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Clinicopathological impact of Anti Smooth Muscle Antibodies in patients with Nonalcoholic Fatty Liver Disease

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Abstract

Introduction: Previous studies examining the prevalence of serum autoantibodies in patients with NAFLD reported a wide range of prevalence rates depending on the threshold values used for an abnormal titer. We aimed to evaluate the association of anti-smooth muscle antibodies (ASMAbs) with pathological changes including liver fibrosis in NAFLD patients. Patients and methods: 124 patients were enrolled and classifed on ASMAbs positive (>1/40) or negative (= or <1/40). AST, ALT, total bilirubin, albumin, triglycerides, prothrombin time were measured by standard routine methods. In addition to liver biopsy and ultrasonography, autoimmune markers (ANA, ASMAbs, and gammaglobulins were assessed. Results: Analysis of histological features showed significant difference between groups with tendency of patients with positive ASMAbs to have higher steatosis percent compared to those with negative ASMA ($P < 0.001$); also current study demonstrated that patients with fatty liver and positive ASMA have a significant difference as regard necro inflammation , liver fibrosis and NAS score when compared to patients with fatty liver and negative ASMAbs ($P= 0.004$, $P <0.001$ and $P= 0.002$) respectively. The result of Logistic regression for prediction of liver fibrosis (early versus significant fibrosis) showed that ASMA is a significant predictor for fibrosis with OR (95% CI): 6.29 (1.95-20.23). Conclusion: ASMAbs are frequently present in NAFLD and have an impact on the histopathology of this liver disease.

Keywords: NASH; ASMA; Significant fibrosis; autoimmune hepatitis; Overlap syndrome; plasma lymphocyte.

Nonalcoholic fatty liver disease (NAFLD) is a public health burden associated with significant liver-related and unrelated morbidity and mortality (1). Results of epidemiological studies suggest that NAFLD is the most prevalent liver disease in the world (2), and in the upcoming decade, it is also expected to be the leading cause of liver transplantation (3). NAFLD exists on a spectrum from simple steatosis to steatohepatitis (nonalcoholic steatohepatitis [NASH]), which can progress to cirrhosis, liver failure, and hepatocellular carcinoma (4). In view of the high and increasing prevalence rates, NAFLD is likely to be superimposed on other chronic liver diseases, such as autoimmune hepatitis (5), and this has a reported worldwide prevalence of 1.8-3.6% (6). Autoantibodies such as anti-nuclear antibodies (ANAs) ANA and anti-smooth muscle antibodies (ASMAbs) are often discovered during the routine evaluation of patients with suspected NAFLD and their presence leads to concerns about autoimmune hepatitis. This non-organ specific autoimmunity occurs in the absence of traditional autoimmune disease, and presents a challenge, both in making the correct diagnoses and in managing coincident and possibly comorbid diseases (7). Therapeutically, it is important to accurately recognize NAFLD in autoimmune hepatitis because the standard treatment for autoimmune hepatitis, glucocorticoids, could potentially worsen NAFLD. Studies examining the relationship between disease severity and the presence of autoantibodies have reported conflicting results. The aim of the current study was to evaluate the association of ASMAbs with the pathological changes, such as liver fibrosis, in NAFLD patients.

We recruited 124 patients with elevated liver enzymes and negative virology, from January 2016 to June 2018, diagnosed to have NAFLD proven by liver biopsy at Tropical Medicine Department, Mansoura University Hospital. Assessment of liver tissue was done blinded to the clinical and biochemical data of the patients. For the evaluation of NAFLD, fibrosis was

scored using a five-grade Ishak scale (8) Steatosis grading was done according to Kleiner et al 2005 into 4 grades (<5%, 5-33%, 33-66%, >66%). Features suggestive of autoimmune hepatitis including prominent interface hepatitis, moderate to severe lymphoplasmacytic infiltrate in portal and periportal areas, prominent bridging necrosis or confluent necrosis with severe lymphoplasmacytic inflammatory changes, and the formation of liver-cell rosettes were excluded (9). Liver histopathology was examined for the presence of lobular inflammation, ballooning, portal fibrosis, interface hepatitis, plasma cell infiltrate and bile duct injury, allowing exclusion of liver diseases other than autoimmune hepatitis. Routine liver biochemical profile and triglycerides were measured by an automated biochemistry analyzer (BT1500; Botecnica instruments S.P.A, Italy). Prothrombin time was measured by routine methods, and, at the time of the liver biopsy, all patients underwent ultrasonography. Autoimmune markers and immunoglobulins were examined by routine methods (commercial kits using chemiluminescence). Patients were classified as ASMAb positive when the titre was >1/40, and negative with a titre $\geq$1/40. Data was analysed using SPSS (Statistical Package for Social Science) program for statistical analysis (version 22; Inc., Chicago. IL). Categorical data are presented in the form of number and percentage, and analysed by chi-squared. The quantitative data are presented as mean and standard deviation, and analysed by Student t test. Spearman rank correlation coefficient was used to study correlation between variables. Logistic regression was done to determine the predictors of fibrosis. $P<0.05$ was considered statistically significant.

There was no statistically significant difference between the groups as regard age, gender, presence of diabetes mellitus and hypertension, AST, ALT, bilirubin, INR or albumin, but total IgG and serum triglycerides were higher in ASMAb positive patients (table

1). The histological index score, liver fibrosis score and NASH score were significantly more adverse in the ASMAb positive group. Stages 1 and 4 steatosis (<5%, >66%) was more prevalent in the ASMAb positive group. Logistic regression for prediction of liver fibrosis (early versus significant fibrosis) showed that only ASMAb titre is a significant predictor with an odds ratio (95% CI) of 6.29 (1.95-20.23)($p<0.001$).

Autoimmune markers have frequently been reported in patients with NAFLD, with a prevalence range from 12 to 48 % (6,10). Data on the clinical and pathological importance of these markers in NAFLD patients are conflicting: it is uncertain whether autoantibodies reflect an immune mediated pathogenic process or whether they are an epiphenomenon (5,6). The current study demonstrates that patients with NAFLD and positive ASMAbs had significant changes as regard necro-inflammatory activity and fibrosis stage than patients with negative ASMAbs, and that the NASH score is higher in positive ASMAb patients, a result consistent with Adams et al. (6). Ravi et al, (11) concluded that autoimmune markers are frequently present among patients with steatohepatitis but do not impact the presentation and course of liver disease. Non-specific antigenic stimulation through the gut-liver axis may probably be mediating this epiphenomenon. Yatsuji et al, (12) also concluded that the presence of ANAs have no prognostic clinicopathological significance, although they failed to assess ASMAbs. Thus previous reports describing the presence of autoantibodies in patients with NAFLD and NASH have not yielded insights into the possible mechanisms for this phenomenon.

Hepatic natural killer T (NKT) cell accumulation is associated with more advanced NAFLD. Concomitant activation of autoantibody production with activation of NKT cells has been previously reported (13) We speculate that autoantibody production in NAFLD subjects may be a consequence of hepatic NKT cell accumulation, whilst another potential mechanism

is of lipid peroxides as, in NAFLD, circulating IgG antibodies against lipid peroxidation products are present. A further mechanism for immune reactions is oxidative stress, and this could be an independent predictor of progression of NAFLD to advanced fibrosis (14), and this could explain the results of the current study. Regulatory T cells (Tregs) are a subset of T cells that are either naturally occurring or inducible. An important role of the CD25 subset of Tregs is their suppression of CD4 and CD8 T cells and thus in a decrease in inflammation. Mouse models have shown that depleting CD25 Tregs results in precipitation of autoimmune disease. In NASH livers, there are increased numbers of Tregs, and these may promote fibrosis.

The problematic diagnosis of autoimmune hepatitis superimposed on NAFLD patients requires a higher titer of autoantibodies (ANA and/or ASMAbs) >1:40 to minimize the potential for false positive. The presence of autoimmune hepatitis-related autoantibodies supports the diagnosis of autoimmune hepatitis. Antibody titers >1:40 have a higher specificity for autoimmune hepatitis (15), whilst ANA and/or ASMAbs are considered requisites for the diagnosis of autoimmune hepatitis. We recognise the limitations that our study is restricted to one center, and that our sample size is not large. Thus, these findings need confirmation from other centers and in larger prospective studies for their generalizability. Our work represents an advance in biomedical science because it demonstrates that ASMAbs are frequently present among patients with NAFLD and are linked to liver fibrosis and other scores of liver disease.

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Table 1: Demographic, laboratory data and histological features of the groups

Demographic data	Fatty liver With positive ASMA: N = 68	Fatty liver With negative ASMA: N= 56	P value
Sex (male/female)	20/48	16/40	0.53
Age (years)	28.08±13.4	25.2±15.00	0.15
Diabetes mellitus (yes/no)	24/44	24/32	0.25
Hypertension (yes/no)	28/40	20/36	0.33
Laboratory data			
AST(U/L)	61.2±29.9	65.8±52.6	0.6
ALT(U/L)	65.4±39.7	71.5±55.3	0.5
Gamma globulin (IgG)(mg/dL)	972.2±1.8	906.5± 1.2	0.002
Bilirubin(µmol/L)	18.8±2.6	17.4±5.5	0.4
Albumin(gm/dl)	3.9±.43	4.1±0.4	0.9
INR	1.1±0.09	1.17±0.3	0.38
Serum triglyceride (mmol/L)	2.331±0.95	2.049±0.35	0.03
Histological activity index			
0	8 (11.8%)	8 (14.3%)	0.004
1	17 (25%)	16 (28.6%)	
2	20 (29.4%)	28 (50%)	
3	12 (17.6%)	4 (7.1%)	
4	11 (16.2%)	0 (0)	
Liver fibrosis			
0	28(41.2%)	16 (28.6%)	<0.001
1	12(17.6%)	24 (42.9%)	
2	8(11.8%)	12 (21.4%)	
3	8(11.8%)	3 (5.4%)	
4	12(11.8%)	1 (1.8%)	
NASH score			
3	40 (58.8%)	44 (78.6%)	0.002
4	20 (29.4%)	12 (21.4%)	
5	80 (11.8%)	0 (0)	
% Steatosis			
1	28 (41.2%)	13 (23.2%)	< 0.001
2	13 (19.1%)	7 (12.5%)	
3	6 (8.8%)	26 (46.4%)	
4	21 (30.9%)	10 (%17.9)	

Fig. 1: (A) Liver biopsy showing mild macrovesicular steatosis and mild portal inflammation (H&E 100x). (B) Liver biopsy showing macrovesicular steatosis with lobular inflammatory cells related to the steatotic cells (H&E 400x). (C) Liver biopsy showing moderate macrovesicular steatosis with focal ballooning of the hepatocytes (H&E 200x). (D) Liver biopsy showing diffuse ballooning of steatotic hepatocytes (H&E 400x).

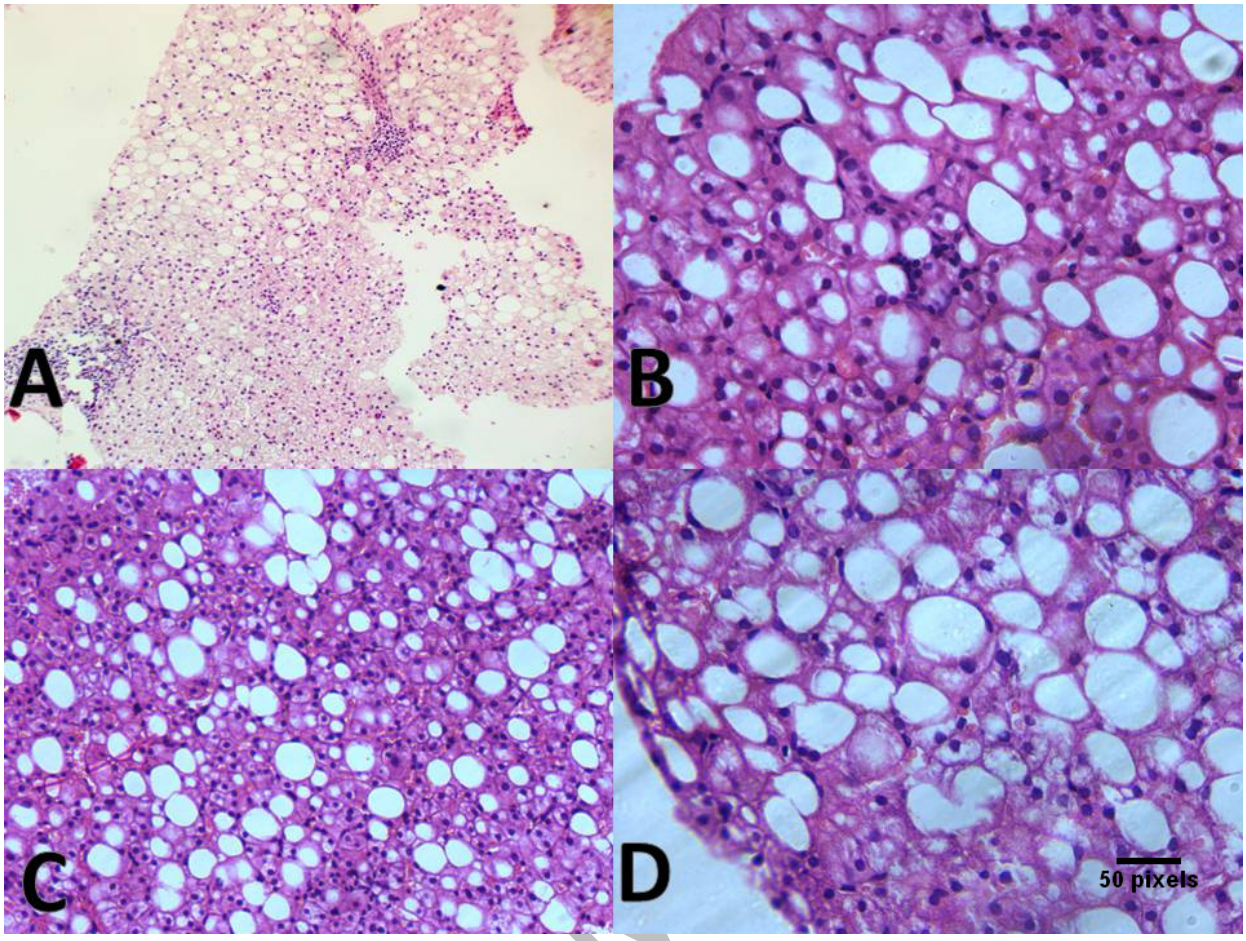

Figure 1