Changes in the balance of Th17/Treg cells and oxidative stress markers in patients with HIV-associated pulmonary tuberculosis who develop IRIS

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Received July 1, 2022; Accepted February 23, 2023

DOI: 10.3892/etm.2023.11970

Abstract. Tuberculosis (TB) is the most common opportunistic infection in patients with acquired immunodeficiency syndrome (AIDS) caused by human immunodeficiency virus (HIV) infection and is one of the primary causes of death from AIDS. The increased accessibility to highly active antiretroviral therapy (HAART) has significantly improved the clinical outcome of patients with HIV infection. However, following ART, rapid restoration of the immune system leads to immune reconstitution inflammatory syndrome (IRIS). Oxidative stress and innate immunity play a role in TB-associated IRIS (TB-IRIS). The present study investigated the changes that occur in oxidative stress markers and T helper (Th)17/regulatory T (Treg) cell balance and their significance in IRIS patients with HIV-associated pulmonary TB. A total of 316 patients with HIV-associated pulmonary TB were treated with HAART and followed up regularly for 12 weeks. Those who developed IRIS were included in the IRIS group (n=60), while the remaining patients were included in the non-IRIS group (n=256). The changes in plasma oxidative stress markers superoxide dismutase (SOD) and malondialdehyde (MDA) were detected with the ELISA, and the ratio of Th17 to Treg cells in whole blood were analyzed before and after treatment through the flow cytometric assay. Following treatment, MDA and Th17 cells levels were significantly increased while SOD and Treg cells levels were decreased in the IRIS group (P<0.05) compared with before treatment. In the non-IRIS group, a non-significant decrease was observed in SOD levels (P>0.05), while the MDA levels significantly decreased compared with before treatment (P<0.05) and the Th17 and Treg cells levels were both significantly increased (P<0.05). After treatment, compared with the non-IRIS group, the IRIS group showed a significant increase in MDA and Th17 cells and decrease in SOD and Treg cells levels (P<0.05). In addition, Th17 cells levels were positively correlated with MDA but negatively correlated with SOD levels. Treg levels were negatively correlated with MDA and positively correlated with SOD levels (P<0.05). The area under the curve values of serum MDA and SOD, Th17 and Treg levels predicting the occurrence of IRIS were 0.738, 0.883, 0.722 and 0.719, respectively (P<0.05). These results indicated that the above parameters have certain diagnostic value for the occurrence of IRIS. The occurrence of IRIS in patients with HIV-associated pulmonary TB may be associated with oxidative stress and Th17/Treg cell imbalance.

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Key words: T helper cell 17, regulatory T cell, oxidative stress, immune reconstitution inflammatory syndrome

Introduction

Acquired immunodeficiency syndrome (AIDS) and tuberculosis (TB) are major serious public health concerns worldwide (1). According to estimates from the Joint United Nations Programme on HIV and AIDS, 1.7 million people were living with HIV in 2018 and there were about 37.9 million cases of HIV/AIDS (2). Patients with AIDS have a compromised immune system and are susceptible to opportunistic infections, such as invasive fungal rhinosinusitis, which without the control of immunosuppression, the infection is aggravated and extends to the orbit and inside the skull base, even with the timely surgical and medical treatment (3). Of opportunistic infections, TB is the most common and one of the primary causes of death in patients with HIV (4,5). In 2019, 208,000 of ~1.4 million TB-related deaths were among HIV-positive people (6). The risk of developing TB for patients

with HIV is 20-30-fold higher than for those without HIV and the synergistic interplay of the two pathogens accelerates the decrease in immunological function (7). Pulmonary TB is the most common form of active TB in patients with HIV (8). Highly active antiretroviral therapy (HAART) is a common treatment for HIV infection that inhibits viral replication and restores the functions of the immune system (9,10). HAART makes HIV infection a controllable chronic disease and plays a key role in HIV infection prevention and treatment (11). However, immune reconstitution leads to an excessive inflammatory response to infectious and non-infectious agents. This phenomenon is known as immune reconstitution inflammatory syndrome (IRIS) (12); it occurs because of the pathological immune inflammatory response triggered by HAART treatment (13,14). TB-associated IRIS (TB-IRIS) is considered to involve *Mycobacterium tuberculosis* antigens and is characterized by unexplained worsening or occurrence of symptoms or signs of TB post-ART initiation (15). For example, The increased clinical blood C-reactive protein (CRP) level and CD4 T-cell count of patients, and worsening of chest imaging included progression to miliary disease, worsening consolidations, enlarged thoracic lymph nodes, and pleural effusions (16). Previous study reported that the overall incidence of IRIS is 18% in patients with HIV-associated TB, with mortality of 2% attributable to TB-IRIS (17) but not all patients will develop IRIS. Risk factors for developing TB-IRIS include low baseline CD4 T cell count before ART initiation, short interval between TB treatment and ART initiation and high mycobacterial burden, also caused by disseminated TB (18). The mechanism of IRIS occurrence remains elusive and it is therefore necessary to find new ideas and treatment approaches to prevent and reverse IRIS in patients with HIV-associated pulmonary TB.

It is clinically reported that IRIS occurs as a pathological, immunological inflammatory response induced in the host following HAART treatment (19). A previous study hypothesized that the combined action of innate and adaptive immune systems increases the risk of IRIS development (20). T helper cell 17 (Th17) and regulatory T cells (Tregs) are two subsets of CD4+ T cells with completely different immune functions (21). Th17 cells are pro-inflammatory, while Treg cells are anti-inflammatory. The balance between these subpopulations is crucial for preventing excessive immune activation and autoimmune responses, which serve a role in IRIS (22,23).

HIV infection is associated not only with increased free radical production through multiple mechanisms that leads to oxidative stress but also with suppression of antioxidant defense systems (24,25). Malonaldehyde (MDA) is a product of lipid peroxidation and a key indicator of oxidative damage in cells (26). High levels of MDA and reactive oxygen species (ROS) in patients with HIV/AIDS co-infected with TB cause systemic cell damage, leading to immune system dysregulation and further deterioration of health (9). Superoxide dismutase (SOD) is an important antioxidant enzyme in living organisms (27).

Oxidative stress is associated with the immune function of patients with HIV, especially immune function of T cells, the imbalance of antioxidant defense system and lymphocyte apoptosis in HIV patients may lead to T cells are sensitive to apoptosis (25). *Mycobacterium tuberculosis* is the primary causative agent of TB in patients with AIDS/HIV infection. A high level of oxidative stress occurs in patients with HIV-TB co-infection because of tissue inflammation and poor nutrition and immunity (28). In patients with AIDS and pulmonary TB, the association between oxidative stress and *M. tuberculosis* and abnormal T cell immune function (29) are associated with development of TB-IRIS (15). This may provide a new direction for treating IRIS in patients with HIV-associated pulmonary TB.

The present study aimed to investigate changes in oxidative stress markers and Th17/Treg cell balance in the development of IRIS in patients with HIV-associated pulmonary TB.

Materials and methods

Study subjects. A total of 316 patients diagnosed with HIV-associated pulmonary TB and admitted to Changsha First Hospital Branch in Changsha, China, from August 2018 to October 2020 were recruited. These patients were treated with HAART and followed up regularly for 12 weeks. Those who developed IRIS during this period were included in the IRIS group (n=60) and the remaining patients were included in the non-IRIS group (n=256). All patients provided written informed consent. The present study was approved by Ethics Committee of Changsha First Hospital (approval no. ky20180103).

The participants were aged 18-84 years old, the male to female ratio is ~1.45:1. Pulmonary TB was confirmed according to the diagnostic criteria of pulmonary TB in the health industry standards of China (WS 288-2008 and WS 288-2017) (30): i) nucleic acid test shows a positive result for *M. tuberculosis* and ii) chest radiological imaging shows typical clinical symptoms of active TB infection. HIV infection was confirmed according to the diagnostic criteria based on the Chinese Guidelines for Diagnosis and Treatment of HIV/AIDS (2018) (31): Nucleic acid test shows a positive result for HIV. IRIS was confirmed according to the diagnostic criteria of the International Network for the Study of HIV-related IRIS consensus case definition (32): i) Patient with HIV; ii) receiving effective ART, as evidenced by a decrease in HIV-1 RNA concentration or an increase in CD4+ T cells from baseline; iii) clinical symptoms consistent with the inflammatory process and iv) clinical course not consistent with the expected course of previously or newly diagnosed opportunistic infection or drug toxicity. The inclusion criteria were as follows: i) Patients with pulmonary TB who received on initial anti-TB treatment and ii) patients with HIV infection who received initial antiretroviral treatment. The exclusion criteria were as follows: i) Patients with other opportunistic infections, (2) pregnant or lactating patients, iii) patients with severe psychiatric or neurological disease, iv) patients with oncological disease and v) patients with severe cardiovascular, cerebrovascular, hepatic, renal or other organ impairment.

Treatment methods. HAART was administered as follows: Lamivudine (300 mg/day), tenofovir (300 mg/day) and efavirenz (600 mg/day) taken orally. The regimen was adjusted based on drug resistance, severe liver and kidney injury or other intolerance.

Anti-TB regimen was as follows: Isoniazid (300 mg/day), rifampicin (450 mg/day), ethambutol (750 mg/day) and pyrazinamide (150 mg/day) taken orally. In the first and second

Table I. General characteristics of each group.

Characteristic	IRIS group (n=60)	Non-IRIS group (n=256)	χ^2/t	P-value
Sex, male/female	36/24	151/105	0.021	0.885
Mean age, years	35.6±9.5	36.5±10.4	0.613	0.540
HIV infection mode (%)			3.142	0.076
Blood transfusion	7 (11.7)	23 (9.0)		
Intravenous drug use	7 (11.7)	15 (5.9)		
Sexual transmission	46 (76.7)	218 (85.2)		
Underlying diseases Y/N				
Diabetes	17/43	86/170	0.612	0.434
Hypertension	12/48	71/185	1.501	0.220
Complications, Y/N				
Pulmonary infection	5/55	22/234	0.004	0.948
Drug-related liver injury	10/50	64/192	1.882	0.170
Drug-induced rash	10/50	54/202	0.590	0.442

IRIS, immune reconstitution inflammatory syndrome; Y, yes; N, no.

months, all four drugs were administered; in the third to ninth months, isoniazid + rifampicin were administered.

ELISA determination of SOD and MDA levels. Blood was collected from all patients before and after HAART at week 12. Patients fasted for 8 h before blood collection. Fasting venous blood (5 ml) was collected at 8:00 AM and stored at 4˚C, then divided into two portions for flow cytometry and preparing serum by centrifugation at 1,200 x g for 15 min at 4˚C, which was stored at -80˚C until use. SOD (cat. no. ab65354) and the MDA Assay kit (cat. no. ab238537) purchased from Abcam were used to determine the levels of serum SOD and MDA with a fully automated biochemical immunoassay analyzer. These kits were used according to the manufacturer's protocol.

Determination of the WBC count, HIV-RNA and CRP levels. CRP was detected by immunoturbidimetric method through C-reactive protein kits (E023, Nanjing Jiancheng Bioengineering). White blood cell (WBC) were detected by automatic blood cell counter (XN-1000V, Sysmex). The patient's 4 ml venous blood was placed in EDTA anticoagulant tube to separate the plasma, and the HIV-1 Qualitative Test kit (Roche, Germany) was used for real-time fluorescence RT-qPCR analyzer (BIO-RAD, Germany) to determine the HIV-RNA load, set positive control and negative control, HIV-RNA copies were converted to geometric mean representation.

Flow cytometric assay to determine ratio of immune cells. Flow cytometric assay was used to determine the ratio of CD4+ T, Th17 and Treg cells in whole blood samples using a CytoFLEX Flow Cytometer (cat. no. B96622) and Kaluza C Analysis Software 2.1.1 (both Beckman Coulter, Inc.). Briefly, 125 μl anticoagulated blood was taken in a flow tube and 125 serum-free medium (12753018, Gibco), 1 PMA/Ionomycin and 1 μl BFA/Monensin Mixture (250X) (70-CS1002) (MultiSciences) were added. Another 125 μl anticoagulated blood and 125 μl serum-free medium were used as control. The mixture was incubated at 37˚C for 4-6 h and shaken every 1-2 h. Peripheral blood mononuclear cells (PBMCs) were isolated by standard Ficoll-Hypaque gradient centrifugation: Whole blood (3 ml) in K3EDTA-diluted PBS was layered over Ficoll-paque PLUS (3 ml; Amersham; Cytiva). After centrifugation at 1,000 x g for 20 min at room temperature, PBMCs were harvested and washed twice with incomplete RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) followed by centrifugation at 700 x g for 10 min at 4˚C. The viable PBMCs were evaluated using 0.2% Trypan blue dye for 3 min at room temperature. Single nucleated cells were resuspended in medium containing 10% fetal bovine serum (16140089, Gibco) to a concentration of $1x10^7$ cells/ml. Next, 250 μl PBMCs in a flow tube was mixed with 1 μl PMA/Ionomycin Mixture (250X) and 1 μl BFA/Monensin Mixture (250X). Samples containing only PBMCs were used as controls. The mixture was incubated at 37˚C for 4-6 h and shaken at 1-2 h intervals. A total of 100 μ cell suspension from the sample and control tubes was mixed with 5 μl Anti-Human CD3, FITC and 5 μl Anti-Human CD8α, PerCP-Cy5.5 (1:100; cat. nos. ab34275, ab34275, ab95591 and ab210329; Abcam) or 5 μl of Anti-Mouse CD3ε, FITC and 5 μl Anti-Mouse CD4, PerCP-Cy5.5 (1:100; cat. nos. ab210317, ab210316, ab218745 and ab210373; Abcam) in a new flow tube. The mixture was shaken and mixed well before incubation for 15 min at room temperature in the dark. A total of 100 μl FIX & PERM Medium A (GAS001S100, Invitrogen) was added to each tube and mixed by shaking. The mixture was incubated at room temperature for 15 min in the dark. Next, 10X Flow Cytometry Staining Buffer (70-S1001, MultiSciences) was diluted to 1X with distilled water. Then, 2 ml pre-chilled 1X Flow Cytometry Staining Buffer was added to each tube. The mixture was centrifuged at 300 x g for 5 min at 4˚C and the supernatant was discarded. Next, 100 μl

Table II. Inflammatory indices, HIV-RNA and CD4+ T cell count.

Group	Treatment point	n	Mean WBC, x10^9	Mean CRP, mg/l	Mean HIV-RNA, g/μl	Mean CD4+ cells/μl
IRIS	Before	60	5.51± 1.26	64.92± 6.10	5.37± 1.73	187.05± 23.97
	After	60	7.51±1.31[a,b]	78.99± 9.05[a,b]	3.86± 1.20[a,b]	277.94± 28.08[a]
Non-IRIS	Before	256	5.22±0.84	67.75±6.85	5.13±1.12	192.5±20.24
	After	256	6.43±1.09[a]	56.32±7.86[a]	3.05±0.78[a]	265.33±33.02[a]

[a]Paired Student's t-test, P<0.05 vs. before treatment; [b]unpaired Student's t-test, P<0.05 vs. non-IRIS. IRIS, immune reconstitution inflammatory syndrome; WBC, white blood cell; CRP, C reactive protein.

Table III. Proportion of Th17 and Treg cells and MDA and SOD levels.

Group	Treatment point	n	Mean Th17, %	Mean Treg, %	Mean MDA, μmol/l	SOD, U/ml
IRIS	Before	60	0.46±0.10	1.61±0.22	48.38±5.61	291.73±32.63
	After	60	0.59±0.09[a,b]	1.09±0.12[a,b]	52.56±8.44[a,b]	139.86±27.33[a,b]
Non-IRIS	Before	256	0.46±0.05	1.49±0.32	50.10±7.33	301.57±41.30
	After	256	0.54±0.08[a]	1.63±0.10[a]	44.05±5.75[a]	299.67±41.23

[a]Paired Student's t-test, P<0.05 vs. before treatment; [b]unpaired Student's t-test, P<0.05 vs. non-IRIS. IRIS, immune reconstitution inflammatory syndrome; Th, T helper; Treg, regulatory T; MDA, malondialdehyde; SOD, superoxide dismutase.

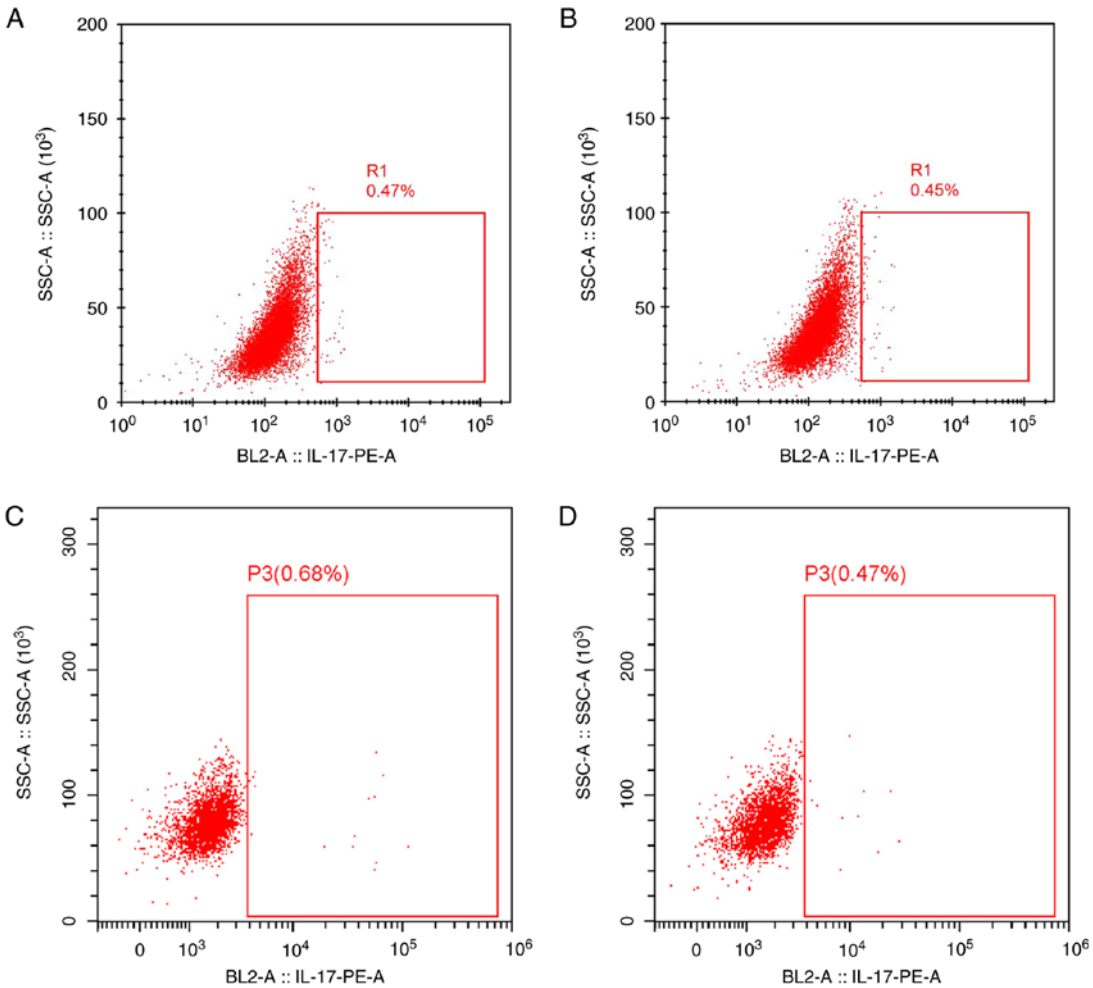

Figure 1. Proportion of T helper 17 cells before and after treatment, as determined by flow cytometry. (A) IRIS and (B) non-IRIS group before treatment. (C) IRIS and (D) non-IRIS group after treatment. IRIS, immune reconstitution inflammatory syndrome.

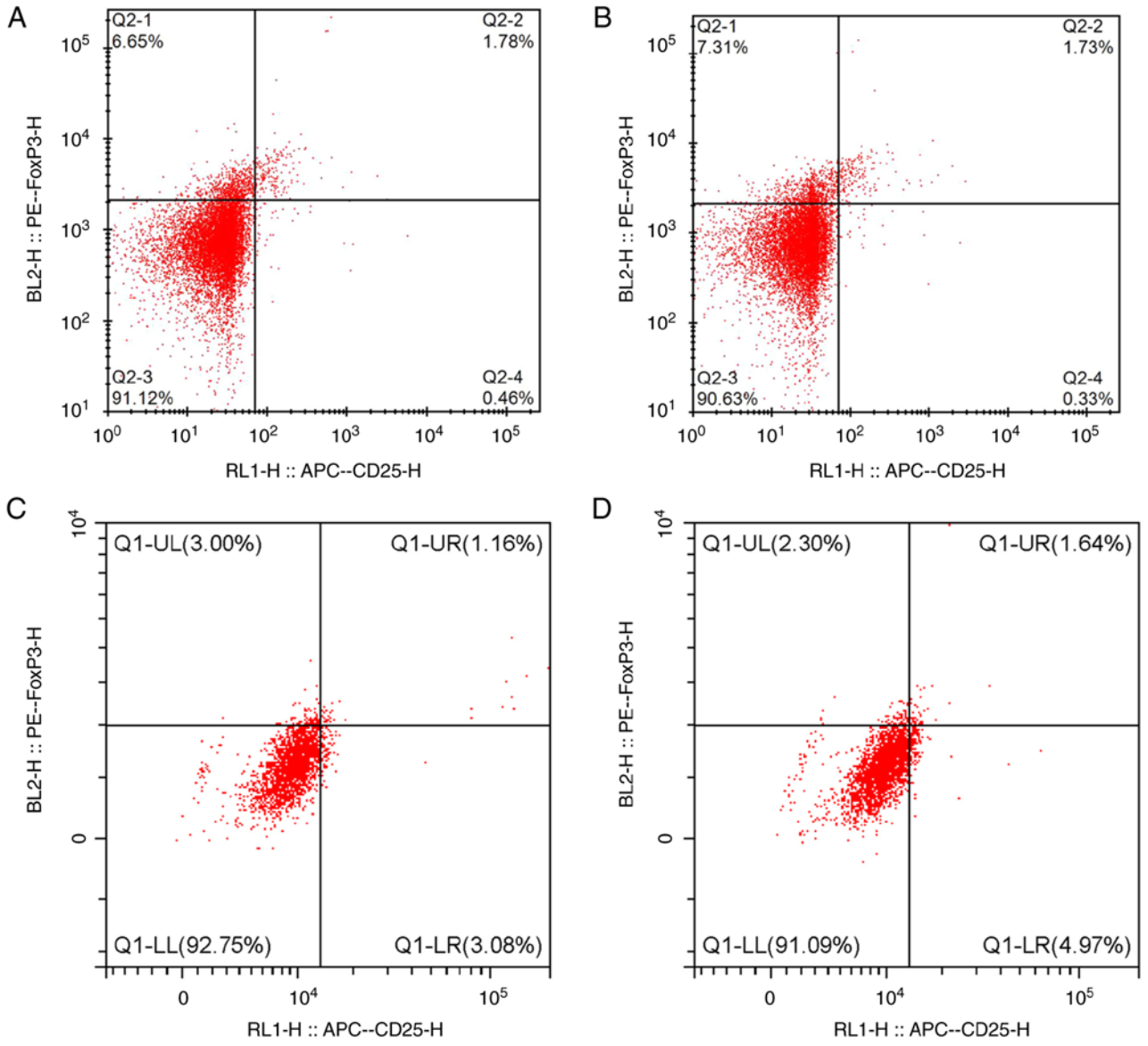

Figure 2. Proportion of regulatory T cells before and after treatment, as determined by flow cytometry. (A) IRIS and (B) non-IRIS group before treatment. (C) IRIS and (D) non-IRIS group after treatment. IRIS, immune reconstitution inflammatory syndrome.

FIX & PERM Medium B (GAS002S5, Invitrogen) and 5 μl Anti-Human IL-17A, PE (1:100; cat. nos. ab189377, Abcam; 560438, BD Pharmingen) or 5 μl Anti-Mouse IL-17A, PE (1:100; cat. nos. ab189377, Abcam; 12-71777-81, eBioscience) were added to each tube. The mixture was incubated at room temperature for 15 min in the dark. Flow Cytometry Staining Buffer (1X, 2 ml) was added to each tube and the mixture was centrifuged at 300 x g for 5 min at 4˚C. The resultant supernatant was discarded. Flow Cytometry Staining Buffer (1X, 500 μl) was added to each tube and the mixture was resuspended and assayed on the flow cytometer.

Statistical analysis. All statistical analysis was performed with SPSS V25.0 (IBM Corp.). Quantitative data with normal distribution are expressed as the mean and standard deviation, each trial was repeated three times. Paired or unpaired t test was used for comparison of two independent samples and χ^2 test was performed for categorical data. Correlation analysis between was performed using Pearson's correlation analysis. $P<0.05$ was considered to indicate a statistically significant difference. In addition, receiver operator characteristic (ROC) curves were used to analyze the predictive value of oxidative stress markers and Th17 and Treg cells for the occurrence of IRIS.

Results

Comparison of clinical characteristics. No statistically significant difference in sex and age was observed between groups (Table I). Compared with the non-IRIS group, the IRIS group did not show statistically significant differences in HIV infection mode, underlying disease or complications (Table I).

Comparison of inflammatory indices, HIV-RNA, and CD4+ T cell count before and after treatment. Before HAART, no statistically significant differences in white blood cell (WBC) count, C reactive protein (CRP) levels, HIV-RNA and CD4+ T cell count were observed between the IRIS and non-IRIS groups (Table II). Following HAART treatment, WBC levels

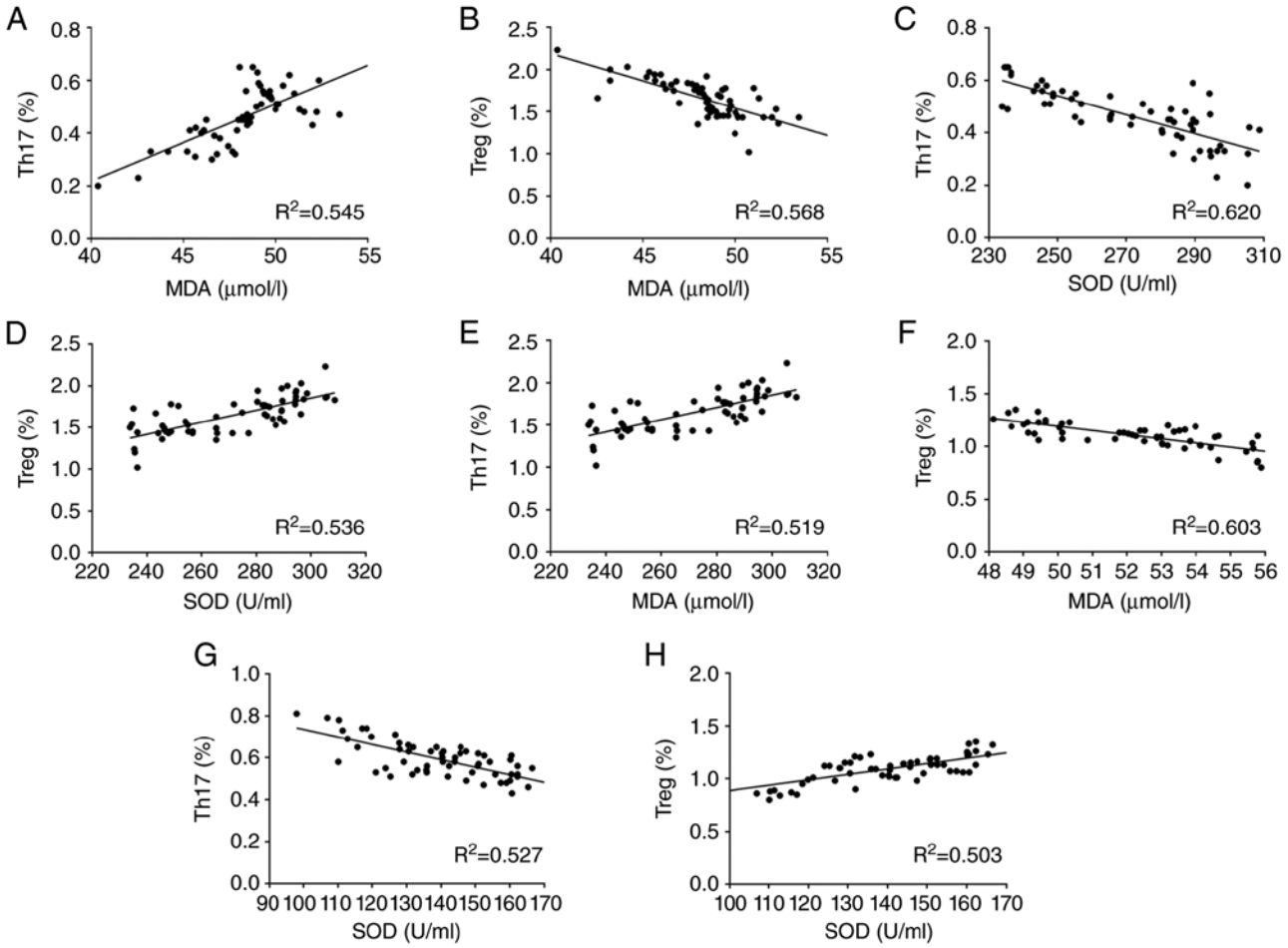

Figure 3. Correlation between oxidative stress markers and Th17/Treg. Correlation between MDA and (A) Th17 and (B) Treg before treatment. Correlation between SOD and (C) Th17 and (D) before treatment. Correlation between MDA and (E) Th17 and (F) Treg after treatment. Correlation between SOD and (G) Th17 and (H) Treg after treatment. Th, T helper; Treg, regulatory T; MDA, malondialdehyde; SOD, superoxide dismutase.

were increased, HIV-RNA was decreased and CD4+ T cell count increased in both groups. After HAART treatment, the CRP level was increased in the IRIS group but decreased in the non-IRIS group and the differences were statistically significant Compared with the non-IRIS group, the IRIS group showed a significant increase in WBC count, CRP level and HIV-RNA after ART but no obvious change was observed in CD4+ T cell count.

Changes in MDA and SOD levels and Th17 and Treg ratio before and after treatment. Before the initiation of HAART, no significant differences in MDA and SOD as well as the Th17 and Treg levels were observed between the IRIS and non-IRIS groups (Table III). Following treatment, the IRIS group showed increased MDA and Th17 but decreased SOD and Treg levels; all these differences were statistically significant. After treatment, the non-IRIS group showed decreased MDA and SOD and increased Th17and Treg levels; except for SOD, the changes of other indicators were significant.

Following HAART, the Th17 and MDA levels in IRIS group were significantly higher than that in the non-IRIS group but the Treg and SOD levels in IRIS group were significantly lower than that in the non-IRIS group (Table III; Figs. 1 and 2).

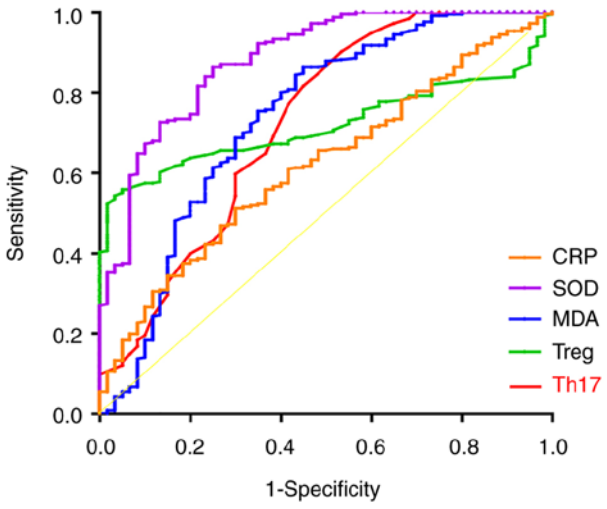

Figure 4. Receiver operating characteristic curves of oxidative stress markers and Th17 and Treg levels for predicting immune reconstitution inflammatory syndrome occurrence. Th, T helper; Treg, regulatory T; MDA, malondialdehyde; SOD, superoxide dismutase; CRP, C reactive protein.

Correlation between oxidative stress markers and Th17 and Treg levels. The correlation between oxidative stress markers and levels of Th17 and Treg cells in the IRIS group was

Table IV. Predictive value of oxidative stress markers and Th17 and Treg for the occurrence of immune reconstitution inflammatory syndrome.

Variable	Truncation value	Sensitivity, %	Specificity, %	Area under the curve	95% CI	P-value
MDA	48.695	86.3	55.1	0.738	0.657-0.819	<0.001
SOD	289.795	86.3	75.2	0.883	0.835-0.932	<0.001
Th17	0.455	90.2	46.7	0.722	0.641-0.804	<0.001
Treg	2.005	54.3	96.7	0.719	0.665-0.773	<0.001
CRP	67.570	51.2	70.3	0.619	0.545-0.693	0.004

Th, T helper; Treg, regulatory T; MDA, malondialdehyde; SOD, superoxide dismutase; CRP, C reactive protein.

analyzed by Pearson's correlation analysis (Fig. 3). Both before and after treatment, MDA had a positive correlation with Th17, but a negative correlation with Treg. SOD had a negative correlation with Th17, but a positive correlation with Treg.

Predictive value of oxidative stress markers and Th17 and Treg for the occurrence of IRIS. The area under the curve (AUC) values of serum MDA and SOD levels and Th17 and Treg were 0.738, 0.883, 0.722 and 0.719, respectively. The differences were statistically significant when receiver operating characteristic curves were plotted (Fig. 4; Table IV). These results showed that the above parameters have certain diagnostic value for the occurrence of IRIS.

Discussion

It is estimated 251,000 people living with HIV infection died from TB, accounting for one third of all HIV-associated deaths and one sixth of all TB-associated deaths, in 2018 (33), thus making TB infection one of the most common causes of death in patients with HIV and accounting for 26% of AIDS-associated deaths (34). Although ART improves the survival of patients with HIV-associated TB and contributes to restoration of immune function, some patients experience worsening of clinical symptoms and exacerbation of the inflammatory response, which lead to development of IRIS. The incidence of IRIS in patients with HIV-associated TB has been reported to be 7-40% (35). Although the pathogenesis, biomarkers and treatment of IRIS have been studied (12-14), the etiology of IRIS is not clear and its treatment is limited.

Patients with HIV are frequently in a state of oxidative stress, marked in part by reduced or altered levels of antioxidants and increased levels of lipid peroxidation products (36). TB is a chronic pathogenic infection that leads to depletion of reducing substances in the body, which increases the occurrence of oxidative stress (37). A previous study (38) reported that higher levels of MDA in patients with AIDS and HIV co-infected with TB result in increased production of ROS and that MDA induces secondary production of ROS and subsequent uncontrolled lipid peroxidation, which may further compromise the immune system and lead to exacerbation of disease in these patients. Here, following treatment, the peripheral serum MDA levels significantly increased, while the SOD levels decreased in the IRIS group. The non-IRIS group showed a slight decrease in SOD levels but a significant decrease in MDA levels, thus suggesting that more severe oxidative stress was present in patients with IRIS compared with the non-IRIS group.

Oxidative stress is associated with abnormal immune function, especially with T cell immune function, in patients with HIV (25). T cell imbalance is an important factor for IRIS development in patients with AIDS. Treg cells are hypothesized to play an important role in immune modulation (38). Th17 cells, which produce cytokines such as IL-17, IL-17F and IL-22, play a key role in sustained inflammatory response and an increase in their number can exacerbate the inflammatory response (39). Thl7 cells promote the inflammatory response, while Treg cells suppress the inflammatory response. The disruption of this balance may amplify the inflammatory response. The present study revealed that the IRIS group showed a significant increase in the CRP levels after HAART compared with the non-IRIS group. Th17/Treg ratio is closely associated with IRIS during HIV treatment (40). Li *et al* (41) revealed that Th17 expression levels are significantly lower and Treg levels are increased in patients with AIDS and TB compared with those in healthy individuals. The Th17/Treg imbalance contributes to the worsening of AIDS combined with TB. In the present study, compared with pretreatment, the IRIS group showed a significant increase in MDA and Th17 levels but a significant decrease in SOD and Treg levels after treatment. The non-IRIS group showed a significant increase in Th17 as well as Treg levels after treatment. These findings suggested that the increase in oxidative stress in the IRIS group was accompanied by Th17/Treg imbalance.

Previous studies have demonstrated that inhibition of Treg-associated markers leads to significantly elevated airway inflammation in patients with acute lung injury (42,43). IL-17A inhibition decreases airway inflammation, while the inhibition of IL-2-inducible T cell kinase signaling reduces airway inflammation by modulating the Th17/Treg immune response and oxidative stress in the lung (44). Yang (45) *et al* reported that in patients with systemic lupus erythematosus (SLE), oxidative stress aggravates inflammation and damage by decreasing Treg cells and inducing the activation of mTORC1 to actively promote Th17 cell differentiation and induction of local chemokine and cytokine production. This suggests that oxidative stress could aggravate SLE in these patients by inducing Th17/Treg imbalance. Yang *et al* (46) showed

decreased Treg/Th17 ratio in peripheral blood of patients with polycystic ovary syndrome, which is involved in the enhancement of systemic inflammatory response, including the activation of oxidative stress.

In the present study, correlation analysis revealed that Th17 cells were positively correlated with MDA but negatively correlated with SOD levels. Treg cells were negatively correlated with MDA and positively correlated with SOD levels. These results suggested that both oxidative stress and Th17/Treg imbalance are related to the development of IRIS in patients with HIV-associated pulmonary TB. In the present study, the AUC values of serum MDA and SOD levels and Th17 and Treg proportions were 0.738, 0.883, 0.722 and 0.719, respectively; thus, these parameters are hypothesized to have some diagnostic value for IRIS occurrence and may be associated with oxidative stress. This may be associated with the effect of oxidative stress on the regulation of Th17/Treg homeostasis. The disruption of Th17/Treg homeostasis in patients with HIV-associated pulmonary TB accelerates the imbalance of the immune system in patients with abnormal oxidative stress (22).

Evidence suggests that the Th17/Treg balance underlies the pathogenic mechanisms of autoimmune diseases (47). Th17/Treg ratio is increased in patients with rheumatoid arthritis (RA), psoriasis, multiple sclerosis and inflammatory bowel disease (48). Monoclonal antibodies against the human IL-6 receptor, namely tocilizumab and sarilumab, are used in patients with RA to decrease Th17 cell levels but increase Treg cell levels (47). The aforementioned studies suggest that the Th17/Treg balance may be a target for IRIS therapy. In patients with HIV, low GSH levels have been shown to induce provirus transcription by NF-κB activation, cell apoptosis and CD4+ T cell depletion. Therefore, replenishment of GSH is considered to be a potential supplement to HAART (49). The present study observed increased MDA and decreased SOD levels in patients with IRIS. Taken together with the findings of previous research, this suggested that the oxidative stress markers may be therapeutic targets for IRIS caused by HAART in patients with HIV-associated pulmonary TB. These results will provide a basis for IRIS clinical treatment.

There were limitations to the present study. The present study does not involve the detection indicators of upstream and downstream signals. The sample size of the study was small. Therefore, further validation is needed through multicenter randomized controlled trials with a larger sample size. In conclusion, following ART treatment, IRIS developed in patients with HIV-associated pulmonary TB because of oxidative stress and Th17/Treg imbalance.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Key Research & Developmental Program of Hunan Province (grant no. 2022SK2047), the Natural Science Foundation of Hunan Province (grant no. 2018JJ2452) and the Natural Science Foundation of Changsha city (grant no. Kp2208448).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LW and ZGZ were responsible for study concept and design. LS and SSW performed experiments. TX and LZ performed data collection, analysis and interpretation. ZGZ was responsible for drafting the manuscript. LW reviewed the manuscript. All authors have read and approved the final manuscript. LW and ZGZ confirm the authenticity of all the raw data.

Ethics approval and consent to participate

All experimental protocols were approved by the Ethics Committee of Changsha First Hospital (ethical approval no. ky20180103). The study subjects provided signed informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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