ÖZGÜN ARAŞTIRMA ORIGINAL RESEARCH

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OXIDATIVE IMBALANCE IN AUTOIMMUNE LIVER DISEASE: EVALUATION OF OXIDANT-ANTIOXIDANT STATUS AND ISCHEMIA-MODIFIED ALBUMIN

OTOİMMÜN KARACİĞER HASTALIĞINDA OKSİDATİF DENGESİZLİK: OKSİDAN-ANTİOKSİDAN DURUMUN VE İSKEMİ MODİFİYE ALBÜMİNİN DEĞERLENDİRİLMESİ

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Öz

Amaç
Bu çalışmada, otoimmün karaciğer hastalığında (AILD) belirteç olarak total antioksidan durum (TAS), oksidatif stres indeksi (OSI), total oksidan durum (TOS), iskemi modifiye albumin (IMA) ve iskemi modifiye albumin oranının (IMAR) yararı değerlendirildi.

Gereç ve Yöntem
Çalışma, 22'si otoimmün hepatit (AIH), 32'si primer biliyer kolanjit (PBC) ve 12'si AIH/PBC örtüşme sendromu olan toplam 66 AILD hastası ve 49 sağlıklı kontrol içermekteydi. Serum TAS, TOS ve IMA düzeyleri analiz edildi. OSI, TOS/TAS olarak hesaplandı ve IMAR, IMA ve Alb'den türetildi.

Bulgular
Serum TAS, TOS, OSI, IMA ve IMAR değerleri AILD grubunda kontrollere göre anlamlı olarak daha yüksekti (sırasıyla p=0.004, <0.001, <0.001, <0.001 ve <0.001). AILD'nin öngörülmesi için TOS, IMA ve IMAR'ın cut-off değerleri sırasıyla 4.1 µmol H2O2 equiv./L, 0.522 absorbans ünitesi (ABSU) ve 0.520 ABSU'dur. TOS, IMA ve IMAR için alıcı işletim karakteristik eğrilerinin altındaki alan sırasıyla 0.760, 0.830 ve 0.858'dir.

Sonuç
Bulgularımız, AILD'de oksidatif stres varlığını ve ayrımında ilgili belirteçlerin yararını göstermiştir.

Anahtar Kelimeler: Otoimmün karaciğer hastalığı, iskemi modifiye albumin, total antioksidan durum, total oksidan durum.

Abstract

Objective
Current study evaluated the utility of total antioxidant status (TAS), oxidative stress index (OSI), total oxidant status (TOS), ischemia-modified albumin (IMA), and ischemia-modified albumin ratio (IMAR) as markers in autoimmune liver disease (AILD).

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Material and Methods
The study included a total of 66 AILD patients, 22 with autoimmune hepatitis (AIH), 32 with primary biliary cholangitis (PBC), and 12 with AIH/PBC overlap syndrome, and 49 healthy controls. Serum TAS, TOS, and IMA levels were analyzed. OSI was calculated as TOS/TAS and IMAR was derived from IMA and Alb.

Results
Serum TAS, OSI, TOS, IMA, and IMAR values were found to be significantly higher in the AILD group compared to controls (p=0.004, <0.001, <0.001, <0.001, and <0.001, respectively). Cut-off values of TOS, IMA, and IMAR for the prediction of AILD were 4.1 μmol H2O2 equiv./L, 0.522 absorbance unit (ABSU), and 0.520 ABSU, respectively. Area under the receiver operating characteristic curves for TOS, IMA, and IMAR was 0.760, 0.830, and 0.858, respectively.

Conclusion
Our findings demonstrated the presence of oxidative stress in AILD and the utility of the related markers for its differentiation.

Keywords: Autoimmune liver disease, ischemia-modified albumin, total antioxidant status, total oxidant status

Introduction

Major autoimmune liver diseases (AILDs) are autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and immunoglobulin G4 (IgG4)-related cholangitis (1,2). In addition to these major groups, some patients diagnosed with AILD exhibit signs characteristic of different AILD subtypes, which is referred to as overlap syndrome (3). AILD, which most commonly presents as AIH and PBC (4), is characterized by self-perpetuating inflammation of the liver with no recognized cause (5). Persistent liver damage seen in these chronic diseases causes sustained inflammation, proliferation of cells, and accumulation of extracellular matrix proteins from portal myofibroblasts and hepatic stellate cells. If left untreated, liver function loss and cirrhosis are inevitable (4,6). Diagnosis of AILD requires a combination of clinical, laboratory, and pathological criteria (7). However, there are no disease-specific clinical features, and autoantibodies are not disease-specific in many cases (8,9). Atypical liver histology also makes diagnosis difficult (10,11).

The pathophysiological processes triggering the progression from first autoimmune attack of AILD to the fibrosis development and eventually cirrhosis and liver failure have not been fully elucidated (12-14). Oxidative injury has been proposed to take part in the pathogenesis of acute and chronic forms of liver disease in animal models, as well as in chronic liver diseases (CLDs) in humans such as alcoholic liver disease and viral hepatitis (15-17). Not many studies have examined the presence of oxidative stress in the various subgroups of AILD and overlap syndrome, and these were limited to the evaluation of individual markers (18,19).

The current study aims to evaluate various parameters of antioxidant defense and oxidative damage in AILD to determine their relationship with the pathophysiology of the disease. For this purpose, we investigated total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), ischemia-modified albumin (IMA), and ischemia-modified albumin ratio (IMAR) in AIH, PBC, and AIH/PBC overlap syndrome. As far as we know, our study presents the first analysis of TAS, TOS, OSI, IMA, and IMAR in AILD.

Materials And Methods

Subjects

A total of 66 AILD patients (22 with AIH, 32 with PBC, and 12 with AIH/PBC overlap syndrome) who were followed up in the AILD outpatient clinic of the Department of Gastroenterology were recruited. All participants were over 18 years of age and diagnosis was based on clinical, serological, endoscopic, and histological criteria. Those with other causes of hepatitis or cholestasis, hemochromatosis, or Wilson's disease were excluded from the study. Mean follow-up of the AIH, PBC, and AIH/PBC overlap syndrome patients were 87.6 ± 57.2, 140.5 ± 90.1, and 97.6 ± 69.1 months, respectively. Seven of the patients had family history of AILD and 10 patients had cirrhosis. Treatment regimens consisted of azathioprine and low-dose steroid therapy for AIH, ursodeoxycholic acid therapy for PBC, and azathioprine, low-dose steroid, and ursodeoxycholic acid therapy for AIH/PBC overlap syndrome. Treatment response was monitored by ensuring liver enzymes were within the reference ranges.

A control group consisting of 49 healthy age- and sex-matched individuals was also recruited. The in-

dividuals in the control group were not pregnant, had no known diseases such as chronic kidney disease, liver disease, cancer, or diabetes mellitus, and had no known history of chronic alcohol use.

Patients and controls did not take antioxidant supplements for at least two months prior to enrollment.

Sample Collection And Preparation

The study protocol was approved by the local ethics committee and each participant provided written informed consent in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. A blood sample was collected from each participant into a clot-activating tube containing gel separator (Ref No: 367955; BD Vacutainer® SST II Advance tube, 5 mL, 13 x 100 mm, NJ, USA). Serum was separated by centrifugation for 10 minutes at 1,500 × g within 1 hour of blood sampling. Serum specimens were aliquoted and stored at −80 °C until measurement of TAS, TOS, IMA, albumin (Alb), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin (TBil), direct bilirubin (DBil), and 25-hydroxyvitamin D (25[OH]D). TAS, TOS, IMA, and Alb levels were analyzed in both the AILD group and controls. In addition, LDH, ALP, AST, ALT, TBil, DBil, and 25(OH)D parameters were analyzed in the AILD group.

Laboratory Assays

Serum Alb, LDH, ALP, AST, ALT, TBil, DBil, and 25(OH)D parameters were analyzed by an automated procedure (Architect C16000 clinical chemistry, and Architect i2000SR immunoassay, Abbott, IL, USA).

A method developed by Erel was implemented to determine serum TAS levels (20). According to this method, the most potent free radical, hydroxyl radical, is generated by mixing a solution of ferrous ion (reagent 1) with hydrogen peroxide (reagent 2) to create a Fenton reaction. The antioxidant effect of the analyzed specimen against the free radical reactions commenced by the produced hydroxyl radical is then measured.

Serum TOS levels were assessed using another method, also developed by Erel (21). Method is based on oxidation of ferrous ion to ferric ion by oxidants in the sample in a reaction medium containing abundant glycerol molecules that enhance the oxidation reaction. In the acidic medium, these ferric ions form a colored complex with xylenol orange that is measured spectrophotometrically to determine the overall amount of oxidant molecule present in the specimen. OSI was calculated as follows: OSI = (TOS / TAS) × 100. Calculation requires the conversion of TAS result unit from mmol Trolox equiv./L to μmol Trolox equiv./L. Serum IMA levels were analyzed spectrophotometrically at 470 nm using the cobalt-binding assay described by Bar-Or (22).

IMAR is used in order to eliminate the impact of albumin concentration on IMA results. In an attempt to correct the IMA values for serum albumin levels, the following formula was applied: (individual serum Alb / median Alb in population) × IMA value (23).

Statistical Analysis

The SPSS version 25 software package (SPSS Inc., Chicago, USA) was the software of choice for statistical analyses performed. Normality of the data distributions was analyzed using Shapiro–Wilk test. For normally distributed data, statistical differences between the groups were evaluated using parametric tests (independent-samples t-test); non-normally distributed data were evaluated with nonparametric tests (Mann–Whitney U). The results were given as mean ± standard deviation or median and interquartile range. Receiver operating characteristic (ROC) curve analyses were performed to determine diagnostic cut-off values and their sensitivity, specificity, and area under curve (AUC) values. For normally distributed data, correlations were evaluated by Pearson's correlation coefficient; Spearman rank correlation coefficient was used for non-normally distributed data. A p value of <0.05 was considered significant.

Ethical Considerations

Local ethics committee approved the study (Resolution Number 2019/17-3, dated November 13, 2019).

Results

Table 1 shows the demographic characteristics and oxidative stress/antioxidant status markers serum levels in the AILD and control groups. No significant difference was found in age or sex between the groups. Serum TAS, TOS, OSI, IMA, and IMAR levels were found to be significantly higher in the AILD group compared to controls.

Levels of oxidative stress/antioxidant markers in the AILD subgroups are presented in Table 2. TAS, TOS, OSI, IMA, and IMAR levels were significantly higher in the AIH and PBC subgroups compared to controls. TOS, IMA, and IMAR levels were significantly higher in AIH/PBC overlap syndrome than in controls. TAS and OSI values were also higher in patients with AIH/PBC overlap syndrome, but the differences were not significant.

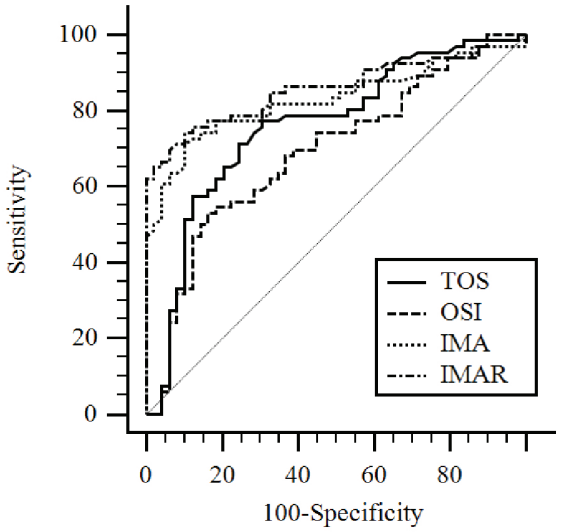

Figure 1
Receiver operating characteristic curves of total oxidative status (TOS), oxidative stress index (OSI), ischemia-modified albumin (IMA), and ischemia-modified albumin ratio (IMAR) to examine their discriminative value in the assessment of autoimmune liver disease.

Serum median LDH, ALP, AST, ALT, TBil, DBil, 25 (OH) D levels in the AILD group were 200.0 (183.0-225.0, IQR) U/L, 106.0 (72.8-157.8) U/L, 27.0 (20.3 -35.0) U/L, 25.0 (18.3-38.8) U/L, 0.70 (0.50-1.00) mg/dL, 0.30 (0.20-0.40) mg/dL, and 18.1 (9.5-31.1) ng/mL, respectively. Correlations between serum oxidative stress markers and serum analytes are shown in Table 3. TOS and OSI were correlated with LDH. IMA was correlated with ALP, AST, ALT, TBil, DBil, and 25(OH)D, while IMAR was correlated with ALP, AST, and DBil.

The diagnostic capacities of the oxidative stress markers were evaluated using ROC curve analysis (Figure 1). Based on the interpretation of AUC proposed by Hosmer and Lemeshow (24), AUC values indicated acceptable discrimination between AILD patients and healthy controls for TOS and excellent discrimination by IMA and IMAR. Cut-off values and diagnostic performance characteristics of TOS, OSI, IMA, and IMAR for the discrimination of AILD from the healthy controls are presented in Table 4. ROC curve analysis showed that the optimal cut-off values for the prediction of AILD were 4.1 µmol H2O2 equiv./L for TOS, 0.522 ABSU for IMA, and 0.520 ABSU for IMAR.

Table 1 Demographic characteristics and oxidative stress/antioxidant status marker levels in the AILD and control groups.

Parameter	AILD group (n=66)	Control group (n=49)	p
Age, years mean ± SD	56.2 ± 12.4	51.6 ± 12.5	0.054
Sex, F/M	59/7	43/6	1.000
Primary diagnosis **Autoimmune Hepatitis**, n (%) **Primary Biliary Cholangitis,** n (%) **AIH/PBC Overlap Syndrome**, n (%)	 22 (33.3) 32 (48.5) 12 (18.2)		
TAS, mmol Trolox equiv./L mean ± SD	1.80 ± 0.20	1.68 ± 0.24	0.004
TOS, µmol H2O2 equiv./L median (IQR)	5.05 (4.04-6.73)	3.78 (3.09-4.15)	<0.001
OSI, arbitrary unit median (IQR)	0.29 (0.22-0.37)	0.22 (0.19-0.26)	<0.001
IMA, ABSU mean ± SD	0.575 ± 0.139	0.423 ± 0.084	<0.001
IMAR, ABSU median (IQR)	0.591 (0.516-0.645)	0.413 (0.367-0.491)	<0.001

Abbr: AILD, autoimmune liver disease; AIH, autoimmune hepatitis; PBC, primary biliary cholangitis; TAS, total anti-oxidative status; TOS, total oxidative status; OSI, oxidative stress index; IMA, ischemia-modified albumin; IMAR, ischemia-modified albumin ratio; ABSU, absorbance unit; SD, standard deviation; IQR, interquartile range (25th–75th percentile).

Table 2 Oxidative stress/antioxidant status marker levels in the AILD subgroups and control group

Parameter	Autoimmune Hepatitis	Primary Biliary Cholangitis	AIH/PBC Overlap Syndrome	Control group	p
TAS, mmol Trolox equiv./L mean ± SD	1.83 ± 0.23	1.79 ± 0.20	1.76 ± 0.18	1.68 ± 0.24	0.012* 0.026† 0.288‡
TOS, μmol H2O2 equiv./L median (IQR)	4.92 (3.84-9.00)	5.14 (4.17-6.49)	5.06 (4.05-6.46)	3.78 (3.09-4.15)	0.001* <0.001† 0.006‡
OSI median (IQR)	0.24 (0.22-0.51)	0.31 (0.22-0.35)	0.28 (0.21-0.39)	0.22 (0.19-0.26)	0.022* 0.001† 0.053‡
IMA, ABSU mean ± SD	0.559 ± 0.131	0.590 ± 0.140	0.560 ± 0.159	0.423 ± 0.084	<0.001* <0.001† 0.013‡
IMAR, ABSU median (IQR)	0.582 (0.506-0.623)	0.600 (0.523-0.660)	0.553 (0.485-0.662)	0.413 (0.367-0.491)	<0.001* <0.001† <0.001‡

*Autoimmune hepatitis vs. control group; †Primary biliary cholangitis vs. control group; ‡ AIH/PBC overlap syndrome vs. control group.Abbr: AILD, autoimmune liver disease; TAS, total anti-oxidative status; TOS, total oxidative status, OSI, oxidative stress index; IMA, ischemia-modified albumin; IMAR, ischemia-modified albumin ratio; ABSU, absorbance unit SD, standard deviation; IQR, interquartile range (25th–75th percentile).

Table 3 Correlations between serum analytes and oxidative stress markers.

Parameter	TOS		OSI		IMA		IMAR	
	r	p	r	p	r	p	r	p
LDH	0.302	0.003*	0.279	0.006*	0.103	0.317	0.118	0.253
ALP	0.080	0.402	0.460	0.629	0.280	0.003*	0.258	0.006*
AST	0.151	0.111	0.111	0.244	0.227	0.016*	0.200	0.034*
ALT	0.032	0.739	-0.003	0.976	0.190	0.044*	0.171	0.070
TBil	0.173	0.082	0.136	0.174	0.199	0.045*	0.171	0.086
DBil	0.085	0.403	0.039	0.705	0.336	0.001*	0.296	0.003*
25(OH)D	0.010	0.938	-0.007	0.959	-0.256	0.043*	-0.223	0.078

Abbr: TOS, total oxidative status, OSI, oxidative stress index; IMA, ischemia-modified albumin; IMAR, ischemia-modified albumin ratio; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TBil, total bilirubin; DBil, direct bilirubin; 25(OH)D, 25-hydroxyvitamin D.
*Statistically significant parameters (p<0.05).

Table 4 Cut-off values and diagnostic performance characteristics of oxidative stress markers in the differential diagnosis of the AILD group from healthy controls.

Parameter	Cut-off value	Sensitivity (%)	Specificity (%)	AUC (95% CI)	p value
TOS, µmol H2O2 equiv./L	>4.1	71.2	75.6	0.760 (0.671-0.834)	<0.001
OSI	>0.289	53.0	83.7	0.692 (0.599-0.775)	<0.001
IMA, ABSU	>0.522	71.2	89.8	0.830 (0.749-0.894)	<0.001
IMAR, ABSU	>0.520	74.2	89.8	0.858 (0.781-0.916)	<0.001

Abbr: AILD, autoimmune liver disease; TOS, total oxidative status, OSI, oxidative stress index; IMA, ischemia-modified albumin; IMAR, ischemia-modified albumin ratio; ABSU, absorbance unit; AUC, area under curve; CI, confidence interval.

Discussion

The major finding from the present study is that oxidative stress markers were elevated in AILD patients. To our knowledge these are the first data on TAS, TOS, OSI, IMA, and IMAR in AILD.

Previous studies in patients with AILD investigated individual oxidative stress parameters such as GSH status and lipid peroxidation products, or accumulation of intrahepatic 3-nitrotyrosine (18,19,25,26). Pemberton et al. reported increased plasma and urine levels of 8-isoprostane, a lipid peroxidation marker, and increased plasma malondialdehyde, another lipid peroxidation marker, in type I AIH patients (18). In a study of 47 patients, Beyazit et al. determined that serum nitric oxide levels were associated with the histological severity of AIH (19). Similar to Pemerton's findings, Aboutwerat et al. also observed elevated urine and plasma 8-isoprostane and plasma malondialdehyde levels in 41 PBC patients (25). Although we evaluated different parameters in the present study, our conclusion was consistent with the studies above.

Reactive oxygen species (ROS) occur normally in all cells and tissues at relatively low levels, acting as messengers in signal transduction pathways and contributing to regulatory processes such as cell proliferation, oncogene expression, and calcium homeostasis (27). However, ROS are highly reactive molecules that overwhelm the antioxidant protective systems when produced in excess and consequently damage cell membranes, disturb mitochondrial function, modify expression of genes, lead to apoptosis or necrosis of liver cells, and promote hepatic fibrosis in CLDs (28,29). ROS can increase existing hepatic fibrosis by activating stellate cells in liver; furthermore, reactive nitrogen species (RNS) can promote apoptosis and hepatocyte loss. Subsequently, fresh ROS and RNS are produced by hepatocyte death, Kupffer cell activation, and the transformation of hepatic stellate cells to myofibroblasts, resulting in self-amplification loops (29). In the present study, the higher oxidative stress marker levels in AILD patient group compared to control group suggest that similar mechanisms may take place in AILD, the pathophysiology of which is still not fully understood.

As measuring the separate oxidant effect of each different molecule is impractical, we evaluated their cumulative oxidant effects using the TOS assay developed by Erel. This method, the main components of which are hydrogen peroxide and lipid hydroperoxide, is fast, easy, stable, reliable, sensitive, and has high linearity (21). TOS has been used as an oxidative stress marker in various pathologies ranging from active pulmonary tuberculosis to necrotizing enterocolitis (30,31). Our study is the first to investigate TOS in AILD and showed that AILD patients have higher TOS compared to healthy controls.

Previous studies have suggested that OSI, which was developed to more clearly define the imbalance between oxidants and antioxidants, may be a useful parameter in the determination of oxidative stress. Previous studies utilized OSI as a marker of oxidative stress and oxidant-antioxidant imbalance in patients with hepatitis B (32), as well as non-alcoholic fatty liver (NAFLD) (33), and experimental liver fibrosis (34). Like TOS, OSI has not been studied previously in AILD. Together with our TOS results, our findings of higher OSI in AILD patients support the role oxidative stress plays in AILD pathophysiology.

Conditions such as ischemia/hypoxia, acidosis, and free radicals result in ROS production which can reduce the ability of the albumin N-terminus to bind with transition metals such as cobalt. This modified albumin is referred to as IMA (35). In recent years, increased IMA levels have been reported in several

chronic illnesses such as diabetes, hyperlipidemia, chronic kidney disease, obesity, and especially ischemic heart disease (36,37,38,39). In addition, a few studies have investigated IMA levels in CLD. Cakir et al. evaluated IMA and IMAR in pediatric patients with CLD and reported that IMA levels were correlated with Child-Pugh criteria, disease severity, and liver fibrosis (40). Kumar et al. suggested that IMA could be used as a marker to evaluate disease severity and prognosis of CLD (41). We observed an increase in IMA in our AILD group consistent with those in TOS and OSI. As the IMA method is based on albumin binding of cobalt, we also calculated IMAR to avoid the effect of interindividual variations in albumin level on IMA results. As in other oxidative stress markers in this study, levels of IMAR were significantly higher in AILD patients.

The fact that all the oxidative stress markers we analyzed were high in AIH and PBC subgroups support the usefulness of TOS, OSI, IMA, and IMAR in differentiating these subgroups from the normal population. Our results suggest that only TOS, IMA, and IMAR can be used as markers in AIH/PBC overlap syndrome.
In the present study, TAS levels were found to be higher in AILD patients compared to controls. Some previous studies present TAS data that conflict with our results. Horoz et al. reported lower levels of TAS in patients with NAFLD compared to controls (42). Sen et al. detected no difference in TAS levels between controls and patients with inactive hepatitis B, whereas chronic hepatitis B patients had lower TAS than controls (43). However, other studies in the literature are consistent with the results of our research. Selek et al. reported that serum TAS concentrations were higher in patients with obsessive-compulsive disorder (OCD) in their study based on literature suggesting the role of free radicals in OCD (44). They suggested that this may be due to a reactive increase in defense mechanisms. Another argument is that antioxidants are not always the “good guys,” which is supported by evidence of higher antioxidant levels in certain diseases, such as polycystic ovary syndrome (45). An alternative explanation is that TAS levels in our patients were influenced by their treatment regimens; for example, ursodeoxycholic acid has antioxidant properties.

We also determined that serum analytes used in the laboratory assessment of AILD were correlated with TOS, OSI, IMA, and IMAR. In addition, the negative correlation between 25(OH)D and IMA was also a noteworthy finding. Vitamin D deficiency plays a key role in many diseases ranging from cancer to autoimmune disease, but very little is known with regards to the role of 25(OH)D in AILD (46). It has been proposed that 25(OH)D is associated with severe fibrosis and inflammation in AIH (47), and one of the mechanisms by which reduced 25(OH)D level may cause these consequences is elevated ROS levels. Findings related to 25(OH)D supplementation and reduced serum MDA levels in CLD also indicate the need for further research into the link between AILD, 25(OH)D, and oxidative stress (48).

In terms of the utility of oxidative stress markers to differentiate patients with AILD from the healthy population, we determined that a TOS value above 4.1 was an acceptable marker for AILD (AUC=0.760, 71.2% sensitivity, 75.6% specificity). Furthermore, IMA above 0.522 (AUC=0.830, 71.2% sensitivity, 89.8% specificity) and IMAR above 0.520 (AUC=0.858, 74.2% sensitivity, 89.8% specificity) were both excellent markers for AILD.

This study has certain limitations. Firstly, the AILD patient group was relatively small. In addition, our analysis was based on a single measurement instead of serial analyses, and LDH, ALP, AST, ALT, TBil, DBil, and 25(OH)D were assessed only in the AILD group.

Conclusion

Consequently, we present the first evaluation of TAS, TOS, OSI, IMA, and IMAR in AILD. Our findings demonstrated the presence of oxidative stress in AILD and the usefulness of these parameters as markers for the differentiation of these patients.

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