
	Median(IQR)	Median(IQR)	Z	p

Platelet count (10^3/mm^3)	320(233.5 – 426.5)	120 (86.5 – 188.5)	-5.127	<0.001**
ALT (u/L)	1050 (800 – 1800)	2800 (1750 – 3450)	-4.251	<0.001**
AST (u/L)	1200 (870 – 1890)	3000(2000 – 4000)	-4.354	<0.001**
Bilirubin	8 (5.25 – 9.35)	20 (15.5 – 24.5)	-5.756	<0.001**
INR	1.3 (1 – 1.4)	4.85(3.7 – 6)	-5.949	<0.001**

t independent sample t test Z Mann Whitney test IQR interquartile range **p≤0.001 is statistically highly significant

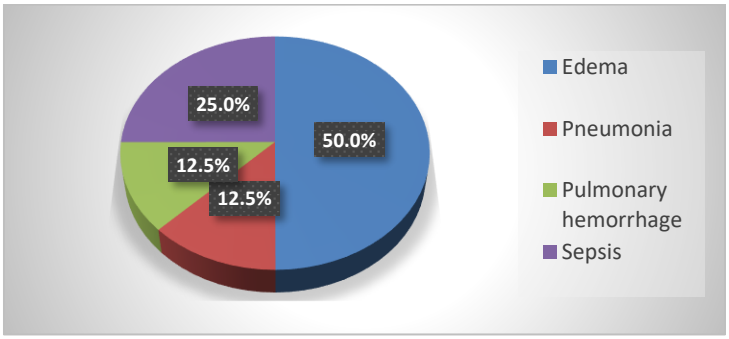

Figure (1): Pie chart showing distribution of patients according to cause of mortality showing that 50 percent of our cases died of cerebral edema, whereas 25%of our cases died of sepsis.

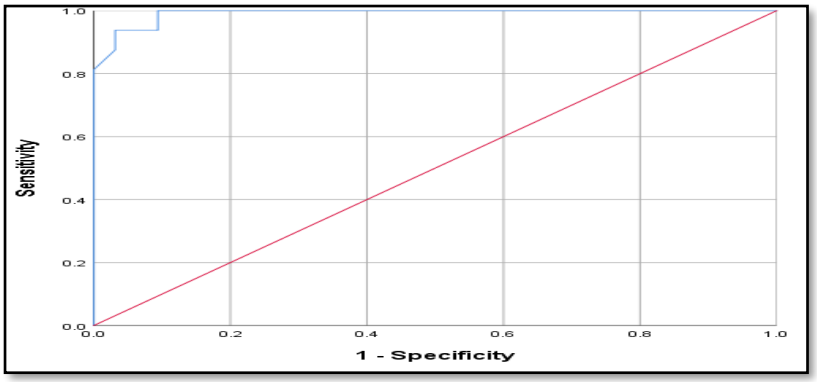

Figure (3): ROC curve showing performance of INR in prediction of mortality among studied patients showing significant positive correlation between INR and the poor outcome of fulminant hepatitis.

Table (3): Performance of IL-10 in prediction of mortality among fulminant hepatitis patients.

Cutoff	AUC	Sensitivity	Specificity	PPV	NPV	Accuracy	P

≥39.5	0.998	95.8%	95.8%	95.8%	95.8%	95.8%	<0.001**

AUC area under curve ,**p≤0.001 is statistically highly significant , PPV positive predictive value,NPV: negative predictive value

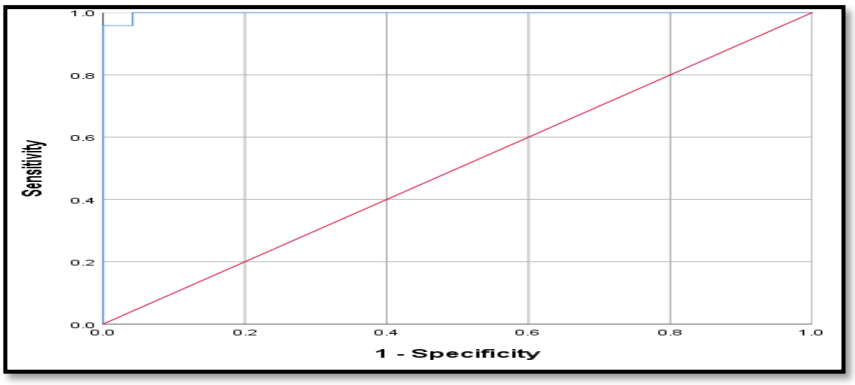

Figure (3): ROC curve showing Performance of IL-10 in prediction of mortality among studied patients showing that interleukin 10 level correlates with the fulminant course of acute viral hepatitis and predicts poor outcome in the fulminant hepatitis group.

Table (4): Comparison between studied groups as regarding IL-10 polymorphism genotypes at A-G transition:

Parameter	Viral hepatitis group N=24(%)	Fulminant liver cell failure group N=24(%)	χ^2	P
Genotype: AA AG GG	 5 (20.8%) 16 (66.7%) 3 (12.5%)	 17 (70.8%) 7 (29.2%) 0 (0%)	12.604§	<0.001**
Alleles: A G	 26 (54.2%) 22 (45.8%)	 41 (85.4%) 7 (14.6%)	8.752	0.003*
COR (95% CI)	4.96 (1.86 – 13.23)			

χ^2chi square test, *p<0.05 is statistically significant, **p≤0.001 is statistically highly significant, COR:Crude odds ratio CI confidence interval §Chi square for trend test, GG homozygous wild type ; AA homozygous mutant type; AG heterozygous mutant type.

Table (5) ;Comparison between survivors and non survivors of fulminant hepatitis group as regarding IL-10 genotype polymorphism at A-G transition among:

	Non-survivors N=16(%)	Survivors N=8(%)	χ^2	P	COR (95% CI)
Genotypes:					
AA	16 (100%)	1 (12.5%)	Fisher	<0.001**	1(reference)
AG	0 (0%)	7 (87.5%)			0
Alleles:					
A	32 (100%)	9 (56.3%)	Fisher	<0.001**	0
G	0 (0%)	7 (43.7%)			

χ^2chi square test ¥Chi square for trend test COR Crude odds ratio CI confidence interval **p≤0.001 is statistically highly significant

Table (6):Comparison between studied groups regarding IL-10 polymorphism genotypes at A-C transition:

Parameter	Viral hepatitis group N=24(%)	Fulminant liver cell failure group N=24(%)	χ^2	p
Genotype:				
AA	14 (58.3%)	2(8,3%)	27.417 §	<0.001**
AC	7(29%)	8(33,3%)		
CC	3 (12,5%)	14 (58.3%)		
Alleles:				
A	35 (72.2%)	12 (24.8%)	32.667	<0.001**
C	13 (27.8%)	36 (75.2%)		
COR (95% CI)	14.44 (5.39– 36.67)			

χ^2chi square test *p<0.05 is statistically significant χ^2chi square test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant COR Crude odds ratio CI confidence interval §Chi square for trend test, AA homozygous wild type, AC homozygous mutant type, CC heterozygous mutant type.

Table (7):Comparison between survivors and non survivors among fulminant hepatitis group and IL-10 genotype polymorphism at A-C transition:

	Non-survivors	Survivors	χ^2	P	COR (95% CI)

	N=16(%)	N=8(%)			
Genotypes: AC CC	 2 (12.5%) 14 (87.5%)	 8 (56.3%) 0 (0%)	Fisher	<0.001**	∞
Alleles: A C	 2 (6.3%) 30 (93.7%)	 8 (71.9%) 8 (28.1%)	Fisher	<0.001**	15 (2.65 – 85.01)*

χ^2chi square test ¥Chi square for trend test COR Crude odds ratio CI confidence interval **p≤0.001 is statistically highly significant

Table (8): Comparison between studied groups as regarding IL-10 polymorphism genotypes at T-C transition:

Parameter	Viral hepatitis group N=24(%)	Fulminant liver cell failure group N=24(%)	χ^2	P
Genotype: CC TC TT	 9(37.5%) 5(20,8%) 10 (41,6%)	 14(66.7%) 9(37.5%) 1 (4.2%)	6.695§	0.01*
Alleles: C T	 23 (47.9%) 25 (52.1%)	 39 (81.3%) 9 (18.7%)	11.658	<0.001**
COR (95% CI)	4.71 (1.88– 11.82)			

χ^2chi square test *p<0.05 is statistically significant χ^2chi square test *p<0.05 is statistically significant **p≤0.001 is statistically highly significant COR Crude odds ratio CI confidence interval §Chi square for trend test, TT homozygous wild type. CC homozygous mutant type. TC heterozygous mutant type

Table (9):Comparison between survivors and non survivors among fulminant hepatitis group and IL-10 genotype polymorphism at T-C transition:

	Non-survivors	Survivors	χ^2	P	COR (95% CI)
	N=16(%)	N=8(%)			
Genotypes:	16 (100%)	0 (0%)	20.361	<0.001**	1 (reference) 0

CC TC TT	0 (0%) 0 (0%)	7 (87.5%) 1 (12.5%)			0
Alleles: C T	32 (100%) 0 (0%)	7 (43.8%) 9 (56.2%)	Fisher	<0.001**	0

χ^2chi square test ¥Chi square for trend test COR Crude odds ratio CI confidence interval **p≤0.001 is statistically highly significant

DISCUSSION:

Fulminant hepatic failure is defined the condition as a clinical syndrome characterized by massive liver necrosis associated with severe impairment of hepatic function, manifesting as progressive jaundice, hepatic coma, and liver atrophy developing within 8 weeks of the onset of the first symptoms of the disease in individuals with no previous history of hepatic disease **(6).**

The Practice Guideline Committee of the American Association for the Study of Liver Diseases(AASLD) defines acute liver failure as "liver disease characterized by the development of hepatic encephalopathy and coagulation abnormalities, usually an international normalized ratio (INR) of ≥1.5 or more, in patients without pre-existing cirrhosis, and an illness of <8 weeks duration" **(7).**

Our study aimed at identifying the role of IL10 level and il10 gene promotor polymorphism and its association with acute viral liver failure.

Our data revealed both groups (acute viral hepatitis group and fulminant hepatitis group) are closely matched regarding their age (mean age for viral hepatitis group 8.07±3.17 whereas the mean for fulminant liver failure group is 9.29 ± 3.21) and gender (11 males and 13 females in the viral hepatitis group in the opposite of 14 males and 10 females in the fulminant hepatitis group) with no statistically significant difference between both groups (***p*** value of 0.4 and 0.9 respectively).

This coincides with the results of **Alina Grama (8)** who reported that sex distribution was 49 boys (50.51%) versus 48 girls (49.48%). The mean age was 7.66 ± 8.18 years, higher in girls (8.03 ± 6.26 years) than in boys (6.10 ± 5.86 years, p = 0.049). However, earlier studies from Greece and some Western communities reported that females are at an increased risk for developing FHF.

A case-control study involving 150 genotype 3 chronic hepatitis C virus (HCV) patients and 150 healthy controls to investigate the association of polymorphisms in the interleukin-10 (IL-10)

gene with chronic HCV infection and the association of these polymorphic variants with the combination of pegylated interferon (Peg-IFN) and ribavirin therapy response revealed that the GG genotype of IL-10 –1082A/G exhibited significant association with genotype 3 chronic HCV infection compared to controls. Treatment response data also showed a significant increase in risk for the GG genotype of IL-10 –1082A/G in response-relapse patients or non-responder patients compared to sustained virological response patients **(9).**

Our study reported a statistically significant difference between studied groups regarding platelet count, hemoglobin and WBCs (significantly higher in viral hepatitis group), ALT, AST, bilirubin, INR (significantly higher in fulminant liver cell failure).

Andogdu et al. (10) reported that patients who did not survive had higher peak total bilirubin level than survivors. **Lee et al.(11)** described a level of 299 mol/L vs. 80 mol/L10. **Rivero-Juarez (12)** reported much higher level among non-survivors, 30 ±19 mg/dL vs. 11±9 mg/dL among survivals. **Li et al. (13)** reported a level of 25.17±14.89 mg/dL vs. 9.89 ±7.41 mg/dL. The rate of serum bilirubin ascend was significantly higher among non-survivors.

In fulminant hepatic failure, all clotting factors synthesized by the liver exhibit depressed plasma activity. Factor II, with a half-life of 2hours, is the first to be depleted with hepatocellular dysfunction and the first to be replaced with recovery. Coagulopathy is often complicated by DIC and thrombocytopenia that can be explained by inadequate thrombopioetin mRNA transcription in the liver. Coagulopathy could be silent or present as a spontaneous and fatal intra cranial bleeding **(14).**

Our results agreed with **Nagaki et al. (15**) who reported that interleukin 10 is significantly higher in patients with FH than in those with Acute viral hepatitis (P<.05,<.05, and <.01, respectively).

This was in accordance with an earlier study by **Berry et al. (16)** which concluded that levels of the antiinflammatory cytokine IL-10 are of equal value to the pro-inflammatory cytokines such as IL-6 and tumor necrosis factor-alpha (TNF-α) in defining the degree of illness in ALF and decompensated cirrhosis.

We analyzed A→G transition resulting in genotypes AA, AG, and GG at position -1082 in the IL-10 gene promoter. Some studies done earlier on IL-10–1082 AA polymorphism indicated its association with depression and tuberculosis. In our study, it was concluded that patients with AA genotype were at a higher risk of developing ALF. In previous studies studying relationship between IL-10–1082 AG polymorphism and various clinical conditions, it was found that patients with this polymorphism were having a greater susceptibility for psoriasis, Alzheimer's disease, coronary artery disease, ankylosing spondylitis, and proliferative diabetic retinopathy.

A meta-analysis demonstrated that rs1800871 (– 819 C/T), rs1800872 (– 592 C/A) and rs1800896 (– 1082 G/A) polymorphisms may influence the risk of viral hepatitis in Asians, while only rs1800896 (– 1082 G/A) polymorphism may influence the risk of viral hepatitis in Caucasians **(17).**

We analyzed A→C transition resulting in genotypes AA,AC, and CC at the IL-10-592 gene promoter position. The AA genotype was found to be more common in the AVH group ((58.3%) as compared to ALF (8,3%) group. Thus, we postulate that individuals with the AA genotype may have a lesser susceptibility to ALF. The AC genotype was found in 33,3% of ALF patients and 29 % of AVH ; hence, patients with the AC genotype had significantly increased susceptibility to ALF . We also found that individuals with the CC genotype had a significant increased susceptibility to ALF and more severe liver injury in the same patients.

We analyzed T→C transition resulting in genotypes TT, TC, and CC at position -819 in the IL-10 gene promoter. The CC genotype was found in 37,5% AVH patients, 66,7% ALF. The difference was found to be statistically significant when the ALF group was compared with the AVH group. Thus, it was postulated that patients with the CC genotype may have a higher risk of developing ALF and may predict the severity of liver injury once hepatitis has occurred. On analyzing the TC genotype, a statistically significant relationship was found when the ALF group (37,5%) was compared against the AVH group (20%).

In our study, we concluded that the TT genotype had a statistically significant protective effect from developing ALF as compared to the control.

On the other hand a case-control study was conducted to investigate the association between three common SNPs in IL-10 gene (rs1800896, rs1800871 and rs1800872) and the development of liver cirrhosis in a Chinese population. Between January 2013 and December 2014, a total of 318 patients with liver cirrhosis and 318 health control subjects were enrolled into the study and found that individuals with the AA genotype and GA+AA genotype of IL-10 rs1800896 were more likely to have an increased risk of liver cirrhosis when compared with the GG genotype **(18).**

CONCLUSION:

Regulatory polymorphism in the IL-10 gene promoter has a possible and significant association with the severity and the outcome in patients with AVH and ALF.

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Author contribution: Authors contributed equally in the study.
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