MITOCHONDRIAL STRESS IN PATIENTS WITH CHRONIC HEPATITIS B AND ADVANCED FIBROSIS

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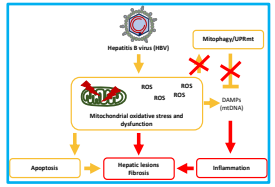
BACKGROUND & AIMS

Around **257 million people are chronically infected by Hepatitis B virus** (HBV). Despite the availability of an effective prophylactic vaccine, new infections still occur.[1] Chronic Hepatitis B (CHB) infection can lead to some complications including fibrosis, cirrhosis and hepatocellular carcinoma (HCC).

Mitochondria are the main source of reactive oxygen species (ROS) within hepatocytes. HBV can interact with complex IV of the respiratory chain and increase mitochondrial oxidative stress (Fig. 1). Mitochondrial DNA (mtDNA) is particularly sensitive to oxidative stress beacause it lacks protective histones and due to its proximity to the respiratory chain complexes.[2]

We postulated that HBV may cause oxidative damage to mtDNA resulting in mitochondrial dysfunction and altered mitochondrial dynamics, all of which might account for fibrosis progression in CHB patients (Fig. 1).

Figure 1. Work hypothesis



PATIENTS & METHODS

136 naïve patients with CHB and 12 control liver specimen (absence of inflammation and pathological pattern), were included in the present study (**Table 1**). CHB patients were HBV mono-infected for more than 6 months, had no excessive alcohol or tobacco consumption.

Total DNAs, RNAs and proteins are isolated from biopsies of these patients. mtDNA damages were investigated by Southern-Blot and PCR. The expression of the main genes of interest was studied by RT-qPCR and Western-Blot.

We were particularly interested in the expression of the following genes: subunits 1 (MT-CO1), 2 (MT-CO2) and 4i1 (COX4i1) of the complex IV (cytochrome c oxidase) of the respiratory, LonP1 peptidases, HSP60, HSP70 chaperones (mitochondrial unfolded protein response, UPRmt), Parkin and PINK1 (mitophagy). Our statistical analysis was performed using the Mann Whitney T-test.

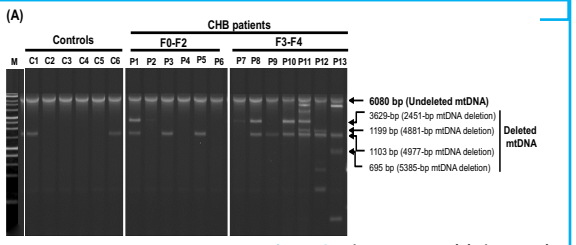
	mtDNA Damage Analysis		mRNA Analysis		Protein Analysis	
Metavir Score	F0-F1-F2	F3-F4	F0-F1-F2	F3-F4	F0-F1-F2	F3-F4
Number (n)	6	7	65	25	15	9
Gender M/F (n)	2/4	5/2	53/12	20/5	10/5	6/3
Age (years) (mean ± SEM)	42 ± 2.8	45 ± 4.1	41 ± 1.4	46 ± 2.7	45 ± 2.7	48 ± 3.6
BMI (Kg/m²) (mean ± SEM)	26.3 ± 2,2	24.5 ± 1.9	25.3 ± 0.7	24.8 ± 0.9	25,2 ± 1.2	23.7 ± 1
Serum levels at liver biopsy						
ALT (UI/L) (mean ± SEM)	110 ± 30	108 ± 16	107 ± 17	115 ± 32	82 ± 20	70 ± 25
AST (UI/L) (mean ± SEM)	75 ± 13	78 ± 10	71 ± 10	70 ± 14	86 ± 28	56 ± 17
γ-GT (UI/L) (mean ± SEM)	98 ± 22	75 ± 18	95 ± 21	62 ± 16*	155 ± 76	60 ± 13
Total cholesterol (g/L; mean ± SEM)	0.8 ± 0.3	1.0 ± 0.3	0.8 ± 0.1	1.1 ± 0.2	0.7 ± 0.1	0.7 ± 0.2
Triglycerides (mmole/L; mean ±SEM)	0.8 ± 0,4	0.9 ± 0,3	1.0 ± 0,1	1.3 ± 0,2	1,2 ± 0,4	0,7 ± 0,1
Glucose (mmole/L) (mean ± SEM)	5.2± 0,5	4.8 ± 0,3	4.9 ± 0.2	5.0 ± 0,3	5.4 ± 0,3	4.9 ± 0.2
Histological analysis						
Activity Grade (METAVIR A0,A1,A2, A3)	2, 3, 1, 0	0, 4, 2, 1	19, 37, 9, 0	0, 12, 8, 5	9, 6, 0, 0	0, 4, 4, 1
Fibrosis stage (METAVIR F1,F2,F3,F4)	2, 2, 2, 0, 0	0, 0, 0, 3, 4	19, 33, 13, 0, 0	0, 0, 0, 14, 11	4, 8, 3, 0, 0	0, 0, 0, 5, 4

RESULTS

mtDNA deletions and mtDNA levels in patients with CHB

MtDNA is a 16.5 kBp, double stranded, circular and supercoiled DNA that encodes for 7 respiratory chain proteins, 2 rRNAs and 22 tRNAs required for the intra-mitochondrial translation. CHB Patients with advanced fibrosis (F3-F4) exhibited either a single or multiple mtDNA deletions. Four different 2451-bp, 4881-bp, 4977, and 5385-bp deletions were observed (Figure 2A).

In addition, we observed a significant decrease by 46% in the mtDNA/nDNA ratio in CHB patients. mtDNA/nDNA significantly decreased by 36% and 76% in CHB patients with moderate to minimal fibrosis (F0-F2) and with advanced fibrosis/cirrhosis (F3-F4) respectively, when compared to controls (*p<0.05*) (Figure 2B).



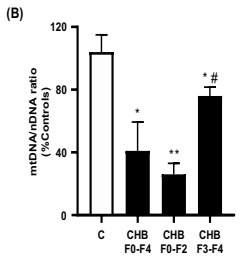
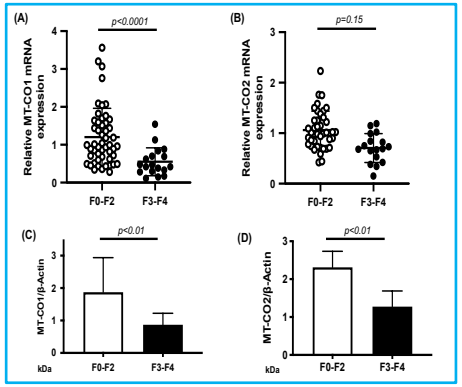


Figure 2. Liver mtDNA deletions and levels. (A) Long PCR detection of mtDNA deletions. Lanes C1-C6: hepatic DNA from six non-CHB controls with normal liver histology (C1-C3), mild fibrosis (C4-C5) or steatosis (lane C6). Lanes P1-P13: CHB patients with F0-F2 fibrosis (P1-P6) or F3-F4 fibrosis (P7-P13). (B) mtDNA/nDNA ratios. Results from 6 patients with F0-F2 fibrosis and 5 patients with F3-F4 fibrosis are expressed as percentages of 6 control values. *Different from control subjects, *p<0.05, **p<0.01.* [#]Different from patients with F0-F2 fibrosis, *p<0.05*.

mtDNA-encoded cytochrome *c* oxidase subunits 1 and 2 decrease in CHB patients with advanced fibrosis

Cytochrome *c* oxidase catalyzes the reduction of oxygen to water. Compared to CHB patients with F0-F2 fibrosis, patients with advanced fibrosis/cirrhosis showed a significant decrease for both MT-CO1 mRNA (1,20 ± 0,75 *vs* 0,55 ± 0,36 *p<0.0001*) and protein expression (3,44 ± 0,80 *vs* 2,55 ± 0,88 *p<0.01*) (Figure 3A, C).

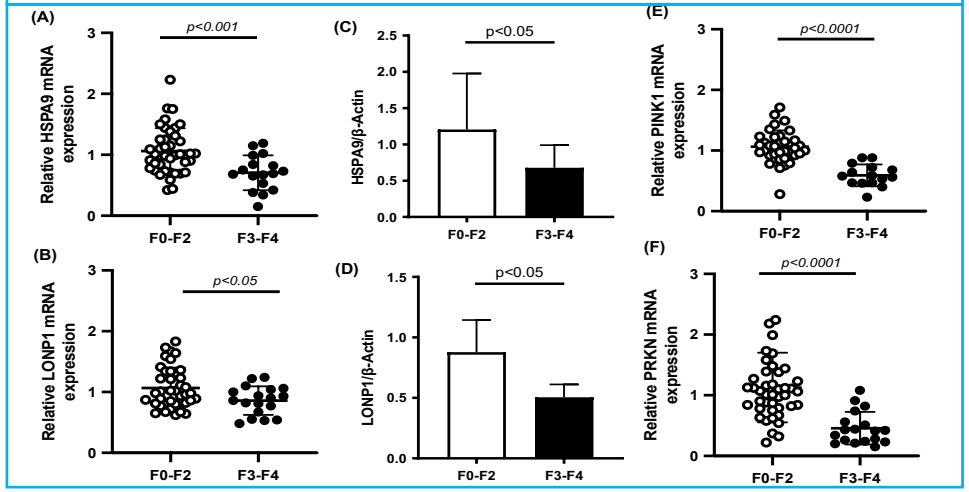
However, MT-CO2 proteins significantly decrease in F3-F4 fibrosis but mRNA expression remains unchanged whatever the stage of fibrosis (Figure 3B, D).



Altered mtUPR and Mitophagy in CHB patients with advanced fibrosis/cirrhosis

HSPA9 folds mitochondrial respiratory chain complexes and mitochondrial peptidases degrade mitochondrial protein aggregates. The imbalance of the stoichiometric ratio between mtDNA-encoded and nuclear DNA-encoded respiratory chain complexes may induce the transcription of HSPD1 and HSPA9 as well as LONP1 peptidase to activate the mitochondrial unfolded protein response (UPRmt). The relative mRNA expression of HSPA9 mRNA (F0-F2, n=42; 1,06 ± 0,37 *vs* F3-F4, n=17; 0,70 ± 0,28 *p<0.001*) and protein expression (F0-F2, n=11; 0.67 ± 0.31 and F3-F4, n=6; 1.20 ± 0.77; *p<0.05*) decreased significantly in advanced fibrosis (F3-F4) compared to patients with F0-F2 fibrosis (Figure 4A, C). LONP1 peptidase mRNA expression (F0-F2, n=40; 1,0 ± 0,33 *vs* F3-F4, n=17; 0,83 ± 0,22 *p<0.05*) and LONP1 protein expression (F0-F2, n=6; 0.87 ± 0.51 *vs* F3-F4, n=5; 0.67 ± 0.31, *p<0.05*) decreased signficantly in advanced fibrosis/cirrhosis (Figure 4B, D).

Mitophagy is the selective degradation of damaged or altered mitochondria. We evaluated two main markers of this process: PRKN and PINK1. The relative mRNA expression of both, PRKN (1,13 ± 0,57 *vs* 0,45 ± 0,27 *p<0.0001*) and PINK1 (1,06 ± 0,27 *vs* 0,59 ± 0,18 *p<0.0001*) decreased significantly in F3-F4 patients compared to F0-F1-F2 patients (Figure 4E, F). 6



CONCLUSION

Massive mtDNA damage associated with alterations of the mitochondrial function, mtUPR and mitophagy.

The extent of these mitochondrial lesions is associated with advanced HBV-mediated liver fibrosis. HBV has been shown to increase mitochondrial oxidative stress and mitochondrial ROS are known to degrade mtDNA.

Combined together, such mitochondrial alterations concomitantly contribute to a major mitochondrial dysfunction that may play an important role in the pathophysiology of CHB and fibrosis progression.

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