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Effects of Infliximab on Liver Function Test

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ABSTRACT

Background and Objective: Autoimmune diseases are a significant problem due to their chronic nature and prevalence in young populations at the peak of their working and reproductive years. Biological therapy is a revolutionary step in the treatment of autoimmune diseases, especially in rheumatology. Infliximab is a recombinant chimeric antibody that is effective in the treatment of autoimmune diseases and has many applications. However, its effect on liver damage is controversial. The aim of this study was to investigate the effect of infliximab on liver function tests.

Methods: This cross-sectional study was conducted among 100 patients with autoimmune diseases. They had normal liver function test at enrollment, and all of them were on infliximab. Patients had been examined every 3 months for liver function tests, anti-smooth muscle antibodies (ASMA), antinuclear antibodies (ANA), along with clinical assessment. Elevation of any liver enzyme or the positivity for both antibodies was considered as an indication for liver injury.

Findings: Eighteen patients (18%) had abnormal liver function test represented by elevated alanine transaminase (ALT) or aspartate aminotransferase (AST) or both. Abnormality in liver function tests was significantly associated with disease duration, treatment with methotrexate, diabetes and the presence of jaundice and autoantibodies. ALT and AST had a positive significant with BMI and disease duration (r=0.237, p=0.018 and r=0.218, p=0.029, respectively).

Conclusion: The results of the study showed that the use of infliximab interfered with liver function tests.

Keywords: *Autoimmune Disease, Infliximab, Liver Function Test, Liver Injury*.

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Introduction

Autoimmune diseases are a spectrum of diseases ranging from those that are organ-specific to non-organ specific or systemic diseases (1). Autoimmune diseases impose a significant clinical burden because of their chronic nature, the associated healthcare cost, and their prevalence in young populations during the peak of their working years and reproductive years (2). Autoimmune diseases affect 3-5% of the population, with autoimmune thyroid disease and type I diabetes (T1D) being the most common of these conditions (3, 4). Rheumatoid arthritis (RA) is a chronic autoimmune disease. This disease is characterized by the presence of long-standing inflammation of the joint's symmetric polyarthritis and synovial membrane hypertrophy with progressive joint damage (5).

Inflammatory bowel disease (IBD) is a chronic intestinal disease, characterized by repetited episodes of inflammation of the gastrointestinal tract caused by an abnormal immune response to the endogenous microbiota of the intestine. The primary risk factor for IBD is family history, with a risk of 10% for the first-degree relatives of affected persons. Inflammatory bowel disease includes two types: ulcerativecolitis (UC) and Crohn's disease (CD) (6, 7). Both disorders are not curable and they both carry enormous morbidity and risk for colorectal cancer (8).

Ankylosing spondylitis (AS) is a chronic inflammatory disease of the axial spine that manifests with various clinical signs and symptoms. Chronic back pain and progressive spinal stiffness are the most common features of the disease (9). Psoriatic arthritis (PsA) is a chronic inflammatory arthritis associated with psoriasis and found in about 20 to 30% of such patients (10). Behçet disease was first described in 1937 by Hulusi Behçet from Istanbul, who described three patients with oral and genital ulcerations, uveitis, and erythema nodosum (11). Biologic therapy is a revolutionized step in the treatment of autoimmune diseases, especially in rheumatology. The major targets of most biologic therapies are cytokines. Anti-cytokines include anti-tumor necrosis factor (TNF)-α, anti-interleukin (IL)-1, and anti-IL-6 agents (12).

(TNF)-α is generally considered as a master pro-inflammatory cytokine that plays a critical role in the pathogenesis of autoimmune inflammatory diseases (13). The Anti-TNF, Infliximab, is a recombinant chimeric antibody that is generated by mouse myeloma cells (14, 15). Although anti-TNF biologics are effective in the treatment of autoimmune inflammatory diseases, but not all patients respond equally well to the treatment. Up to 40% of patients have no response to anti-TNF treatment. There are several adverse effects associated with anti-TNF biologics; there is also some evidence that anti-TNF treatment is associated with increased risk of malignancy (16, 17).

Based on the effect of drug on liver tissue, autoimmune hepatitis (AIH) is categorized into three categories. The first category is referred to as "AIH with DILI", where DILI refers to drug-induced liver injury. In this state, patients already have documented AIH along with possible advanced fibrosis on histology at the time of exposure to a drug, with subsequent liver injury. The second category is "drug-induced AIH". It includes patients who probably have subclinical and undiagnosed AIH or those that have a predisposition for AIH (18). The third category is the "immune-mediated DILI" group. These patients have no signs or symptoms of AIH prior to medication exposure. Typical characteristics of symptoms and markers of liver damage and abnormal serology are absent and appear only after exposure to an offending medication (19).

One of the main effects of infliximab includes induction of autoantibodiesincluding antinuclear (ANA), anti-smooth muscle Abs (ASMA) and anti-doublestranded DNA autoantibodies (anti-dsDNA Abs) (20). This study aims to investigate the potential liver injury induced by infliximab in patients diagnosed with various autoimmune diseases, demonstrating on its biochemical and serological effect.

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Methods

This prospective cohort study was ethically approved by the Arab Council for Medical Specializations (No. of ethical approval 12344 in 6/5/2021). This study includes all patients with autoimmune diseases (with the exclusion of autoimmune hepatitis) who referred to the Department of Gastroenterology, Baghdad Medical City during the period from July 2021 to end of April 2022. The diagnosis of particular autoimmune disease was based on clinical manifestations, laboratory investigations and radiological imaging if necessary. During the study period, a total of 100 patients were enrolled in the study. Patients who were negative for anti-smooth muscle antibodies (ASMA), antinuclear antibodies (ANA) and liver kidney microsomal type 1 (LKM1) antibodies, and had normal liver function test were enrolled. All patients were on regular use of infliximab within their treatment protocol.

Inclusion criteria: All patients, males and females diagnosed with autoimmune diseases, negative for autoimmune hepatitis specific antibodies and with normal liver function test.

Exclusion criteria: Patients with elevated liver enzymes, patients with any acute or chronic liver disease (including fatty liver disease) and patients positive for autoimmune hepatitis specific antibodies.

Data Collection: Demographic data including the age, gender, family history, residence, body mass index (BMI) and smoking status were collected through direct interview. Clinical characteristics including type of autoimmune disease, and duration of infliximab were gathered from patients' records.

Patients were followed up for 10 months after first enrollment. Then, they were examined every 3 months for liver function tests, ANA, and anti-SMA antibodies, along with clinical assessment. Elevation of any enzyme or the positivity for either antibody was considered as an indication for liver injury. Liver test abnormalities were defined as the elevation of the following liver enzymes in serum: ALT>40 U/L, AST>40 U/L, alkaline phosphatase (ALP)>135 U/L, and total serum bilirubin (TSB)>17.1 μmol/L. Patients with normal liver function and abnormal liver function after taking infliximab were assessed. For data analysis, SPSS software version 26, descriptive and inferential statistics via chi-square test, and Pearson's correlation were used and $p<0.05$ was considered significant.

Results

Demographic Characteristics of the Patients: A sample of 100 patients with different autoimmune diseases receiving infliximab in a dose ranging from 200 to 400mg every 8 weeks were studied to assess the clinical and biochemical effect of infliximab on liver function through measurement of antinuclear antibodies (ANA) and anti-smooth muscle antibodies (ASMA). The mean age of the patients was 36.16±10.21 years (range 18-55 years) and females accounted for 56% of patients. The patients had a mean BMI of 27.42±3.3 kg/m^2 (range 22.33-35.65 kg/m^2). 61% were urban residents (Table 1).

Clinical Characteristics of the Patients: The mean serum concentration of ALT and AST was 49.66±38.8 U/L and 42.54±27.45 U/L, respectively. Disease duration was variable and ranged between 6 to 96 months with a mean of 23.88±20.46 months. The most common autoimmune disease was IBD, accounting for 29 % of patients (divided into 16% and 13% for UC and CD, respectively) followed by AS (27%) and RA (21%). On the other hand, Bechet's disease was the least common autoimmune disease accounting for only 9% of the patients (Figure 1). The majority of patients (92%) had no specific symptoms for hepatitis. Jaundice was found in 8% of the patients. Hypertension and DM as comorbidities were reported in 6% and 5% of the patients, respectively. Only 4 patients (4%) were using methotrexate as treatment. Auto-antibodies (ANA and ASMA) were reported in 5% of the patients (Table 2).

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Table 1. Demographic characteristics of the patients (n=100)

Variables	Values
Age, years	
Mean±SD	36.16±10.21
Range	18-55
Gender, Number(%)	
Male	44(44)
Female	56(56)
Body mass index, kg/m^2	
Mean±SD	27.42±3.3
Range	22.33-35.65
Residence, Number (%)	
Urban	61(61)
Rural	39(39)

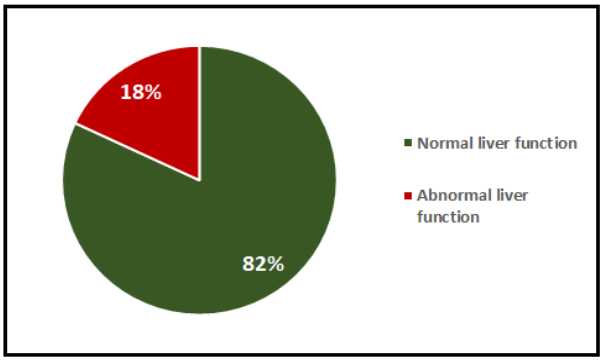

Figure 1. Proportion of patients that developed abnormal liver function

Table 2. Clinical characteristics of the patients (n=100)

Variables	Values
ALT, U/L	
Mean±SD	49.66±38.8
Range	21-176
AST, U/L	
Mean±SD	42.54±27.45
Range	18-120
Disease duration, months	
Mean±SD	23.88±20.46
Range	6-96
Diagnosis, Number (%)	
Ankylosis spondylitis	27(27)
Rheumatoid arthritis	21(21)
Ulcerative colitis	16(16)
Psoriatic arthritis	14(14)
Crohn disease	13(13)
Behcet's disease	9(9)

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Chief complain, Number(%)	
No complain	92(92)
Jaundice	8(8)
Comorbidity, Number(%)	
No comorbidity	89(89)
Hypertension	6(6)
Diabetes	5(5)
Treatment, Number(%)	
No treatment	96(96)
Methotrexate	4(4)
ANA and ASMA auto-antibodies, Number(%)	
Negative	95(95)
Positive	5(5)

The proportion of abnormal liver function: Out of 100 patients included in the study, 18 (18%) had abnormal liver function test represented by elevated AST or ALT (Figure 1).

Association of Abnormal liver function with Demographic Characteristics of the Patients: Although the mean age of patients with abnormal liver function was higher than that with normal liver function (39.0±11.34 years versus 35.54±9.96 years), the difference was not significant (Table 3).

Table 3. Association of abnormal liver function with demographic characteristics of the patients

Variables	Normal liver function (n=82)	Abnormal liver function (n=18)	p-value
Age, years			
Mean±SD	35.54±9.96	39.0±11.34	0.194
Range	18-55	23-55	
Gender, Number(%)			
Male	36(43.9)	8(44.44)	0.967
Female	46(56.1)	10(55.56)	
BMI, kg/m^2			
Mean±SD	27.2±3.14	28.36±3.93	0.181
Range	22.33-34.58	24.23-35.65	
Residence, Number(%)			
Urban	52(63.41)	9(50)	0.291
Rural	30(36.59)	9(50)	

Association of Abnormal liver function with Clinical Characteristics of the Patients: Mean disease duration in patients with abnormal liver function was 34.67±24.83 months which was significantly higher than that of normal liver function (21.51±18.73 months). The frequency of jaundice was 33.33% and 0% in patient with abnormal and normal liver function, respectively, with a highly significant difference. Diabetes, in particular, was more frequent in abnormal than normal liver function patients (22.22% and 1.22%, respectively), with highly significant difference. Three patients (16.67%) with abnormal liver function were using methotrexate compared with one patient (1.22%) among normal liver function group, with a highly significant difference.

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Finally, 27.78% of patients with abnormal liver function were positive for ANA and ASMA auto-antibodies compared with none among normal liver function group, with a highly significant difference (Table 4).

Table 4. Association of abnormal liver function with clinical characteristics of the patients

Variables	Normal liver function (n=82)	Abnormal liver function (n=18)	p-value
Disease duration, months			
Mean±SD	21.51±18.73	34.67±24.83	0.013
Range	6.0-96.0	12.0-96.0	
Diagnosis, Number(%)			
Ankylosis spondylitis	21(25.61)	6(33.33)	0.694
Rheumatoid arthritis	17(20.73)	4(22.22)	
Ulcerative colitis	9(10.96)	0(0)	
Psoriatic arthritis	11(13.41)	2(11.11)	
Crohn disease	12(14.63)	4(22.22)	
Behcet's disease	12(14.63)	2(11.11)	
Chief complain, Number(%)			
No complain	80(97.56)	12(66.67)	<0.001
Jaundice	2(2.44)	6(33.33)	
Comorbidity, Number(%)			
No comorbidity	76(92.68)	13(72.22)	0.001
Hypertension	5(6.1)	1(5.56)	
Diabetes	1(1.22)	4(22.22)	
Treatment, Number(%)			
No treatment	81(98.78)	15(83.33)	0.002
Methotrexate	1(1.22)	3(16.67)	
ANA and ASMA auto-antibodies, Number(%)			
Negative	82(100)	13(72.22)	<0.001
Positive	0(0)	5(27.78)	

Correlation between AST and ALT with Age, Duration and Body Mass Index: Pearson's correlation test was used to explore the possible correlation between AST and ALT with age, disease duration and BMI. ALT and AST had a positive significant with disease duration (r=0.237, p=0.018 and r=0.218, p=0.029, respectively). AST, per se, had a strong correlation with ALT (r=0.952, p<0.001) as shown in table 3-5, figures 2-4.

Table 5. Pearson's correlation between AST and ALT with age, duration and body mass index

Variables	ALT		AST	
	R	p-value	R	p-value
Age	0.096	0.344	0.140	0.166
Duration	0.143	0.156	0.218	0.029
Body mass index	0.237	0.018	0.138	0.171
AST	0.952	<0.001		

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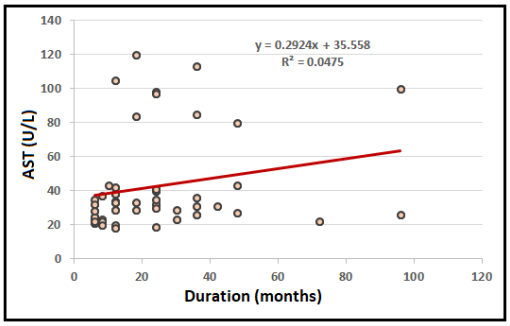

Figure 2. Scatter plot and line of regression between autoimmune disease duration and serum concentration of AST

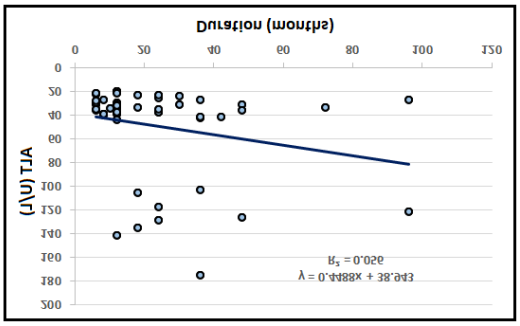

Figure 3. Scatter plot and line of regression between autoimmune disease duration and serum concentration of ALT

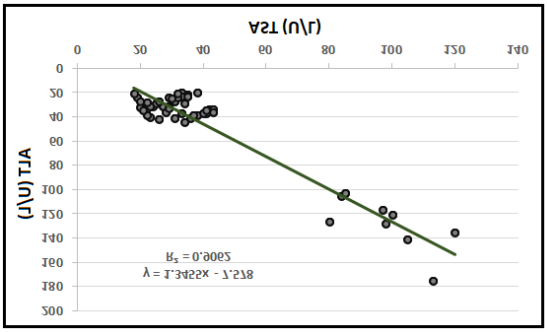

Figure 4. Scatter plot and line of regression between serum concentration of AST and ALT

Follow up of patients with abnormal liver function test (LFT): The present study revealed that 18 out of 100 patients have developed adverse hepatic events represented by abnormal LFT with infliximab use. Subsequently, all of these patients have discontinued infliximab and then rechecked after 3 months to assess their LFT. It was found that 17 (94.4%) of them recovered with normal LFT (Table 6).

Table 6. Follow up of patients with abnormal LFT after discontinuation of infliximab

Variables	Abnormal LFT (n=1) Number (%)	Normal LFT (n=17) Number (%)	Total (n=18) Number (%)
No methotrexabte use	0(0)	15(83.3)	15(83.3)
With methotrexabte use	1(5.6)	2(11.1)	3(16.7)

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Discussion

According to the results of the present study, 18% of patients with autoimmune disease receiving infliximab had abnormal LFT represented by elevated AST and/or ALT. Abnormalities in LFT range from transient abnormal ALT and AST, and cholestatic picture to life threatening (18). As early as 2004 when infliximab was issued by the FDA for the first time, severe hepatitis reaction was reported in 35 volunteers who were used to study the possible side effect of the drug (19). Since then, the same organization has declared more than 130 cases of liver injury resulting from either infliximab or etanercept treatment in post-marketing surveillance program.

In a small Icelandic study, Björnsson et al. (19) reported that in 8 out of 11 patients, the relationship between liver damage and infliximab was highly probable. In the same country, a prospective study in the general population identified 96 patients with DILI. Of note, the highest risk of liver injury was due to infliximab and azathioprine (20). Mancini et al. (21) stated that infliximab can induce both immune-mediated and/or direct liver injury after only one or two doses.

However, the present rate of abnormal LFT is relatively low compared with many other studies. In a retrospective study including 176 patients with IBD treated with infliximab for the previous 5 years, Parisi et al. (22) reported that 39% of those patients have elevated liver enzyme. It is obvious that the treatment period and disease duration are among the main factors responsible for this variation in the rate of abnormal LFT.

The mechanism by which infliximab influences the LFT is not clearly illustrated. Hepatotoxicity may not be a classical effect of infliximab treatment. Rather, this injury may be related to the development of antibodies against infliximab (23). Supporting this assumption are the studies that demonstrated the development of autoimmune damage with autoantibodies (i.e., ANA, ASMA, and anti-LKM antibody), along with classic histologic characteristics of autoimmune hepatitis (i.e., interface hepatitis, lymphoplasmacytic infiltrate, and bridging fibrosis) (24). Drug-induced autoimmune hepatitis (DIAIH) seems to be indistinguishable from classic AIH regarding clinical signs, symptoms, autoantibodies and histology (25).

In a British study, 23% and 16%, respectively, of patients receiving infliximab for RA developed ANA and anti-double-stranded DNA antibodies versus 6 and 0% of placebo recipients (26). Individuals with T2DM have a higher incidence of LFT abnormalities than individuals who do not have diabetes. Mild chronic elevations of transaminases often reflect underlying insulin resistance (27).

In a meta-analysis, we included a total of 32 studies with 13177 participants, 6877 of whom were prescribed methotrexate for RA, psoriasis, PsA, or IBD. Liver adverse events were common with a cumulative incidence of 11.2% in methotrexate treated patients and 6.3% in the control group. This translated to an incidence rate of liver adverse events of 20/100 patient-years in methotrexate treated patients compared to 9/100 patient-years in the comparators giving an attributable risk of 11/100 patient years in methotrexate treated patients (28).

Interestingly, there was no significant association between a particular autoimmune disease (AID) and abnormal LFT in the present study. This implies that all AIDs may have an even impact on liver function and other factors (e.g. disease duration, comorbidities and concurrent use of the other drugs).

In the present study, ALT and AST had a positive significance with BMI and disease duration ($r=0.237$, $p=0.018$ and $r=0.218$, $p=0.029$, respectively). Regarding disease duration, it is reasonable to assume that liver dysfunction is developed (and thus abnormal LFTs) with the prolonged treatment. For BMI, this result is in accordance with many previous studies which demonstrated that increased BMI is associated with elevated liver enzymes, especially AST and ALT (29).

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Patients with autoimmune disease receiving infliximab should be regularly checked up for liver function test. Whenever there is abnormal level in these tests, infliximab treatment should be stopped. About one-fifth of the patients with autoimmune disease receiving infliximab had abnormal liver function test. Abnormality in liver function tests is significantly associated with disease duration, treatment with methotrexate, diabetes and the presence of jaundice and autoantibodies.

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