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Nonalcoholic fatty liver disease: a potential consequence of tumor necrosis factor-inhibitor therapy

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Abstract

Introduction—Although tumor necrosis factor inhibitors (TNFi) might be expected to protect against nonalcoholic fatty liver disease (NAFLD), we have seen patients who appeared to develop NAFLD during TNFi treatment. We aimed to explore risk factors for this TNFi complication in a case–control study.

Methods—We reviewed clinic records at our VA hospital to identify patients with inflammatory diseases who developed aminotransferase elevations during TNFi therapy and who had liver biopsies showing NAFLD. These patients were matched with patients in each of three control groups: (i) inflammatory disease controls: patients on TNFi treatment with normal aminotransferase levels, (ii) nonalcoholic steatohepatitis (NASH) controls: patients with biopsy-proven NASH with no other inflammatory disease, and (iii) healthy controls. Genotyping was performed for *PNPLA3*, a gene predisposing to NASH.

Results—We identified eight cases (five steatohepatitis, three steatosis); elevated aminotransferase levels were first observed 1–63 months into TNFi therapy (average 12 months). TNFi therapy was stopped in five patients, whose aminotransferase levels then normalized within 2–8 months. There were no significant differences between cases and inflammatory disease

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Conflicts of interest

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controls in the frequency of features of metabolic syndrome. Cases had more methotrexate exposure than inflammatory controls (50 vs. 12.5%, $P=0.28$). *PNPLA3* genotyping revealed mutations in 75% of cases, 38% of inflammatory controls, 88% of NASH controls, and 63% of healthy controls ($P=NS$).

Conclusion—Our findings suggest that NAFLD can be a side effect of TNFi therapy, and that methotrexate exposure and *PNPLA3* gene mutations might be risk factors. Further studies are needed to determine how TNFi causes NAFLD and to confirm these risk factors.

Keywords

inflammatory diseases; nonalcoholic steatohepatitis; *PNPLA3*; tumor necrosis factor inhibitor

Introduction

Nonalcoholic fatty liver disease (NAFLD) spans a spectrum of liver injuries ranging from simple hepatic steatosis to nonalcoholic steatohepatitis (NASH) with cirrhosis. NAFLD is now the most common chronic liver disease in the USA. In the Dallas Heart Study, almost one-third of patients were found to have NAFLD [1]. Severe obesity and the metabolic syndrome (a disorder of energy storage and utilization characterized by central obesity, hypertriglyceridemia, low high-density lipoprotein levels, elevated fasting glucose levels, and high blood pressure) are strongly associated with NAFLD [2,3]. Up to 90% [4] of severely obese adults and 70% [5] of adults with diabetes have NAFLD. Fortunately, most patients with NAFLD have an excellent prognosis, and only a minority develop NASH that progresses to cirrhosis, liver failure, and/or liver cancer. The metabolic syndrome is an important risk factor for NASH in patients with NAFLD [6].

Metabolic syndrome has also been associated with the activity of a number of immune-mediated inflammatory diseases, such as rheumatoid arthritis [7,8], psoriasis, psoriatic arthritis [9,10], and ankylosing spondylitis [11]. Even in inflammatory bowel diseases that were historically associated with cachexia, recent studies have shown a high prevalence of obesity and metabolic syndrome, comparable to that in the general population [12]. All of these inflammatory disorders are associated with overexpression of the proinflammatory cytokine tumor necrosis factor α (TNF α), and the disorders respond to treatment with TNF inhibitors (TNFi). TNF α also appears to be a key mediator of fatty liver disease and hepatic fibrosis [13], and TNF α has been proposed as a potential therapeutic target for patients with NASH. Although these data suggest that TNFi treatment might prevent NAFLD, we have observed a number of patients with immune-mediated inflammatory diseases to develop NAFLD during treatment with TNFi, which resolved when TNFi therapy was stopped. In this case-control study, we have explored potential risk factors for the development of NAFLD during TNFi therapy.

Methods

We reviewed the medical records of patients seen at our inflammatory bowel disease and rheumatology clinics to identify those who developed abnormal alanine aminotransferase (ALT) levels during TNFi therapy and whose liver biopsies showed NAFLD (NASH or

steatosis). We also identified three control groups: (i) inflammatory disease controls: patients on TNFi treatment with normal ALT levels, matched to patients on the basis of the type of underlying inflammatory disease, race, sex, age, and BMI, (ii) NASH controls: patients with biopsy-proven NASH who had no other apparent inflammatory disease, matched to cases on the basis of race, and (iii) healthy controls: patients without diabetes or liver disease who were recruited (and matched for race and sex to our cases) from among healthy veterans undergoing screening colonoscopy and among healthy medical staff members at our VA hospital. Patients meeting the study inclusion criteria were invited to participate, and informed consent was obtained. Patients were recruited for participation between April 2013 and April 2014. Data on demographics, lipid panels, and clinical features of metabolic syndrome were obtained for all patients. Patients were considered to be alcohol users if they consumed at least one alcoholic drink per day. For methotrexate users, total cumulative exposure was considered significant if they received a total dose of 3 g or more.

Liver biopsies of the patients and the controls with NASH were reviewed by a single expert pathologist (T.C.) in conjunction with an expert hepatologist (G.B.). Only six of the NASH controls were available for review. Biopsies were reviewed using Brunt's criteria and the NASH Clinical Research Network pathologic criteria for the diagnosis of NASH [14,15].

For each patient and matched control, genotyping was performed for *PNPLA3*, a polymorphism known to confer susceptibility to NAFLD [7], using a specific 5'-nucleotidase assay for the rs738409 single-nucleotide polymorphism of *PNPLA3* (rs738409[G], encoding I148M; Life Technologies). The single-nucleotide polymorphism assay was performed on an HT7900 Real-Time PCR system, using TaqMan reagents (Life Technologies, Grand Island, New York, USA).

Statistical analysis

Analyses were carried out using Fisher's exact test for categorical variables and the unpaired *t*-test (normally distributed) or the Mann-Whitney test (not normally distributed) for continuous variables. For comparison of multiple groups, analysis of variance was used. Statistical analysis was carried out using GraphPad Instat 3 (GraphPad Software, La Jolla, California, USA).

All authors had access to the study data and reviewed and approved the final manuscript. The institutional review board of Dallas VA Medical Center approved this study.

Results

Cases and inflammatory controls were predominantly middle-aged, obese men with features of metabolic syndrome

We enrolled eight cases (patients with inflammatory diseases who developed abnormal ALT levels during TNFi treatment and whose liver biopsies revealed NAFLD), whom we matched with eight inflammatory controls (inflammatory disease patients who did not develop liver disease during TNFi therapy) on the basis of the type of underlying inflammatory disease, race, sex, age, and BMI. All of the cases and matched inflammatory disease controls were men. There were no significant differences between cases and inflammatory disease controls

in any baseline characteristic including the frequencies of weight gain during treatment, the presence of diabetes mellitus type 2, alcohol use, and hypertriglyceridemia (Table 1). Methotrexate use (50 vs. 12.5%) and hypertension (63 vs. 38%) were more common among the cases than among the inflammatory disease controls, but these differences were not statistically significant (Table 1).

The cases included patients with Crohn's disease, rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis who were treated with either infliximab, adalimumab, or etanercept (Table 2). Interestingly, 50% of the cases were of Hispanic background, whereas the rest were nonHispanic Whites. Of note, abnormal liver enzyme levels during TNFi treatment were not observed in any African-American patient. The average peak ALT was 111 (range 69–180) and the peak AST was 63 (range 38–88). In the majority of cases, bilirubin and alkaline phosphatase levels remained normal. The time to the development of abnormal liver tests after starting TNFi therapy ranged from 1 to 63 months, with an average of 12 months. Three patients elected not to stop the therapy; among those who did stop the therapy, the liver tests normalized within 2–8 months (average 6 months). An illustrative case with the time course of drug exposure and aminotransferase levels is shown in Fig. 1. In this case, aminotransferase levels showed an increase 6 months after the initiation of infliximab therapy, with rapid improvement when infliximab was stopped. When treatment was started with another TNFi, adalimumab, aminotransferase levels rapidly increased. When this second TNFi was stopped, aminotransferase levels declined slowly.

Liver histology

Liver biopsy revealed NASH in five cases (four with portal fibrosis and one with cirrhosis) and steatosis in three cases (one with portal fibrosis and two with no fibrosis; Fig. 2 and Table 3). Only the NASH controls (six of eight) had liver biopsies available for comparison with the cases. Cases and NASH controls showed different patterns of hepatic steatosis; four of eight cases showed sparing of steatosis around the central vein (zone 3), whereas the NASH controls showed a more uniform distribution of steatosis. Of note, the majority of cases (88%) and controls (66%) had only minimal hepatic inflammation. In addition, the majority of cases (88%) and NASH controls (84%) had either zone 3 fibrosis and portal fibrosis or portal fibrosis alone (Table 3). Interestingly, granulomas were seen in 75% of the cases but in none of the NASH controls.

Genotyping

Genotyping for I148M *PNPLA3* revealed mutations in one or both alleles in 75% of cases, compared with only 38% of inflammatory controls who also received TNFi therapy but did not have liver function test abnormalities ($P=0.31$). Interestingly, 88% of patients with NASH had mutations in one or both alleles, whereas 63% of healthy, race-matched (50% of Hispanic origin) controls had mutations in one or both alleles (Fig. 3).

Discussion

TNFi therapy is widely used to treat immune-mediated diseases like rheumatoid arthritis and inflammatory bowel disease, and one might expect this therapy to have beneficial effects

against NAFLD. However, we describe a series of eight patients who developed features of NAFLD during TNFi therapy, most of whom had not gained weight and whose ALT levels normalized when TNFi treatment was stopped. Of note, three patients elected to continue TNFi therapy after risk–benefit discussions: two had no further increase in transaminase levels, and transaminase levels normalized in the third case. Earlier reports of patients who developed abnormal liver test results during TNFi therapy have described liver biopsies showing features of autoimmune hepatitis [16–18] or drug-induced liver injury [19]. However, none of our cases had biopsies typical of autoimmune hepatitis or drug-induced liver injury. More recent reports have demonstrated NAFLD in patients on TNFi therapy. In a case series of seven patients with Crohn’s disease and elevated liver enzyme levels (six of whom were on biologics or immunomodulators), liver biopsies showed NAFLD in all seven patients [20]. However, six of the seven patients in the study had gained weight, the temporal relationship between the transaminase rise and the start of biologic therapy was unclear, and the authors attributed NAFLD to weight gain. In another study of 48 patients with psoriatic arthritis who had hepatic steatosis before TNFi treatment, hepatic steatosis worsened in patients whose arthritis symptoms persisted during TNFi treatment, whereas it remained unchanged in those whose arthritis symptoms responded to TNFi therapy [21]. Our study differs substantially from these two investigations in at least two important ways. First, all our cases had normal aminotransferase levels documented before the start of TNFi therapy, and all aminotransferase elevations occurred during TNFi therapy. Second, we included three pertinent control groups to better understand the etiology of this seemingly paradoxical TNFi effect.

Our three control groups (matched to our cases) included patients with similar inflammatory diseases who were treated with TNFi but did not develop abnormal transaminase levels, patients who had biopsy-proven NASH without other inflammatory diseases, and healthy controls with no known inflammatory disease or liver disease. There were no significant differences in the frequency of risk factors for metabolic syndrome between our cases and the control patients on TNFi who did not develop liver abnormalities. However, exposure to methotrexate was more common among cases than among inflammatory disease controls (50 vs. 12.5%, *P*-value not significant). Methotrexate liver injury can mimic NAFLD and might have contributed to the ALT elevations in our cases. We also found more *PNPLA3* polymorphism I148M (a DNA sequence variation strongly associated with increased hepatic fat levels and hepatic inflammation) in our cases than in inflammatory disease controls on TNFi who did not develop liver abnormalities. *PNPLA3* polymorphism I148M has emerged as the key genetic determinant of NAFLD, with a recent systematic review confirming its strong association with increased hepatic fat content and increased risk for elevated serum alanine aminotransferase levels, NASH, and fibrosis, irrespective of alcohol consumption, BMI, and diabetes [22]. The I148M *PNPLA3* allele is most common in Hispanics (49% in Hispanics compared with 23% in Whites and 17% in Blacks), the group most susceptible to NAFLD [23]. It is also interesting to note that, in our study, four of eight of our cases were Hispanics, whereas Hispanics comprise only a small minority of our patient population.

The mechanisms underlying the development of NAFLD in patients receiving TNFi therapy are not clear. Indeed, our findings are particularly surprising in light of reports that have documented elevated serum TNF α levels and hepatic TNF α messenger RNA (mRNA)

expression in patients with NASH [24,25]. In a mouse model of NASH, treatment with anti-TNF α antibodies improved liver histology, reduced hepatic total fatty acid content, and decreased serum ALT levels [26]. Moreover, studies on the use of pentoxifylline, which inhibits the production of TNF α , as a treatment for NASH have noted some improvement in aminotransferase levels [27,28].

The development and progression of NASH are multifactorial processes, and NASH may evolve through varying pathways depending upon the immunological milieu or environment [29]. The liver histopathological findings in the majority of our cases were consistent with early NASH, as evidenced by sparing of steatosis around the central vein in one-half of the cases [30]. It is also interesting to note that we found granulomas in 75% of our cases but in none of the NASH controls, even though granulomas can often be found in the liver of patients with rheumatologic disorders. Although their presence may be the consequence of having a rheumatologic disorder, the importance of these granulomas as a marker or potential modular of NASH in patients with autoimmune diseases treated with TNFi therapy is not clear.

Our study has several major strengths. First, our cases were reviewed by a multidisciplinary group including a gastroenterologist, a hepatologist, a rheumatologist, and a pathologist. Second, we utilized liver histology rather than less specific imaging techniques to document NAFLD. Third, we were able to evaluate all of our cases and controls for polymorphisms in the *PNPLA3* gene. Limitations of our study include the small sample size and the retrospective nature of data collection. We did not have uniform documentation of inflammatory disease activity at the time of peak ALT levels, and thus were unable to clearly define the contribution of inflammatory disease activity to liver abnormalities. Another limitation is that liver biopsy was not repeated to document resolution of the NAFLD when TNFi therapy was discontinued. Moreover, our cases were all male and the findings may not be applicable to female individuals. Nevertheless, our findings are relevant as elevation in aminotransferase levels during TNFi therapy might warrant consideration of stopping TNFi therapy in patients who have disabling inflammatory disease symptoms. Moreover, awareness of potential risk factors for the development of NAFLD during TNFi therapy might identify patients who warrant especially close monitoring of aminotransferase levels.

Conclusion

In our case series of patients with immune-mediated diseases receiving TNFi therapy who developed elevations in ALT levels, we found that NAFLD/NASH were the common findings on liver biopsies. However, the mechanisms underlying this association remain incompletely understood. On the basis of our data, potential risk factors for ALT elevation during TNFi therapy include concurrent methotrexate use, Hispanic race, and a predisposing polymorphism of the *PNPLA3* gene. Clearly, further studies are warranted to identify the underlying mechanisms and to confirm these risk factors.

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Fig. 1.

Time course of transaminitis in a single patient who was exposed to two TNFi sequentially. TNFi, tumor necrosis factor inhibitors.

Fig. 2. Histological appearance of the liver of patients (cases) who have received TNFi. (a) Evidence of central lobular sparing of the steatosis (solid arrow represents central vein). (b) Significant steatosis observed in the majority of case patients. (c) Granulomas (identified by the solid arrow) were present in half of the cases. (d) Lipogranulomas (identified by the solid arrow) were also observed. Liver tissue was obtained at the time of transaminase elevation.

Fig. 3.

Genotyping results for I148M *PNPLA3* for the cases and the three control groups. II, homozygous wild type; IM, one mutated allele and one wild-type allele; MM, homozygous mutated alleles.

Table 1
Baseline characteristics including features of metabolic syndrome in cases and controls

	Cases	Inflammatory controls	NASH controls	Healthy controls	P-value
Age (average)	50.1 ± 9.6	54 ± 16.1	50 ± 15.3	49.5 ± 13.3	0.91
Sex (male)	8 (100%)	8 (100%)	8 (100%)	7 (88%)	0.38
BMI	34.4 ± 4.7	30.8 ± 6.6	36.5 ± 5.5	31.2 ± 6.1	0.15
Alcohol use	1/8 (12.5%)	0/8 (0%)	0/8 (0%)	0/8 (0%)	0.38
Methotrexate use (>3 g)	4/8 (50%)	1/8 (12.5%)	–	–	0.28
Weight gain on anti-TNF	3/8 (38%)	3/8 (38%)	–	–	1.0
Diabetes	3/8 (38%)	3/8 (38%)	6/8 (75%)	0/8 (0%)	0.19
Hypertension	5/8 (63%)	3/8 (38%)	5/8 (63%)	3/8 (38%)	0.57
Hyperlipidemia	5/8 (63%)	4/8 (50%)	6/8 (75%)	4/8 (50%)	0.70
Elevated LDL (>100 mg/dl)	2/8 (25%)	3/7 (43%)	3/8 (38%)	NA	0.75
Elevated TG (>150 mg/dl)	4/8 (50%)	3/7 (43%)	6/8 (75%)	NA	0.41

LDL, low-density lipoprotein; NASH, nonalcoholic steatohepatitis; TG, triglyceride; TNF, tumor necrosis factor.

Table 2

Summary of details from cases with abnormal LFTs after anti-TNF use

Age (years)	Disease	Ethnicity	Anti-TNF	Anti-TNF dosing	Duration of therapy at time of LFT rise	Peak ALT (U/l)	Peak AST (U/l)	Peak bilirubin (mg/dl)	Peak Alk phos (U/l)	Time to improvement after stopping
56	PA	White	Infliximab	3 mg/kg Q8 weeks	5 months	75	38	0.6	125	2 months
47	RA	Hispanic	Etanercept	50 mg Q week	1 month	112	65	0.8	117	4 months
50	CD	Hispanic	Infliximab	5 mg/kg Q8 weeks	8 months	112	54	0.7	81	7 months
55	RA	White	Adalimumab	40 mg Q2 weeks	3 months	104	88	1.0	121	7 months
66	PA	White	Etanercept	25 mg Q2 weeks	12 months	69	63	0.7	72	Did not stop
45	RA	Hispanic	Etanercept	50 mg Q week	4 months	180	75	0.9	116	Did not stop
33	AS	White	Infliximab	3 mg/kg Q10 weeks	1 month	71	41	1.6	76	Did not stop
49	RA	Hispanic	Adalimumab	40 mg Q2 weeks	63 months	163	76	1.5	172	8 months

Alk phos, alkaline phosphatase; ALT, alanine transaminase; AS, ankylosing spondylitis; AST, aspartate transaminase; CD, Crohn's disease; LFT, liver function test; PA, psoriatic arthritis; RA, rheumatoid arthritis; TNF, tumor necrosis factor.

Table 3

Liver biopsy characteristics

Histopathological features						
	Cases			NASH controls		
Steatosis percentage	>66% (<i>n</i> =2)	33–66% (<i>n</i> =4)	<33% (<i>n</i> =2)	>66% (<i>n</i> =3)	33–66% (<i>n</i> =2)	<33% (<i>n</i> =1)
Steatosis location	Uniform (<i>n</i> =4)	Central lobular sparing (<i>n</i> =4)		Uniform (<i>n</i> =5)	Central lobular sparing (<i>n</i> =0)	Portal vein sparing (<i>n</i> =1)
Brunt system						
Brunt grade ^a	Grade 1 (<i>n</i> =3)	Grade 2 (<i>n</i> =3)	Grade 3 (<i>n</i> =2)	Grade 1 (<i>n</i> =3)	Grade 2 (<i>n</i> =3)	Grade 3 (<i>n</i> =0)
Brunt stage ^b	Stage 0–1 (<i>n</i> =2)	Stage 2 (<i>n</i> =5)	Stage 3 or 4 (<i>n</i> =1)	Stage 1 (<i>n</i> =3)	Stage 2 (<i>n</i> =2)	Stage 3 or 4 (<i>n</i> =1)
NCRN ^c						
Inflammation	0–1 (<i>n</i> =7)	2 (<i>n</i> =1)	3 (<i>n</i> =0)	0–1 (<i>n</i> =4)	2 (<i>n</i> =2)	3 (<i>n</i> =0)
Steatosis	0–1 (<i>n</i> =2)	2 (<i>n</i> =4)	3 (<i>n</i> =2)	0–1 (<i>n</i> =1)	2 (<i>n</i> =2)	3 (<i>n</i> =3)
Ballooning	0–1 (<i>n</i> =6)	2 (<i>n</i> =2)	3 (<i>n</i> =2)	0–1 (<i>n</i> =5)	2 (<i>n</i> =1)	3 (<i>n</i> =0)
Fibrosis	0–1 (<i>n</i> =3)	2 (<i>n</i> =3)	3&4 (<i>n</i> =1)	0–1 (<i>n</i> =3)	2 (<i>n</i> =2)	3 (<i>n</i> =1)
Other findings						
Granulomas	<i>n</i> =4			<i>N</i> =0		
Lipogranulomas	<i>n</i> =2			<i>N</i> =0		

NCRN, Nonalcoholic steatohepatitis Clinical Research Network; NASH, nonalcoholic steatohepatitis.

^aBrunt grade (numerical score of three features: inflammation, steatosis, and ballooning (1, mild; 2, moderate; and 3, severe).^bBrunt STAGE (numerical score based on the severity of fibrosis: zone 3 fibrosis, 1; zone 3 and portal fibrosis, 2; bridging fibrosis, 3; and cirrhosis, 4).^cNCRN [summation of scores for [steatosis (0–3), inflammation (0–3), and ballooning (0–1)] and fibrosis (portal fibrosis, 1 a–c), (perisinusoidal and portal fibrosis, 2), (bridging fibrosis, 3), and (cirrhosis, 4)].