Original Article

Improvement of the histopathology on a maintenance regimen in children with autoimmune hepatitis.

El-Sayed I. Salama[a] , Nermine A. Ehsan[b], Behairy E. Behairy[a], Mohsen H. Hussein[a]
Menan E.Salem[a]

[a]*Department of Pediatrics, National Liver Institute, Menofia University, Egypt*
[b]*Department of Pathology, National Liver Institute, Menofia University, Egypt*

Abstract. Autoimmune hepatitis (AIH) is a chronic liver disease characterized histologically by interface hepatitis and fibrosis. Recent studies have reported that hepatic fibrosis and cirrhosis may be reversible by treatment in some patients. Combined low dose prednisolone and azathioprine regimen was rarely studied in children with AIH. Twenty children with median age 8±3.5 yrs (9 girls, 11 boys), 18 AIH type I and two were AIH type II, who were in clinical and biochemical remission for at least 6 months, and who had a diagnostic and a follow-up liver biopsy, were included in this study. Different histological stains were used for assessing the grade of necroinflammatory activity (HAI) and for evaluating the stage of fibrosis according to Ishak scoring system. Morphometric analysis using LeicaQ500IS image analyzer was applied on Perl's stained liver sections to assess the percentage of liver fibrosis. Data revealed significant decrease in the median HAI from 8.85 to 3.6 (p=0.001). The median fibrosis score showed significant reduction from 3.9 to 2.4 (p=0.001) and the median fibrosis percentage decreased from 28.7 to 12.8 (p=0.001). These data provide evidence for regression of fibrosis in AIH in children who responded to the combined low dose immunosuppressive prednisolone and azothioprine regimen.

Key words: Autoimmune hepatitis, low dose prednisolone combined regimen, fibrosis regression, children

1. Introduction

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease characterized serologically by the presence of non-organ and liver specific autoantibodies and histologically by plasmalymphocytic infiltrate, interface hepatitis and liver cell damage (1). AIH is divided into two subtypes according to seropositivity for smooth muscle and/or antinuclear antibody (SMA/ANA, type 1) or liver kidney microsomal antibody (LKM1, type 2) (2).

*Correspondence: Dr: El-Sayed Ibrahim Salama. MD, PhD.
7 Sinai str. App # 3, from wadi-el-Nile, Almohandessin, 12411, Giza, Egypt.
Mobile: 02-0191221403
Tel: 02-33533303
Email: elsayedsalama4@yahoo.com
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There is a female predominance in both types. AIH patients usually respond to immunosuppressive treatment, which should be instituted as soon as diagnosis is made (3). There are three clinical patterns of disease onset: (i) in at least 40% of patients, the presentation is indistinguishable from that of an acute viral hepatitis (non-specific symptoms of malaise, nausea/vomiting, anorexia, and abdominal pain, followed by jaundice, dark urine, and pale stools), some children, particularly those who are anti-LKM-1 positive, develop acute hepatic failure within 2-8 wk from onset of symptoms; (ii) in 25%-40% of patients, the onset is insidious, with an illness characterized by progressive fatigue, relapsing jaundice, headache, anorexia, and weight loss, lasting from several months to even years before diagnosis; (iii) in about 10% of patients, there is no history of jaundice, and the diagnosis follows presentation with complications of portal hypertension, such as splenomegaly, hematemesis from esophageal

varices, bleeding diathesis, chronic diarrhea, and weight loss (4). The mode of presentation of AIH in childhood is therefore variable and the course of the disease may be fluctuating with flares and spontaneous remissions (5,6). Evidence suggests that liver injury in a patient with AIH is the result of cell-mediated immune attack directed against genetically predisposed hepatocytes (7). Aberrant display of human leukocyte antigen (HLA) class II on the surface of hepatocytes facilitates the presentation of liver cell membrane constituents to antigen presenting cells (8).

Hepatic fibrosis occurs in response to many types of chronic hepatic injury including AIH (9). The major component of fibrous accumulation in chronic liver injury is due to the phenotypic modulation of hepatic stellate cells (10). It is predominantly the cytokines and soluble mediators secreted by Kupffer cells, other inflammatory cells and injured cells that stimulate stellate cells to divide, shift from the resting lipocyte phenotype to a transitional myofibroblast phenotype with increased capacity for synthesis and secretion of extracellular matrix (ECM) (10). As fibrosis progresses to cirrhosis, shunted blood flow through fast vascular channels leaving the remainder of the hepatic parenchyma devoid of blood supply contributing to ongoing hepatocellular necrosis (11).

Recent data has been reported that fibrosis and even cirrhosis are reversible in chronic liver diseases including AIH (12,13), Wilson's disease (14), hemochromatosis (15), hepatitis B virus infection (16), hepatitis C virus infection (17) and primary biliary cirrhosis (18). Primary therapeutic approach to reversal of fibrosis focuses on treatment of the underlying disease (11). Two treatment regimens are comparable to each other in the management of AIH. Prednisolone alone or a lower dose of prednisolone with azathioprine (19). The combination regimen is associated with a lower occurrence of corticosteroid-related side effects than the higher dose prednisolone regimen (20). Effective therapy of AIH is believed to improve the degree of necroinflammatory activity and may lead to reversibility of fibrosis or cirrhosis (21). The response to immunosuppressant therapy is determined on the basis of clinical, biochemical and histological findings (22). This is practically comprised of two phases: induction of remission and maintenance of remission (23). Liver biopsy findings can evaluate the adequacy of histological response, the risk of subsequent relapse, the need for additional treatment before drug withdrawal and it should be considered before terminating therapy (24).

In this study, we investigated liver histopathology of twenty children with AIH who responded clinically and biochemically to the combined low dose prednisolone and azathioprine regimen for regression of fibrosis.

2. Materials and methods

2. 1. Study population and patient selection criteria

This study included 20 pediatric patients who satisfy the international criteria for the definite diagnosis of AIH according to the International Autoimmune Hepatitis Group (1). These patients were selected retrospectively (11 cases) and prospectively (9 cases) from all AIH cases (74 patients) attending the Pediatrics Department at the National Liver Institute, Menofia University, between January 1998 until May 2008. The study population received the combined low dose regimen of prednisolone and azathioprine. Prednisolone was given in a dose of (1mg/kg, not more than 20-30mg/day) and azathioprine in a dose of 1mg/kg/day. During the first 6-8 weeks of treatment, liver tests were checked weekly to allow a frequent fine-tuning. When complete normalization of liver enzymes or at least less than two folds elevation of transaminases was achieved, gradual withdrawal of prednisolone tailored to reach the maintenance dose of 10mg/day.

Criteria of selection of patients included in this study were: 1. patients who had a diagnostic liver biopsy, 2. those who had shown clinical and biochemical criteria of remission (absence of signs and symptoms of liver disease, transaminases and gamma globulin levels within reference levels) for at least 6 continuous months after starting treatment with the combined low dose regimen, 3. those who had a second follow up liver biopsy. An informed consent was obtained from the guardians of the children.

Exclusion criteria included: cases with chronic Hepatitis B infection, chronic Hepatitis C infection, decompensated liver disease, history of previous antiviral therapy and absolute contraindication to liver biopsy.

2. 2. Clinical and laboratory assessment

All patients were subjected to full history taking including history of drug exposure, autoimmune features, blood transfusion and risk factors for viral infection. Thorough clinical examination and abdominal ultrasonogrphy were done. The following tests were done: alanine amino transferase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), protein electrophoresis, gamma glutamyl transpeptidase

(GGT), total proteins, serum albumin, total bilirubin, prothrombin time and concentration, complete blood count, hepatitis B surface antigen (HBsAg), Anti-hepatitis B core antigen (IgG and IgM), hepatitis C virus antibody (anti- HCV) in serum and HCV- RNA by quantitative PCR for +ve HCV-ab.

2. 3. Autoantibodies

A key criterion for the diagnosis of AIH was the detection of ANA, SMA, and anti-LKM-1 by indirect immunofluorescence on a freshly prepared rodent substrate that included kidney, liver, and stomach to allow the detection of ANA, SMA, anti-LKM-1 as well as anti-liver cytosol type 1 and anti-mitochondrial antibody (AMA). In children, the titers of 1/20 for ANA and SMA and 1/10 for anti-LKM-1 were clinically relevant (4).

2. 4. Liver biopsy

Liver biopsies (whether for diagnosis or follow up) were obtained by ultrasound percutaneous puncture (True-cut needle, Baxter International, Deerfield, IL). It was done for diagnosis before treatment and 6 months after start of remission (control liver biopsy). The remission range time was 2-3 mo**.**

2. 5. Histopathological evaluation

Liver fragments were fixed in 10% formalin buffered saline, processed according to the routine histological techniques and embedded in paraffin. Five micron thick liver sections were cut from paraffin blocks and mounted on glass slides. Liver sections were stained with Hematoxylin and Eosin (H&E) for grading the necro-inflammatory activity, histological activity index (HAI). Masson's Trichrome and Orcein stained sections were used for scoring the stage of fibrosis. Liver histopathology was assessed according to Ishak scoring system for grading and staging chronic hepatitis (25). The histological activity index (HAI) 0-18, which grades necrosis and inflammation, (periportal or periseptal interface hepatitis, 0-4; confluent necrosis, 0-6; focal spotty necrosis, 0-4; portal inflammation, 0-4). Fibrosis scale from 0-6, 0=no fibrosis, 1-2=portal fibrosis, 3-4=bridging fibrosis and 5-6= cirrhosis (developing and established cirrhosis). Perls' stained liver sections were used to evaluate the percentage of liver fibrosis by computed assisted LeicaQ500IS image analyzer (26). Perls' stain aided the demarcation of fibrosis content stained red among a pale whitish parenchyma. Briefly, quantitation assessment of fibrosis content per biopsy surface area was performed with computed assisted program that determine the mean, standard deviation and standard error of fibrosis percentage analyzed per slide in total areas. Mean fibrosis percentage for each liver biopsy was calculated and used as a numerical score for statistical analysis.

2. 6. Statistical analysis

Data was statistically analyzed using SPSS (statistical package for social science) program version 13.0. Results were shown as mean±SD, range, confidence interval (95% CI), frequency and percentage. P-value <0.05 was considered significant. The following tests were applied: Student t test, Wilcoxon test, Kruskal-Wallis test, Pearson's correlation test, Mann Whitney and Fisher exact tests.

The protocol of this research study has been approved by the Institute Research Board (IRB) of the National Liver Institute (NLI), Menofia University, Egypt, and is in accordance with the Helsinki Declaration of 1975.

3. Results

3. 1. Study population profile

Data from this study demonstrated that there was no statistically significant difference between different clinical presentations and histological findings in liver biopsies at diagnosis or at follow-up. Likewise, no statistically significance difference regarding age at presentation, gender of child, patients with serum level of ALT more or less than 500IU/L at presentation regarding HAI, fibrosis score or fibrosis percentage at diagnosis or follow-up liver biopsies. The total number of pediatric cases diagnosed as AIH attending the Pediatrics Department at the National Liver Institute, from January 1988 till May 1988 were 74 cases. Fifty four cases did not meet the inclusive criteria of this study. Twenty children, 11 boys and 9 girls with established diagnosis of AIH based on clinical, serological and histological data using the diagnostic criteria of the International Autoimmune Hepatitis Group were enrolled. Their age at diagnosis ranged from 4.5 to 14.0 years (mean 8±3.5yrs). Autoantibody test at diagnosis, anti-smooth muscle antibodies (ASMA) were positive in 13 patients (65%), anti-nuclear antibodies (ANA) were positive in 4 patients (20%), the concomitant presence of positive ASMA and ANA was detected in one patient (5%) whereas, two patients (10%), a boy and a girl, were positive for anti-liver-kidney microsomal antibodies (LKM-1). Gammaglobulins' levels ranging from 16-44.5 g/L with a mean of 24.3±8.9. AIH score calculated at diagnosis revealed that 15 children (75%) had a score

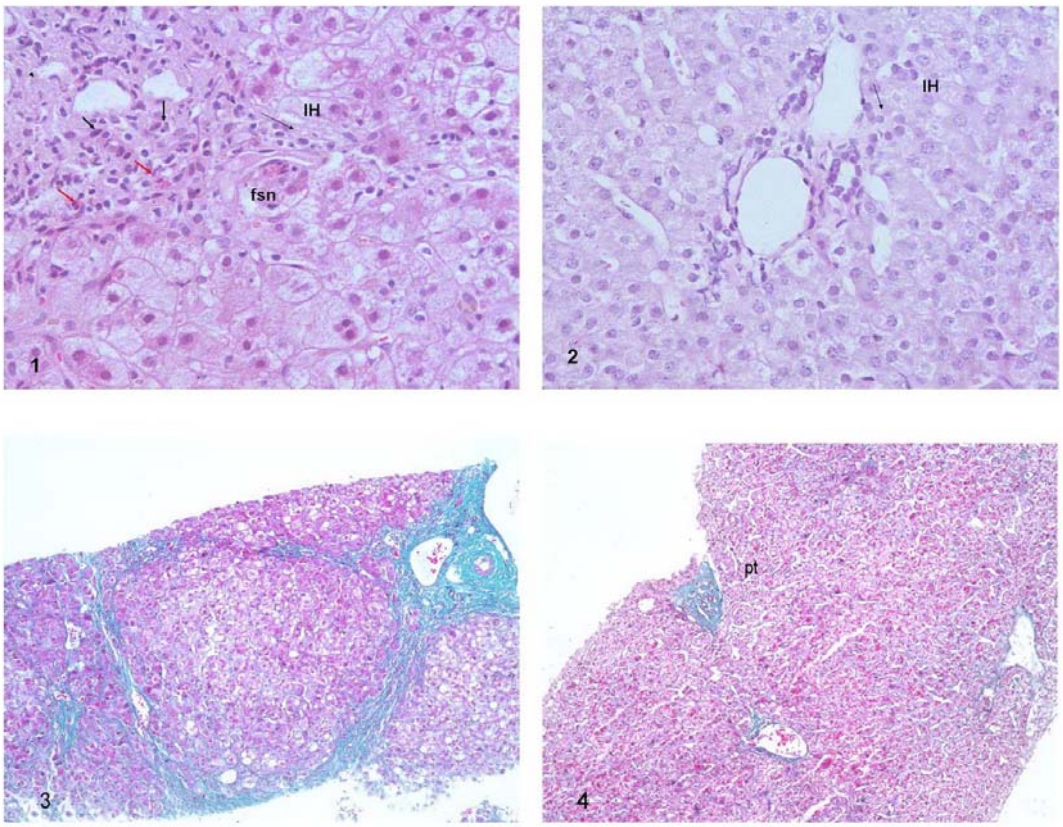


Fig. 1. Photomicrograph of H&E stained liver section from a child with AIH at diagnosis biopsy. The portal tract showed moderate inflammatory infiltrate rich in plasma cells (black arrows) and eosinophils (red arrows). Interface hepatitis (IH) and focal spotty necrosis (fsn).

Fig. 2. H&E stained liver section from the same child at follow-up liver biopsy after 24 months of treatment. The portal tract showed minimal inflammatory infiltrate, minimal focal IH and no parenchymal inflammation. Orginal mag. x20.

Fig. 3. Photomicrograph of Masson Trichrome stained liver section from a child with AIH at diagnosis biopsy showing liver cirrhotic nodule.

Fig. 4. Liver section from the same patient at follow-up biopsy after 54 months of treatment showing profound regression of fibrosis limited to fibrous expansion of portal tracts. Orginal mag.x10.

ranged from 15-23, mean=18± 4 with a definite diagnosis of AIH, while 5 patients (25%) had a score between 10-15 thus probable diagnosis of AIH.

Patients in this study received the combined regimen of prednisolone and azathioprine for treatment and achieved clinical and biochemical remission after a period ranging from 2-24 months in those cases who were selected prospectively. Whereas, the duration of treatment of the retrospectively selected patients ranged from 1-9 years. Liver enzymes were sometimes elevated during the period of treatment with the number of occasions of elevation of transaminases ranging from 0-12 with a mean of 2±3. Eleven patients (55%) presented by jaundice only with insidious onset and intermittent course, 5 patients (25%) presented by picture of acute hepatitis (jaundice with dark colored urine and abdominal pain), 2 patients (10%) presented with abdominal enlargement of gradual onset and progressive course while another 2 patients (10%) were asymptomatic and diagnosed incidentally. Although 74 cases were diagnosed as AIH for the last 10 years in the Pediatrics Department, NLI, only 20 cases that met the inclusive criteria for this study. The other 54 cases comprised the following: 7 patients had coagulopathy at presentation and therefore did not have an initial biopsy for diagnosis. Sixteen patients did not achieve normalization of liver enzymes either due to compliance or treatment failure. Seven patients had their biopsies not assessable, one patient died from acute hepatic failure on top of

Hepatitis A virus infection, 4 patients had under gone follow-up outside the National Liver Institute, 9 patients had no regular follow-up and 10 patients could not be reached.

3. 2. Histological evaluation of liver biopsies at presentation for diagnosis

H&E stained liver sections from AIH children at initial diagnosis biopsy revealed consistent pathological features of AIH including interface hepatitis, plasmalymphocytic infiltrate in portal tracts and liver cell inflammation. The extent of interface hepatitis varied in different biopsies according to the magnitude of necro-inflammatory activity. One biopsy revealed focal interface hepatitis, five biopsies demonstrated mild interface hepatitis, 12 biopsies revealed involvement more than 50% of the circumference of the limiting plate by interface hepatitis, while one biopsy exhibited bridging necrosis where the inflammatory process encroached along the whole circumference of the limiting plate extending to neighboring portal tracts. The portal inflammatory infiltrate varied from mild to moderate to severe inflammation. The nature of the inflammatory infiltrate comprised mainly of plasma cells with prominent eosinophils in a background of scattered mononuclear inflammatory cells as shown in Fig.1. Hepatic parenchyma displayed variable grades of inflammatory activity ranging from focal spotty necrosis, scattered foci of spotty necrosis, focal confluent necrosis, zone 3 necrosis and pan acinar necrosis. Inconsistent pathological features of AIH that were observed in some liver biopsies of children at initial diagnosis included: cholestasis detected in 4 cases as intracellular and intracanalicular cholestasis with cholestatic rosettes, macrovesicular steatosis in two cases (2 girls that was mild in one case, 10% of hepatocytes showing lipid vacuoles) and moderate in the other case (30-60% of hepatocytes showing lipid vacuoles). The biopsy of one case (a girl) showed giant cell hepatitis and one case (a girl) presented with features suggestive of sclerosing cholangitis. The stage of fibrosis was as follows: 11 cases had cirrhosis (5-6/6), 3 cases had bridging fibrosis (3-4/6) and 6 cases had mild fibrosis (2/6). The percentage of liver fibrosis as evaluated by LeicaQ500IS Image Analyzer revealed that the highest fibrosis percentage was 48.2% while the lowest fibrosis percentage at initial diagnosis biopsy was 7.9%.

Table 1. Histological features in liver biopsies of children with AIH before and after treatment with the combined low dose prednisolone and azathioprine regimen

Histological features		Before treatment No. of biopsies	After treatment No. of biopsies
Interface hepatitits	Minimal	1	13
	Mild	5	7
	Moderate	12	0
	Severe	2	0
Parenchymal infla.	Focal spotty necrosis (<5/10x)	1	13
	Focal spotty necrosis (>5/10x)	6	7
	Focal confluent necrosis	11	0
	Zonal and acinar necrosis	2	0
Fibrosis stage	0	0	2
	1	0	5
	2	6	6
	3	1	2
	4	2	4
	5-6	11	1
Inconsistent finding	Cholestasis	4	0
	Giant cell-transformation	1	0
	steatosis	2	1
	Features suggestive of sclerosing cholangitis	1	0

3. 3. Histological evaluation of follow-up liver biopsies

Microscopic examination of follow-up liver biopsies revealed control of the necro-inflammatory activity. There was minimal inflammatory infiltrate in the portal tract comprised mainly of mononuclear cells and devoid of plasma cells or eosinophils (Fig. 2). There were no or focal interface hepatitis. No or occasional foci of spotty necrosis occurred in the hepatic parenchyma. The histological activity index ranged from 2-3/18 in those cases. Seven cases out of 20 had mild inflammatory activity 4-7/18. Table 1. demonstrated the histological features associated with liver biopsies of children with AIH before and after treatment. There were remarkable improvement in fibrosis scores and fibrosis percentages. Four cases of cirrhosis showed regression in the fibrosis score to fibrous expansion of portal tracts without septa formation (stage 2/6) as shown in Figures 3,4. Six cases of cirrhosis showed reduction in stage of fibrosis to bridging fibrosis with fibrous septa extending from portal tracts to neighboring portal tracts. Only one cirrhotic case in this study series that did not show regression in the stage of fibrosis and cirrhosis persisted even after achieving clinical and biochemical remission and presence of minimal necro-inflammatory activity in liver histopathology. Most cases with variable stages of fibrosis ranging from mild to moderate fibrosis showed improvement in score of the fibrosis stage. Statistical analysis revealed that there were significant improvement in HAI that has dropped from a mean of 8.85±2.7 before treatment to a mean of 3.6±1.5 after treatment. The fibrosis score improved from a mean of 3.9±1.4 before treatment to a mean of 2.4±1.3 after treatment.

Table 2. Statistical results of histological findings associated with liver biopsies of children with AIH before and after treatment with the combined low dose prednisolone regimen

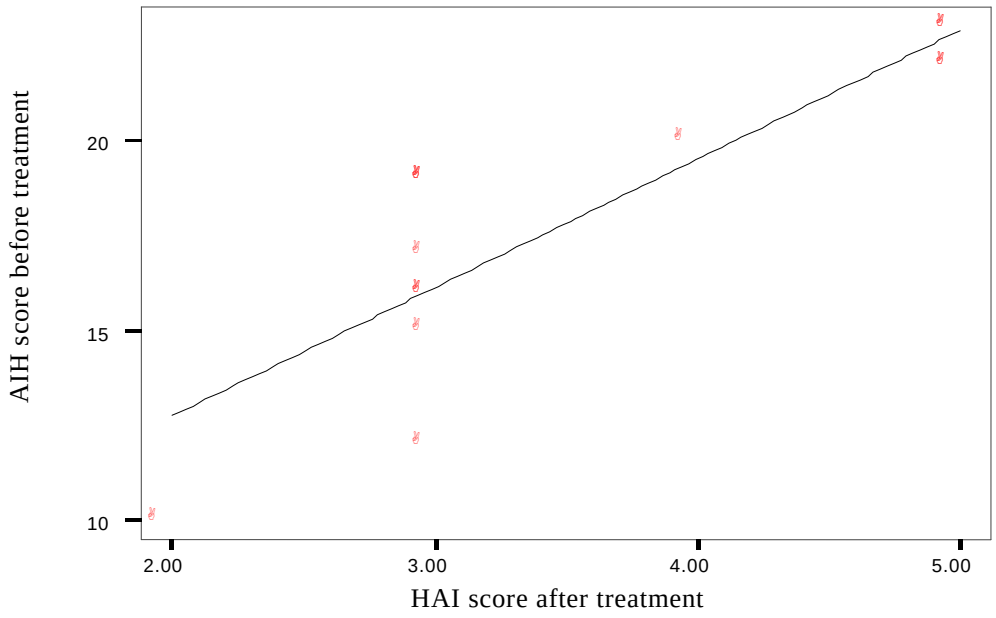
Studied variables	Pre treatment (no. 20) Mean ± SD	Post treatment (no. 20) Mean ± SD	Test of significance Wilcoxon test	p-value
HAI	8.85 ± 2.7	3.6 ± 1.5	3.84	< 0.001
Fibrosis score	3.9 ± 1.4	2.4 ± 1.3	3.7	< 0.001
Fibrosis percentage	28.7 ± 15.5	12.8 ± 8.8	3.4	< 0.001

Table 3. Pearson's correlation between calculated AIH score before treatment and liver pathology findings before and after treatment with the combined low dose corticosteroid regimen

	AIH score at initial biopsy	R	p-value
Before treatment	Fibrosis score	0.005	> 0.05
	HAI	- 0.18	> 0.05
	Fibrosis percent	- 0.28	> 0.05
After treatment	Fibrosis score	0.05	> 0.05
	HAI	0.48	< 0.05*
	Fibrosis percent	- 0.09	> 0.05

Table 4. Pearson's correlation between time to biochemical remission (time taken for enzymes to be normal) and liver histopathology findings before and after treatment and between number of occasions of elevated enzymes during period of treatment and liver histopathology before and after treatment

	Studied pathological variables	Time to remission (in months)		Number of occasions of elevated enzymes during period of treatment	
		r	p- value	r	p- value
Before treatment	Fibrosis score	- 0.41	> 0.05	0.03	> 0.05
	HAI	- 0.1	> 0.05	- 0.27	> 0.05
	Fibrosis %	- 0.03	> 0.05	- 0.09	> 0.05
After treatment	Fibrosis score	- 0.26	> 0.05	0.49	< 0.05*
	HAI	0.58	< 0.05*	- 0.05	> 0.05
	Fibrosis %	0.04	> 0.05	0.31	> 0.05



Graph 1. Correlation between AIH score before treatment and histological activity index (HAI) in liver biopsies after treatment with the combined low dose prednisolone and azathioprine regimen.

Likewise the fibrosis percentage dropped from a mean of 28.7%±15.5 before treatment to a mean of 12.8±8.8 after treatment (Table 2). These results were highly statistically significant with a p value <0.001.

Other pathological findings that had disappeared upon treatment with the combined prednisolone and azathioprine regimen included cholestasis, giant cell hepatitis and features suggestive of sclerosing cholangitis. One case of mild steatosis had subsided, whereas the other case of moderate steatosis showed mild improvement of steatosis (10-30% of hepatocytes showing lipid vacuoles) after treatment.

Pearson's correlation revealed positive correlation between calculated AIH score before treatment and grade of necro-inflammatory activity, HAI, in follow up liver biopsy ($p < 0.05$) as shown in Table 3 and graph 1. Patients in this study achieved clinical and biochemical remission after 2-24 months, in the prospective group. Table 4 showed that there was statistically positive correlation between time to remission and HAI in follow-up liver biopsy (p value <0.05), and also statistically positive correlation between a number of occasions of elevated enzymes during period of treatment and fibrosis score in the follow up liver biopsy (p value <0.05).

4. Discussion

Twenty children with established diagnosis of AIH who had achieved clinical and laboratory response to the combined Prednisolone regimen were investigated in this study. The principal findings were regression of liver fibrosis and necro-inflammatory activity in liver biopsies obtained 6 months following clinical and biochemical features of remission compared to liver biopsies obtained at presentation for diagnostic purposes. The mounting clinical evidence that fibrosis and even cirrhosis may regress is easier to digest now than in the past two decades (11,27). It is difficult to separate the description of regenerative capacity of the liver from the actual reversal of fibrosis, the two are probably mechanistically linked. Several publications have pointed to the capacity of the injured liver to resorb scar, emphasizing the importance of enzymatic processes to fibrosis regression as well as hepatocyte renewal in order to achieve restoration of the functional parenchymal mass (28). It is well established that the primary approach for fibrosis regression and reversal of cirrhosis is the treatment of the underlying disease (11).

Results from this study revealed a statistically significant reduction in necro-inflammatory activity following clinical and biochemical remission induced by the combined low dose predisolone regimen. An improvement in periportal and periseptal interface hepatitis, focal spotty necrosis as well as portal inflammation were achieved. There were no confluent necrosis or bridging necrosis in liver biopsies of children that showed biochemical and clinical remission. No biopsy showed worsening of intensity of inflammatory activity with treatment. Results from this study are in accordance with Ferreira et al., 2008 (29) who reported similar results in AIH children and adolescents. These findings demonstrate that the treatment effectively acts on the inflammatory cells, and consequently reduces the histopathological alterations induced by their activation. It has been reported that the prognosis of AIH is related to response to corticosteroid therapy and that, failure of laboratory results improvement during therapy, has to be the principal indication of poor outcome (30). Seven children showed mild inflammatory activity in spite of revealing features of clinical and biochemical remission following treatment. In five cases, these findings could be explained by the short duration of immunosuppressant therapy (2-3 months). Whereas, the other two cases belonged to the retrospective group, in spite of the reasonably appropriate duration of treatment (2 and 5 years) the failure might be due to interruption in the course of treatment or cessation of medications by parents or other causes (30). Interestingly, the nature of the inflammatory infiltrate was predominantly plasma cells with scattered eosinophils at initial diagnosis biopsy. Whereas, plasma cells and eosinophils subsided in follow-up biopsies were replaced by occasional mononuclear inflammatory cells. It has been reported that portal plasma cell infiltration is predictive of relapse after drug withdrawal in tissue specimens already satisfying criteria for remission (31). Plasma cells may be indicative of an active antibody-dependent pathogenic mechanism which may explain the higher frequency of relapse that occur in AIH after discontinuation of treatment.

Regarding fibrosis regression, our results revealed profound improvement in fibrosis stage and fibrosis percentage in liver biopsies of AIH children who responded to the combined low dose prednisolone and azathioprine regimen. In this study, the use of orcein stain to demonstrate elastic fibres that outline established fibrosis and accordingly aid to evaluate the stage of fibrosis, helped us to overcome the distortion of liver architecture; as a result of necrosis, collapse and regeneration; that is easily mistaken for fibrosis. The Ishak scoring system has been applied in staging of fibrosis in cases with chronic hepatitis (32).

Cirrhosis has been the most frequent histopathological finding in the diagnosis of AIH in children varying from 56.5% to 100% (29). Data from this study revealed that 36.3% (4 out of 11) of cirrhotic patients at presentation had showed regression of fibrosis to fibrous expansion of portal tracts with no septa formation (stage 2/6) in an average interval of treatment of 54 months. Sixty percent (6 out of 11) of cirrhotic patients had reduction in fibrosis stage

into bridging fibrosis (3-4/6) in an average interval of treatment of 25.4 months. While one child (a girl, belonging to the retrospective study group) with cirrhosis out of 11 (9%) remained in the same stage of cirrhosis after a duration of treatment of 54 months. Although that child had reduction in the necro-inflammatory activity to minimal (3/18), the stage of fibrosis remained at the same score with minimal reduction in fibrosis percentage from 39.5% to 31.1%. The failure in regression of cirrhosis in that case could be explained by interruption in the course of treatment or cessation of medications by parents. Intrinsic pathogenic mechanisms due to host-dependent genetic predisposition that cannot be suppressed by conventional regimens may also explain this phenomenon such as HLA DRB1*03 (30). Functional genetic polymorphisms encoding cytokines, chemokines and their receptors, molecules involved in fibrogenesis and fibrolysis, blood coagulation, antigen presentation, oxidative and antioxidative mechanism are also obstacles for fibrosis regression (11).

Three patients had bridging fibrosis at presentation (diagnosis biopsy), two of them (66%) had shown regression to fibrous expansion to portal tracts with no septa formation in an average interval of treatment of 54 months. While one patient showed early bridging fibrosis with occasional fibrous septa linking neighboring portal tracts, in an interval of treatment of 18 months. It might be speculated that the longer duration taken before obtaining the follow up liver biopsy, the more improvement and regression in the stage of fibrosis is achieved.

Collagen degradation is a slow process and collagen I after its deposition sustains extensive cross-linking that becomes more resistant over time (30). Results from this investigation revealed that six patients had fibrous expansion in most portal tracts (2/6) at initial diagnosis biopsy. One child (a boy) showed total reversibility of fibrosis with no expansion of any portal tracts in an interval of treatment of 10 months. Four patients out of six (66%) had improvement of fibrosis to stage 1/6 where some of the portal tracts demonstrate fibrous expansion, in an average interval of treatment of 39 months. Only one child (belonging to the retrospective group) with stage 2/6 fibrosis remained on the same score of fibrosis after a duration of treatment of 63 months. Some pathological findings observed in liver biopsies at diagnosis such as cholestasis, giant cell transformation and steatosis had subsided after treatment. A likely explanation is that those pathological features were part of the spectrum of the inflammatory process such as cholestasis, steatosis, giant cell transformation and that effective treatment had led to the restoration of the parenchymal anatomical and functional mass of the liver. Whereas, one case of moderate steatosis showed mild improvement in the percentage of steatosis as the patient was overweight with a body mass index equal to 26.

However, there were few cases that did not show regression of fibrosis in spite of the long duration of treatment, which could be explained by intrinsic pathogenic mechanisms, or by host-dependent genetic predisposition that cannot be suppressed by conventional regimens.

Regression of fibrosis was usually accompanied by clinical and biochemical improvement as well as histological improvement of the necro-inflammatory activity.

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