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ORIGINAL PAPER

Malondialdehyde and advanced oxidation protein products are not increased in psoriasis: a controlled study

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Abstract

This study investigated oxidative stress in patients with psoriasis of low and medium disease activity. We measured advanced oxidation protein products (AOPP) and malondialdehyde (MDA) in plasma using UV-spectrophotometry and high performance liquid chromatography connected to a fluorescence detector in 84 patients and 84 matched healthy subjects. AOPP is a marker of protein oxidation due to inflammation, whereas MDA is a hydroxyl radical initiated lipid peroxidation product. Clinico-demographic variables including age, gender, disease severity, and fatigue were assessed in relation to AOPP and MDA. Disease severity was evaluated with the Psoriasis Area and Severity Index and the Dermatology Life Quality Index. Median (interquartile range, IQR) AOPP concentrations were 66 µmol/l (IQR 54–102) in patients and 69 µmol/l (IQR 55–87) in healthy subjects ($P=0.75$). Median plasma MDA concentrations were significantly lower in patients than in healthy subjects (0.68 µM, IQR 0.54–0.85 vs. 0.76 µM, IQR 0.60–0.97; $P=0.03$). Plasma levels of AOPP and MDA did not indicate oxidative stress in patients with mild psoriasis. Higher AOPP concentrations were associated with male gender, high body mass index, and high hemoglobin values. Elevated MDA concentrations were associated with advanced age and male gender. No associations with disease severity were detected. Although, the two selected biomarkers do not provide a complete measure of oxidative damage, our study demonstrates that a number of physiological and methodological factors influence the levels of MDA and AOPP. Such methodological issues are important to consider when interpreting results using these biomarkers in patients with psoriasis.

Keywords Oxidative stress · Advanced oxidation protein products · AOPP · Malondialdehyde · MDA · Psoriasis

Abbreviations

AOPP	Advanced oxidation protein products
MDA	Malondialdehyde
ROS	Reactive oxygen species
IQR	Interquartile range
PASI	Psoriasis area and severity index
QoL	Quality of life
DLQI	Dermatology life quality index
fVAS	Fatigue visual analog scale
HADS-D	The depression subscale of the Hospital Anxiety and Depression Scale
TBARS	Thiobarbituric acid reactive substances
HPLC-F	High-performance liquid chromatography connected to a fluorescence detector

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Introduction

Psoriasis vulgaris is a chronic, immune-mediated inflammatory skin disease, characterized by sharply demarcated erythematous, scaly plaques and a predilection for extensor prominences and the scalp. The etiology and pathogenesis is incompletely understood, but increasing evidence points

to a dysregulation of T cells that become activated by an unknown autoantigen, or by innate immunity cells, such as dendritic cells and macrophages, that respond to danger signals [8]. Psoriasis is associated with low systemic inflammatory activity [11]. A number of conditions such as obesity, atherosclerosis, cardiovascular disease, diabetes type 2, and depression are related to psoriasis [41]. It is yet to be established the role of systemic inflammation in forming these relations.

It has been suggested that reactive oxygen species (ROS) might be involved in psoriasis pathophysiology [7]. In the psoriatic lesions there are abnormal differentiation of keratinocytes and infiltration of inflammatory cells including activated neutrophils that produce large amounts of superoxide anion (O_2^-), hydrogenperoxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$) [10]. ROS are constitutively produced in normal physiological aerobic metabolism. Under inflammatory conditions, these highly reactive molecules are produced and released in excess. The unstable nature of ROS promotes oxidation and molecular targets for potential damage include proteins, lipids and DNA. This may result in cell damage or inactivation. The term "oxidative stress" describes the domination of ROS over anti-oxidant defense mechanisms [32]. ROS interfere with production of proinflammatory cytokines, such as the nuclear factor kB transcription factor pathway, that is known to be involved in the psoriasis pathogenesis [28]. Dimethylfumarate, a drug used for treatment of moderate to severe psoriasis for decades, is known to upregulate antioxidative pathways [15]. Although the data are somewhat conflicting, most studies on oxidative stress in psoriasis have reported increased levels of oxidation products often associated with psoriasis severity [2–4, 17, 38, 49]. Furthermore, it has been demonstrated that oxidative stress increases the incidence of cardiovascular disease and its related complications in patients with psoriasis [2].

To study redox balance, precise measurement of oxidative biomarkers is desirable. An important caveat in measurements of oxidative stress is the lack of applicable methods for analyzing ROS directly in humans [19]. Instead, indirect measures are obtained by quantifying the damage or impact that reactive molecules have on lipids, proteins, DNA, or the anti-oxidant defense system. These analytes are typically referred to as biomarkers of oxidative damage.

Currently, although no available methods meet the analytical validation criteria for an "ideal" biomarker, some are considered better than others [19]. Previous studies on psoriasis and oxidative stress have frequently measured the level of lipid oxidation by applying simple spectrophotometry without chromatographic separation [3, 17, 22, 38]. These methods are inexpensive and easy to perform, but are interfered by other absorbing or light scattering compounds such as lipids [9, 13]. Concern has, therefore, been raised over the analytical and clinical validity of oxidative stress biomarkers [9, 18, 21]. Patients with psoriasis have a propensity for obesity, and dyslipidemia seems to be associated with the severity of psoriasis [1, 24, 37]. This potential source of interference might, therefore, be of particular importance to consider when spectrophotometric methods are applied in this patient group.

To reduce interference from other fluorescent compounds, we used HPLC connected to a fluorescence detector for MDA analysis [9], one of the most prevalent degradation product of $^{\cdot}OH$ oxidation of polyunsaturated lipids [6]. Furthermore, we measured advanced oxidation protein products (AOPP) by an optimized UV-spectrometry method where possible interference from lipids was reduced [20]. AOPP is a non-specific measure of oxidized proteins, based on light absorption at 340 nm, and comprises several chromophores, of which proteins cross-linked by dityrosine, pentosidine and carbonyls are important constituents. Formation of dityrosine results from activation of neutrophil granulocytes and the subsequent release of myeloperoxidase and formation of hypochlorous acid (HOCL), and may, therefore, be a useful measure of oxidative stress due to inflammation [10]. Analysis of AOPP by UV-spectrophotometry may suffer from interference from other compounds, but is the current standard monitoring method. To the best of our knowledge, only one other study has focused on AOPP in patients with psoriasis [49]. We compared measures in patients with psoriasis to age- and gender-matched healthy subjects. Furthermore, we aimed to explore potential associations with clinical variables that might influence—or be influenced by—ROS levels; e.g., fatigue and mental depression [34, 40].

Materials and methods

Patients

Eighty-four patients over 18 years of age and with chronic plaque-type psoriasis were included in the study. Exclusion criteria were other non-plaque types of psoriasis; cancer; psoriatic arthritis; untreated hyper- or hypothyroidism; chronic inflammatory rheumatological diseases or autoimmune diseases other than psoriasis. A total of 120 patients were examined by a dermatologist (IMS) and screened for potential participation, of these 84 were included. The majority were consecutively identified based on referral letters to the outpatient clinic, Department of Dermatology, Stavanger University Hospital, from November 6, 2012 to May 19, 2015, and only three patients were recruited from the follow-up clinic. The same cohort of patients were used in a previous study, which has the detailed enrolment process described [39].

Healthy subjects

The 84 healthy control subjects fulfilled the same inclusion and exclusion criteria as the patients, except for the diagnosis of psoriasis, and were matched with patients by age (± 3 years) and gender.

Demographic and clinical characteristics

All participants underwent a general clinical examination. Demographic and clinical data were collected. Medical history, current medication and tobacco smoking were recorded. Hypertension was defined by a systolic blood pressure of ≥ 140 mmHg, a diastolic blood pressure of ≥ 90 mmHg, or receiving antihypertensive therapy at the time of examination. Additionally, information of following comorbidities was recorded: a history of diabetes, overt cardiovascular disease (e.g., myocardial infarction, angina pectoris, stroke, transient ischemic attack, or cardiac dysrhythmia), migraine, or respiratory disease (e.g., asthma or chronic obstructive pulmonary disease). Concomitant depression was assessed with the depression subscale of the Hospital Anxiety and Depression Scale (HADS-D) [29]. We defined depression as a score ≥ 8 on the HADS-D [31].

Clinical assessment and disease activity

To measure the severity of skin disease, we implemented the Psoriasis Area and Severity Index (PASI) [16]. The PASI are subgrouped into mild (PASI < 7), moderate (PASI 7–12), and severe (PASI > 12) [33].

Quality of life (QoL) was evaluated with the Dermatology Life Quality Index (DLQI). This widely used self-reported questionnaire consists of 10-items. The DLQI was used to evaluate the impact of skin disease on QoL. The scores ranged from 0 (no impairment) to 30 (maximal impairment). A score of 10 is considered to represent a significant influence on patient QoL [14].

Measures of fatigue

We measured fatigue severity with the generic, uni-dimensional, widely used fatigue Visual Analog Scale (fVAS), with a scale of 0–100 mm [46]. A score of 0 denoted "no fatigue" and 100 denoted the "worst possible fatigue".

Blood analyses

Blood samples were drawn by venous puncture in the morning after an overnight fast. Serum samples for routine laboratory analyses were separated from blood cells (centrifuged 7 min at 2600×*g* at 22 °C) within 2 h and analyzed within 5 h after collection. Serum creatinine and CRP (C-reactive protein) and were measured on Abbott Architect c16000 analyzer (Abbott Diagnostics, Illinois, USA). Estimated glomerular filtration rate (eGFR) was estimated using the CKD Epidemiology Collaboration (CKD-EPI) equation [26]. Hemoglobin was measured in venous EDTA-blood by XE-5000 (Sysmex, Kobe, Japan).

Peripheral venous EDTA blood samples for AOPP and MDA analyses was centrifuged (15 min at 2500×*g* at 4 °C) within 30 min of sampling, and aliquots of plasma were stored at − 80 °C until analysis.

MDA measurements

MDA was extracted from plasma, after derivatization with thiobarbituric acid (MDA-TBA_2), then analyzed with high performance liquid chromatography connected to a fluorescence detector (HPLC-F, excitation/emission wavelengths 525/560 nm) with a procedure modified from Yagi [48], Lykkesfeldt [27], and Seljeskog [35]. Briefly, calibration standards of 1,1,3,3-tetraethoxypropane (TEP) were prepared in concentrations that ranged from 0.1 to 10 µM. Sulfuric acid (H_2SO_4, 350 µl, 42 mM), butylated hydroxytoluene (BHT, 50 µl, 0.7 mM in 40% ethanol), and thiobarbituric acid (100 µl, 0.67 g/100 ml 50% acetic acid, v/v) were added to standards (25 µl) and plasma samples (25 µl). The mixtures were heated at 95 °C for 1 h and cooled on ice Butanol (500 µl) and phosphotungstic acid (10 µl, 10% v/v) were added, and the samples were mixed and centrifuged (16,000×*g* at 4 °C, 3 min). The supernatant (400 µl) was evaporated to dryness and dissolved in a mixture of mobile phase A and methanol (90:10), prior to HPLC-injection (10 µl onto a Gemini® 3 µm NX-C18 110 Å, 2 mm × 50 mm LC-column; Phenomenex, USA). The HPLC mobile phase consisted of (A) 10% methanol in 50 mM potassium phosphate buffer (pH 7.0) and (B) 90% methanol; the elution profile was 10–75% B in 8 min. The flow rate was 0.25 ml/min. The reproducibility of the method was monitored by analyzing aliquots of a plasma control sample with each assay. This analysis demonstrated < 10% intra- and inter-assay coefficients of variation for MDA ($n = 4$). The limit of detection (LOD) and the limit of quantification (LOQ) were 0.06 µM and 0.10 µM, respectively.

AOPP measurements

AOPP was analyzed according to Hanasand et al. [20], with minor modifications. Briefly, 20 µl plasma was transferred (in triplicate) to wells in a 96-well microplate (Costar®, UV-transparent bottom flat), and 180 µl 0.2 M citric acid was added. Different concentrations of chloramine-T (2–75 µmol/l) diluted in citric acid were used as calibration standards and potassium iodide (10 µl 1.19 M) was added to develop color. After 10 min on a microplate shaker, the

absorbance of AOPP was read at 340 nm, and the relative amount of turbidity in the samples was detected at 630 nm. The calibration curve was constructed with chloramine-T concentration as one variable and the subtracted absorption (340–630 nm) as the other variable. The curve was fitted with least-squares regression ($r^2 > 0.99$). Consequently, AOPP concentrations are expressed as μmol/l chloramine-T equivalents. AOPP intra- and inter-assay coefficients of variation were < 3.8% ($n = 6$) and 6.1% ($n = 5$), respectively, determined by analyzing freshly thawed aliquots of a control plasma sample. The LOD and LOQ were 0.68 and 2.27 μM, respectively. The total protein concentrations in plasma were determined with the Bradford assay [5], according to the 96-well protocol described by the manufacturer (Sigma-Aldrich).

Statistics

Most continuous data were not normally distributed; thus, they are presented as the median (IQR) or as counts and percentages, for categorical data. For pairwise comparisons, the Mann–Whitney *U* test was used, when data were not normally distributed, and the *t* test was used, when data were normally distributed. To improve model fitting, we used natural log-transformed values in linear regression analyses that assessed associations between measures of oxidative stress and potential influential factors. We determined the estimated effect (beta) and 95% confidence interval (CI); the *P* value, for Wald tests of hypotheses of no effect; and the R^2 value, to evaluate model fits. Variables that showed *P* values < 0.25 in univariable analyses were subsequently selected for multivariable regression analyses. The final regression models included only explanatory variables with $P < 0.05$. We performed a stepwise backward selection, with age and gender forced into the model. We determined that no excluded variable would attain this significance level and/or substantially change other effect estimates, when subsequently included in a forward selection. In the regression analyses, data for one patient and one healthy subject were excluded, due to missing HADS-D scores. In addition, the data for one patient were deemed highly influential, based on Cook's distance; thus, those data were excluded from the regression analyses. All statistical analyses were performed with IBM SPSS Statistics v. 23.

Results

Compared to healthy controls, patients exhibited a somewhat higher BMI and significantly higher rate of smoking. The patient and control groups were similar with regards comorbidities except for hypertension and depression. The median duration of fasting (counting from midnight) were 10.3 h and 8.8 h, in patients and healthy subjects, respectively (difference = 1.5 h; $P = 0.001$). The most common times for blood sample sampling were 11:15 am for patients and 9:45 am for healthy subjects (difference = 1.5 h; $P < 0.001$).

Seventy percent of patients had mild disease, with a median (IQR) PASI of 6.0 (4.5–7.3). The median DLQI score was 10 (IQR 6–13). The median disease duration of psoriasis was 14.0 years, with an interquartile range (IQR) of 8–24 years. Only two patients were taking oral medications (one methotrexate and the other acitretin). The remaining 82 patients received, at most, topical treatment, but no systemic treatment. All patients, except one, were naïve to biological medications. Demographic, clinical and biochemical data, comorbidities, and current medications of patients and healthy subjects are given in Table 1.

AOPP and MDA concentrations

Plasma concentrations of AOPP and MDA in patients and healthy subjects are shown in Fig. 1. Median (IQR) plasma AOPP concentrations were 66 μmol/l (54–102) in the patient group and 69 μmol/l (55–87) in the healthy group ($P = 0.75$). MDA concentrations in patients with psoriasis were approximately 11% lower than in healthy subjects (0.68 μM, IQR 0.54–0.85 vs. 0.76 μM, IQR 0.60–0.97; $P = 0.03$).

AOPP and MDA concentrations in relation to gender

AOPP and MDA concentrations were considerably higher in male patients compared to female patients (84 μmol/l, IQR 63–137 vs. 56 μmol/l, IQR 46–68; $P < 0.01$ and 0.76 μM, IQR 0.59–0.93 vs. 0.60 μM, IQR 0.47–0.75; $P < 0.01$, respectively). Likewise, healthy males had significantly higher AOPP and MDA concentrations than healthy females (Fig. 1).

AOPP and MDA concentrations in relation to disease severity

Patients with PASI ≥ 7 had higher AOPP concentrations than patients with PASI < 7 (77 μmol/l, IQR 57–173 vs. 64 μmol/l, IQR 53–91; $P = 0.05$). MDA concentrations were not significantly different between patients with PASI ≥ 7 and those with PASI < 7 (0.67 μM, IQR 0.54–0.89 vs. 0.69 μM, IQR 0.54–0.85; $P = 0.78$).

Associations between AOPP and selected variables in psoriasis patients

Selected demographic and clinical variables of patients were tested for associations with AOPP concentrations in univariable linear regression analyses. We found that male

Table 1 Comparison of demographic and clinical characteristics between patients with psoriasis and healthy subjects (controls)

Characteristic	Patients with psoriasis ($N=84$)	Control subjects ($N=84$)	P
Demographics			
Age, years	45 (33–55)	44 (34–55)	
Male sex	51 (61%)	51 (61%)	
Psoriasis duration, years	14 (8–24)		
Current smoking	25 (30%)	13 (16%)	0.03
No. of cigarettes	0 (0–7)	0 (0–0)	0.02
Clinical data			
BMI, kg/m^2	27 (24–30)	26 (23–29)	0.08
HADS-D ($N=83$)	4.0 (1–7)	1.0 (0–3)	< 0.001
fVAS	50 (22–67)	11 (3–20)	< 0.001
PASI	6.0 (4.6–7.3)		
PASI < 7	59 (70%)		
DLQI	10 (6–13)		
Biochemical data			
CRP, mg/l	2.2 (0.7–4.9)	1.3 (0.5–3.3)	0.01
Hb, g/100 ml	15 (1.1)	15 (1.4)	0.71
GFR, ml/min/1.73 m^2	98 (14.0)	94 (16.5)	0.18
Comorbid disease			
Hypertension	44 (52%)	28 (33%)	0.01
Diabetes	4 (5%)	1 (1%)	0.17
CVD	5 (6%)	1 (1%)	0.10
Respiratory disease	8 (10%)	5 (6%)	0.39
Depression (HADS-D ≥ 8) ($N=83$)	20 (24%)	1 (1%)	< 0.001
Medication			
Antidepressants	4 (5%)	0 (0%)	0.04
Levothyroxine	1 (1%)	0 (0%)	0.32
Immunosuppressive drugs	2 (2%)	0 (0%)	0.16
Beta-blockers	8 (10%)	2 (2%)	0.05

Continuous data are presented as mean (standard deviation), for Hb and GFR, and as the median (interquartile range) for other variables. Categorical data are presented as the number (percentage)

BMI body mass index, *HADS-D* depression subscale of the Hospital Anxiety and Depression Scale, *fVAS* fatigue Visual Analog Scale, *PASI* Psoriasis Area and Severity Index, *DLQI* Dermatology Life Quality Index, *CRP* C-reactive protein, *Hb* hemoglobin, *GFR* glomerular filtration rate, *CVD* cardiovascular disease

gender, elevated BMI, hypertension, elevated hemoglobin, and diabetes were significantly associated with elevated AOPP concentrations (Table 2). There were no associations between AOPP and age or disease duration, nor between AOPP and smoking. Next, we selected variables with significance levels < 0.25 in univariable regression analyses including age and gender, for inclusion in multiple regression analyses. In stepwise backward and forward selections, we identified a final model that explained 39% of the variance in AOPP (Table 3). This model indicated that when adjusted for age, male gender, a high BMI, and elevated hemoglobin levels were the only variables associated with increased plasma AOPP concentrations.

Associations between MDA and selected variables in psoriasis patients

Among our patients, simple linear regression analyses revealed three factors that were independently associated ($P < 0.05$) with increasing MDA concentrations: male gender, increasing age, and hemoglobin levels (Table 4). There were no associations between MDA and disease duration, or between MDA and smoking. The final multiple regression model, which explained 18% of the variance in MDA, indicated that only age and male gender were significant factors (Table 5).

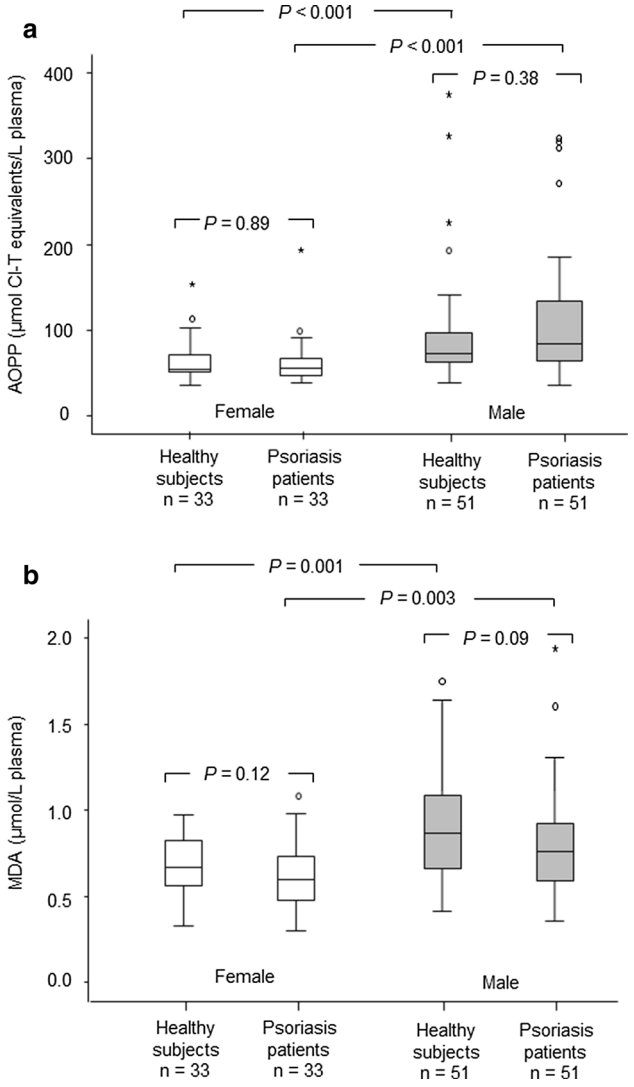

Fig. 1 Plasma oxidation product concentrations in patients with psoriasis and age- and gender-matched healthy subjects. Results are stratified by gender. (Top) AOPP levels show protein oxidation expressed in chloramine-T (Cl-T) equivalents; (bottom) MDA levels show lipid peroxidation. *AOPP* advanced oxidation protein products, *MDA* malondialdehyde

Associations with fatigue and depression in psoriasis patients

Linear regression analyses indicated that neither AOPP nor MDA were associated with fatigue ($P = 0.80$ and $P = 0.27$, respectively) or with depression ($P = 0.70$ and $P = 0.30$, respectively).

Effects of fasting time, age and gender on significant results

The difference in AOPP levels between patients with PASI ≥ 7 and PASI < 7 became insignificant in multivariable regression analyses adjusted for the fasting duration, the time of day for blood sample collection, age, and gender (Supplementary Table 1).

Differences in fasting durations and the time of day that blood samples were drawn explained some of the differences in MDA levels between patients and healthy subjects. After adjustments, the differences became insignificant (Supplementary Table 2).

Discussion

The main finding in this study was that plasma AOPP and MDA concentrations were not different between patients with psoriasis and healthy subjects. This implies that systemic inflammation in patients with psoriasis of low to medium disease severity is not reflected in oxidative stress as measured by AOPP and MDA. Our finding contrasted with the majority of previous studies that advocated a state of oxidative stress in these patients. An explanation for this discrepancy could be that, although psoriasis is generally characterized by mild systemic inflammation, the amounts of ROS produced and released were not sufficient to detect oxidative stress with our methods. The other noteworthy finding was the lack of association between disease activity and AOPP or MDA levels.

Notably, AOPP and MDA concentrations were strongly dependent on gender; higher levels were found in males than in females, both among patients and healthy subjects. When adjusted for age and gender, only a high BMI and elevated hemoglobin levels were associated with high concentrations of AOPP. None of the tested variables were associated with MDA, after adjusting for age and gender. Depression or fatigue was not associated with plasma AOPP nor by MDA levels.

Concise data on oxidative stress-marker levels in different genders are scarce. Previous studies found elevated levels of anti-oxidants, such as vitamin E and glutathione, in females compared to males, and estrogens are known to upregulate anti-oxidant enzymes [44]. Those findings might explain the lower concentrations of AOPP and MDA we found in females compared to males. Aging was also related to oxidative stress in numerous previous studies [45]. In this study, we found a significant association between age and MDA, but not between age and AOPP. Accumulation of oxidative damage during the course of a chronic inflammatory disease could possibly accelerate the complexity between age and measures of oxidative stress; however, in this patient group we could not detect any association between disease duration and the chosen biomarkers of oxidative stress.

Obesity is known to be associated with elevated plasma AOPP and MDA concentrations. In one study, diet

Table 2 Associations between plasma AOPP concentrations and selected demographic and clinical variables in 82 patients with plaque psoriasis

Characteristics	Model results[a]		
	B (95% CI)	R^2	*P*
Demographics			
Age (per decade)	−0.07 (−0.02 to 0.15)	0.03	0.12
Male sex	0.49 (0.26 to 0.68)	0.20	< 0.001
Psoriasis duration (per decade)	0.05 (−0.04 to 0.14)	0.02	0.28
No. of cigarettes (per 5 cigarettes)	0.02 (−0.06 to 0.11)	0.00	0.58
Clinical data			
BMI, kg/m^2	0.05 (0.02 to 0.07)	0.18	< 0.001
Hypertension	0.30 (0.09 to 0.52)	0.09	0.01
PASI	0.04 (−0.00 to 0.07)	0.04	0.06
DLQI	− 0.00 (−0.02 to 0.02)	0.00	0.69
HADS-D	0.01 (−0.03 to 0.04)	0.00	0.70
fVAS (per 20 units)	− 0.01 (−0.09 to 0.07)	0.00	0.80
Biochemical data			
CRP, mg/l	0.01 (−0.02 to 0·04)	0.00	0.57
Hb, g/100 ml	0.22 (0.13 to 0.32)	0.23	< 0.001
GFR, ml/min/1.73 m^2 (per 10 units)	−0.06 (−0.14 to 0.03)	0.02	0.19
Comorbid disease			
Diabetes	0.58 (0.07 to 1.09)	0.06	0.03
CVD	0.00 (−0.47 to 0.48)	0.00	0.99
Respiratory disease	0.02 (−0.36 to 0.40)	0.00	0.92
Procedure differences			
Time of day blood sample obtained	−0.02 (−0.10 to 0.07)	0.00	0.72
Time since last meal	−0.03 (−0.07 to 0.00)	0.04	0.06

AOPP advanced oxidation protein products, *BMI* body mass index, *PASI* Psoriasis Area and Severity Index, *DLQI* Dermatology Life Quality Index, *HADS-D* depression subscale of the Hospital Anxiety and Depression Scale, *fVAS* fatigue Visual Analog Scale, *CRP* C-reactive protein, *Hb* hemoglobin, *GFR* glomerular filtration rate, *CVD* cardiovascular disease

[a]Simple linear regression analyses performed with log-transformed AOPP values

Table 3 Final multiple regression model with variables adjusted for age that significantly influenced AOPP plasma concentrations in 82 patients with psoriasis

Variable	Model results[a]	Significance
	B (95% CI)	*P*
Age (per decade)	0.05 (−0.23 to 0.11)	0.19
Male sex	0.25 (0.01 to 0.50)	0.04
BMI	0.03 (0.01 to 0.05)	< 0.01
Hemoglobin	0.12 (0.01 to 0.24)	0.03

$R^2 = 0.39$, *BMI* body mass index, *AOPP* advanced oxidation protein products

[a]The model used log-transformed AOPP values

interventions in juveniles that displayed obesity resulted in significant reductions in oxidative stress [23].

Hemoglobin is known to participate in redox reactions with various metal complexes, which generate hydroxyl radicals [43]. It remains speculative whether this property of hemoglobin was relevant to the association found in this study between hemoglobin and AOPP levels.

Our results showed that patients with psoriasis displayed lower MDA levels than healthy subjects. The use of topical corticosteroids for treating psoriasis was previously reported to be associated with low MDA concentrations and might explain our findings [47]. It has been suggested that smoking plays a role in pathogenesis of psoriasis by oxidants delivery and results in oxidative stress [36]. We did not find an association between smoking and AOPP levels, or between smoking and MDA levels among psoriasis patients. This could be explained by the relative low number of cigarettes reported by patients.

Study strengths and limitations

The strengths of this study included the matched case–control design, which prevented confounding effects of age and gender. Moreover, we evaluated MDA levels with HPLC-F after sample purification. This method was reported to be

Table 4 Associations between plasma MDA concentrations and selected demographic and clinical variables in 82 patients with plaque psoriasis

Variables	Linear regression results[a]		
	B (95% CI)	R^2	*P*
Demographics			
Age, (per decade)	0.07 (0.02 to 0.13)	0.08	0.01
Male sex	0.23 (0.08 to 0.39)	0.10	< 0.01
Psoriasis duration, (per decade)	0.02 (−0.05 to 0.08)	0.00	0.57
No. of cigarettes, (per 5 cigarettes)	−0.00 (−0.06 to 0.05)	0.00	0.92
Clinical data			
BMI, kg/m^2	0.01 (−0.01 to 0.03)	0.02	0.20
Hypertension	0.15 (−0.01 to 0.30)	0.04	0.06
PASI	−0.00 (−0.03 to 0.02)	0.00	0.85
DLQI	−0.01 (−0.02 to 0.01)	0.01	0.30
HADS-D	−0.01 (−0.03 to 0.01)	0.01	0.35
fVAS (per 20 units)	−0.03 (−0.09 to 0.03)	0.01	0.30
Biochemical data			
CRP, mg/l	−0.01 (−0.03 to 0.02)	0.01	0.53
Hb, g/100 ml	0.08 (0.01 to 0.15)	0.07	0.02
GFR, ml/min/1.73 m^2 (per 10 units)	−0.02 (−0.08 to 0.04)	0.00	0.55
Comorbid disease			
Diabetes	0.24 (−0.12 to 0.60)	0.02	0.19
CVD	0.06 (−0.27 to 0.39)	0.00	0.71
Respiratory disease	0.16 (−0.11 to 0.42)	0.02	0.24
Procedure differences			
Time of day blood sample obtained	−0.03 (−0.08 to 0.03)	0.01	0.32
Time since last meal	−0.01 (−0.03 to 0.02)	0.00	0.59

MDA malondialdehyde, *BMI* body mass index, *PASI* Psoriasis Area and Severity Index, *DLQI* Dermatology Life Quality Index, *HADS-D* depression subscale of the Hospital Anxiety and Depression Scale, *fVAS* fatigue Visual Analog Scale, *CRP* C-reactive protein, *Hb* hemoglobin, *GFR* glomerular filtration rate, *CVD* cardiovascular disease

[a]Linear regression analyses performed with log-transformed MDA values

Table 5 Final multiple regression model with variables that significantly influenced MDA plasma concentrations in 82 patients with psoriasis

Variables	Model results[a]	Significance
	B (95% CI)	*P*
Male sex	0.22 (0.07–0.37)	< 0.01
Age (per decade)	0.07 (0.02–0.12)	0.01

$R^2 = 0.18$, *MDA* malondialdehyde

[a]The model used log-transformed MDA values

one of the most reliable approaches for assessing lipid peroxidation [6].

Our study also had some limitations. First, the relative low number of participants may have been too small to allow the detection of differences. Furthermore, the patients mainly had mild disease. Including patients with a broader spectrum of disease activity might have resulted in higher AOPP and MDA levels. However, we could not detect any differences in AOPP and MDA concentrations when we compared patients with low disease activity to patients with moderate disease activity, after adjusting for age and gender. Furthermore, previous studies have reported an effect of systemic therapy on the measured levels of biomarkers of oxidative stress [4, 12]. In the present study psoriatic patients with predominantly mild disease not on systemic therapy were included. Alcohol is known to promote generation of ROS [30]. Unfortunately we did not have accurate data regarding alcohol consumption and could, therefore, not adjust for this. However, it is reasonable to believe the patient included were not heavy drinkers because of the relatively low cigarette consume reported. To reliably assess the associations of these biomarkers of oxidative stress with diseases, multiple sampling from each participant at different timepoints would be preferable. The cross-sectional design of this study precludes conclusions of trends in time and the potential effect of psoriasis treatment on these biomarkers as no repeated sampling within the individuals where performed. MDA and AOPP concentrations are influenced

by fasting; higher concentrations have been detected in non-fasting states [25, 42]. Although, in this study, fasting times were significantly different between patients and healthy subjects, we had a precise record of the sampling times, and we could adjust for the difference in our statistical analyses. Finally, AOPP concentrations are influenced by interference from other absorbing compounds and light scattering particles, such as lipids [20]. Although we subtracted the signal from a reference cuvette at 630 nm, interference due to light scattering at lower wavelengths could have led to concentration overestimations. The absence of oxidative stress as measured by AOPP and MDA in psoriasis patients does not necessarily mean that ROS do not play a role in the psoriasis pathophysiology. Biomarkers measured in plasma may not reflect the local situation in the psoriatic tissue.

In conclusion, applying AOPP and MDA as biomarkers of oxidative damage, we could not confirm the presence of oxidative stress in psoriasis patients with mild disease activity. However, these two biomarkers cannot exclude the presence of mild oxidative stress that do not lead to distinct oxidative damage of proteins, lipids, or other substances. Our results suggested that confounding factors and shortcomings of commonly used analytical methods are important factors that might cloud the understanding of this issue.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

Ethical approval This study was approved by the Regional Committee for Medical Research Ethics in Norway (REK vest 2010/1455). All participants provided written informed consent, and the study was conducted in accordance with the latest revision of the Helsinki Declaration.

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