Original paper

Immunoreactive hepatic stellate cells in biopsy material in children with chronic hepatitis B. The first report in pediatric patients

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The research objective was to identify and quantify the immunohistochemically (IHC) stained hepatic stellate cells (HSCs) in children with chronic hepatitis B (CHB), including staging (S), location in the hepatic lobule, and correlation with hepatocyte count.
Retrospective morphological analysis was based on liver biopsies obtained from 70 CHB children before antiviral treatment. To determine fibrosis stage, the Batts and Ludwig scoring system was applied. Immunohistochemical examinations used monoclonal antibodies against α-SMA.
IHC observations in CHB children revealed a significant positive correlation between the mean number of α-SMA immunopositive HSCs within the hepatic lobule ($r = 0.518$; $p < 0.001$) and fibrosis stage. In biopsy specimens with intensive fibrosis, most HSCs had an elongated shape and demonstrated evidently strong immunoexpression of cytoskeletal protein α-SMA. The mean counts of HSCs/100 hepatocytes (in high power field) in 4 study groups, i.e. with S-0, S-1, S-2, S-3, were 5.00; 5.98; 9.80; 12.19, respectively. Interestingly, in most groups the highest count of immunoreactive HSCs/100 hepatocytes was in the intermediate zone, indicating its high metabolic activity in liver fibrogenesis.
Immunohistochemical and statistical investigations of HSCs in children with CHB showed a close positive correlation of cell count with fibrosis intensity, which may have prognostic implications in this pathology.

Key words: hepatic stellate cells, immunohistochemistry, α-SMA, chronic hepatitis B, children, fibrosis.

Introduction

Chronic hepatitis is the main cause of liver fibrosis, including cirrhosis. Its major etiologies involve hepatotropic viral infections (HBV, HCV), excessive alcohol consumption, and metabolic and autoimmune liver disorders [1, 2, 3, 4, 5, 6, 7]. The incidence of chronic hepatitis B (CHB) in Poland has increased in recent years. Chronic hepatitis B was most frequently diagnosed among pediatric patients aged 15-19, which is most likely associated with the very high incidence observed among children under 4 years in the early 1990s [8].

The process of liver fibrosis is characterized by excessive accumulation of extracellular matrix (ECM) components, which is the result of disturbances in the dynamic balance between synthesis (fibrogenesis) and degradation (fibrolysis). The course of fibrosis

involves deposition of excessive quantities of ECM proteins in the space of Disse, which initiate collagen fibroplasia and constitute the earliest morphological form of this pathology [1, 5, 9, 10, 11, 12, 13]. It is assumed that hepatic stellate cells (HSCs), building the so-called nonparenchymal hepatic cells (NPCs) that are topographically bound to the sinusoidal wall, make the greatest contribution to fibrogenesis. It is the HSCs which being transformed to metabolically active transitional HSCs and myofibroblastic cells are responsible for the synthesis of the extracellular matrix and play a key role in this pathology [2, 13, 14, 15, 16, 17, 18].

The hepatology literature concerning the morphogenesis and development of liver fibrosis in the course of CHB refers mainly to adult patients and experimental studies [4, 5, 7, 9, 13, 15, 17]. Up to now, the microscopic picture of HSCs assessed in biopsy material obtained from large groups of pediatric patients has not been documented. Therefore, the major objective of this study was identification and quantitative evaluation of HSCs stained for immunohistochemistry (IHC) with a protein marker for these cells, α-smooth muscle actin (*Monoclonal Mouse Anti-Human Alpha Smooth Muscle Actin*), in biopsy material obtained from children with chronic hepatitis B. We took into consideration selected morphological parameters – stage of fibrosis, location of HSCs within the hepatic lobule, intensity of biomarker expression in the cells, and their relationship with hepatocyte count. The current study is a continuation of the morphological research conducted in our center on biopsy material obtained from children inhabiting northeastern Poland, who suffer from various liver disorders, especially those accompanied by liver fibrosis [6, 19, 20, 21, 22, 23].

Material and methods

Patients

In all study cases, the retrospective morphological analysis of liver bioptates was performed using biopsy material collected in the Department of Medical Pathomorphology, Medical University of Bialystok.

Liver oligobiopsy specimens were obtained from 70 children with type B chronic hepatitis (46 boys and 24 girls), aged 3-17 years (mean 11), who were qualified for interferon α or lamivudine therapy before antiviral treatment in the Department of Pediatrics, Gastroenterology and Allergology, Medical University of Bialystok.

The patients had a documented HBV infection (HBsAg- and HBeAg-positive) of > 6 months duration. Patients infected with HCV, with other liver disorders (autoimmune hepatitis, chosen metabolic diseases) and with liver cirrhosis were excluded from the study.

The study was approved by the Bioethics Committee, Medical University of Bialystok.

Collection and preparation of tissue material

For morphological analysis (histological and IHC), liver oligobiopsy specimens were obtained from each patient by blind biopsy. The tissue material was fixed in 4% buffered formalin for 24 hours at 4°C. The sections were stained histologically (routinely with Mayer's hematoxylin and eosin [HE] and additionally for connective tissue stroma components: according to Gomori, by the Azan method according to Mallory in the Heidenhain modification, according to Masson-Goldner). A semiquantitative histologic scoring system according to Batts and Ludwig was used to determine the extent of liver fibrosis (staging – S) [24].

Immunohistochemical investigations

In order to reveal the presence, intracellular distribution and intensity of α-SMA expression within the stellate cells, liver biopsy specimens obtained from children with CHB were subjected to IHC reaction with mouse monoclonal antibody (Clone 1A4, N 1584, Dako) against epitopes of the human α-SMA molecule. Following incubation of preparations with the antibody for 20 minutes in a humid chamber, at room temperature, the reaction was performed using the detection system En Vision (K 8012/K 8013 kit). In order to visualize the antigen-antibody complex, the preparations were incubated in a 3,3-diaminobenzidine solution (chromogen DAB, Dako). Then for better visualization of the cell picture they were contrasted in hematoxylin solution for 2 min. After dehydration in ethanol at increasing concentrations and passage through a series of xylenes, the preparations were closed in a Canadian balsam and subjected to microscopic investigations.

Considering the intensity of IHC staining, showing α-SMA expression in the cytoplasm of the cells studied, HSCs demonstrated weak, moderate and strong immunoreactivity.

Statistical analysis

For statistical analysis performed in high power field (400×), all the α-SMA stained HSCs present within the respective zones, i.e. 1 – periportal, 2 – intermediate and 3 – central (perivenous), of three randomly chosen hepatic lobules and all hepatocytes found within the same zones were added up. In each of the lobular zones, the mean numbers of hepatocytes and HSCs were calculated for one patient and then for all study patients.

The IHC findings were subjected to statistical analysis using nonparametric tests: Kruskal-Wallis test and Mann-Whitney test; Spearman's correlation coefficient was also calculated. The level of $p < 0.05$

was considered statistically significant. The calculations were based on SPSS Statistics (Statistical Package for the Social Sciences).

Results

The semiquantitative numerical scoring system evaluating staging according to Batts and Ludwig (24) was used in microscopic investigations to distinguish 4 study groups: group I (S-0) – 5 patients (2 boys; 3 girls) aged 8-13 years (mean 10.8 years); group II (S-1) – 27 patients (18 boys; 9 girls) aged 3-17 years (mean 11 years); group III (S-2) – 31 patients (21 boys; 10 girls) aged 6-17 years (mean 11.5 years); group IV (S-3) – 7 patients (5 boys; 2 girls) aged 4-13 years (mean 10.7 years).

Immunohistochemical assessment of α-SMA positive HSCs

Group I (S-0)

In the patients with CHB in the S-0 group, α-SMA positive HSCs were markedly dispersed throughout the hepatic lobule, mostly in the perisinusoidal region. They showed poorly enhanced expression of the biomarker, which was manifested by a delicate cytoplasmic IHC reaction, with a tendency to microgranular submembranous accumulation of the protein (Fig. 1A). The mean number of immunoreactive HSCs in zone 1 (periportal zone) calculated per mean number of hepatocytes (147.0 ±36.2) was 6.27 ±2.34. In zone 2 (intermediate), the mean number of liver parenchymal cells (145.4 ±41.78) corresponded to an average of 8.27 ±4.28 HSCs. In zone 3 (central; perivenous), the mean number of hepatocytes (147.8 ±45.35) was accompanied by 7.47 ±3.48 α-SMA positive HSCs on average (Table I). The mean numbers of HSCs and hepatocytes in the respective zones of the hepatic lobule did not differ statistically significantly (Table I). This is consistent with the calculation per 100 liver parenchymal cells: zone 1 – 4.27, zone 2 – 5.69 (the highest) and zone 3 – 5.05. On average, there were 5.0 immunopositive HSCs in the whole hepatic lobule per 100 hepatocytes.

Group II (S-1)

In children with CHB in the S-1 group, α-SMA immunoreactive HSCs were found not only in the perisinusoidal location, dispersed throughout the hepatic lobule, but also within fibrotic foci in the portal and periportal areas. Most cells demonstrated moderately or strongly positive cytoplasmic staining for protein, mainly with submembranous accumulation of the biomarker. They were more frequently found in the intermediate part of the hepatic lobule (Fig. 1B). The number of HSCs in zone 1 was significantly lower as compared to zones 2 and 3 ($p < 0.001$). The mean number of hepatocytes in zone 1 was 191.14 ±73.3, being accompanied by 9.28 ±4.15 immunoreactive HSCs. In zone 2, the mean number of hepatocytes (191.69 ±69.53) corresponded to the mean number of HSCs (12.79 ±4.74). In zone 3, the mean number of hepatocytes (192.59 ±75.65) went along with 12.32 ±4.34 HSCs with a positive reaction to α-SMA protein (Table I). The above data correlated with the mean number of HSCs/100 hepatocytes in the respective zones of the hepatic lobule. In the periportal zone, their number was 4.86 (the smallest), in the intermediate zone 6.67 (the largest), and in the central zone 6.4. On average, there were 5.98 α-SMA immunopositive HSCs per 100 hepatocytes.

Group III (S-2)

Most α-SMA immunopositive HSCs in the S-2 group showed a far stronger IHC reaction to fibrosis protein. The color reaction product was located in the cell cytoplasm, with a tendency to accumulate mainly in submembranous areas. Immunopositive HSCs were found more often than in group II; some cells were elongated. They were situated both in the perisinusoidal region, mainly around the central vein of the lobule and within the intermediate zone of the hepatic lobule (Fig. 1C), and within the foci of intensive periportal fibrosis or single porto-portal bridges. The study showed a significantly lower number of HSCs in zone 1 of the hepatic lobule as compared to zones 2 and 3 ($p < 0.001$). In zone 1, the mean number of immunopositive hepatic stellate cells was 14.6 ±7.26 per mean number of 180.7 ±65.76 hepatocytes. However, in zone 2, the mean number of HSCs was 18.99 ±7.83 per mean number of 179.45 ±67.3 hepatocytes. In turn, in zone 3, the mean number of hepatocytes was 179.27 ±63.3 per mean number of 19.25 ±6.86 HSCs (Table I). The above data correlated with the mean number of HSCs/100 hepatocytes, being the smallest in zone 1 (8.08), whereas in zone 2 it was 10.58, and it was slightly higher in zone 3 (10.74). With reference to the whole hepatic lobule, the mean number of immunopositive HSCs/100 hepatocytes was 9.8.

Group IV (S-3)

Among all the groups with type B chronic hepatitis, S-3 patients had the highest number of α-SMA positive HSCs, both in perisinusoidal and in the extraperisinusoidal area, i.e. situated within the foci of intensive fibrosis (bridging fibrosis with architectural disorders). Most perisinusoidal α-SMA positive HSCs were found in the intermediate part of the lobule (Figs. 1D and 1E). The quality of the IHC picture of these cells was similar to that in the S-2 patients. Most

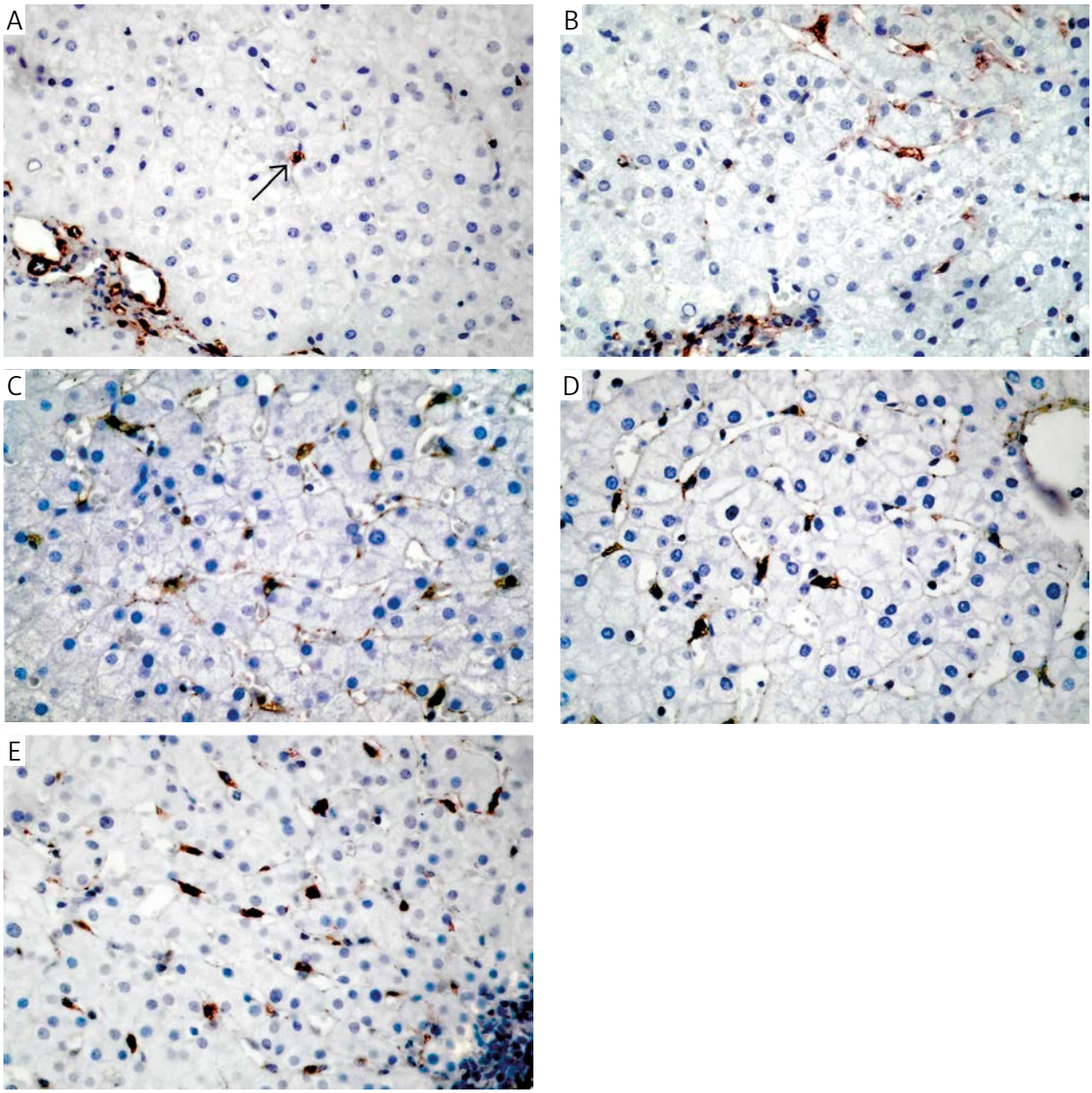

Fig. 1. The picture of immunoreactive HSCs of the liver showing positive IHC reaction for α-SMA protein in children, in the respective groups of patients with type B chronic hepatitis. **A)** Fragment of a hepatic lobule with a visible single regularly-shaped HSC (arrow), located on the border of zone 1 (periportal) and zone 2 (intermediate) of the lobule, showing delicate cytoplasmic pattern of IHC staining. Also mesenchymal components of the hepatic triad, strongly immunoreactive towards α-SMA protein, can be seen in the lower part of the figure. Group I (S-0). **B)** A few α-SMA positive HSCs, with well-preserved shape, located on the border of the periportal and intermediate zone and within the intermediate zone of the hepatic lobule. The cells show moderately positive cytoplasmic IHC staining, mainly with submembranous accumulation of the biomarker. The immunopositive cells are also visible in the dilated portal tracts. Group II (S-1). **C)** Relatively numerous perisinusoidal HSCs present in the intermediate zone of the hepatic lobule, demonstrating moderately and strongly positive cytoplasmic pattern of IHC staining, with distinct submembranous accumulation of the biomarker. Group III (S-2). **D, E)** Numerous distinctly elongated, perisinusoidal HSCs, showing evidently strong expression of alpha-SMA protein, situated mainly in the intermediate zone of the hepatic lobule and less numerous in the vicinity of the central vein of the lobule (vc). At the bottom of Fig. **E**, fragment of a massive inflammatory infiltrate into the portal space. Group IV (S-3). A-E) original magnification 400×

HSCs demonstrated evidently strong reaction for the cytoskeletal protein, showing a cytoplasmic staining pattern. These cells were often elongated (Figs. 1D and 1E). In the periportal zone of the hepatic lobule, the mean number of immunopositive HSCs was 19.67 ±6.82 per mean number of 167.62 ±58.4 hepatocytes. In the intermediate zone, the mean number of HSCs (23.48 ±9.63) corresponded to that

Table I. Quantitative distribution of the mean number of HSCs depending on the location in the respective zones of the hepatic lobule and the stage of fibrosis (S) in children with CHB

	Zones of hepatic lobule			p value*		
	zone 1	zone 2	zone 3	I vs. II	I vs. III	II vs. III
Mean number of HSCs in group I (S-0)	6.27 ±2.34	8.27 ±4.28	7.47 ±3.48	NS	NS	NS
Mean number of HSCs in group II (S-1)	9.28 ±4.15	12.79 ±4.74	12.32 ±4.34	< 0.001	< 0.001	NS
Mean number of HSCs in group III (S-2)	14.6 ±7.26	18.99 ±7.83	19.25 ±6.86	< 0.001	< 0.001	NS
Mean number of HSCs in group IV (S-3)	19.38 ±8.19	23.48 ±9.63	19.67 ±6.82	NS	NS	NS

HSCs – hepatic stellate cells; S – stage of fibrosis; NS – not statistically significant

of hepatocytes (172.14 ±58.37). In the perivenous zone, the mean number of HSCs was 19.38 ±8.19 (Table I). The mean number of HSCs/100 hepatocytes was 11.73 in the periportal zone, 13.64 (the highest) in the intermediate zone and 11.21 (the lowest) in the central zone. On average, throughout the hepatic lobule, 12.19 immunoreactive HSCs were found per 100 hepatocytes. In all the patients, irrespective of the extent of fibrosis, immunoreactive HSCs were the least common near the limiting plate of the lobule. There was a significant positive correlation between fibrogenesis intensity and the increase in the mean number of immunopositive HSCs ($r = 0.518$; $p < 0.001$).

Discussion

The immunohistochemical study of α-SMA conducted on the liver biopsy material obtained from children with CHB showed a substantial effect of chronic viral infection on the number, lobular distribution and picture of the population of immunopositive HSCs.

Biopsy investigations of the liver, both histological and immunomorphological ones, have long been considered the gold standard in the determination of fibrosis progression [9, 11, 19, 25, 26, 27, 28] which, in children and adults, is the most common sequel of chronic infection caused by hepatotropic viruses, i.e. HBV and HCV. Although attempts are made worldwide, including our center, to determine noninvasive serous markers of liver fibrosis [4, 7, 29, 30, 31, 32], their diagnostic advantage over blind biopsy of the organ has not yet been proven [7, 9, 19, 25, 26, 27, 28, 29].

The current IHC observations of liver biopsy specimens from children with CHB in group I (without features of fibrosis) showed that α-SMA immunopositive HSCs were markedly dispersed throughout the lobule, being located in the perisinusoidal region and demonstrating a poorly expressed cytoplasmic expression of the fibrosis biomarker. However, the IHC pictures of HSCs, being qualitatively similar in groups II, III and IV, were characterized by strong (group II) and/or markedly strong expression of the cytoskeletal protein, α-SMA (group III and IV). The immunoreactive HSCs, frequently elongated, were located mainly perisinusoidally and within the new and already formed fibrotic foci. Most cells demonstrating evidently strong immunoreaction to the fibrosis biomarker were found in group IV. The extent of liver fibrosis in the Batts and Ludwig numerical scale [24] was proportional to the number of α-SMA immunoreactive HSCs in the biopsy material. The mean numbers of HSCs/100 hepatocytes in the study groups, i.e. with S-0; S-1; S-2 and S-3, were: 5.00; 5.98; 9.80; 12.19, respectively. Interestingly, in 3 groups, namely with S-0; S-1 and S-3, the highest percentage of HSCs/100 hepatocytes was observed in zone 2, i.e. intermediate, defined in the literature as the zone of variable metabolism. The lowest numbers of HSCs with a positive reaction to α-SMA protein were found in zone 1 of the lobule, which indicates that this area in the process of fibrosis in children with CHB is least metabolically active.

The current results of immunohistochemical and statistical analyses are consistent with our preliminary findings presented recently at the 19th Congress of the Polish Society of Pathologists [33].

The present study is the first extensive morphological work on HSCs biology in chronic hepatitis B, performed on biopsy material in pediatric patients. There are no reports concerning quantitative morphometry of these cells in adult patients, except for a study by Mak and Lieber [34] on alcohol disease, which involved a considerably smaller amount of material. In our opinion, the quantitative method applied in the current study to assess immunoreactive HSCs, although more laborious, is more objective and easier to interpret than the semiquantitative tool used in adult patients [35, 36, 37].

A topographically similar picture of α-SMA positive HSCs in the course of chronic HBV infection in biopsy material was observed in the studies conducted by Guido *et al.* [35], Shi *et al.* [38], and Tomanović *et al.* [39] in adult patients. Both the current IHC study in children with CHB and analogical observations performed by the quoted researchers in adult patients indicate that the majority of perisinusoidal HSCs with strong or very strong α-SMA expression can be found in the intermediate zone of the hepatic lobule and then in its central zone. Thus, it was zone 2, intermediate, that was most metabolically active and involved in the development of changes. On the other hand, the fewest α-SMA positive stellate cells were found in zone 1, i.e. periportal [35, 38, 39].

A separate group of literature reports have discussed the biology of stimulated HSCs, with special regard to α-SMA immunoexpression, in other types of liver diseases [28, 34, 36, 37, 40, 41, 42]. Tomanović *et al.* [37], who performed a semiquantitative assessment of α-SMA positive HSCs in chronic hepatitis C in adult patients, noted their increased number in all regions of the hepatic lobule studied, especially in the portal space, as well as in the fibrous septa. They observed a positive correlation between the number of activated HSCs within the portal spaces and fibrous septa and the stage of fibrosis, which they consider to play a role in the development of progressing fibrosis and cirrhosis in chronic viral HCV infection. A significant increase in the number of immunopositive HSCs in zone 1 of the lobule in patients with chronic type C hepatitis topographically differentiates this type of hepatitis from type B chronic hepatitis, where the fewest cells were found, both in adults [35, 38, 39] and in children in the current study.

Summing up, the immunohistochemical assessment of HSCs within different areas of hepatic tissue showed an increase in the number of cells with enhanced intensity of liver fibrosis, which confirms their crucial role in the development and progression of liver fibrosis, and can be a useful prognostic factor of unfavorable termination of the disease.

The highest mean number of immunoreactive HSCs/100 hepatocytes was found in the intermediate zone, which indicates that this area of the hepatic lobule is most metabolically active in the process of fibrosis in children with CHB. However, the smallest count of activated HSCs was observed in the periportal zone, which suggests that this area of the hepatic lobule is the least metabolically active in the course of liver fibrogenesis.

Conclusions

The immunochemical assessment of HSCs within the hepatic lobule in children with CHB, combined with statistical assessment, showed a close positive correlation of cell count with fibrosis intensity. The analysis of HSC activity within specified areas of liver tissue, being the first extensive morphological work on the biology of these nonparenchymal hepatic cells in CHB pediatric patients, may lead to new perspectives in early diagnosis of liver fibrosis and have prognostic implications in this pathology.

The study may also contribute to the clinical development and validation of antifibrotic therapies in patients with chronic liver diseases.

The authors declare no conflict of interest.

References

1. Lee Y, Friedman SL. Fibrosis in the liver: acute protection and chronic disease. Prog Mol Biol Transl Sci 2010; 97: 151-200.
2. Maia JM, Maranhão Hde S, Sena LV, et al. Hepatic stellate cell activation and hepatic fibrosis in children with type 1 autoimmune hepatitis: an immunohistochemical study of paired liver biopsies before treatment and after clinical remission. Eur J Gastroenterol Hepatol 2010; 22: 264-269.
3. Czaja AJ. Hepatic inflammation and progressive liver fibrosis in chronic liver disease. World J Gastroenterol 2014; 20: 2515-2532.
4. Popov J, Schuppan D. Targeting liver fibrosis: strategies for development of validation of anti fibrotic therapies. Hepatology 2009; 50: 1294-1306.
5. Tacke F, Weiskirchen R. Liver fibrosis – pathogenesis and novel therapeutic approaches. Internist (Berl) 2010; 51: 21-29.
6. Sobaniec-Lotowska ME, Lotowska JM, Lebensztejn DM. Ultrastructure of oval cells in children with chronic hepatitis B, with special emphasis on the stage of liver fibrosis: the first pediatric study. World J Gastroenterol 2007; 13: 2918-2922.
7. Zhou K, Lu LG. Assessment of fibrosis in chronic liver diseases. J Dig Dis 2009; 10: 7-14.
8. Stepień M, Czarkowski MP. Hepatitis B in Poland in 2011. Przegl Epidemiol 2013; 67: 239-245.
9. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005; 115: 209-218.
10. Lee UE, Friedman SL. Mechanisms of hepatic fibrogenesis. Best Pract Res Clin Gastroenterol 2011; 25: 195-206.
11. Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 2008; 88: 125-178.
12. Friedman SL. Evolving challenges in hepatic fibrosis. Nat Rev Gastroenterol Hepatol 2010; 7: 425-436.
13. Hernández-Gea V, Friedman SL. Pathogenesis of liver fibrosis. Annu Rev Pathol 2011; 6: 425-456.
14. Mallat A, Lotersztajn S. Reversion of hepatic stellate cell to a quiescent phenotype: From myth to reality? J Hepatol 2013; 59: 383-386.
15. Nagatsuma K, Hano H, Murakami K, et al. Hepatic stellate cells that coexpress LRAT and CRBP-1 partially contribute to portal fibrogenesis in patients with human viral hepatitis. Liver Int 2014; 34: 243-252.
16. Nepomnyashchikh GI, Aidagulova SV, Nepomnyashchikh DL, et al. Ultrastructural and immunohistochemical study of hepatic stellate cells over the course of infectious viral fibrosis and cirrhosis of the liver. Bull Exp Biol Med 2006; 142: 723-728.
17. Elpek GÖ. Cellular and molecular mechanisms in pathogenesis of liver fibrosis: An update. World J Gastroenterol 2014; 20: 7260-7672.
18. Wang Y, Li J, Wang X, Sang M, et al. Hepatic stellate cells, liver innate immunity, and hepatitis C virus. J Gastroenterol Hepatol 2013; 28 Suppl. 1: 112-115.

19. Lebensztejn DM, Kaczmarski M, Sobaniec-Lotowska ME, et al. Blind liver biopsy in children – diagnostic significance and complications in authors' own material. Med Sci Monit 2000; 6: 1155-1158.
20. Sobaniec-Lotowska ME, Lebensztejn DM. Ultrastructure of hepatocyte mitochondria in nonalcoholic steatohepatitis in pediatric patients: Usefulness of electron microscopy in the diagnosis of the disease. Am J Gastroenterol 2003; 98: 1664-1665.
21. Lotowska JM, Sobaniec-Lotowska ME, Lebensztejn DM. Electron microscopic alterations in intermediate hepatocyte-like cells in children with chronic hepatitis B. The first report in pediatric patients. Eur J Gastroenterol Hepatol 2010; 22: 741-747.
22. Sobaniec-Lotowska ME, Lebensztejn DM, Lotowska JM, et al. Ultrastructure of liver progenitor/oval cells in children with nonalcoholic steatohepatitis. Adv Med Sci 2011; 56: 172-179.
23. Lotowska JM, Sobaniec-Lotowska ME, Lebensztejn DM. The role of Kupffer cells in the morphogenesis of nonalcoholic steatohepatitis – ultrastructural findings. The first report in pediatric patients. Scand J Gastroenterol 2013; 48: 352-357.
24. Batts KP, Ludwig J. Chronic hepatitis. An update on terminology and reporting. Am J Surg Pathol 1995; 19: 1409-1417.
25. Afdhal NH. Biopsy or biomarkers: is there a gold standard for diagnosis of liver fibrosis? Clin Chem 2004; 50: 1299-1300.
26. Afdhal NH, Nunes D. Evaluation of liver fibrosis: a concise review. Am J Gastroenterol 2004; 99: 1160-1174.
27. Arends JE, de Knegt RJ, Van Erpecum KJ, et al. Diagnosis of hepatic fibrosis and cirrhosis. Ned Tijdschr Geneeskd 2007; 151: 1502-1506.
28. Ionescu AG, Streba LA, Vere CC, et al. Histopathological and immunohistochemical study of hepatic stellate cells in patients with viral C chronic liver disease. Rom J Morphol Embryol 2013; 54: 983-991.
29. Lebensztejn DM, Skiba E, Kaczmarski M, et al. Serum cystatin C concentration does not predict advanced liver disease in children with chronic hepatitis B. Clinica Chim Acta 2004; 347: 227-228.
30. Lebensztejn DM, Sobaniec-Lotowska ME, Kaczmarski M, et al. Matrix-derived serum markers in monitoring liver fibrosis in children with chronic hepatitis B treated with interferon alpha. World J Gastroenterol 2006; 12: 3338-3343.
31. Lebensztejn DM, Skiba E, Sobaniec-Lotowska ME, et al. Serum hyaluronan and laminin level in children with chronic hepatitis B during long-term lamivudine treatment. Hepatogastroenterology 2007; 54: 834-838.
32. Castera L. Hepatitis B: are non-invasive markers of liver fibrosis reliable? Liver Int 2014; 34 Suppl 1: 91-96.
33. Lotowska JM, Lebensztejn DM. α-SMA immunoreactive hepatic stellate cells (HSCs) in the course of chronic hepatitis B. The first report in pediatric patients. 19th Congress of Polish Society of Pathologists. Pol J Pathology 2013; 64 (Suppl. 1): 103.
34. Mak KM, Lieber CS. Lipocytes and transitional cells in alcoholic liver disease: a morphometric study. Hepatology 1988; 8: 1027-1033.
35. Guido M, Rugge M, Chemello L, et al. Liver stellate cells in chronic viral hepatitis: the effect of interferon therapy. J Hepatol 1996; 24: 301-307.
36. Schmitt-Gräff A, Krüger S, Bochard F, et al. Modulation of alpha smooth muscle actin and desmin expression in perisinusoidal cells of normal and diseased human livers. Am J Pathol 1991; 138: 1233-1242.
37. Tomanović NR, Boricić I, Brasanac DC, et al. Activated liver stellate cells in chronic viral C hepatitis: histopathological and immunohistochemical study. J Gastrointestin Liver Dis 2009; 18: 163-167.
38. Shi J, Hao JH, Ren WH, et al. Pathogenesis of liver fibrosis in patients with chronic hepatitis B. Zhonghua Gan Zang Bing Za Zhi 2009; 17: 443-445.
39. Tomanović NR, Boricić I, Brasanac D. Immunohistochemical analysis of alpha-SMA and GFAP expression in liver stellate cells. Vojnosanit Pregl 2006; 63: 553-557.
40. Jou J, Choi SS, Diehl AM. Mechanisms of disease progression in nonalcoholic fatty liver disease. Semin Liver Dis 2008; 28: 370-379.
41. Okanoue T, Burbige EJ, French SW. The role of the Ito cell in perivenular and intralobular fibrosis in alcoholic hepatitis. Arch Pathol Lab Med 1983; 107: 459-463.
42. Page A, Paoli PP, Hill SJ, et al. Alcohol directly stimulates epigenetic modifications in hepatic stellate cells. J Hepatol 2015; 62: 388-397.

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