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ORIGINAL ARTICLE

Mitochondrial alterations in children with chronic liver disease

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Abstract *Background:* Over recent years it has become apparent that the hepatocyte mitochondrion functions both as a cause and as a target of liver injury. Resultant dysfunction of mitochondria yields deficient oxidative phosphorylation, increased generation of reactive oxygen species, impairment of other metabolic pathways and activation of both necrotic and apoptotic pathways of cellular death. *Methods:* This study was conducted on 26 children and adolescents with chronic liver disease who presented to or were following up in the Pediatric Hepatology Clinic, Children's Hospital, Ain-Shams University. They were divided into three groups according to the aetiology of liver disease (GI = patients with Wilson's disease (WD), GII = patients with chronic hepatitis C, GIII = patients with chronic liver disease other than Wilson's and chronic hepatitis C). Ultrasound-guided gun liver biopsies were performed, under local anaesthesia for all the 26 patients, using a modified 18-gauge truecut needle. Two liver biopsy cores were taken from each patient. One for light and electron microscopic examinations and the other was immediately immersed in liquid nitrogen to be frozen and used for studying mitochondrial DNA deletions by PCR.

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Results: Liver steatosis was higher in the group of patients with Wilson's disease and other liver disease. Electron microscopic examination of the mitochondria revealed significant mitochondrial pleomorphism in patients with Wilson's disease and patients with chronic hepatitis C infection. Enlarged mitochondria were found to be more prevalent among patients with chronic hepatitis C infection. Three of our patients (11.53%) had mitochondrial DNA deletions. We developed scoring system for mitochondrial affection in our patients, 7 patients (32%) were considered to have mild mitochondrial affection, 9 patients (41%) had moderate mitochondrial affection, while 6 patients (27%) had severe mitochondrial affection. Four of the studied patients had no mitochondrial affection.

Conclusion: Mitochondria affection is common in chronic liver disease. This mitochondrial affection might be responsible for some of the chronic liver disease manifestation such as easy fatiguability and steatosis.

1. Introduction

Chronic hepatitis is an ongoing injury to the liver cells which lasts for longer than 6 months. It may be caused by many factors such as: viruses (B, C and D), drugs, autoimmune disease, metabolic disease and other disorders. The hepatitis C virus (HCV) is one of the most important causes of chronic liver disease. It accounts for 60–70% percent of chronic hepatitis cases, and up to 50% of cirrhosis, end-stage liver disease and liver cancer [1]. An inherited defect in the metabolism of copper is known to cause chronic hepatitis (copper-associated hepatitis/copper toxicosis) [2].

Mitochondrial medicine is a new and rapidly developing medical subspeciality. Researchers discovered that mitochondria have their own DNA or "blue print" (mtDNA), which is different than the nuclear DNA (nDNA) found in the cell's nucleus. Mitochondria are the power house of the cell being responsible for processing oxygen and converting substances from the foods we eat into energy for the essential cell functions [3]. Mitochondrial and metabolic medical conditions are now referred to as mitochondrial cytopathies, which actually include more than 40 different identified diseases that have different genetic features [4].

Hepatic involvement is a common feature in childhood mitochondrial hepatopathies particularly in the neonatal period, and may manifest as neonatal acute liver failure, steatohepatitis, cholestasis, or cirrhosis with chronic liver failure of insiduous onset. In recent years, specific molecular defects (mutations in nuclear genes such as SCO1, BCS1L, POLG, DGUOK and MPV17 and the deletion or rearrangement of mitochondrial DNA) have been identified, with the promise of genetic and prenatal diagnosis [5]. Studies assessing mitochondrial function and structure in livers from humans with chronic liver disease, including liver cirrhosis, revealed a variety of alterations in comparison with normal subjects. Depending on the aetiology of chronic liver disease, the function of electron transport chain and/or ATP synthesis was found to be impaired, leading to decreased oxidative metabolism of various substrates and to impaired recovery of the hepatic energy state after a metabolic insult. The most important strategies to maintain an adequate mitochondrial function per liver are mitochondrial proliferation and increase in the activity of critical enzymes or in the content of cofactors per mitochondrion [6].

Hepatocyte mitochondrion functions are a cause as well as a target of liver injury [7]. In primary disorders, mitochondrial defect is the primary cause of liver disease often producing fatal hepatic failure in infancy or childhood. In secondary disorders, insult to mitochondria is caused by either a gene defect that affects non-mitochondrial proteins or by an exogenous injury to mitochondria [8].

Treatment of these disorders is currently empirical involving agents that may improve the redox status of mitochondria, promote electron flow, or act as mitochondrial antioxidants. Liver transplantation has occasionally been successful in patients who lack other systemic involvement [9].

The objective of the present study is the assessment of mitochondria in chronic liver disease among Egyptian children and adolescents through studying their morphology by light microscopy, electron microscopy and the deletion of mitochondrial DNA.

2. Subjects and methods

This study included 26 children and adolescents with chronic liver disease who presented to or were following up in the pediatric hepatology clinic, children's Hospital, Ain-Shams University in the period from 2006 to 2009. They were 14 males and 12 females with male to female ratio 1.6:1. Their age ranged from 3 months to 17 years with a mean age of 9 ± 3.8 years.

2.1. Inclusion criteria

Children and adolescents eligible to be included were:

1. Patients with chronic HCV infection, Wilson's disease or any other chronic liver disease (diagnosed clinically, biochemically and/or histologically).
2. Patients having single liver pathology.
3. Recently diagnosed patients who did not start treatment yet and had a clear definite diagnosis.

2.2. Exclusion criteria

Children and adolescents who were excluded were:

1. Patients who have started treatment.
2. Patients who presented with acute liver disease.
3. Patients suffering from more than one pathology directly affecting the liver (e.g., thalassemics).
4. Patients who proved to have primary mitochondrial disease.

5. Patients who were treated with anti-convulsants or other drugs that might affect mitochondria.
6. Patients having coagulopathy till being controlled.
   - Patients were classified into three groups based on the aetiology of liver disease:

Group I: Patients with Wilson's disease (11 patients).
Group II: Patients with chronic hepatitis C (6 patients).
Group III: Patients with chronic liver diseases other than Wilson's and chronic hepatitis C (9 patients), 2 with type 1 diabetes, 2 with glycogen storage disease, 2 with lipid storage disease, one with type I autoimmune hepatitis, one patient with congenital hepatic fibrosis and one patient with Budd-chiari syndrome.

2.3. Methods

All included patients were chosen after doing the following:

(A) Baseline assessment which included:

I. Full history taking: with special emphasis on duration of liver disease, symptoms suggestive of advanced liver disease (gastro-intestinal bleeding, jaundice, ascites, neurological manifestations, fatigue, any other system affection, etc.).
II. Thorough clinical examination with special emphasis on signs of chronic liver disease (jaundice, clubbing, spider naevi, edema, distended abdominal wall veins, etc.), abdominal examination (ascites, liver and spleen size), signs of hyperdynamic circulation, and full neurological examination.
III. Investigations: complete blood picture, liver functions tests which included; serum ALT, AST, alkaline phosphatase, total bilirubin, direct bilirubin, albumin and prothrombin time (INR), HCV antibody, HCV RNA by PCR for patients with positive HCV-antibody, serum ceruloplasmin, abdominal doppler U/S with special emphasis on size of liver and spleen, texture of liver, portal circulation, presence or absence of collaterals and slit lamp examination of the eye for Kayser Fleisher ring.

2.3.1. Liver biopsy

Ultrasound-guided liver biopsies were performed, under local anaesthesia for all the 26 patients, using a ultrasound guided modified 18-gauge truecut needle. Two liver biopsy cores were taken from each patient. One for light and electron microscopic examinations and the other core was immediately immersed in liquid nitrogen to be frozen and used for studying mitochondrial DNA by PCR.

2.3.2. Light microscopy

Liver biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. Six serial sections (thickness, 4–5 μm) were cut and stained with haemotoxylin-eosin and Van Gieson stains. Histological findings and fibrosis degree were scored according to the numerical scoring system by Knodell et al. [10]. Histological findings were blindly interpreted by a senior histopathologist.

2.3.3. Electron microscopy

An aliquot of liver biopsy specimen was fixed in 0.5% glutaraldehyde plus 4% paraformaldehyde, then postfixed in osmium tetroxide. The liver biopsy specimen was then dehydrated in graded alcohol and embedded in araldite. After uranyl acetate and lead citrate staining the thin sections were observed with a Philips 400 T transmission electron microscopy. Histological findings were interpreted by a senior histopathologist.

2.3.4. Mitochondrial DNA PCR

All the DNA samples were extracted from liver biopsies (frozen section) and whole blood (10 ml heparinized whole blood) according to Goto et al. [11]. We quantitated mitochondrial DNA for deletion by the method described by Goto [12]. Approximately 2 μg DNA aliquot from patient and from one control (wild DNA and plasmid with known mitochondrial DNA deletion) were used in two polymerase chain reactions each amplifying a part of the mitochondrial DNA sequence; the first reaction amplifying 11.2 kb while the second reaction amplifying 5.3 kb, to check the whole mitochondrial DNA sequencing for any deletions. Long PCR was done in each reaction using forward and reverse primers. The first set P1001, P1004, covered the longer portion of mitochondrial DNA, while the second set P1003, P5204 covered the smaller portion of mitochondrial DNA. The template DNA from all patients was amplified using thermal cycler (PJ 9600) (Perkin Elmer). The amplified DNA fragments were electrophoretically checked on 1% agarose gel to check for the deleted DNA.

2.3.5. Statistical analysis

The results of light microscopic, electron microscopic examination and mitochondrial DNA-PCR assessment of the three groups were tabulated and statistically analyzed using personal computer with SPSS software package. We used repeated measures ANOVA, Pearson χ^2-test and Z-test. A cut point of 0.05 was used to designate statistical significance and 0.01 to designate high significance.

For inclusion in the study, an informed written consent was obtained from the children's guardians. The study protocol was approved by the hospital's ethical committee.

3. Results

The results of present study could be summarized in the following points:

3.1. Results of light microscopy

Results of light microscopic examination of the liver biopsy are shown in Tables 1a and b. The number of patients with disturbed hepatic architecture is significantly higher in patients with Wilson's disease than in patients with chronic hepatitis C or patients with other chronic liver disease. As regards portal tract fibrosis or inflammation, disruption of the limiting plate, hepatocyte ballooning, liver cirrhosis, micro-vesicular steatosis, macro-vesicular steatosis, there is insignificant statistical difference between the three groups.

Table 1a Comparison between patients with Wilson's disease (WD) vs. patients (HCV) as regards light microscopic findings.

Light microscopic findings	Group	*N*	%	Chi-square		
				X^2	*P*-value	Significance
Disturbed hepatic architecture	Wilson's	8/11	72.73	0.243	0.62	$P > 0.05$
	HCV	1/6	16.67			Non-significant
Portal tract fibrosis	Wilson's	9/11	81.82	2.941	0.230	$P > 0.05$
	HCV	4/6	66.67			Non-significant
Portal tract inflammation	Wilson's	8/11	72.73	2.235	0.327	$P > 0.05$
	HCV	6/6	100.00			Non-significant
Disruption of the limiting plate	Wilson's	5/11	45.45	0.118	0.943	$P > 0.05$
	HCV	3/6	50.00			Non-significant
Hepatocyte ballooning	Wilson's	11/11	100.00	4.156	0.041	$P < 0.05$
	HCV	4/6	66.67			Significant
Liver cirrhosis	Wilson's	6/11	54.55	2.300	0.129	$P < 0.05$
	HCV	1/6	16.67			Non-significant
Micro-vesicular steatosis	Wilson's	6/11	54.55	2.059	0.560	$P > 0.05$
	HCV	2/6	33.33			Non-significant
Macro-vesicular steatosis	Wilson's	2/11	18.18	4.353	0.113	$P > 0.05$
	HCV	0/6	0.00			Non-significant

Comment: Disturbed hepatic architecture and liver cirrhosis, were more common in WD > HCV. Portal tract inflammation was more in HCV > WD, steatosis (micro-vesicular and macro-vesicular) more in WD.

Table 1b Comparison between the patients with Wilson's disease and patients with hepatitis C virus (HCV) versus patients with other chronic liver diseases as regards light microscopic findings.

Light microscopic findings	Group	*N*	%	Chi-square		
				X^2	*P*-value	Significance
Disturbed hepatic architecture	Wilson's & HCV	9/17	52.94	4.462	0.216	$P > 0.05$ Non-significant
	Other CLD	7/9	77.78			
Portal tract fibrosis	Wilson's & HCV	13/17	76.47	0.58	0.445	$P > 0.05$ Non-significant
	Other CLD	8/9	88.89			
Portal tract inflammation	Wilson's & HCV	14/17	82.35	11.846	0.008	$P < 0.01$ Highly significant
	Other CLD	5/9	55.56			
Disruption of the limiting plate	Wilson's & HCV	8/17	47.06	3.231	0.357	$P > 0.05$ Non-significant
	Other CLD	3/9	33.33			
Hepatocyte ballooning	Wilson's & HCV	15/17	88.24	0.193	0.660	$P > 0.05$ Non-significant
	Other CLD	8/9	88.89			
Liver cirrhosis	Wilson's & HCV	7/17	41.18	3.846	0.279	$P > 0.05$ Non-significant
	Other CLD	6/9	66.67			
Microvesicular steatosis	Wilson's & HCV	8/17	47.06	2.615	0.455	$P > 0.05$ Non-significant
	Other CLD	4/9	44.44			
Macrovesicular steatosis	Wilson's & HCV	2/17	11.76	16.154	0.001	$P < 0.01$ Highly significant
	Other CLD	3/9	33.33			

Table 2a Comparison of patients with Wilson's disease versus patients with HCV as regards electron microscopic findings.

Electron microscopic findings	Group	*N*	%	Chi-square		
				X^2	*P*-value	Significance
Mitochondrial pleomorphism	Wilson's	10/11	90.91	7.176	0.028	$P < 0.05$ Significant
	HCV	6/6	100.00			
Mitochondrial enlargement	Wilson's	7/11	63.64	0.824	0.662	$P > 0.05$ Non-significant
	HCV	6/6	100.00			
Mitochondrial dense metrical granules	Wilson's	8/11	72.73	6.294	0.098	$P > 0.05$ Non-significant
	HCV	5/6	83.33			
Degeneration of mitochondrial cristae	Wilson's	5/11	45.45	1.98	0.159	$P < 0.05$ Significant
	HCV	1/6	16.67			
Widening of mitochondrial inter-crystal space	Wilson's	3/11	27.27	2.235	0.327	$P > 0.05$ Non-significant
	HCV	0/6	0.00			
Lysosomal enlargement	Wilson's	5/11	45.45	1.409	0.235	$P > 0.05$ Non-significant
	HCV	1/6	16.67			
Dilatation and vesiculation of the endoplasmic reticulum	Wilson's	3/11	27.27	4.898	0.27	$P < 0.05$ Significant
	HCV	5/6	83.33			

N.B. The electron microscopy of one patient (who had lipid storage disease and severe cachexia) could not be obtained as the biopsy was very small revealed only portal tract and the general condition of the patient did not allow another biopsy.

Table 2b Comparison of patients with Wilson's disease and patients with HCV versus patients with other CLD as regards electron microscopic findings.

Electron microscopic findings	Group	*N*	%	Chi-square		
				X^2	*P*-value	Significance
Mitochondrial pleomorphism	Wilson's and HCV	16/17	94.12	13.520	0.001	$P < 0.01$ Highly significant
	Other CLD	4/8	50.00			
Mitochondrial enlargement	Wilson's and HCV	13/17	76.47	0.040	0.841	$P > 0.05$ Non-significant
	Other CLD	4/8	50.00			
Mitochondrial dense metrical granules	Wilson's and HCV	13/17	76.47	10.040	0.018	$P < 0.05$ Significant
	Other CLD	3/8	37.50			
Degeneration of mitochondrial cristae	Wilson's and HCV	6/17	35.29	1.520	0.468	$P > 0.05$ Non-significant
	Other CLD	0/8	0.00			
Widening of mitochondrial inter-crystal space	Wilson's and HCV	3/17	17.65	0.107	0.74	$P > 0.05$ Non-significant
	Other CLD	1/8	12.50			
Lysosomal enlargement	Wilson's and HCV	6/17	35.29	8.120	0.044	$P < 0.05$ Significant
	Other CLD	1/8	12.50			
Dilatation and vesiculation of the endoplasmic reticulum	Wilson's and HCV	8/17	47.06	0.080	0.961	$P > 0.05$ Non-significant
	Other CLD	4/8	50.00			

Comment: Mitochondrial E/M changes were more common in WD and HCV than other CLD.

Table 3 The mitochondrial affection scoring system.

Degree of mitochondrial affection	Number of affected mitochondrial parameters	Number of affected patients with chronic liver disease
Mild	1–2	7/22 (32%)
Moderate	3	9/22 (41%)
Severe	4–5	6/22 (27%)

3.2. Results of electron microscopy

Results of electron microscopy of liver biopsy are shown in Tables 2a and b. Twenty of the studied patients (80%) had mitochondrial pleomorphism. The number of chronic hepatitis C patients with mitochondrial pleomorphism is significantly higher than that of patients with Wilson's disease or of patients with other chronic liver disease. There is insignificant difference among the three groups as regards the number of patients.

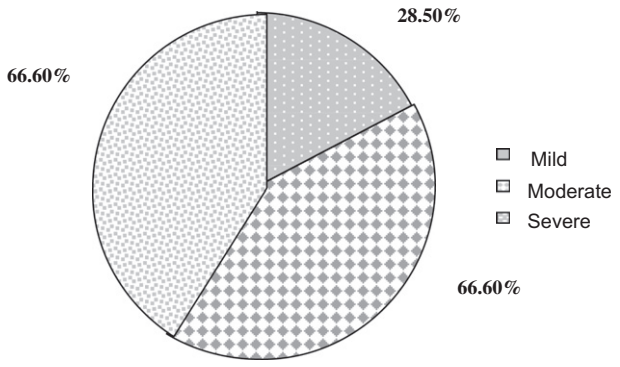

Figure 1 The presence of hepatic steatosis among patients with different degrees of mitochondrial affection.

3.3. Result of mitochondrial PCR

Mitochondrial DNA deletions was found in three patients (12%) (two with Wilson's disease and one with lipid storage disease).

Table 4 Relation of degree of mitochondrial affection to hepatic steatosis.

Degree of mitochondrial	Hepatic steatosis						Chi square value	*P*-value
	Present		Absent		Total			
	No.	%	No.	%	No.	%		
Mild	2/7	28.57	5/7	71.5	7/22	31.8	1.281	0.257
Moderate	6/9	66.6	3/9	33.4	9/22	40.9		$P > 0.05$ Non-significant
Severe	4/6	66.6	2/6	33.4	6/22	27.2		

Although there was no statistical difference between the three studied groups (classified according to degree of mitochondrial affection) yet there is an increase in severity with increase frequency of steatosis.
N.B.: Four of the studied patients had no mitochondrial affection.

We developed a scoring system for mitochondrial affection in our patients depending on the degree of the previously mentioned ultra-structural mitochondrial changes. Four of the studied patients had no mitochondrial affection (see Fig. 1 and Tables 3 and 4).

4. Discussion

Mitochondria are important cellular organelles responsible for energy production through oxidative phosphorylation (OXPHOS). Mitochondrial dysfunction yields deficient oxidative phosphorylation, increased generation of reactive oxygen species (ROS), accumulation of hepatocyte lipid, impairment of other metabolic pathways and activation of both apoptotic and necrotic pathways of cellular death [13]. The only DNA outside the nucleus is the mitochondrial DNA, it is characterized by its high mutability and by being only maternally inherited [14]. Mitochondrial disorders were once regarded as neuromuscular diseases, however 33–56% of patients with mitochondrial disorders have non-neuromuscular manifestations at presentation [15]. Sokol and Treem [16] have proposed a classification scheme for mitochondrial hepatopathies into primary and secondary hepatopathies. In primary disorders, mitochondrial defect is the primary cause of liver disease. In the secondary disorders hepatic mitochondria undergo injury or dysfunction caused by another pathologic process [17], including diseases of uncertain aetiology (e.g. Reye's syndrome), exposure to endogenous and exogenous toxins, drugs (such as nucleoside analogues), or metals, and other conditions in which mitochondrial oxidative injury or abnormal electron transport may be involved (e.g. cholestasis, NASH) [15,16].

Among our 26 chronic liver disease patients 22 patients (84.6%) showed evidence of mitochondrial affection in the form of ultra-structural mitochondrial morphological changes, steatosis, and or mitochondrial DNA deletions. As all our patients were with a well defined aetiological diagnosis thus, the mitochondrial affection of the patients could not be explained by a primary mitochondrial disease; all were secondary mitochondrial affection. We divided our patients into three groups (Group I: patients with Wilson's disease, Group II: patients with chronic hepatitis C, Group III: patients with chronic liver disease other than Wilson's and chronic hepatitis C). Clinically there were no differences between the three groups except in the frequency of hepatomegaly which was higher in Wilson's disease patients (10 out of 11, 99.9%). This hepatomegaly could be explained by the early glycogen deposition and fatty infiltration of the liver in WD [18]. Pathologically cirrhosis and hepatic architecture disturbance were higher among Group I and Group III (8 out of 11, 72.73%) (7 out of 9 77.78%), compared to patients with Group II (1 out of 6, 16.6%, respectively). This could be explained based on the natural history of liver affection in Wilson's disease that usually peaks at ages 10–13 years [19]. Irrespective of the initial symptoms, almost all patients will have some evidence of cirrhosis on liver biopsy reflecting the response to years of hepatic copper accumulation before clinical symptoms [20]. In our study chronic hepatitis C patients were freshly diagnosed with short disease duration, thus explaining the low frequency of cirrhosis in these patients (6–12 months from diagnosis). This was in consistency with other studies held by Kage et al. [21] who studied the pathology of chronic hepatitis C in children and they reported no case of cirrhosis and only 3.9% of children had architectural distortion and bridging fibrosis (mean duration of infection in this study was 2–6 years), and that of Goodman et al. [22] who published that inflammation, fibrosis, steatosis and cirrhosis were mild, in HCV infected children. They added that, there is a positive correlation of inflammation with the duration of infection.

Mitochondrial affection in these three groups of patients showed some variabilities. All 11 patients with Wilson's disease had morphological evidence of mitochondrial affection in the form of mitochondrial pleomorphism in 10 out of 11 (90.91%), mitochondrial enlargement in 7 out of 11 (63.64%), mitochondrial dense metrical granules in 8 out of 11 (72.73%), degeneration of mitochondrial cristae in 5 out of 11 (45.45%) and widening of mitochondrial inter-crystal space in 3 out of 11 (27.27%). Also steatosis (micro-vesicular and macro-vesicular) was present in 6 out of 11 of WD patients. Wilson's disease is caused by diverse mutation of nuclear gene encoding a copper-transporting ATPase. Decreased elimination of copper into the bile causes progressive accumulation of copper into hepatocytes. Mitochondrial involvement in Wilson's disease has been implicated since the early observation by Stenlieb et al. [23] of abnormal mitochondrial morphology so characteristic of this disorder of copper metabolism. Changes include decreased matriceal density enlarged inter-membranous spaces, dilatation and vacuolization of cristae, crystalline inclusion and vacuoles in the matrix. This was directly proportionate to the age and was more prevalent among patients with hepatic variety than in patients with the neurological variety. Copper selectively accumulates within mitochondria during copper overload leading to mitochondrial dysfunction and increasing mitochondrial reactive oxygen species (ROS) production. Mitochondrial ROS target proteins, and patients with Wilson's disease have decreased activity of respiratory chain complexes, which may further increase mitochondrial oxidative pathway [24]. Sokol and Narkewicz [25] mentioned that in the early stages of Wilson's disease, hepatocellular mitochondria are pleomorphic and abnormally large. This is because patients with Wilson's disease have decreased activity of respiratory chain complex. Findings are consistent with findings of Abdel Ghaffar et al. [26] who studied Wilson's disease in Egyptian children. They found that 85.7% of their patients with Wilson's disease had mitochondrial alteration in form of pleomorphism in shape and size, with the presence of giant forms and finely granular and coarse electron dense material. These results were strengthened by the findings of Brewer [27], who described the abnormal mitochondrial morphology characteristic of Wilson's disease which included, intermembranous space dilation and vacuolization of cristae, crystalline inclusions and vacuoles in the matrix. Also he documented disappearance of cristea and widening of the inter-crystal space of the hepatic mitochondria. Gu et al. [28] who studied oxidative phosphorylation in Wilson's disease and showed evidence of severe mitochondrial dysfunction in livers of patients with Wilson's disease. In their study, enzyme activities were decreased as follows: complex I by 62%, complex II + III by 52%, complex IV by 33% and aconitsae by 71%. On the contrary, they reported that high copper concentrations found in the liver of the studied patients with primary biliary (PBC) and primary sclerosing cholangitis (PSC) were not associated with the mitochondrial fraction. Mitochondrial respiratory chain func-

tions and aconitsae activity were normal in those patients supporting the suggestion that it is the intra-mitochondrial localization of copper which results in deficiency of these enzymes in Wilson's disease. They proposed that WD protein might function as a copper transporter at both the trans-Golgi network and at the mitochondrial membrane, and mutations in ATP7B gene may cause mitochondrial copper overload through defective export. In our study lysosomal enlargement was found in 5 out of 11 (45.4%) patients with Wilson's disease compared to only two patients in each of other two groups. This lysosomal enlargement could be explained by the accumulation of copper within the lysosomes and denotes later stages of the disease as Liu et al. [29] studied copper distribution inside and outside lysosomes in the Wilson's disease. They found that in the early stage of hepatolenticular disease, copper is accumulated outside lysosome, which is paralleled with increase of metallothionein-like protein (copper and sulfur-binding protein). With the development of the disease, more copper is deposited inside lysosome than outside lysosome. They concluded that the up-regulation expression of copper and sulfur-binding protein and copper accumulation in lysosomes may play an important role in lowering the ATP-7B gene mutation-included toxic effects of free copper on the cell.

All six patients with hepatitis C virus (HCV) in our study also had mitochondrial morphological changes in the form of mitochondrial pleomorphism in 6 out of 6 (100%), mitochondrial enlargement in 6 out of 6 (100%), mitochondrial dense metrical granules in 5 out of 6 (83.3%), degeneration of mitochondrial cristae in 1 out of 6 (16.67%). None had widening of mitochondrial inter-crystal space, also micro-vesicular steatosis was present in 2 out of 6 HCV patients (33.3%). These findings were supported by that of Calza et al. [30] who reported that, mitochondrial alterations were demonstrated in HCV infected subject and the most frequent alteration was mitochondrial pleomorphism. Barbaro et al. [31] found enlarged irregular mitochondria in 92% of studied patients with hepatitis C (genotype Ia). Barbaro et al. [32] also reported that 25% of studied patients with hepatitis C infection had dense mitochondrial granules. This granules are osmiophilic granules which are able to be involved in haemostasis of calcium. Calza et al. [30] found reduction or loss of cristae in (20.5%) of cases with HCV infection. In our study micro-vesicular steatosis was detected in liver biopsy of 2 out of 6 (33.3%) with chronic hepatitis C patients. This is nearly in agreement to the result of Zubair et al. [33] who observed micro-vesicular steatosis in 21 out of 46 cases with hepatitis C (HCV) virus infection. They reported that steatosis is a common histologic feature of chronic hepatitis C (CHC). This complication of CHC may have implication on its long-term prognosis. Steatosis can be explained by the findings of Salem et al. [34] they documented enhanced lipid peroxidation in all patients by assaying lipid peroxidation products in plasma malondialdehyde (MDA). Another study held by Bortolotti et al. [35] found that 27.5% of the studied 80 children with chronic hepatitis C infection had steatosis. The mechanism by which the hepatitis C virus (HCV) causes steatosis is unknown. Ramalho [36] suggested that both host and viral factors may play an important role in its development. He suggested a direct effect of certain viral sequence on the pathogenesis of hepatic steatosis. Okuda [37] studied the mitochondrial affection induced by hepatitis C core protein. They found that core protein also increased lipid peroxidation products and induced antioxidant gene expression as well. A mitochondrial electron transport inhibitor prevented the core-induced increase in ROS. A fraction of expressed core protein was localized to the mitochondria and was associated with redistribution of cytochrome C from mitochondrial to cytosolic fractions. Therefore they concluded that hepatitis C has direct effect on mitochondria. Another possible mechanism could be drawn from the study of Barbaro et al. [38] in this study, chronic hepatitis patients showed depletion of glutathione (H-GSH, P-GSH and L-GSH). The value of P-GSH and L-GSH were found to reflect directly the hepatic stores of GSH. They proved an inverse correlation between GSH and MDA that reflects an increase in lipid peroxidation in patients with hepatitis C. They attributed this deficiency in glutathione to increased free radical production as well as mitochondrial dysfunction. It is essential also to mention the work of Badizadegan et al. [39] who compared the histopathologic features of liver in children and adults with hepatitis C virus infection. They documented that steatosis is higher in adults (54–72%) with chronic hepatitis C infection than in children (50%). This was attributed to longer duration of disease. The finding of altered mitochondrial structure in HCV-related liver disease would help to explain the steatosis that is so prevalent in this disease. These mitochondrial changes were found significantly more often in patients with genotype 1b than in patients with genotype 2a/C or 3a. All mitochondrial alterations were associated with a depletion of tissue glutathione store, a key cellular antioxidant. Collectively, the data suggest that genotype-dependent mitochondrial alterations are a prominent feature of liver cell injury by HCV [40].

Still in patients with other chronic liver disease mitochondrial affection was present but less frequently, while steatosis was found in 66.6% of patients with either moderate or severe mitochondrial affection, only 28.5% of patients with mild mitochondrial affection had hepatic steatosis, thus relating steatosis to the degree of mitochondrial affection. In secondary steatohepatitis such as in Wilson's disease, the vicious cycles of hepatic lipogenesis and hepatic steatosis are further aggravated by the causative disease itself. Wilson's disease increase ROS formation, ROS may oxidize fat deposits, releasing lipid peroxidation products that damage mitochondrial DNA and proteins to partially block the flow of electrons along the respiratory chain, thus further increasing mitochondrial ROS formation. ROS may also deplete antioxidants and cause the formation of tumor necrosis factor-α (TNF-α), 2 effects that may further impair the flow of electrons and increase mitochondrial ROS formation leading to steatosis and so on. Another evidence of subcellular level of affection in hepatitis C is the disruption in rough endoplasmic reticulum. This is consistent with findings of Barbaro et al. [32] who reported dilated vesiculated endoplasmic reticulum in 64% of the examined patients with hepatitis C virus (genotype 1-a). Also Tardif et al. [41] documented that hepatitis C virus (HCV) gene expression disrupts normal endoplasmic reticulum (ER) functions and induce ER stress. ER stress results from accumulation of unfolded or misfolded proteins in the ER.

In our study mitochondrial DNA deletion was found in three patients (11.5%) (two with Wilson's disease (7.69%) and one with lipid storage disease (3.84%). The two patients with Wilson's disease were a 13 year old boy and a 12 year old girl. Both were child C at time of presentation (both

had ascites, hypoalbuminemia below 2.5 g/dl and prolonged prothrombin time). The boy had some neurological manifestations of Wilson's disease (mask like face, rigidity, salivations but no involuntary movements). They both had positive kayser-Fleisher ring. Their liver biopsies revealed cirrhosis and marked mitochondrial morphological changes. Both of them had other affected sibs. Mansouri et al. [42] found that oxidant damage in hepatic mitochondria also leads to deletions in mtDNA in young adults with Wilson's disease. Copper is a powerful prooxidant that generates the hydroxyl radicals and other reactive species. Copper forms Cu–DNA complexes, this reactive species are generated close to DNA, making it an elective target. Copper cause single strand breaks, double strand breaks, intra-strand links between adjacent bases, oxidized bases, base substitutions, as well as adducts between DNA. DNA strand breaks can cause misannealing during replication, leading to DNA deletions [43]. Our results revealed that the prevalence of micro-vesicular steatosis seemed to be two times greater in patients with mitochondrial DNA deletion than in those without (2 of 2 vs. 4 of 9). However, these numbers were small and not statistically significant. This is in consistency with the findings of Mansouri et al. [42] who reported close proportions (3 of 8 vs. 1 of 8). Chinnery et al. [44] mentioned that, the mother of a proband with a mtDNA deletion is usually unaffected and does not have mtDNA deletions in her tissues; however, exceptions occur. The risk to the sibs of a proband is usually extremely low. Offspring of a female proband are usually not at risk of inheriting the mutation. Prenatal testing is available on a clinical basis, although interpretation of results is difficult. Emphasized by presence of mitochondrial DNA deletion in 3 of our patients, mitochondrial DNA deletion are not inherited from mother to children, it may occur as a new events.

5. Conclusion

We thus conclude that secondary mitochondrial hepatopathy occurs frequently in patients with chronic liver disease especially if it is due to Wilson's disease or HCV infection. This hepatopathy could be explained by aetiological agent (Copper in the case of Wilson's disease, HCV genome in the case of HCV infection) but may also be compounded by the presence of the cirrhosis in itself.

Conflict of interest

The authors declare that there is no conflict of interest.

References

[1] Jau-Shin Wu. Chronic hepatitis (definition) (Taiwan Medical Network-TMN) Old Doc wu'Series posta; 1996; Revised 12, 2009.

[2] Manolaki N, Nikolopoulou G, Daikos GL, Panagiotakaki Eleni, Tzetis M, Roma Eleftheria, et al.. Wilson disease in children: analysis of 57 cases. J Pediatr Gastroenterol Nutr 2009;48(1):72–7.

[3] Treem WR, Sokol RJ. Disorders of the mitochondria. Semin Liver Dis 1998;18(3):237–53.

[4] Lee WS, Sokol RJ. Liver disease in mitochondrial disorders. Semin Liver Dis 2007;27(3):259–73.

[5] Lee WS, Sokol RJ. Mitochondrial hepatopathies: advances in genetics and pathogenesis. Hepatology 2007;45:1555–6.

[6] Massimo Z, Stefano Di. Mitochondrial disorders. Brain 2004;127(10):2153–72. doi:10.1093/brain/awh259.

[7] Bandyopadhyay SK, Dutta A. Mitochondrial hepatopathies. J Assoc Physicians India 2005;53:973–8.

[8] Schapira AH. New mitochondrial disorders. J Neurol Neurosurg Psychiatry 2002;72:144–9.

[9] http://my.clevelandclinic.org/disorders/Mitochondrial_Disease/hic_Mitochondrial_Di.

[10] Knodell RG, Ishak KG, Block WC, et al.. Formulation, application of numerical scoring system for assessing histological activity in a symptomatic chronic active hepatitis. Hepatology 1991;4:431.

[11] Goto Y, Koga Y, Horai SU, Nonaka I. Detection of mitochondrial DNA. J Neurol Sci 1990(100):63–96.

[12] Goto Y. Detection of DNA encompassing the deletion junction of mitochondrial genome. Biochem Biophys Res Commun 1996;1(222):215–9.

[13] Moslemi AR, Darin N. Molecular genetic and clinic aspects of mitochondrial disorders in childhood. Mitochondrion 2007;7(4):241–52.

[14] Schwartz M, Vissing J. Paternal inheritance of mitochondrial DNA. N Engl J Med 2002;347:576–80.

[15] Munnich A, Rotig A, Chretien D, et al.. Clinical presentation of mitochondrial disorders in childhood. J Inherit Metab Dis 1996;19:521–7.

[16] Sokol RJ, Treem WR. Mitochondrial hepatopathics. In: Suchy FJ, Sokol RJ, Balistreri WF, editors. Liver disease in children. Philadelphia: Lippincott. Williams & Wilkins; 2001. p. 787–810.

[17] Chinnery PF, Turnbull DM. Epidemiology and treatment of mitochondrial disorders. Am J Med Genet 2001;06:94–101.

[18] Ettinger MJ. Hepatic copper metabolism. In: Zakim D, Boyer T, editors. Hepatology textbook of liver disease, 3rd ed., vol. 80(3). Philadelphia, Toronto, London, Montere, Sydney: W.B Saunders Company; 2001. p. 554–61.

[19] Shah R, Piper MH. Wilson disease: emedicine gastroenterology; 2009. http://www.emedicine.com/med/topic2413.htm.

[20] Loudianos G, Gitlin JD. Wilson's disease. Semin Liver Dis 2003;20(3):353–64.

[21] Kage M, Fujisawa T, Shiraki K, Tanaka T, Kimura A, et al.. Pathology of chronic hepatitis C in children. Hepatology 1997;26:771–5.

[22] Goodman ZD, Makhlouf HR, Liu L, et al.. Pathology of chronic hepatitis C in children: liver biopsy findings in the Peds-C Trail. Hepatology 2008;47(3):836–43.

[23] Stenlieb I, Quintana N, Volenberg I, Schilsky ML. An array of mitochondrial affection in the hepatocytes of long-Evans Cinnamon rats. Hepatology 1995;22(6):1782–7.

[24] Schapira AH. Primary and secondary defects of mitochondrial respiratory chain. J Inherit Metab Dis 2002;25(3):207–14.

[25] Sokol RJ, Narkewicz MR. Copper and iron storage disorders. In: Suchy FJ, Sokol RJ, Balistrei WF, editors. Liver disease in children. 2nd ed.. Lippincott William & Wilkins; 2001.

[26] Abdel Ghaffar TY, El Monaiery MS, Badr SH, Abdel Ghaffar AY, Seif E. Wilson's disease in Egyptian children: clinical, biochemical, histological and ultra-structural characterization. Egypt J Pediatr 1998;15(30):433–50.

[27] Brewer GJ. Recognition diagnosis and management of Wilson's disease. Proc Soc EX Bio Med 2000;223:39–46.

[28] Gu M, Copper JM, Butter P, Walker AP, Mistry PK, Dooley JS, et al.. Oxidative–phosphorylation defects in liver of patients with Wilson's disease. Lancet 2000;356(9228):469–74.

[29] Liu W, Li JY, Jin J, Zuo J. Detection of distribution of copper inside and outside of lysosomes in cultured hepatolenticular degeneration fibroblasts by electron prob X-ray microanalysis. Hepatobiliary Pancreat Dis Int 2003;2(2):270–3.

[30] Calza L, Verucchi G, Biagetti C, Attard L, Costigliola P, Manfredi R, et al. Liver mitochondrial abnormalities associated with HCV-monoinfection, HIV–HCV-coinfection, and antiretroviral therapy in a chohort of 68 adult patients. In: 9th European Aids Conference (EACS) 1st EACS Resistance and Pharmacology Workshop, 2003, Warsaw, Poland.

[31] Barbaro G, Di Lorenzo G, Asti A, Ribersani M, Belloni G, Grisorio B, Filice G, et al.. Hepatocellular mitochondrial alterations in patients with chronic hepatitis C: ultrastructural and biochemical findings. Am J Gastroenterol 1999;94(8):2198–205.

[32] Barbaro G, Di Lorenzo G, Soldini M, et al.. Hepatic glutathione deficiency in chronic hepatitis C: quantitative evaluation in patients who are HIV positive and HIV negative and correlation with plasmic and lymphocytic concentrations and with the activity of the liver disease. Am J Gastroenterol 2007;91:2569–73.

[33] Zubair A, Jamal S, Mubarik A. Morphometeric analysis of hepatic steatosis in chronic hepatitis C infection. Saudi J Gastroenterol 2009;15:11–4.

[34] Salem M, El-serafy O, Abdel El-Rahman Hamouda, Arnaout H. Lipid peroxidation in Wilson disease. Egypt J Med Human Genet 2001;2(1):33–41.

[35] Bortolotti F, Jara P, Diaz C, Vajro P, et al.. Post-transplantation and community hepatitis C in childhood. J Pediatr 2008;124: 709–13.

[36] Ramalho F. Hepatitis C virus infection and liver steatosis. Antiviral Res 2003;60(2):125–7.

[37] Okuda K. Chronic hepatitis. In: Okudak, Mitchel DG, Itai Y, Ariyama J, editors. Hepatobiliary diseases: pathophysiology and imaging. Canada and Australia: Blackwell Science USA; 2001. p. 103–18.

[38] Barbaro G, Di Lorenzo G, Soldini M, et al.. Hepatic glutathione deficiency in chronic hepatitis C. Quantitative evaluation in patients who are HIV positive and HIV negative and correlation with plasmic and lymphocytic concentrations and with the activity of the liver disease. Am J Gastroenterol 1996;91: 2569–73.

[39] Badizadegan K, Ott MJ, Nelson SP, Perez-Atayde AR. Histopathology of the liver in children with chronic hepatitis C viral infection. Hepatology 1998;28(5):1416–23.

[40] Rust C, Gores G. Does hepatitis C cause liver injury by pathways associated with mitochondrial dysfunction? Am J Gastroenterol 1999;94(8):2003–5.

[41] Tardif KD, Morik, Kaufman RJ, Siddiqui A. Hepatitis C virus suppresses the IRE1-XBP1 pathway of the unfolded protein response. J Biol Chem 2004;279(17):17158–64.

[42] Mansouri A, Graou I, Fromenty B, Berson A, et al.. Premature oxidative aging of hepatic mitochondrial DNA. Gastroenterology 1997;113:599–605.

[43] Sokol R. Mechanisms of acute and chronic liver disease: implications for diagnosis, pathogenesis and treatment 2002. In: AASLD Conference.

[44] Chinnery PF, DiMauro S, Shanske S, Schon EA, Zeviani M, Mariotti C, et al.. Risk of developing a mitochondrial DNA deletion disorder. Lancet 2004;364:592–6.