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CD95 & CD178 Signaling Pathways and Associated Genetic Polymorphisms in the Patients with Chronic Viral Hepatitis B (VHB) Infection

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Abstract In Chronic Viral Hepatitis B (VHB), the mechanisms of the CD95-CD178 signaling pathway, and the associated genetic polymorphism, together with the cirrhotic process remains unclear. We evaluate the involvement of CD95-CD178 and associated genetic polymorphisms in viral persistence and hepatic cytolysis in patients with chronic VHB. Whole blood from 343 Chronic VHB patients were collected and, CD95 and CD178 levels were performed by Flow cytometry and ELISA, respectively. Genotyping of CD95-670 A/G, CD95-1377 G/A and CD178-844 C/T polymorphisms was performed using PCR-RFLP. The searches and quantifications of the DNA of the HBV were carried out by qPCR. Data were analyzed using GraphPad PRISM 7.0, with the significance threshold set at $p \leq 0.05$ and a 95% confidence interval. Out of 343 patients, 105 (30.61%) were healthy and 238 (69.39%) were HBV-infected. Plasma levels of CD95 and CD178 were significantly elevated in HBV-infected patients compared to healthy patients, and in cirrhotic patients compared to non-cirrhotic patients. There were statistically significant correlations between CD95 and fibrosis scores, between CD95 and HBV viral load, between CD178 and fibrosis scores, between CD178 and HBV viral load, and between HBV viral load and the fibrosis score. The genotypic frequencies of CD95-670 A/G and CD178-844 C/T were significantly different between infected and healthy patients. Increased expression of CD95-670 A/G AG genotypes, CD95-1377 G/A GG genotypes, and CD178-844 C/T CT genotypes are associated with the severity of hepatic fibrosis, overexpression of CD95 and CD178 in patients infected with HBV, and by implication, the increase and persistence of the viral load of the Hepatitis B virus (HBV).

Keywords: *VHB, CD95-CD178, genetic polymorphism, HVB viral load, fibrosis, cirrhosis*

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1. Introduction

Chronic Viral Hepatitis B (VHB) is an inflammatory disease of the liver, caused by the Hepatitis B Virus (HBV), whose surface antigen has persisted in the host for at least six months [1]. HBV does not directly exert a cytolytic action on infected hepatocytes; The hepato-cytopathogenicity associated with HBV is mainly attributed to the persistence of this virus in hepatocytes. However, the mechanisms responsible for the persistence of HBV in hepatocytes, and its escape from cell-mediated immune responses in infected patients, remain to be elucidated.

The persistence of HBV in hepatocytes leads to repeated attempts to eliminate them by the immune system. These attempts to eliminate infected hepatocytes is mediated by several immune mechanisms including apoptosis of infected hepatocytes, which is an active and genetically programmed phenomenon of cell death, characterized by a unique sequence of events, with morphological features distinct from necrosis [2]. The triggering of this "programmed death" would be done by

the activation of a specialized signaling pathway, namely the CD95 & CD178 pathway [3].

CD95 belongs to the TNF-R / NGF-R family [4]. Members of this family are characterized by the presence of cysteine-rich domains in their extra cytoplasmic portion. The CD95 & CD178 system is composed of two isoforms of the CD95 receptor and their CD8 natural ligands [5]. The "death receptor" subfamily is distinguished at the intracytoplasmic portion, that contains a domain of about 80 amino acids called the "death domain".

CD95 is the CD178 receptor. The induction of apoptosis by CD95 follows its oligomerization by an agonist monoclonal antibody or by its natural ligand CD178. The latter belongs to the superfamily of TNF / NGF [3,4]. The CD95 molecule is expressed in many tissues (liver, heart, hematopoietic tissue). However, a complete lack of expression of the CD95 protein in humans has no direct consequences on the lymphoid system [3]. CD95 is expressed on the surface of activated T- and B-lymphocytes.

The CD95:CD178 interaction represents a preferred route of control of the immune response, and in particular of the regulation of autoimmune proliferations [6]. CD95-mediated T-cell apoptosis has been known for a long time as a mechanism for contraction of T-cell responses for the prevention of immunopathogenesis and the maintenance of immune tolerance, and this is described as an immune checkpoint mechanism.

CD95 is a type-I membrane protein, and its gene consists of nine exons mapped on the chromosome 10q23 [7]. CD178 (FasL or CD95L) is a type-II membrane protein which its gene is mapped on chromosome 1q23 in humans with four exons [8]. There are several Single Nucleotide Polymorphisms (SNPs) in the promoter region of CD95 gene including -670 (A/G) (rs1800682) and -1377 (G/A) (rs2234767) that change Stimulatory Protein-1 (SP-1) and to the signal transducer and activator of transcription-1 (STAT-1) binding sites [9], and in CD178 gene in the promoter region at position -844 (C/T) (rs763110) that reduces the interaction of transcription factor CAAT/enhancer binding protein β (C/EBPβ) with promoter [10]. CD95-670 (GG), -1377 (AA) and CD178-844 (TT) genotype decrease promoter activity and CD95 and CD178 gene expression [10]. The -844C allele has twice the basal activity of the -844T allele and results in a significantly higher basal expression of CD178 [10]. Alteration in the levels of CD95 and CD178 expression is implicated in the pathogenesis of several liver diseases including viral hepatitis by B and C viruses [11,12], autoimmune hepatitis [13], and alcoholic liver disease [13].

The objective of this study was to investigate involvement of CD95-CD178 and associated genetic polymorphisms in viral persistence and hepatic cytolysis in patients with chronic VHB.

2. Methods

2.1. Ethical Considerations

Ethical approval to conduct the study was obtained from the Institutional Ethics and Research Committee for Human Health (N°2019/0803/CEIRSH/ESS/MIM). Written and verbal informed consent was given by all participants. The study was conducted according to the ethical principles guidelines and guidelines of the international Declaration of Helsinki 2013. All procedures were standard and presented minimal risk to participants.

Sampling was performed after informed consent was obtained from each patient included in the study to use the samples and clinical data for research purposes after being informed about the nature of the study. All procedures were standard and presented minimal risk to participants.

2.2. Study Design

We performed a cross-sectional study from September 2019 to December 2021 of patients admitted to the hepatology and gastroenterology department at the Yaoundé General Hospital, Yaoundé, Cameroon. The recruitment was consecutive and not probabilistic. The results of the biological analysis were returned to the patients and incorporated into their medical records.

2.3. Study Population

Included in our study were all patients willing to participate in the study, who were naive to all treatment, and who were “healthy” (considered here as being control subjects) on the one hand, for controls, and who were diagnosed as positive only HBV infection on the other hand (472 patients initially included). Patients with liver diseases of other aetiologies or those having HBV infection and a history of autoimmunity, drug-dependence or co-infection with other viruses, including hepatitis C virus (HCV), hepatitis D virus (HDV), HIV and human T-cell leukemia virus type 1 (HTLV-1), were excluded from the study (129 excluded patients).

There were no gender restrictions in patient recruitment, but patients recruited had to be between 18 and 60 years old.

The selection criteria for the control participants were not having a medical history of HBV, HCV, HDV, HTLV-1 or HIV infection, autoimmunity and not having consumed alcohol in the past 7 years. These control participants were selected from a population of blood donors at the Yaoundé General Hospital and screened for these viral infections and diseases.

All the required investigations were carried out for confirmation of the final diagnosis. The laboratory investigations included CBC (Complete Blood Count) and evaluation of alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, albumin, prothrombin, HBe-antigen, CD95, CD178 and HBV viral load.

From the fibrotest examination, patients were divided based on their METAVIR fibrosis score into cirrhotic and non-cirrhotic HBV infected patients. Still based on the METAVIR fibrosis score, cirrhotic patients were distributed over five stages (scoring from F0-F4): F0: Absence of Fibrosis; F1: Minimal fibrosis; F2: Moderate fibrosis; F3: Severe fibrosis; F4: Cirrhosis.). Social characteristics (age and sex) and clinical information (cirrhosis statute, levels of CD95 and CD178, viral load of HBV) were collected for each participant using a standard questionnaire.

2.4. Sample Collection and Analysis Site

Eight milliliters (8 mL) of whole blood were collected under standard conditions, into sterile endo-toxin-free vacuum blood collection tubes on potassium Ethylene Di-amine Tetra Acetate (EDTA-K2) and transported at room temperature to the Immuno-virology Laboratory of the Center for the Study and Control of Communicable Diseases (CSCCD) of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1 (FMBS/UY1). These samples were centrifuged at 5000 rpm for 5 minutes; the plasmas were extracted, aliquoted in duplicate and stored at -20 °C for the subsequent determination of the HBV viral load, CD95 and CD178. METAVIR scores of fibrosis were collected from patients' medical records.

2.5. Detection of Serological HBV Markers

HBs-Ag screening tests were performed by enzyme-linked immunosorbent assay (ELISA) (Behring, Marburg, Germany). Anti-HBc screening tests were performed by a manual microplate Enzyme Immunoassay (EIA) using an anti-HBc commercial kit (Radim, Pomezia, Italy). The present method is based on a competitive EIA. All of the samples were also screened by ELISA (Radim) for possible HCV, HDV, HIV, and HTLV-1 infections.

2.6. Determination of CD95 Plasma Levels

Determination of CD95 was performed using monoclonal antibody (Dako, Denemark). This test depends on the ability of a monoclonal antibody to bind to the surface of the cells expressing CD95 lymphocytes and measured by flow cytometry (Becton Dickinson FACSCalibur. Becton, Dickinson and Company, BD Biosciences, San Jose).

2.6.1. Sample Preparation

Two volumes of EDTA blood were layered carefully on top of one volume of ficol and then centrifuged for 20 min at 1800 rpm. The mononuclear cells were deposited in a white band at the interference between plasma and ficol and then separation of mononuclear layer was carried out in a separate tube. It was washed with 3 ml of PBS and then centrifuged for 5 min at 3200 rpm. The supernatant was decanted, and the sediment was resuspended in 500 ml of PBS.

2.6.2. Sample Staining

For each sample two tubes were prepared: one for the test and the other for unstained control.

A volume of 10 ml of diluted monoclone was added to 100 ml of the previously prepared cell suspension, mixed well, and incubated at 2-8 °C for 30 min; the cells were then washed in 2 ml of PBS.

Finally, the cells were resuspended in 200 ml of PBS for final flow cytometric analysis.

All samples were analyzed using a flow cytometer (Becton Dickinson FACSCalibur).

2.7. Determination of CD178 Plasma Levels

The determination of the levels of CD178 was performed using plasma, by the sandwich enzyme-linked immune-sorbent assay (ELISA) technique, by the R&D system (Quantikine®, R&D Systems, United Kingdom), scrupulously respecting the manufacturer's instructions. The optical densities were measured at 450 nm wavelength, using an ELISA reader (Sunrise Tecan, Austria), and all assays were conducted in duplicate, and the means concentrations of CD178 were calculated. The CD178 levels in the samples were determined by extrapolating the results from a standard curve.

2.8. Genotyping for Polymorphisms of CD95-670 A/G and -1377 G/A, and CD178-844 C/T

Genomic DNA was extracted from each 5 ml sample of whole blood collected in EDTA tubes from HBV infected patients and healthy controls, using a salting out method [14]. Genomic DNA concentration was determined using BioPhotometer (Eppendorf-Germany). Genotyping for CD95-670A/G and -1377 G/A, and CD178-844 C/T polymorphisms was performed by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assays [15]. The PCR primers for the amplification of the CD95 and CD178 promoter variants had been previously designed to amplify a region of 1 kilobases upstream of the transcription start site of the human CD95 (NM_000043.5) and CD178 (NM_000639.2) promoters. Restriction enzymes and digestion schemes are shown in Table 1.

Table 1. PCR primers for amplification of the CD95 and CD178 promoter variants, specificities, restriction enzymes and digestion patterns

Location	Primer sequences (5' → 3')	Annealing temperature °C	PCR products (pb)	Restriction enzyme used	Genotype
CD95-670 A/G		61	193	ScrFI	AA: 193 bp; AG: 193 + 136 + 57 bp; GG: 136 + 57 bp
F:	ATA GCT GGG GCT ATG CGA TT				
R:	CAT TTG ACT GGG CTG TCC AT				
CD95-1377 G/A		61	122	BstUI	GG: 104 + 18 bp; GA: 122 + 104 + 18 bp; AA: 122 bp
F:	TGT GTG CAC AAG GCT GGC GC				
R:	GCA TCT GTC ACT GCA CTT ACC ACC A				
CD178-844 C/T		61	401	BsrDI	CC: 233 + 168 bp; CT: 401 + 233 + 168 bp; TT: 401 bp
F:	CAG CTA CTC GGA GGC CAA G				
R:	GCT CTG AGG GGA GAG ACC AT				

Source: Data compilation of our reagents.

The amplification was carried out using the Thermocycler (Eppendorf, Germany) under the following conditions: each reaction mixture of 50 µL containing 100 ng of template DNA, 0.5 µM of each primer, 0.2 mM of each deoxy-nucleoside 5' triphosphates, 2.0 mM of $MgCl_2$ and 2.5 units of Taq DNA polymerase (CinnaGen, Iran), in PCR reaction buffer with 1X ammonium sulphate.

At the beginning, an initial denaturation step was carried out for 5 minutes, at 94°C. Then, 35 cycles were carried out, including 30 seconds at 94°C. (for the denaturation), 30 seconds at 62° C. (for the annealing of the primers) and 45 seconds at 72°C. (for the polymerization). Finally, a final stage of final polymerization was carried out for 7 minutes at 72°C. The restriction endonucleases ScrFI, BstUI, and BsrDI (New England Biolabs, USA) were used to digest the CD95-670 A/G, CD95-1377 G/A, and CD178-844 C/T PCR amplicons respectively. The CD95-670AA product had no restriction site for ScrFI and amplicon remained undigested (193 bp). Inversely, two fragments of 136 and 57-bp were produced by ScrFI digestion on CD95-670GG PCR amplicon. BstUI digestion for CD95-1377 G/A polymorphism generated two fragments of 104 and 18 bp for CD95-1377GG genotype, and a 122 bp undigested fragment for CD95-1377AA. The CD178-844C allele had a BsrDI restriction endonuclease site that resulted in two fragments of 233 and 168 bp, but the T allele lacked this site and therefore only a 401 bp undigested band was generated (Table 1). The digested PCR products were separated on 3% agarose gel containing ethidium bromide and visualized under the UV transilluminator.

2.9. Determination of Viral Load of HBV

The search and quantification of HBV DNA was performed by quantitative Polymerase Chain Reaction (qPCR) on Cobas (Cobas 8 800 Roche) with a quantization domain of between 10 and 1 000 000 000 IU/mL (1-9 log) on plasma collected in a sterile endo-toxin-free vacuum blood collection tube on potassium EDTA. The results of quantitative HBV PCR were expressed as international units per milliliter.

2.10. Statistical Analysis

Statistical evaluation of data from this study were recorded in the Microsoft Office Excel 2016.

Software (Microsoft Corporation, Redmond, Washington, United States), and statistical analysis were performed using Graph Pad PRISM 5.0 software package (Graph Pad Software Inc., La Jolla, California, United States), and the IBM SPSS Statistics V22.0 software (International Business Machines Corporation, Armonk, New York, United States). Continuous variables were expressed as median or mean ± standard deviation (SD); categorical variables were expressed as number (percentage). Comparisons between Viral load, CD95 and CD178 within different groups, were performed using the non-parametric test of Mann-Whitney and Kruskal-Wallis [16,17]. The correlations between CD95, CD178, viral load and the fibrosis score were established using the Spearman's correlation coefficient (r). All values of $p \leq 0.05$ were considered statistically significant, for a confidence interval of 95%.

Genotype frequency differences of CD95 and CD178 promoter polymorphisms were analyzed between the infected and uninfected patients, and between the different groups of fibrosis stage, using Chi-squared test.

The genetic trait association between the groups was measured by odds ratio (OR) and the exact confidence intervals (CI) of 95% were obtained. To assess the consistency of genotype distributions with the Hardy-Weinberg equilibrium, Chi-squared test was used.

3. Results

3.1. Socials and Clinical Characteristics of Healthy Patients and HBV-Infected Patients

Out of 343 recruited patients, 105 (30.61%) were healthy, and 238 (69.39%) were HBV-Infected. Among healthy patients, 64 (32.49%) were male, 41 (28.08%) were female, and the average age was 35.72 ± 13.45 years old. Among HBV-infected patients, 133 (67.51%) were male, 105 (71.92%) were female, and the average age was 32.14 ± 11.15 years old. The difference of the average age and the gender between these two groups were not statistically significant, with a p value = 0.066, and p value = 0,382 respectively.

Fibrosis stage of all of the Healthy patients were F0; and the fibrosis stage of HBV-infected patients were F0 for 49 (20.59%) patients, F1 for 21 (8.82%) patients, F2 for 35 (14.71%) patients, F3 for 45 (18.91%) patients, and F4 for 88 (36.97%) patients.

So, 105 (100%) of the healthy patients were non-cirrhotic; 88 (36.97%) of the HBV-infected patients were cirrhotic, and 150 (63.03%) of those were non-cirrhotic.

The mean of total bilirubin was 6.40 ± 3.21 mg/L among the healthy patients, and 17.01 ± 13.62 mg/L among the HBV-infected patients; the difference between these two groups was statistically significant, with a p value < 0.001. The mean of direct bilirubin was 3.10 ± 1.45 mg/L among the healthy patients, and 10.96 ± 9.39 mg/L among the HBV-infected patients; the difference between these two groups was statistically significant, with a p value < 0.001. The mean of undirect bilirubin was 5.02 ± 1.62 mg/L among the healthy patients, and 6.05 ± 4.39 mg/L among the HBV-infected patients; the difference between these two groups was not statistically significant, with a p value = 0.869.

The mean of Albumin was 2.91 ± 1.25 g/dL among the healthy patients, and 3.46 ± 1.04 g/dL among the HBV-infected patients; the difference between these two groups was statistically significant, with a p value < 0.001.

The mean of ALT was 18.91 ± 8.34 U/L among the healthy patients, and 145.09 ± 96.10 U/L among the HBV-infected patients; the difference between these two groups was statistically significant, with a p value < 0.001.

The mean of AST was 17.21 ± 8.76 U/L among the healthy patients, and 61.63 ± 57.63 U/L among the HBV-infected patients; the difference between these two groups was statistically significant, with a p value < 0.001.

Table 2. Socials and clinical characteristics of healthy patients and HBV-Infected patients at Yaoundé General Hospital, Cameroon, September 2019 – December 2021

Parameters	Clinical status n (%)		*p*
	Healthy patients	HBV-Infected patients	
Number	105 (30.61%)	238 (69.39%)	
Age (years)			
Mean ± SD	35.72 ± 13.45	32.14 ± 11.15	0.066
Median	32	30	
Range	30-34	30-34	
Gender			
Male 197 (57.43%)	64 (32.49%)	133 (67.51%)	0,382
Female 146 (42.57%)	41 (28.08%)	105 (71.92%)	
Fibrosis stage			…
F0	105 (100%)	49 (20.59%)	…
F1	…	21 (8.82%)	…
F2	…	35 (14.71%)	…
F3	…	45 (18.91%)	…
F4	…	88 (36.97%)	…
Hepatitis state			…
Cirrhotic	…	88 (36.97%)	
Non-cirrhotic	105 (100%)	150 (63.03%)	
Total bilirubin (mg/L)			
Mean ± SD	6.40 ± 3.21	17.01 ± 13.62	< 0.001
Median	6	13	
Direct bilirubin (mg/L)			
Mean ± SD	3.10 ± 1.45	10.96 ± 9.39	< 0.001
Median	3	8	
Undirect bilirubin (mg/L)			
Mean ± SD	5.02 ± 1.62	6.05 ± 4.39	0.869
Median	5	5	
Albumin (g/dL)			
Mean ± SD	2.91 ± 1.25	3.46 ± 1.04	< 0.001
Median	3	5	
ALT (U/L)			
Mean ± SD	18.91 ± 8.34	145.09 ± 96.10	< 0.001
Median	17.25	50	
AST (U/L)			
Mean ± SD	17.21 ± 8.76	61.63 ± 57.63	< 0.001
Median	17	40.10	
TP (%)			
Mean ± SD	88.35 ± 8.34	76.03 ± 14.89	< 0.001
Median	84	40.10	

Source: Compilation of our basic data.

The mean of TP was 88.35 ± 8.34% among the healthy patients, and 76.03 ± 14.89% among the HBV-infected patients; the difference between these two groups was statistically significant, with a p value < 0.001. (Table 2).

3.2. Comparing the Levels of CD95 and CD178 in HBV-infected patients and Healthy Patients at Yaoundé General Hospital, Cameroon, September 2019 - December 2021

The level of CD95 in HBV-infected patients ranged from 1.35 to 8.5 pg/mL, with a median of 6 pg/mL. The level of CD95 in healthy patients ranged from 0.91 to 2.18 pg/mL, with a median of 1.35 pg/mL. The difference between these two groups was statistically significant, with a p value < 0.001 (Figure 1A). The level of CD178 HBV-infected patients ranged from 2.28 to 4.9 pg/mL, with a median of 3.28 pg/mL. The level of CD178 in healthy patients ranged from 0.12 to 2.33 pg/mL, with a median of 1.76 pg/mL. The difference between these two groups was statistically significant, with a p value < 0.001 (Figure 1B).

3.3. Comparing the Levels of CD95 and CD178 in HBV-infected Patients, Sorting by Fibrosis Score, at Yaoundé General Hospital, Cameroon, September 2019 - December 2021

The levels of CD95 and CD178 in HBV-infected patients were progressively increasing with the progression of liver impairment from F0 to F4. For CD95, the median values were 2.9; 4; 5.85; 6 and 6.96 pg/mL, from F0 to F4 respectively. The difference between these five groups was statistically significant, with a p value <0.001 (Figure 2). For CD178, the median values were two; 3; 3.36; 3.43 and 4.2 pg/mL, from F0 to F4 respectively. The difference between these five groups was statistically significant, with a p value <0.001 (Figure 2).

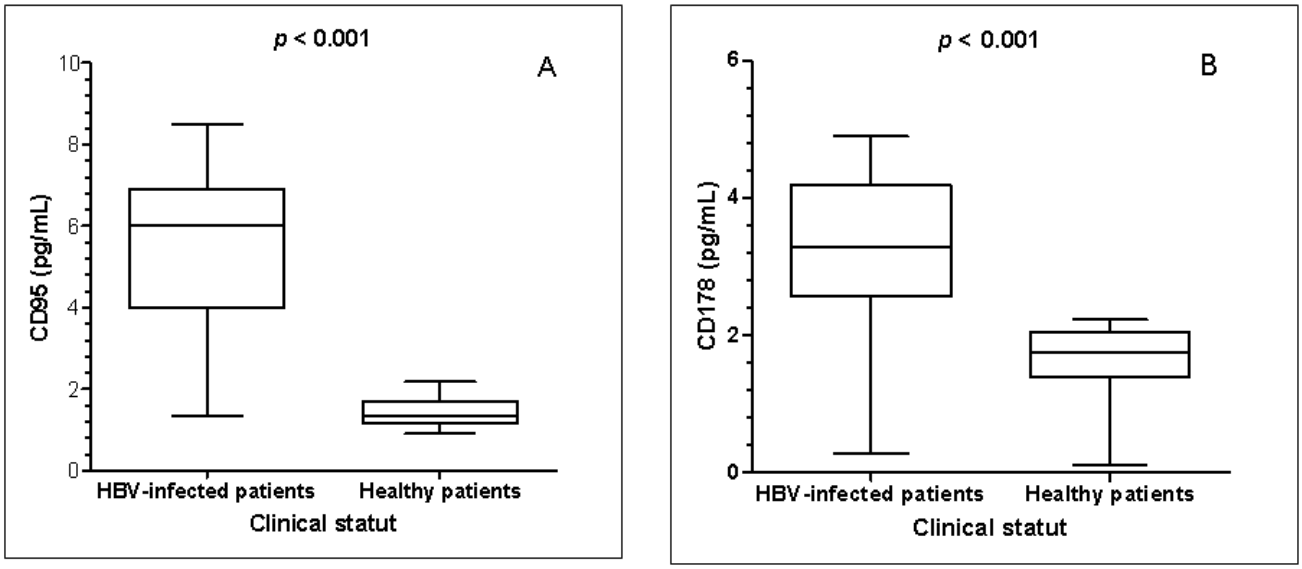

Figure 1. Levels of CD95 and CD178 in HBV-infected patients and healthy patients at Yaoundé General Hospital, Cameroon, September 2019 – December 2021 (*Source*: Compilation of our basic data)

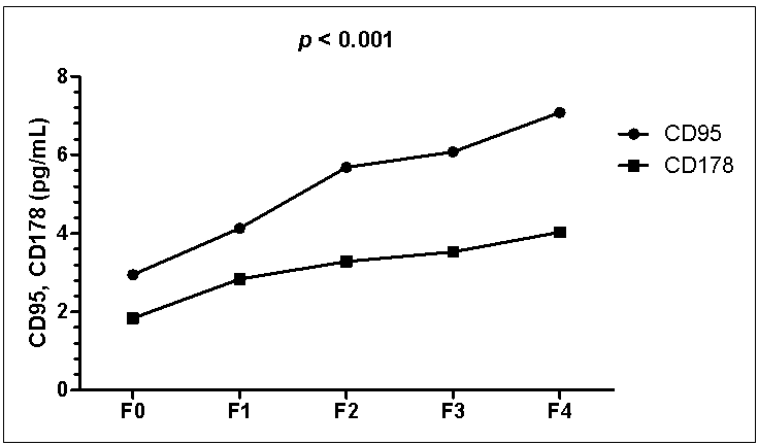

Figure 2. Comparing the levels of CD95 and CD178 in HBV-infected patients, sorting by fibrosis score, at Yaoundé General Hospital, Cameroon, September 2019 – December 2021 (*Source*: Compilation of our basic data)

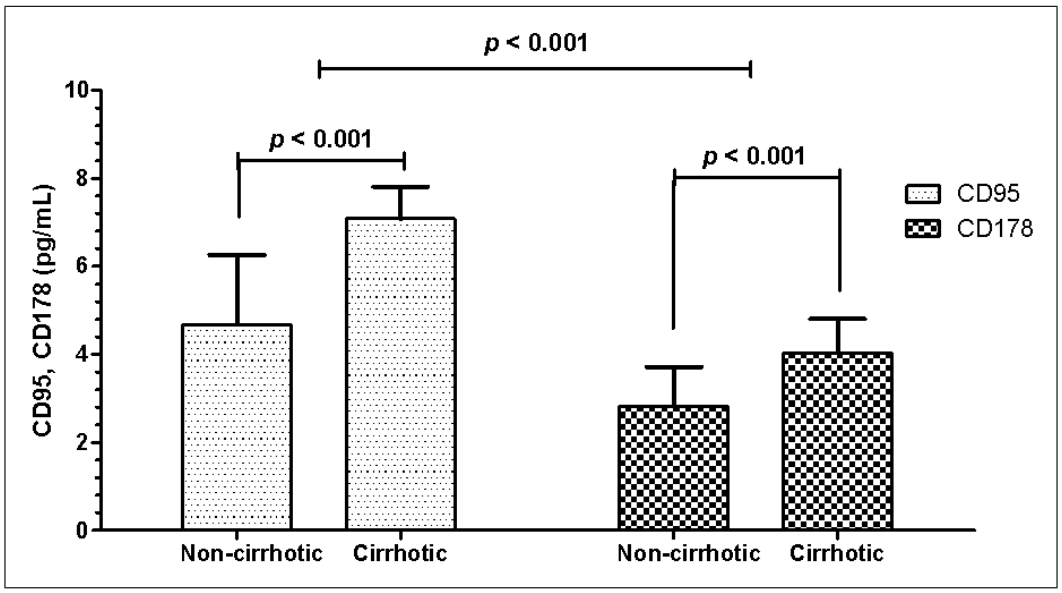

Figure 3. Comparing the levels of CD95 and CD178 in HBV-infected patients, sorting by hepatitis state, at Yaoundé General Hospital, Cameroon, September 2019 – December 2021 (*Source*: Compilation of our basic data)

3.4. Comparing the Levels of CD95 and CD178 in HBV-infected Patients, Sorting by Hepatitis State, at Yaoundé General Hospital, Cameroon, September 2019 - December 2021

The level of CD95 in cirrhotic HBV-infected patients ranged from 5.42 to 8.5 pg/mL, with a median of 6.96 pg/mL. The level of CD95 in non-cirrhotic HBV-infected patients ranged from 1.35 to 7.1 pg/mL, with a median of 5.1 pg/mL. The difference between these two groups was statistically significant, with a p value < 0.001 (Figure 3). The level of CD178 in cirrhotic HBV-infected patients ranged from 2.3 to 4.9 pg/mL, with a median of 4.2 pg/mL. The level of CD178 in non-cirrhotic HBV-infected patients ranged from 0.28 to 4.51 pg/mL, with a median of 3.07 pg/mL. The difference between these two groups was statistically significant, with a p value < 0.001 (Figure 3).

3.5. Correlation between Fibrosis Score, CD95, CD178, and HBV Viral Load in HBV-infected Patients at Yaoundé General Hospital, Cameroon, September 2019 - December 2021

There was a statistically significant and positive correlation between CD95 and CD178 (r = 0.715, $p < 0.001$), between CD95 and fibrosis score (r = 0.864, $p < 0.001$) and between CD95 and HBV viral load (r = 0.414, $p < 0.001$). We found a statistically significant and positive correlation between CD178 and fibrosis score (r = 0.757, $p < 0.001$), and between CD178 and HBV viral load (r = 0.384, $p < 0.001$). We also found a statistically significant and positive correlation between fibrosis score and HBV viral load (r = 0.383, $p < 0.001$) (Table 3).

3.6. Distribution of CD95 and CD178 Polymorphisms in HBV-infected Patients and Healthy Patients at Yaoundé General Hospital, Cameroon, September 2019 - December 2021

The frequencies of AA, AG and GG genotypes for CD95-670 A/G polymorphism were respectively 13.33%, 53.33%, and 33.33% among the healthy patients, and 22.27%, 64.29%, and 13.45% in HBV-infected patients respectively. For CD95-670 A/G polymorphism, frequencies of A and G allele were respectively 60% and 40% among the healthy patients, and 78.15% and 21.85% in HBV-infected patients respectively. Therefore, the distribution of genotypes and allele frequencies for CD95-670 A/G polymorphism was statistically different between healthy patients and HBV-infected patients (Table 4).

The frequencies of AA, GA and GG genotypes for CD95-1377 G/A polymorphism were respectively 6.67%, 20%, and 73.33% among the healthy patients, and 6.72%, 13.45%, and 79.83% in HBV-infected patients respectively. For CD95-1377 G/A polymorphism, frequencies of A and G allele were respectively 6.67% and 99.33% among the healthy patients, and 12.61% and 87.39% in HBV-infected patients respectively. Distribution of genotypes and allele frequencies for CD95-1377 G/A polymorphism was not statistically different between healthy patients and HBV-infected patients (Table 4).

The frequencies of CC, CT and TT genotypes for CD178-844 C/T polymorphism were respectively 33.33%, 60%, and 6.67% among the healthy patients, and 22.27%, 53.36%, and 18.79% in HBV-infected patients respectively. For CD95-670 A/G polymorphism, frequencies of C and T allele were respectively 73.33% and 26.67% among the healthy patients, and 70.59% and 29.41% in HBV-infected patients respectively. Therefore, the distribution of genotypes for CD178-844 C/T polymorphism was statistically different between healthy patients and HBV-infected patients, and the distribution of allele frequencies was not statistically different (Table 4).

3.7. Distribution of CD95 and CD178 Polymorphisms in HBV-infected Patients Sorting by Fibrosis Score, at Yaoundé General Hospital, Cameroon, September 2019 - December 2021

Among the HBV-infected patients raged from F0 to F4, the frequencies of AA, AG and GG genotypes for CD95-670 A/G polymorphism were respectively 42.68%, 14.29% and 42.86% among the F0 patient; 33.33%, 66.67% and 0% among the F1 patients; 40%, 60% and 0% among the F2 patients; 24.44%, 75.56% and 0% among the F3 patients; and 0%, 87.50% and 12.50% among the F4 patients. For CD95-670 A/G polymorphism, frequencies of A and G allele were respectively 57.14% and 42.86% among the F0 patient; 100% and 0% among the F1 patient; 100% and 0% among the F2 patient; 71.11% and 28.89% among the F3 patient; and 79.55% and 20.45% among the F4 patient (Table 5).

The frequencies of AA, GA and GG genotypes for CD95-1377 G/A polymorphism among the HBV-infected patients raged from F0 to F4, were respectively 14.29%, 42.66% and 42.86% among the F0 patient; 33.33%, 33.33% and 33.33% among the F1 patients; 0%, 0% and 100% among the F2 patients; 4.44%, 8.89% and 86.67% among the F3 patients; and 0%, 0% and 100% among the F4 patients. For CD95-1377 G/A polymorphism, frequencies of A and G allele were respectively 28.57% and 71.43% among the F0 patient; 66.67% and 33.33% among the F1 patient; 0% and 100% among the F2 patient; 4.44% and 95.56% among the F3 patient; and 0% and 100% among the F4 patient (Table 5).

The frequencies of CC, CT and TT genotypes for CD178-844 C/T polymorphism among the HBV infected patients raged from F0 to F4, were respectively 28.57%, 42.86% and 28.57% among the F0 patient; 33.33%, 33.33% and 33.33% among the F1 patients; 0%, 60% and 40% among the F2 patients; 53.33%, 26,67% and 20,00% among the F3 patients; and 25%, 75% and 0% among the F4 patients. For CD178-844 C/T polymorphism, frequencies of C and T allele were respectively 57.14%

and 42.86% among the F0 patient; 66.67% and 33.33% among the F1 patient; 60% and 40% among the F2 patient; 55.56% and 44.44% among the F3 patient; and 90.91% and 9.09% among the F4 patient (Table 5).

Therefore, the distribution of genotypes and alleles for CD95-670 A/G, CD95-1377 G/A and CD178-844 C/T polymorphisms were statistically different stages of fibrosis among HBV-infected patients (Table 5).

Table 3. Correlation between fibrosis score, CD95, CD178, and HBV viral load in HBV-infected patients at Yaoundé General Hospital, Cameroon, September 2019 – December 2021.

Immunologic, hepatic and viral parameters	HBV viral load (UI/mL)		Fibrosis score		CD178 (pg/mL)		CD95 (pg/mL)	
	r	*p*	r	*p*	r	*p*	r	*p*
CD95 (pg/mL)	0.414	< **0.001**	0.864	< **0.001**	0.715	< **0.001**		
CD178 (pg/mL)	0.384	< **0.001**	0.757	< **0.001**	1	---		
Fibrosis score	0.383	< **0.001**	1	---	---	---		
HBV viral load (UI/mL)								
< 10^3	---	---	0.281	< **0.001**	-0.134	0.219	0.238	< **0.001**
10^3 to 10^5	---	---	0.162	0.144	0.137	0.216	0.222	< **0.001**
10^5 to 10^7	---	---	-0.227	0.159	0.024	0.884	-0.169	0.297
10^7 to 10^9	---	---	-0.988	< **0.001**	-0.903	< **0.001**	-0.855	< **0.001**
> 10^9	---	---	---	---	---	---	---	---

Source: Compilation of our basic data.

Table 4. Distribution of CD95 and CD178 Polymorphisms in in HBV-infected patients and healthy patients at Yaoundé General Hospital, Cameroon, September 2019 – December 2021

Genotypes and Alleles	Healthy patients (n = 105)	HBV-infected patients (n = 238)	P-Value	OR	95% CI	Chi-Squared
CD95-670 A/G						
Genotype						
AA	14 (13.33%)	53 (22.27%)				
AG	56 (53.33%)	153 (64.29%)	< **0.001**	---	---	19.165
GG	35 (33.33%)	32 (13.45%)				
Allele						
A	63 (60%)	186 (78.15%)	< **0.001**	2.3846	1.451 - 3.920	
G	42 (40%)	52 (21.85%)				
CD95-1377 G/A						
Genotype						
AA	7 (6.67%)	16 (6.72%)				
GA	21 (20%)	32 (13.45%)	0.298	---	---	2.421
GG	77 (73.33%)	190 (79.83%)				
Allele						
A	7 (6.67%)	30 (12.61%)	0.131	2.0192	0.858 - 4.758	
G	98 (99.33%)	208 (87.39%)				
CD178-844 C/T						
Genotype						
CC	35 (33.33%)	67 (22.27%)				
CT	63 (60%)	127 (53.36%)	**0.0176**	----	---	8.084
TT	7 (6.67%)	44 (18.79%)				
Allele						
C	77 (73.33%)	168 (70.59%)	0.697	0.8727	0.522 - 1.460	
T	28 (26.67%)	70 (29.41%)				

Source: Compilation of our basic data.

Table 5. Distribution of CD95 and CD178 Polymorphisms in HBV-infected patients sorting by fibrosis score, at Yaoundé General Hospital, Cameroon, September 2019 – December 2021

Genotypes and alleles	HBV-infected patients (n = 238)					P-Value
	F0 (n = 49)	F1 (n = 21)	F2 (n = 35)	F3 (n = 45)	F4 (n = 88)	
CD95-670 A/G						
Genotype						
AA	21 (42.86%)	7 (33.33%)	14 (40%)	11 (24.44%)	0 (0%)	
AG	7 (14.29%)	14 (66.67%)	21 (60%)	34 (75.56%)	77 (87.50%)	< **0.001**
GG	21 (42.86%)	0 (0%)	0 (0%)	0 (0%)	11 (12.50%)	
Allele						
A	28 (57.14%)	21 (100%)	35 (100%)	32 (71.11%)	70 (79.55%)	< **0.001**
G	21 (42.86%)	0 (0%)	0 (0%)	13 (28.89%)	18 (20.45%)	
CD95-1377 G/A						
Genotype						
AA	7 (14.29%)	7 (33.33%)	0 (0%)	2 (4.44%)	0 (0%)	
GA	21 (42.66%)	7 (33.33%)	0 (0%)	4 (8.89%)	0 (0%)	< **0.001**
GG	21 (42.86%)	7 (33.33%)	35 (100%)	39 (86.67%)	88 (100%)	
Allele						
A	14 (28.57%)	14 (66.67%)	0 (0%)	2 (4.44%)	0 (0%)	< **0.001**
G	35 (71.43%)	7 (33.33%)	35 (100%)	43 (95.56%)	88 (100%)	
CD178-844 C/T						
Genotype						
CC	14 (28.57%)	7 (33.33%)	0 (0%)	24 (53,33%)	22 (25%)	
CT	21 (42.86%)	7 (33.33%)	21 (60%)	12 (26,67%)	66 (75%)	< **0.001**
TT	14 (28.57%)	7 (33.33%)	14 (40%)	9 (20,00%)	0 (0%)	
Allele						
C	28 (57.14%)	14 (66.67%)	21 (60%)	25 (55.56%)	80 (90.91%)	< **0.001**
T	21 (42.86%)	7 (33.33%)	14 (40%)	20 (44.44%)	8 (9.09%)	

Source: Compilation of our basic data.

4. Discussion

The CD95 and CD178 system is an important route of apoptosis in the liver [3,13]. This study focused on investigation of the involvement of CD95-CD178 and associated genetic polymorphisms in viral persistence and hepatic cytolysis in patients with chronic VHB.

Concentrations of CD95 and CD178 were higher in HBV-infected patients than in healthy patients, with a statistically significant difference between these two groups. These results correlate those of Azebaze et al. [3], and Peter et al. [16], who had observed a low constitutive expression of CD95 in healthy people, compared with patients with chronic HBV infection. The results obtained in the present study are also in line with literature data, according to which the expression of CD95 is overexpressed during HBV infection [11]. This result would be justified by the fact that the expression of the CD95 would be increased in response to a primary stimulus and make the hepatocytes more susceptible to stimulation by CD178. This hypothesis is supported by the observation that induction of CD95 expression occurs as a result of chronic lymphohistiocytic inflammation in different cells [17], thus liver cells in our case. Moreover, with regard to the increase of CD178, its expression is activated by the T-Cell Receptor (TCR), costimulatory molecules, and cytokine receptors [18].

We have noted progressive increases in CD95 and CD178 with the progression of F0 to F4 fibrosis in HVB-infected patients. These results suggest that the CD95-CD178 apoptotic signaling pathway would be implicated in the cytolysis of hepatocytes, seen in HVB-infected patients. These results corroborate those obtained by Nagata and O'Connell in 1999, who had noted a positive correlation between the level of serum CD178 and fibrosis progression, leading them to suggest that CD95-CD178 system induces the apoptosis of hepatocytes [19,20]. Otherwise, in the CD95-mediated apoptotic pathway, binding of CD178 drives CD95 clustering and binding of CD95 to the Fas Associated Death Domain (FADD). FADD recruits caspase-8 and caspase-10 to form the death-inducing signaling complex (DISC) [21]. The Death Inducing Signaling Complex (DISC) is activated by specific post-translational modifications of the Death Receptor (DR), such as palmitoylation and O-linked glycosylation [22]. The DISC mediates autocatalytic processing and activation of caspase-8 and caspase-10, which propagate the death signal, which would be responsible for the consequent destruction of hepatocytes

The concentrations of CD95 and CD178, higher in cirrhotic patients than in non-cirrhotic patients, corroborate the observations of Peter et al. [16], who had noted a low constitutive expression of CD95 in non-cirrhotic patients with chronic VHB, compared to patients with cirrhosis related to HBV. The results obtained in this study are also in accordance with literature that CD95 is overexpressed during HBV infection [23]. This expression of CD95 would be increased in response to a primary stimulus, and would make hepatocytes more susceptible to stimulation by CD178. This hypothesis is corroborated by the observation that the induction of CD95 expression

occurs as a result of chronic lymph histiocytic inflammation in different epithelial cells [24]. These results suggest that liver destruction in HBV infected patients may primarily involve the destruction of hepatocytes by T-cells using the CD95-CD178 receptor-ligand system.

There was a statistically significant positive correlation between the fibrosis score and the CD95 and CD178 levels in these HBV infected individuals; this reflected an association between liver injury and activation of the CD95-CD178 apoptosis pathway. These results corroborate those obtained by Peter et al. in 1995 in a study of CD95 receptor and ligand involvement in hepatic injury, where CD95 receptor expression was very high in hepatocytes [16]. These results are also in parallel with those obtained in the case of infections with the hepatitis C virus by Hayashi and Mita [25], where the expression of CD95 was upregulated according to the severity of inflammation of the liver.

We also found a statistically significant positive correlation between the concentration of CD95, CD178 and viral load of HBV in infected individuals. These results suggest that the increase in CD95 expression was positively associated with an increase in viremia. This could be justified by the destruction of activated lymphocyte cells responsible for viral clearance by the CD95-CD178 signaling pathway, thus leading to free viral proliferation. In our study, there was a statistically significant positive correlation between fibrosis score and HBV viral load in HBV-positive patients; this suggests that the increase in HBV viremia was related to a worsening of cirrhotic hepatocyte lesions because the factor mainly associated with the risk of cirrhosis is clearly the level of viral load [26]. The higher the viral load, the higher the relative risk of cirrhosis or cancer in these patients initially without cirrhosis, irrespective of transaminase activity [26].

The results obtained from this study allowed us to highlight the predominance of AA and AG genotypes for CD95-670 A/G polymorphism, in HBV-infected patients, compared with healthy patients. These results corroborate those obtained in 2016 by Liao et al. In a study on HCV [27]. However, we noted a low representation of the genotype GG for CD95-670 A / G polymorphism, in HBV-infected patients, compared to healthy patients. These results marked by differences all statistically significant, lead us to suggest that the genotypes AA and AG for CD95-670 A/G polymorphism are involved in the strong expression of CD95 in HBV-infected patients.

We also noted a decrease in the expression of CC and CT genotypes for CD178-844 C/T polymorphism, in HBV-infected patients, compared with healthy patients, and an increase in TT genotype expression in HBV-infected patients. As observed in a similar study in 2003 by Wu et al, but focusing on HCV, our measurements lead us to suggest that TT genotypes for CD178-844 C/T polymorphism are involved in the overexpression of CD178 in HBV-infected patients.

Although Jung et al. Showed in a 2007 study that there was no significant association between CD178 polymorphism (-844 C/T) and clearance of HBV in patients with hepatitis Chronic [28], we nevertheless point out a significant difference in the distribution of CC, CT and CC genotypes between healthy patients and HBV-infected patients. This discrepancy between our results and those of Jung [28] could be explained by genetic differences between the populations studied. Our results also corroborate those of Asadollah et al. obtained in 2015, in a study of CD95 and CD95-ligand polymorphisms in Hepatitis B virus infection [29], in which they noted that polymorphism CD178-844 C/T was significantly different in the naturally restored group and in patients with HBV infection.

Many studies have shown that the A allele and the AA genotype of CD95-670 are associated with liver diseases, such as autoimmune hepatitis and the development of cirrhosis [30], the rejection of a liver transplant [31] and hepatitis C [32]. In the same idea as Zamani et al. who showed that the CD95-670 A/G polymorphism was associated with chronic HBV infection, unlike the CD95-1377 G/A polymorphism; [33], we suggest that the CD95-670 A/G polymorphism is associated with chronic HBV infection.

Moreover, concerning the polymorphisms CD95-670 A/G and CD95-1377 G/A, the differences in non-significant distribution observed between the genotypes could be explained by the substitution of the A allele for G in the CD95-670 polymorphism. 670 polymorphism CD95-1377.

Moreover, concerning the polymorphisms CD95-670 A/G and CD95-1377 G/A, the differences in non-significant distribution observed between the genotypes could be explained by the substitution of the A allele in the CD95-670 polymorphism, by the G allele in CD95-1377 polymorphism.

Among the immune responses to HBV, apoptosis has the role of eliminating hepatocytes infected with HBV. Exaggeration and lack of apoptosis are associated with chronic complications of HBV. The absence of apoptosis will lead to the persistence of HBV in hepatocytes and the evasion of the immune system, whereas exaggerated apoptosis is associated with an inflammatory reaction in the liver, leading to severe inflammation associated with HBV infection.

We underlined a statistically significant overexpression of the AG genotype, and of the G allele of CD95-670 A/G among fibroses of higher score (F3, F4). These results are in accordance with those obtained by Wang et al., McIlroy et al., and Liao et al. [27,34].

These data suggest that the AG genotype of CD95-670 A/G may be associated with fibrosis severity, and occurrence of cirrhosis due to HBV infection. Moreover, the linear significance between the expression of CD95 in the liver tissue and these genotypes suggests that these genotypes could be considered as markers indicative of the degree of hepatic injury, thus liver fibrosis [27,35].

It is important to note that the A allele and AA and GA genotypes of CD95-1377 G/A showed a low risk of HBV fibrosis, while the G allele and the GG genotype of CD95-1377 G/A were suggestive of an increased risk of hepatic cirrhosis. It is also apparent from our study that the T allele and the TT genotype of CD178-844 C / T indicated a low risk of HBV fibrosis, while the C allele and G/A CT genotype of CD95-1377 suggested an increased risk of hepatic cirrhosis.

Thus, the correlation between CD95, CD178 genotypes and hepatic fibrosis degrees provides a data set indicating that CD95-mediated hepatocyte apoptosis would contribute significantly to the pathology of chronic Viral hepatitis B. Genetic polymorphisms that modify the level of expression of CD95 and CD178 in hepatocytes are therefore likely to modify the apoptosis of hepatocytes and thus affect the degree of fibrosis in HBV-infected patients, as in the case of HCV [34].

The genetic polymorphism of the CD95 gene could therefore explain some of the histopathological variability during chronic Viral Hepatitis B.

5. Conclusion

The results of the present study highlight the involvement of CD95 and CD178 and associated genetic polymorphisms in viral persistence and hepatic cytolysis in patients with chronic VHB. It results mainly that the increase of the expression of genotypes AG of CD95-670 A/G; GG genotypes of CD95-1377 G/A and CT genotypes of CD178-844 C/T is associated with the severity of liver fibrosis, with the overexpression of CD95 and CD178 in HVB-infected patients, and by implication, with the increase and the persistence of HVB viral load.

Although apoptosis and cellular immune responses are essential for maintaining homeostasis of the liver and for the elimination or persistence of viral infections, cirrhosis is the result of inflammation and chronic liver damage. Therefore, changes in the expression of CD95 / CD178 in the liver can be harmful and destructive.

The present results may reveal that the study of CD95 / CD178 expression related to its genotypes can be used to guide the clinical staging of the patient and can be considered as a new prognostic marker.

6. Limitations

Our study did not evaluate the activation of the CD95 & CD178 signaling pathway depending on time, during the evolution of Hepatitis B Virus infection in infected patients.

Abbreviations

VHB, Viral Hepatitis B;
HVB, Hepatitis B Virus;
AICD, Activation-Induced Cell Death;
ALPS, Autoimmune Lymphoproliferation Syndrome;
bp, base pair;
cFLIP, caspase-8-Like Inhibitory Protein;
DISC, Death Inducing Signaling Complex;
DR, Death Receptor;
FADD, Fas Associated Death Domain;
Mach, Mort-1 associated Ced-3 homologue;
NF-κB, Nuclear Factor-kappa β;
ng, nanogram;
qPCR, quantitative PCR;
TCR, T-Cell Receptor;
TNF-α, tumor necrosis factor-alpha;
TRAF2, TNF-Receptor-Associating Factor 2;
TRAIL, TNF-related apoptosis-inducing ligand;
SD, Standard Deviation;
CD178, Cluster of Differentiation 178;
CD95, Cluster of Differentiation 95;
CD95L, Cluster of Differentiation 95 Ligand;
mL, milliliter;
pg, picogram;
CD4, Cluster of Differentiation 4;
CD8, Cluster of Differentiation 8;
µL, microliter;
r, Spearman's correlation coefficient;

Implication for Health Policy/Practice/Research/Medical Education

This article was a research article in the medical field concerned with patients with liver disease particularly those with liver cirrhosis caused by Hepatitis B Virus (HBV). This article tried to clarify the role of CD95 and CD178 and associated genetic polymorphisms in viral persistence and hepatic cytolysis in patients with chronic VHB.

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Conflict of Interest

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References

[1] Dane, D.S., Cameron, C.H. and Briggs, M, "Virus-like particles in serum of patients with Australia-antigen-associated hepatitis," *Lancet*, vol. 1, no. 7649, pp. 695-698, 1970.

[2] Alkahtani, S, "Hepatitis C infection and apoptosis in hepatocellular carcinoma," *Pak J Biol Sci*, vol. 12, pp. 804-808, 2009.

[3] Azebaze, A.F.S., Talla, P., Okomo, A.M.C., Jacobs, G., Mbakam, C.H., Guiedem, E., Mesembe, M.T., Lyonga, E. and George, M.I, "Involvement of CD95 and ligand in CD4+ T-cell and CD8+ T-cell depletion and hepatic cytolysis in patients with chronic viral hepatitis B," *African Journal of Laboratory Medicine*, vol. 10, p. 6, 15 March 2021.

[4] Maryam, K., Seyed, M. and Heidar, S, "Comparison of Serum Hepatitis B Virus DNA and HBsAg Levels Between HBeAg-Negative and HBeAg-Positive Chronic Hepatitis B Patients," *Jundishapur Journal of Microbiology*, vol. 8, no. 3, p. e21444, 2015.

[5] Zeisel, M.B., Fofana, I., Fafi-Kremer S. and al., "Hepatitis C virus entry into hepatocytes: molecular mechanisms and targets for antiviral therapies," *J Hepatol*, vol. 54, p. 566-76, 2011.

[6] Pizon, M., Rampanarivo, H., Rampanarivo, S., Chaigne-Delalande, B., Daburon, S., Castroviejo, M., Moreau, P., Moreau, J.F. and Legembre, P, "Actin-independent exclusion of CD95 by PI3K/AKT signalling: implications for apoptosis," *The Journal of Immunology*, vol. 41, no. 8, pp. 2368-2378, 2011.

[7] Behrmann, I., Walczak, H. and Krammer, P.H, "Structure of the human APO-1 gene," *Eur J Immunol*, vol. 24, no. 12, p. 3057-3062, 1994.

[8] Takahashi, T., Tanaka, M., Inazawa, J., Abe, T., Suda, T. and Nagata, S, "Human Fas ligand: gene structure, chromosomal location and species specificity," *Int Immunol*, vol. 6, no. 10, p. 1567-1574, 1994.

[9] Huang, D.C., Hahne, M., Schroeter, M., Frei, K., Fontana, A., Villunger, A., Newton, K., Tschopp, J. and Strasser, A, "Activation of Fas by FasL induces apoptosis by a mechanism that cannot be blocked by Bcl-2 or Bcl-x(L)," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, p. 14871-14876, 1999.

[10] Wu, J., Metz, C., Xu, X., Abe, R., Gibson, A.W., Edberg, J.C. and al., "A novel polymorphic CAAT/enhancer-binding protein beta element in the FasL gene promoter alters Fas ligand expression: a candidate background gene in African American systemic lupus erythematosus patients," *J Immunol*, vol. 70, no. 1, pp. 132-138, 2003.

[11] Lee, J.Y., Chae, D.W., Kim, S.M., Nam, E.S., Jang, M.K., Lee, J.H., Lee, H.Y. and Yoo, J.Y, "Expression of FasL and perforin/granzyme B mRNA in chronic hepatitis B virus infection," *Journal of Viral Hepatitis*, vol. 11, p. 130-135, 2004.

[12] Bortolami, M., Kotsafti, A., Cardin, R. and Farinati, F, "Fas / FasL system, IL-1beta expression and apoptosis in chronic HBV and HCV liver disease," *J Viral Hepat*, vol. 15, no. 7, p. 515-522, 2008.

[13] Guicciardi, M.E. and Gores, G.J, "Apoptosis: a mechanism of acute and chronic liver injury," *Gut*, vol. 54, no. 7, p. 1024-1033, 2005.

[14] Miller, S.A., Dykes, D.D. and Polesky, H. F, "A simple salting out procedure for extracting DNA from human nucleated cells," *Nucleic Acids Res*, vol. 13, no. 3, p. 1215, 1988.

[15] Sun, T., Miao, X., Zhang, X., Tan, W., Xiong, P. and Lin, D, "Polymorphisms of death pathway genes FAS and FASL in esophageal squamous-cell carcinoma," *J Natl Cancer Inst*, vol. 96, no. 3, p. 1030-1036, 2004.

[16] Mann, B.H. and Withney, R.D, "On a Test of Whether one of Two Random Variables is Stochastically Larger than the Other," *The Annals of Mathematical Statistics*, vol. 18, n° %11, pp. 50-60, March 1947.

[17] William, H.K. and Wallis, A.W, "Use of Ranks in One-Criterion Variance Analysis," *Journal of the American Statistical Association*, vol. 47, n° %1260, pp. 583-621, 1952.

[18] Peter, R.G., Walter, J.H., Henning, W., Heinz, S., Gerd, O., Wolfgang, S., Peter, H.K. and Laura, R, "Involvement of the CD95 (APO-1/Fas) Receptor and Ligand in Liver Damage," *Journal of Experimental Medicine*, vol. 182, pp. 1223-1230, November 1995.

[19] Leithäiuse, F., Dhein, J., Mechtersheimer, G., Koretz, K., Briiderlein, S., Henne, C., Schmidt, A., Debatin, K.M., Krammer, P.H. and Möller, P, "Constitutive and induced expression of APO-1, a new member of the NGF/TNF receptor superfamily, in normal and neoplastic cells," *Laboratory Investigation*, vol. 69, pp. 415-429, 1993.

[20] Arakaki, R., Yamada, A., Kudo, Y., Hayashi, Y. and Ishimaru, N, "Mechanism of activation-induced cell death of T cells and regulation of FasL expression," *Critical Reviews in Immunology*, vol. 34, p. 301-314, 2014.

[21] Nagata, S, "Fas ligand-induced apoptosis," *Annual Review of Genetics*, vol. 33, p. 29-55, 1999.

[22] O'Connell, J., Bennett, M., O'Sullivan, G.C., Collins, J.K. and Shanahan, F, "The Fas counterattack: cancer as a site of immune privilege," *Immunology Today*, vol. 20, pp. 46-52, 1999b.

[23] Wilson, N.S., Dixit, V. and Ashkenazi, A, "Death receptor signal transducers: nodes of coordination in immune signaling networks," *Nature Immunology*, vol. 10, p. 348-355, 2009.

[24] Wagner, K.W., Punnoose, E.A., Januario, T., Lawrence, D.A., Pitti, R.M., Lancaster, K., Lee, D., Von Goetz, M., Yee, S.F., Totpal, K., Huw, L., Katta, V., Cavet, G., Hymowitz, S.G., Amler, L. and Ashkenazi, A, "Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL," *Nature Medicine*, vol. 13, p. 1070-1077, 2007.

[25] Liu, W., Lin, Y.T., Yan, X.L., Ding, Y.L., Wu, Y.L., Chen, W.N. and Lin, X, "Hepatitis B virus core protein inhibits Fas-mediated apoptosis of hepatoma cells via regulation of mFas/FasL and sFas expression," *FASEB J*, vol. 29, no. 3, pp. 1113-1123, 2015.

[26] Mueller, D.L, "Mechanisms maintaining peripheral tolerance," *Nature Immunology*, vol. 11, p. 21-27, 2010.

[27] Hayashi, N. and Mita, E, "Fas system and apoptosis in viral hepatitis," *Journal of Gastroenterology and Hepatology*, vol. 12, no. 9-10, pp. S223-S226, 1997.

[28] Chen, C.J., Yang, H., Su, J., Jen, C.L., You, S.L., Lu, S.N., Huang, G.T. and Iloeje, U.H, "Risk of hepato-cellular carcinoma across a biological gradient of serum hepatitis B virus DNA level," *Journal of the American Medical Association*, vol. 295, pp. 65-73, 2006.

[29] Liao, G.S., Lo, C.H., Chen, T.W. and al., "Fas/FasL polymorphisms are associated with hepatitis C related cirrhosis and serum Alpha-Fetoprotein with hepatocellular carcinoma patients," *J Cancer Res Prac*, 2016.

[30] Jung, Y.J., Kim, Y.J., Kim, L.H. and al., "Association présumée de polymorphismes des gènes Fas et FasL avec les résultats cliniques de l'infection par le virus de l'hépatite B," *Intervirologie*, vol. 50, pp. 369-376, 2007.

[31] Asadollah, M., Nader, T., Alireza, S.-H., Seyed, M.A., Zohreh, S. and Lida, J, "FAS and FAS-Ligand Promoter Polymorphisms in Hepatitis B Virus Infection," *Hepat Mon*, vol. 15, no. 10, p. e26490, October 2015.

[32] Agarwal, K, Czaja, A.J. and Donaldson, P.T, "A functional Fas promoter polymorphism is associated with a severe phenotype in type 1 autoimmune hepatitis characterized by early development of cirrhosis," *Tissue Antigens*, vol. 69, no. 3, p. 227-235, 2007.

[33] Marin, M., Muro, M., Moya-Quiles, M., Miras, M. Minguela, M., Bermejo and al., "Étude des polymorphismes de Fas (CD95) et de FasL (CD178) chez des greffés du foie," *Tissu Antigens*, vol. 67, no. 2, p. 117-126, 2006.

[34] Aguilar-Reina, J., Ruiz-Ferrer, M., Pizarro, M.A. and Antinolo, G, "Le polymorphisme -670A> G dans la région promotrice du gène FAS est associé à une nécrose des zones périortales chez les patients atteints d'hépatite chronique C," *J Hépat viral*, vol. 12, no. 6, p. 568-573, 2005.

[35] Zamani, A.G., Barlas, I.O., Durakbasi-Dursun, G., Oural, O., Erdal, E. and Yildirim, M.S, "Evaluation of death pathway genes FAS and FASL polymorphisms in chronic HBV infection," *Int J Immunogenet*", vol. 40, no. 6, p. 482-487, 2013.

[36] McIlroy, D., Thodorou, I., Ratziu, V. and al., "FAS promoter polymorphisms correlatewith activity grade in hepatitis C patients," *Eur J Gastroenterol Hepatol*, p. 1081-1088, 2005.