Ozone autohemotherapy elevates PPAR-γ expression to lower blood lipid in treatment for psoriasis

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Research

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Abstract

Background: Psoriasis is widely accepted as a metabolic syndrome with significantly abnormal lipid metabolism and high blood lipids keep patients in a persistent low level of inflammatory condition. Hyperlipidemia and associated inflammatory reaction are believed to be the major risk factors contributing to the onset and recurrence of psoriasis. Peroxisome proliferator activated receptor-gamma (PPAR-γ) can effectively control the blood lipid level and inhibit inflammatory reaction.

Methods: Ozone autohemotherapy (OAHT) was applied to treat psoriatic patients. The psoriasis area and severity index (PASI) score and blood lipid level were used to evaluate the efficacy. PPAR-γ expression level and correlation analysis were used to determine the OAHT target involving psoriasis.

Results: We found that OAHT significantly decreased patients' PASI scores and lipid blood level. Furthermore, we found that PPAR-γ expression in CD4+ T cells from patients with psoriasis was significantly lower than healthy controls, furtherly, we detected PPAR-γ expression upregulated after treatment compared with before treatment. There, we performed the correlation analysis on PPAR-γ level and patients' PASI scores or lipid blood level. These results suggested OAHT might obviously improve patients' PASI scores and decrease lipid blood level by regulating PPAR-γ expression.

Conclusions: We found that OAHT can attenuate the condition of psoriatic patients and lower blood lipid by inducing PPAR-γ expression, suggesting that OAHT is effective in treating psoriasis and is worthy of further evaluations for its clinical applications.

Background

Psoriasis is a T-cell–mediated inflammatory cutaneous disease that is triggered by the combination of genetic, immune, obesity, and many other environmental factors. Hyperlipidemia and the associated inflammatory reaction are identified as the major risk factors contributing to the onset and recurrence of psoriasis[1]. White fat, extensively existing in the human body and containing adipocytes and immune cells [macrophages and dendritic cells (DC_S)], can secrete inflammatory and anti-inflammatory markers such as C-reactive protein (CRP), adiponectin, leptin, resistin, and lipoprotein-related phospholipase A2 (Lp-PLA2) to accelerate the occurrence and recurrence of psoriasis[2]. Previous studies showed that high-density lipoprotein cholesterol (HDL-C) could inhibit antigen or toxins to contact antigen-presenting cell and leukocyte adhesion to endothelial cells. In addition, apolipoprotein (a) (Apo(a)), lipoprotein (a) (Lp(a)), and low-density lipoprotein cholesterol (LDL-C) particles could promote the inflammatory response in patients with psoriasis[3]. These evidences indicate that the inflammatory response caused by hyperlipidemia is a key trigger in psoriasis. Admittedly, peroxisome proliferator activated receptor-gamma (PPAR-γ) can regulate the expression of certain enzymes involved in lipid metabolism and gene transcription, so as to regulate blood lipid levels and adipocyte differentiation[4, 5]. Thus, targeted regulation of PPAR-γ expression to control blood lipid and inflammatory response is a potential novel therapeutic strategy for psoriasis.

Ozone has been widely used in dermatology, including for infectious skin diseases, allergic diseases, erythema scaly diseases, and wound healing and ulcer recovery, and its therapeutic mechanism refers to antimicrobial effect, immune regulation, antioxidant defense, and apparent regulation[6]. The clinical application forms of ozone are also constantly enriched and improved. At present, local ozone-therapy strategies mainly include ozone bagging, ozone water soak, ozone oil smear, ozone point injection, and joint injection. The systemic application only means ozone autohemotherapy (OAHT). Studies have found that ozone can induce and activate the body's antioxidant enzyme system to produce superoxide dismutase (SOD), which can remove part of the free radicals produced in the inflammatory reaction and interfere with the inflammatory factors produced in the process of disease development[7]. In animal experiments, it has been found that low-dose ozone can improve the level of blood lipid and inhibit oxidative stress to alleviate the progress of atherosclerotic diseases[8]. However, how ozone regulates blood lipid has not been fully elucidated.

In this study, we observed the short-term therapeutic effects of OAHT on psoriasis. In addition, we investigated the mechanism of OAHT action on psoriasis by regulating the expression of PPAR-γ. In addition, we tried to identify that OAHT upregulated the expression of PPAR-γ to decrease lipid blood level and alleviated inflammatory response to treat psoriasis, indicating a potential therapeutic method for psoriasis.

Material And Methods

Enrolled patients

This study was approved by the IRB committee of the Third Xiangya Hospital, Central South University, China. A total of 30 patients who were diagnosed with psoriasis vulgaris and 30 healthy volunteers were enrolled in this study; we screened 12 psoriatic patients with hyperlipidemia to participate in this trial. All of the participants signed informed consent. Psoriasis disease activity was assessed by psoriasis area and severity index (PASI) score. The inclusion criteria included the following: age of 18 to 60 years old and diagnosed with psoriasis vulgaris by pathologic examinations and with hyperlipidemia [total cholesterol (TC)≧6.2 mmol/L; triglycerides (TG)≧2.3 mmol/L; LDL-C≧4.1 mmol/L; and HDL-C1.0 mmol/L). The exclusion criteria included the following: allergic to ozone; severe cardiovascular disease; local vessel intolerable treatment; abnormal coagulation; and systemic or local topical corticosteroid, immune inhibitor, or vitamin D3 derivative therapy within the past 2 weeks.

Medical ozone major autohemotherapy

All subjects were treated with OAHT, as follows: 100 to 150 mL of venous blood was mixed with medical pure oxygen and ozone (20 μg/mL) (Humares ozone therapy device, Germany), then transported back into the body, once every other day for 10 times in a course of treatment.

Blood lipid test

All participants needed to undergo blood lipid tests before and after treatment. Venous blood was collected in the morning before treatment after a night of fasting and the next day after treatment, and the serum was used for determination of TC, TG, HDL-C, and LDL-C according to protocols provided by the manufacturer of Hitachi 7060 automatic biochemical analyzer.

Evaluation of clinical photographs and reflectance confocal microscope images of skin lesions

All subjects received free treatment with OAHT only, and received no other drugs or treatments during the trial. Clinical photographs, PASI scores, and reflectance confocal microscope (RCM) images were used by the same professional physicians to evaluate disease severity. The PASI score contains skin lesion area, erythema color, erythema infiltration depth, and scale thickness according to the literature[9]. Each subject was assessed by RCM images from three different skin lesion sites. The scanned total thickness of skin was 51 layers ×3.05 μm vertically for every scan. Under RCM, epidermal thickness and infiltrated inflammatory cells were also evaluated before and after treatment.

Total $CD4^+$ T-cell isolation

Peripheral blood mononuclear cells (PBMCs) were separated from the blood of all patients before and after treatment with density gradient centrifugation (GE Healthcare, Switzerland). $CD4^+$ T cells were isolated by a positive selection using CD4 beads (Miltenyi, Germany) according to the manufacturer's instructions; the purity was generally greater than 95%. Then, the isolated $CD4^+$ T cells were collected for subsequent experiments.

RNA isolation and quantitative PCR (qPCR)

Total RNA was extracted from $CD4^+$ T cells according to the manufacturer's instructions of Trizol reagent (Invitrogen, USA). The mRNA was reverse-transcribed with the PrimeScript®RT reagent kit (TaKaRa Biotech Co., China), and each test consumed 1 μg of total RNA. The reaction mixture in RT-PCR contained 2 μL of cDNA, 10 μL of SYBR Premix Ex Taq™ (TaKaRa Biotech Co., China), and 400 nM sense and antisense primers to a total volume of 20 μL. qPCR was performed on a LightCycler®96 (Roche, Switzerland) thermocycler. The quantity of gene expression was calculated using comparative cycle thres-hold(CT) methods and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primers are listed in supplement table 1.

Western blotting

$CD4^+$T cells were lysated and proteins were extracted by Nuclear Extraction Reagent (Boster, China). Proteins were quantified by the Bradford assay (HyClone-Pierce, USA) followed by 10% vertical dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes (Millipore, USA). The PVDF membrane was blocked in 5% skim milk for 1 hour at room temperature and then incubated with an antibody against PPAR-γ (Abcam, China) for 12 to 16 hours at 4°C, followed by mouse anti-rabbit immunoglobulin G (IgG) antibody (H&L) (GenScript, USA) for

1 hour at room temperature. Proteins were detected with an ECL western blot detection kit (Thermo Scientific, USA). Band intensity was quantified using an ImageQuant^{TM} LAS 4000 mini (GE-Healthcare). Quantification of PPAR-γ was normalized to GAPDH by densitometry.

Statistical analysis

All of the diagrams and graphs reporting cumulative data were made using GraphPad Prism 6.0. The data are represented as the mean ± standard deviation SD or standard error of the mean (SEM). Distributions of the means were analyzed with non-parametric tests (SPSS 18.0, USA). Differences in individual treatments were analyzed by unpaired or paired t test. Statistical significance (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$) was assessed using a two-tailed unpaired Student *t* test for comparisons between two groups and one-way analysis of variance (ANOVA) with relevant post-hoc tests for multiple comparisons.

Results

In our clinic, we fortuitously discovered that psoriatic patients with hyperlipidemia had a remission of erythema, scales, and itching after OAHT. To confirm the curative effect of ozone therapy in psoriasis, we screened 12 patients diagnosed with psoriasis to participate in this trial, and we rigorously evaluated the disease severity of psoriatic patients with PASI scores before and after treatment. In the study, we assessed skin lesions by clinical photographs and RCM images. Notably, the skin lesions of patients with psoriasis improved with visible clinical changes (Fig. 1A). And, we observed apparent attenuation of inflammatory erythema and scales from RCM images (Fig. 1B). In addition, we further found the epidermis was markedly thinned and infiltrating inflammatory cells were decreased. Simultaneously, to quantify the thickness of the epidermis, we showed the statistical data of epidermis thickness with RCM with a count of the vertical swept layers in all participants (Fig. 1C). Correspondingly, to assess the effect of ozone therapy on psoriasis severity, we estimated with PASI score, and the PASI score markedly decreased after 10 treatments (Fig. 1D).

Based on previous clinical experience, the therapeutic potential of OAHT was demonstrated in patients with severe peripheral arterial disease, coronary disease, cholesterol embolism, severe dyslipidemia, Madelung disease, and sudden deafness of vascular origin[20]. Thus, we screened 12 patients with psoriasis with hyperlipidemia to detect the serum lipid before and after treatment. As expected, we observed a marked decrease in TC, TG, HDL-C, and LDL-C. The results showed that OAHT could lower the serum level of TG and TC (Table 1 and Fig. 2A, B) significantly, and increase the level of HDL-C (Table 1 and Fig. 2C). However, it had no effect on the level of LDL-C (Table 1 and Fig. 2D). These data demonstrated ozone action on psoriasis by decreasing the generation of circulating lipid in serum, although the exact mechanism of OAHT on lipid metabolism is unclear.

First, we measured PPAR-γ expression in peripheral blood $CD4^+$ T cells from patients with psoriasis and healthy controls with quantitative RT-qPCR and western blotting methods and found that it is

downregulated in $CD4^+$ T cells from patients with psoriasis compared with healthy controls (Fig. 3A&B). Notably, we observed a significant correlation between the PPAR-γ expression in $CD4^+$ T cells from patients with psoriasis and PASI scores in serum (Fig. 3C). Thus, we further analyzed the relevance between PPAR-γ expression level and lipid level, and found it was negatively correlated with circulating TG level in serum (Fig. 3D), whereas it had no significant relationship with TG and LDL-C levels (Fig. 3E&F). Visually, it suggested a positively relevant trend of HDL-C without statistical significance (Fig. 3G).

Based on the aforementioned studies, we detected PPAR-γ expression in peripheral blood $CD4^+$ T cells from patients with psoriasis pre- and post-treatment to further investigate the mechanism of ozone to regulate the level of serum lipid in psoriasis. As assessed by qPCR and western blot techniques, PPAR-γ expression was upregulated in $CD4^+$ T cells from patients with psoriasis after treatment compared with before treatment (Fig. 4A and B). Simultaneously, we performed RT-PCR to detect the relative expression levels of key cytokines [tumor necrosis factor-alpha (TNF-α), IL-6, transforming growth factor-β (TGF-β), IL-17a, and IL-23] in the pathogenesis of psoriasis. Altogether, the levels of TNF-α, IL-6, and IL-17a in $CD4^+$ T cells from patients with psoriasis were decreased by OAHT, whereas it had no effect on the expression of TGF-β and IL-23 (Fig. 4C). Therefore, according to these research outcomes, we preliminarily state that OAHT increased PPAR-γ expression in psoriatic $CD4^+$ T cells to reduce inflammation to relieve psoriatic disease.

Discussion

Emerging studies indicated that there was significant abnormal lipid metabolism in patients with psoriasis, which seriously affected the disease outcome and the quality of life[10]. It is generally believed that hyperlipidemia keeps patients in a state of low inflammatory response, inducing or aggravating the progression of the disease. The inflammation response in local skin lesions of psoriasis is accompanied by a systematic cascade reaction. Immune cells released into systemic circulation lead to increased risk factors for metabolic diseases; conversely, systemic inflammation induced by obesity, metabolic syndrome, and other diseases can also aggravate local skin lesions, forming a vicious cycle[11, 12]. A study conducted by Naldi et al.[13] found that obesity was a modifiable, independent, psoriasis-associated risk factor, accounting for 16% of all psoriatic episodes. A further study by Wolk and Sabat[14] stated that the risk of developing psoriasis increased by 9% for every unit increase in body mass index (BMI). In obesity-related metabolic diseases, the number of macrophages, $CD8^+$ T cells, and helper T (Th) 1 cells increases, and these secrete a large amount of IL-6, TNF cytokines, interferon-gamma (IFN-γ), and other pro-inflammatory cytokines[15]. Whereas in psoriasis, the activation of Th1, Th17, and Th22 cells prompts the lymphocytes and keratinocytes in local skin lesions to produce a variety of inflammatory mediators, which is mainly initiated by pro-inflammatory cytokines and adipokines produced by adipose tissue; this process leads to insulin resistance and endotheliocytes injury, further motivating glucose and lipid metabolism disorders, vascular dysfunction, and immune cell infiltration[16, 17]. Thus,

hyperlipidemia and associated inflammatory reaction are believed to be the major risk factors contributing to the onset of psoriasis.

Ozone therapy is playing an increasing role in many inflammatory diseases due to its bacteriostasis, oxidative stress, immune regulation, and epigenetic regulation. Our previous studies have found that topical ozone therapy is safe and effective for the treatment of stable psoriasis vulgaris, with an efficacy equivalent to that of intermediate-acting glucocorticoids, and we found that ozonated oil is suitable for the long-term care and management of psoriasis[18]. A study showed that the application of OAHT had achieved an inspiring response in the field of anti-oxidation and aging delay[19]. Since medical OAHT was initiated in Germany in the 1950s, some scholars have applied it for the treatment of chronic fatigue and anti-aging, due to its anti-oxidation action. In 2001, Tylicki innovatively confirmed that atherosclerotic ischemic diseases benefit from OAHT treatments. In his trial, he explained the mechanisms by referring to lowering fibrinogen concentration and blood viscosity and decreasing plasma cholesterol levels[20]. Collectively, multiple studies have shown that ozone can improve the level of lipid peroxidation and increase the activity of lipid metabolic enzymes, thus participating in lipid metabolism and decelerating blood lipids in the body. In this study, we further clarify the specific mechanism that ozone decreases blood lipid level action on regulating PPAR-γ expression to treat psoriasis, which will provide more theoretical basis for ozone therapy in psoriasis.

PPAR-γ is a kind of nuclear transcription factor activated by ligand with adipose tissue specificity. PPAR-γ can be induced by fatty acids and exogenous peroxisome proliferator to regulate the expression of certain enzymes involved in lipid metabolism and gene transcription, so as to regulate blood lipid levels and adipocyte differentiation[21]. In addition, it also mediates the expression of multiple nuclear target genes, such as nuclear factor (NF)-κB[22], signal transducer and activator of transcription (STAT),[23] and activator protein (AP)-1[24], thus blocking the transcription of pre-inflammatory factors and exhibiting multiple biological effects. Studies have confirmed that PPAR-γ plays a critical role in regulating Th17/Treg cell balance. PPAR-γ agonists inhibit the differentiation of Th17 cells while promoting Treg polarization, and suppress inflammatory responses by inducing the production of IL-10 and inhibiting the generation of IL-17a, IL-17f, IL-22, and IL-23[25]. The mouse models experiment showed that activation of PPAR-γ action on Treg cells can alleviate inflammatory response and increased insulin sensitivity[26]. Our data verified that the expression level of PPAR-γ in $CD4^+$ T cells in psoriasis vulgaris was significantly decreased compared with the normal control group. After ozone treatment, PPAR-γ level significantly increased in psoriasis, and inflammatory factor expression level also decreased obviously. Moreover, there was a marked improvement in skin lesions, which might be associated with ozone-induced PPAR-γ expression to inhibit the transcription and expression of inflammatory factors. The study substantiated that ozone-mediated PPAR-γ level plays a key role in controlling the state of the inflammatory response to treat psoriasis.

Limitations

The limitations of this study include: 1) insufficient sample size that is unable to reduce variances due to individual variables in dietary habit; and 2) technological limitations of ozone autohemotherapy applied in cell and animal experiments to further verify the mechanisms.

Conclusion

Collectively, we found that OAHT can lower blood lipid and attenuate inflammatory responses in psoriasis by upregulating the expression of PPAR-γ, suggesting that OAHT is an effective treatment for psoriasis and is worthy of further clinical evaluation and application.

Abbreviations

PPAR-γ, Peroxisome proliferator activated receptor-gamma; TG, triglycerides; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; NF-κB, nuclear factor-κB; OAHT, ozone autohemotherapy; PASI, psoriasis area and severity index; RCM, reflectance confocal microscopy; SOD, superoxide dismutase; DCs, dendritic cells; Lp-PLA2, lipoprotein-related phospholipase A2.

Declarations

Authors' contributions

Study design: Jinrong Zeng, Jianyun Lu, Shu Ding. Data acquisition: Jinrong Zeng, Jianyun Lu, Zhen Tang, Jianhua Dou. Data analysis: Shu Ding, Xiaoliang Tong, Lihua Gao. Data interpretation: Zhen Tang, Xiaoliang Tong, Jianhua Dou, Lihua Gao. Drafting of the manuscript: Jianyun Lu, Shu Ding. Revision of the manuscript: Jinrong Zeng, Lihua Gao. All authors contributed significantly to this work and agreed to be accountable for the work. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

This study was approved by the IRB committee of the Third Xiangya Hospital, Central South University, China

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

Not applicable.

Availability of data and materials

The data of the current study are available at request for scientists wishing to use them with kind full permission.

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553.

Table

Due to technical limitations, table 1 is only available as a download in the supplemental files section.

Figures

Figure1.

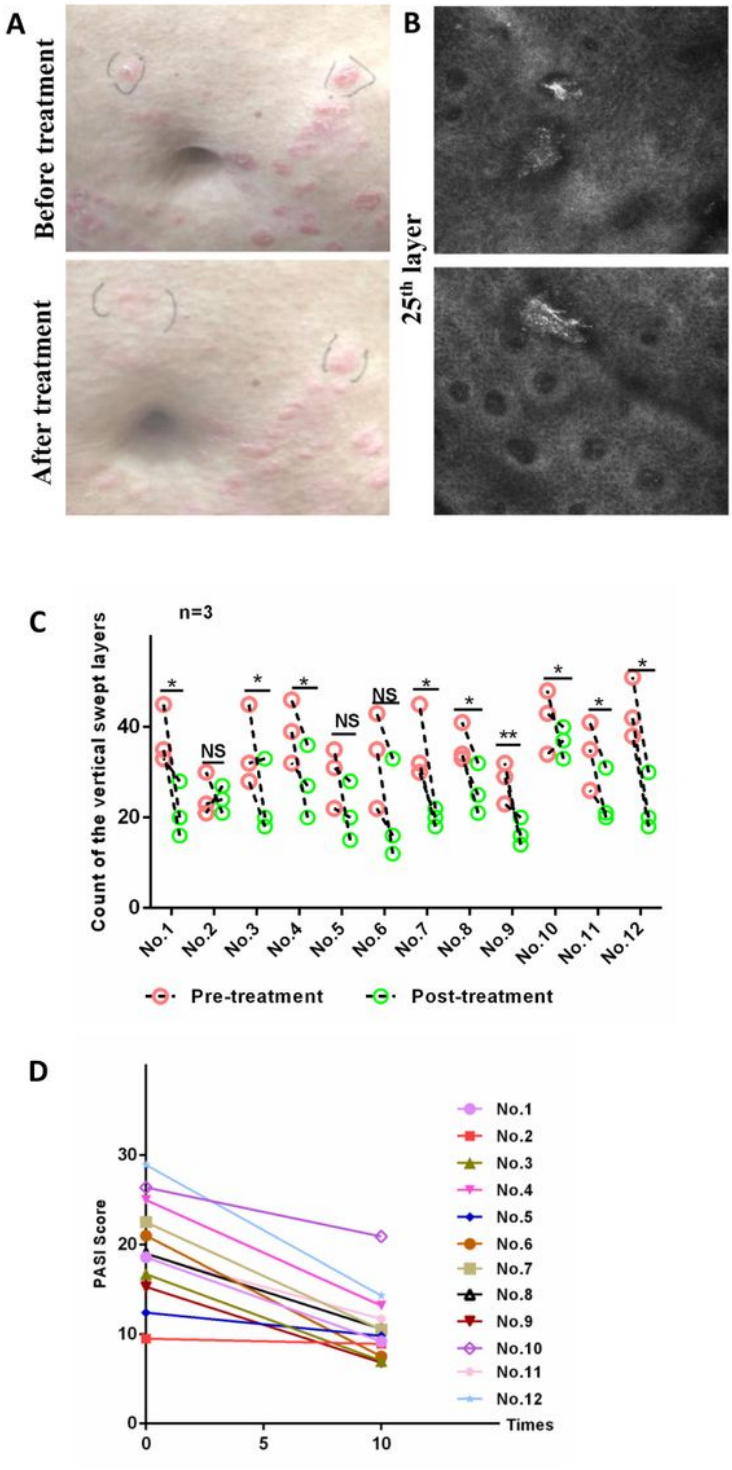

Figure 1

OAHT improves the condition of psoriatic skin lesions (A) Clinical photographs of a psoriatic skin lesion before and after treatment with OAHT. (B) Evaluation of RCM images showing the 25 scanning layers before and after treatment. (C) Vertically swept layers of quantitative RCM images for an assessment of thickness of the epidermis; (D) PASI scores for all participants. Note: * = P < 0.05; ** = P < 0.01; *** = P < 0.001; NS = no statistical significance.

Figure2.

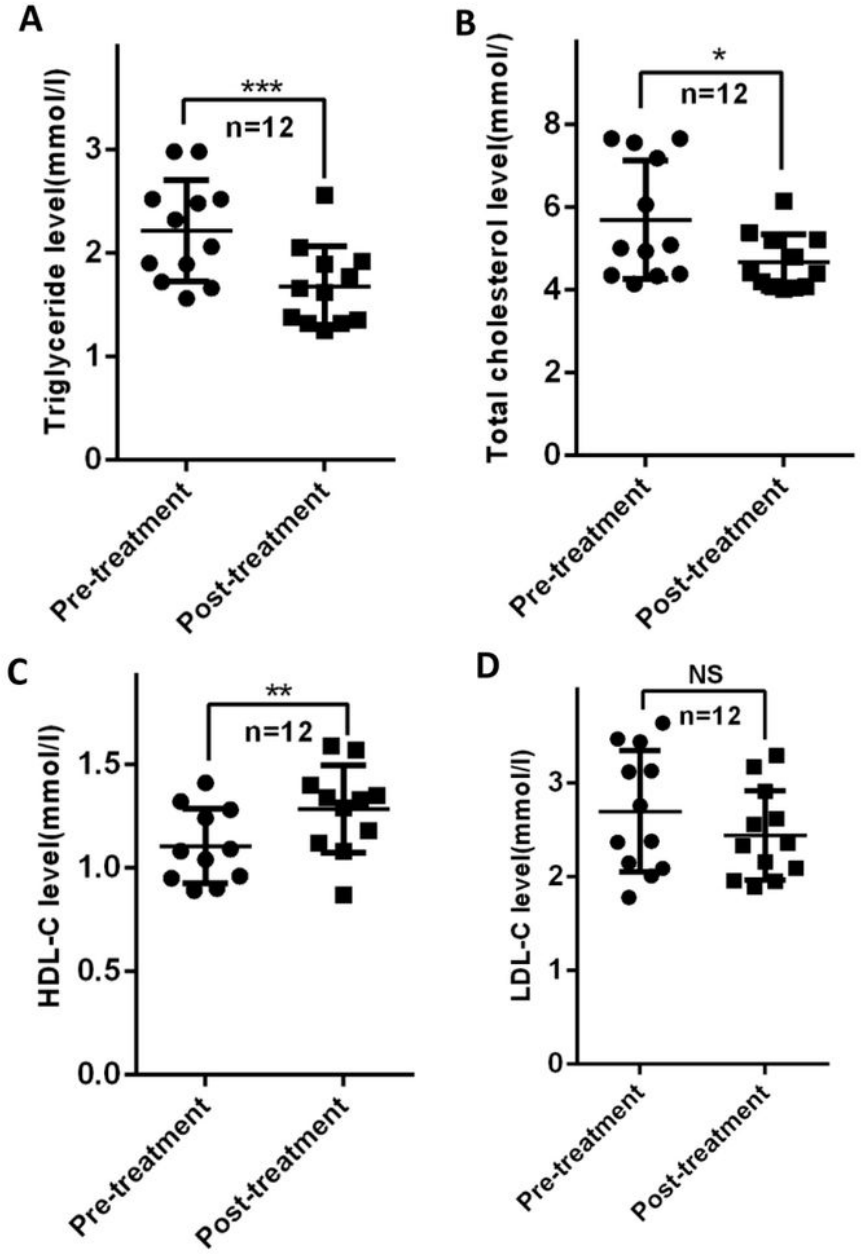

Figure 2

Statistical analysis of TG, TC, HDL-C, and LDL-C in serum before and after treatment. Statistical analysis results of TG (A), TC (B), HDL-C (C), and LDL-C (D) in serum from enrolled patients before and after treatment.

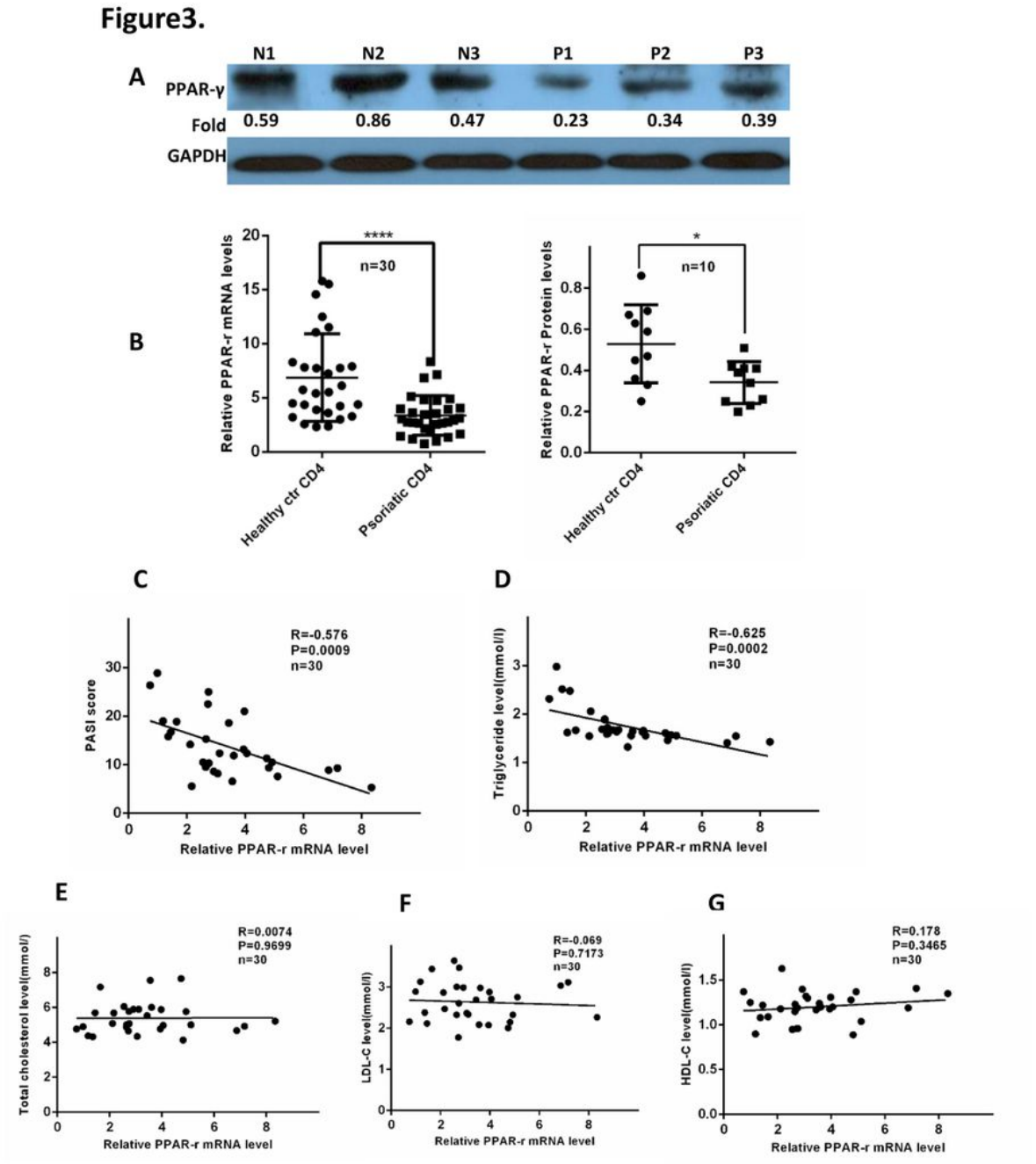

Figure 3

PPAR-γ expression level and correlation analysis in CD4+ T cells from the peripheral blood of patients with psoriasis. (A) PPAR-γ protein expression level in CD4+ T cells from the peripheral blood of patients with psoriasis and normal controls by western blot. (B) Statistical analysis results of PPAR-γ expression level in CD4+ T cells from the peripheral blood of patients with psoriasis and normal controls by qPCR

and western blot. Correlation analysis of PPAR-γ expression level and PASI score (C), triglyceride (D), total cholesterol (E), LDL-C (F), and HDL-C (G) before treatment.

Figure4.

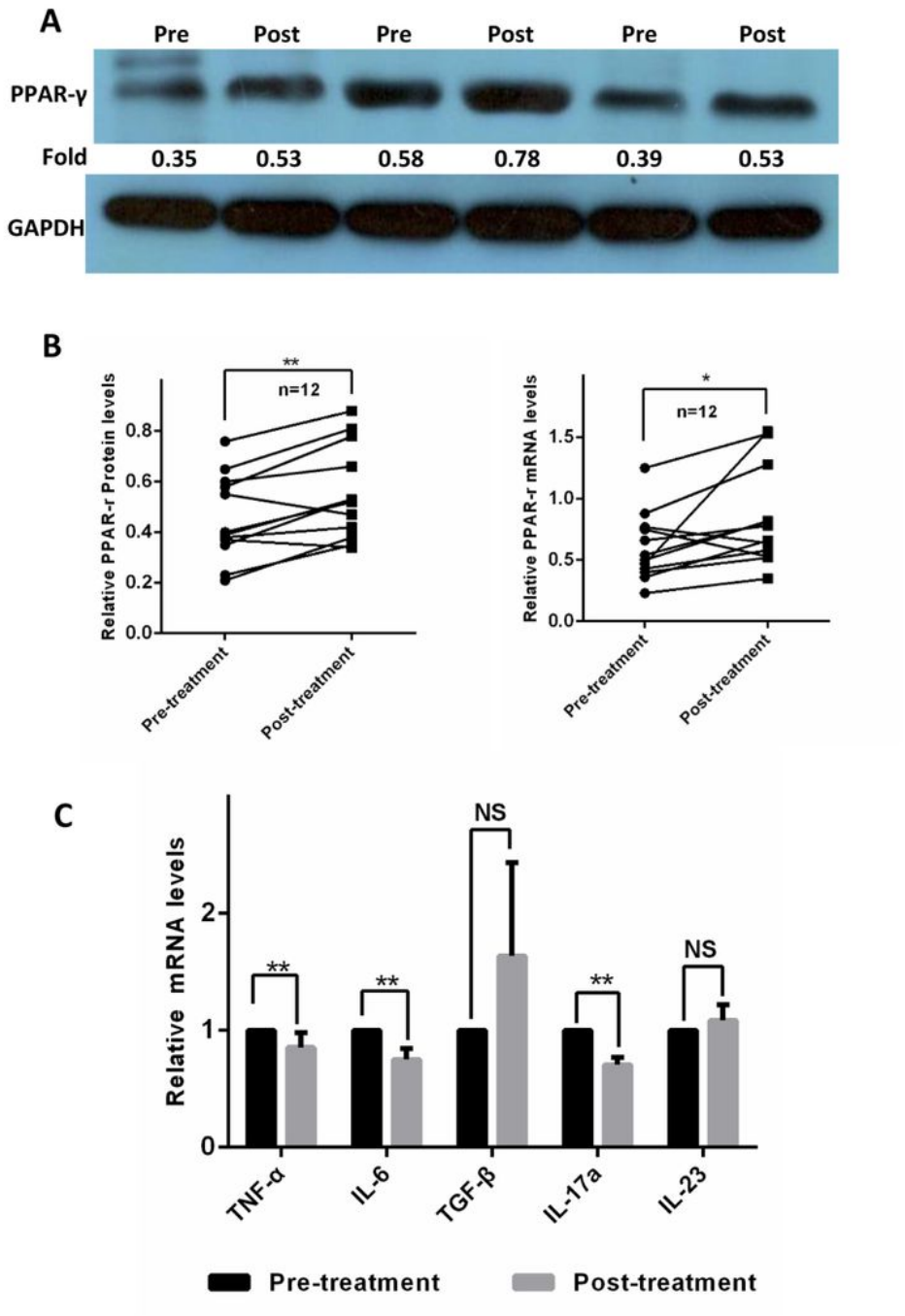

Figure 4

OAHT may increase PPAR-γexpression to relieve psoriatic disease (A) Western blot to detect expression levels of PPAR-γin CD4+ T cells from peripheral blood of patients with psoriasis before and after treatment. (B) PPAR-γ expression level statistical graph of enrolled patients with psoriasis in before and

after treatment by qPCR and western blot. (C) q-PCR to detect relative cytokines in CD4+ T cells from peripheral blood of patients with psoriasis before and after treatment.

Supplementary Files

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- SupplementaryTable1.docx
- Table1.jpg