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RESEARCH ARTICLE

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Oxidative stress in patients with asthma and its relation to uncontrolled asthma

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Abstract

This study aims to evaluate markers of oxidative stress in Tunisian asthmatic patients and investigate whether their markers are correlated with uncontrolled asthma.

This prospective cohort study was conducted on 48 healthy subjects and 60 patients with asthma (34 patients with controlled asthma and 26 patients with uncontrolled asthma). The levels of malondialdehyde (MDA), advanced oxidation protein products (AOPP), and glutathione (GSH), as well as the activities of glutathione peroxidase (GPx) and superoxide dismutase (SOD), were estimated in plasma by spectrophotometry.

Asthmatic patients have significantly higher plasmatic levels of MDA and AOPP than healthy controls ($p < 0.001$). Lower GSH level and GPx activity were found in patients with asthma compared to controls ($p < 0.001$). In contrast, higher SOD activity was noted in asthmatic patients ($p < 0.001$).

The comparison among the patients with controlled asthma and uncontrolled asthma revealed increased MDA and AOPP levels and SOD activity ($p < 0.001$) as well as a decreased GSH level and GPx activity ($p = 0.004$, $p = 0.019$) in patients with uncontrolled asthma. Spirometry level was significantly correlated with SOD activity ($r = 0.447$; $p = 0.010$), whereas no significant correlations were found with the other parameters (MDA, AOPP, GSH, and GPx).

Asthmatic patients, especially those with uncontrolled asthma, suffer a high degree of reactive oxygen species (ROS) formation causing considerable oxidative stress. Increased MDA level and SOD activity and reduced GPx activity were predictors of poorly controlled asthma.

KEYWORDS
asthma, malondialdehyde, oxidative stress, superoxide dismutase, uncontrolled asthma

1 | INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways that affects people of all ages.[1] The inflammatory process in asthma causes vascular hyperresponsiveness, airway edema, airway remodeling, and bronchoconstriction, and it results in the recruitment episodes of airway obstruction.[2] Prominent symptoms include wheezing, shortness of breath, chest tightness, and coughing, especially at

night.[2] Asthma prevalence has increased significantly worldwide.[3] It is defined as a public health issue that touches more than 3.6% of the Tunisian people.[4]

Clinical manifestations of asthma may be controlled by appropriate medication. However, when asthma is uncontrolled, it can place severe exacerbations in daily life and it is sometimes fatal.[5] Hence, inadequate asthma control leads to a difficult economic burden for the patient and the health care establishments. Asthma control measures include a periodic assessment of clinical and functional manifestations such as limitations in daily activity, breathing difficulty, nocturnal awakenings, and rescue medications used.[5] Guidelines on asthma management published by the Global Initiative for Asthma (GINA) proposed a new asthma classification based on the Asthma Control Test (ACT) Questionnaire.[3,6]

The expression of asthma is a complex and interactive process that depends on the interplay between host factors (e.g., innate immunity, genetics, and sex) and environmental exposures (e.g., airborne allergens and viral respiratory infections).[7] The sustained activation of phagocytic cells and inflammatory mediators in the airways of asthmatic patients lead to high levels of oxidative stress in the lungs.[8–10] This increased oxidative stress affects mucus hypersecretion and alters capillary endothelium, which may cause a leak of reactive oxygen species (ROS) into the systemic circulation.[11] Retaining strong oxidizing abilities, ROS damage proteins, lipids, and DNA inducing a loss of function of these molecules and a change of cellular responses.[12] Several antioxidant defensive systems are available to block the harmful effects of ROS in cells like glutathione peroxidase (GPx), superoxide Dismutase (SOD), catalase (CAT), glutathione (GSH), and vitamins A, C, and E.[13]

So far, the impact of ROS in the inflammatory process of asthma has received considerable attention. Some investigations found a relationship between inflammation and oxidative stress in asthma progression.[14–16] Although a Tunisian study has looked into oxidative stress biomarkers in asthmatic patients, it did not conclude to a significant difference between patients with different severity for the disease.[17] Furthermore, only one study has evaluated patients by subgrouping them according to their ACT scores.[18] In this study, we aimed to evaluate oxidant/antioxidant status in asthmatic patients compared to healthy controls and to investigate the correlation with the level of asthma control.

2 | MATERIALS AND METHODS

2.1 | Study population

Sixty asthmatic patients monitored in the Pneumology Department of Hedi Chaker University Hospital, Sfax, Tunisia, from January 2018 to March 2018, were investigated cross-sectionally in our study. The included patients were required to meet the following conditions: (i) diagnosed with asthma for at least 6 months, (ii) more than 18 years, and (iii) treated with an inhaled corticosteroid and oral or inhaled beta2-agonist. Exclusion criteria consisted of (i) patients diagnosed with respiratory infections or immune deficiency requiring specific therapy or any other diseases that may influence the asthma evolution, (ii) smokers, and (iii) patients on systemic steroid therapy in the preceding 2 weeks.

Forty-eight healthy people matched according to their age and sex were included as controls. All of them were non-smokers and were not taking any vitamin supplements.

To avoid the risk of potential bias, the gender and age of healthy controls were matched to the asthmatic patients.

The experimental protocol was established in accordance with the guidelines of the Declaration of Helsinki, and informed consent was obtained from all participants.

2.2 | Data collection

Demographic characteristics of all participants were collected. For asthmatic patients, specifically, clinical data were collected, including body mass index (BMI), spirometry level (measures the rate of airflow and estimates lung size), skin prick test positivity (defined by a mean wheel diameter ≥3 mm than the negative control for at least one of 10 allergens), and duration of illness (defined as the time since asthma diagnosis).

2.3 | Asthma control measurement

Asthma control and the quality of life were estimated using the ACT questionnaires.[6] Asthma is defined as "controlled" if the ACT score is ≥20, "partly controlled" if the ACT score is between 16 and 19, and "uncontrolled" if the ACT score is ≤15.[6] For this purpose, our patients were divided into two subgroups: controlled asthma (n = 34) and uncontrolled asthma (n = 26).

2.4 | Collection of plasma samples and storage

Heparinized blood samples were collected by venepuncture from asthmatic patients and healthy subjects. The plasma layer was separated by centrifugation at 4042 g for 10 min. Samples were immediately frozen and stored at −80°C in a small aliquot until analysis.

2.5 | Malondialdehyde level assay

Malondialdehyde is an end product of lipid peroxidation. It is estimated in plasma by the thiobarbituric acid reactive species (TBARS) assay.[19] MDA reacts with thiobarbituric acid (TBA) at acidic conditions to form a pink-colored complex measurable spectrophotometrically at a wavelength of 532 nm. The plasmatic MDA level was expressed as nmol MDA/mg of protein.

TABLE 1 Basic characteristics of asthmatic patients

Characteristics	Asthmatic patients (n = 60)	Controlled asthma (n = 34)	Uncontrolled asthma (n = 26)	VIF	p-Value
Age at diagnosis (mean ± SD) (years)	51.7 ± 13.1	57.7 ± 12.9	49.8 ± 11.2	1.19	0.075
Gender (M/F)	37/23	19/15	18/8	1.10	0.149
Body mass index (mean ± SD) (Kg/m^2)	27.3 ± 5.8	26.6 ± 5.4	28.1 ± 5.9	1.04	0.973
Spirometry (mean ± SD) (L)	2.64 ± 0.8	2.63 ± 0.8	2.65 ± 0.9	1.32	0.214
Skin Prick Test positivity (%)	55	70.6	34.6	1.16	0.429
Duration of illness (mean ± SD) (years)	15.2 ± 5.3	16.4 ± 6.2	14.0 ± 6.8	1.08	0.300

Note: ANCOVA test applied for the comparisons between controlled and uncontrolled asthma patients.
Abbreviation: VIF, variance inflation factor.

TABLE 2 Comparison of oxidative profile between healthy controls and asthmatic patients (controlled asthma or not)

	Healthy controls (n = 48)	Asthmatic patients (n = 60)	Controlled asthma patients (n = 34)	Uncontrolled asthma patients (n = 26)	p-value
MDA level	0.07 ± 0.01	0.15 ± 0.04	0.12 ± 0.02	0.16 ± 0.03	<0.001[a,b,c,d]
AOPP level	0.18 ± 0.01	0.21 ± 0.04	0.18 ± 0.02	0.21 ± 0.04	<0.001[a,b,d]
GSH level	2.99 ± 0.78	1.26 ± 0.34	1.37 ± 0.30	1.14 ± 0.28	<0.05[a,b,c,d]
GPx activity	0.02 ± 0.006	0.013 ± 0.002	0.014 ± 0.002	0.012 ± 0.003	<0.05[a,b,c,d]
SOD activity	146.82 ± 23.87	263.53 ± 30.24	185.42 ± 49.91	243.78 ± 40.11	<0.001[a,b,c,d]

Note: Data presented as mean ± S.E.M. and ANOVA test applied for the comparisons, $p < 0.05$ was considered statistically significant.
Abbreviations: AOPP, Advanced Oxidation Protein Products (expressed as nmol AOPP/mg of protein); GPx, Glutathione Peroxidase (expressed as nmol GSH consumed/min/mg of protein); GSH, Glutathione (expressed as nmol GSH/mg of protein); MDA, Malondialdehyde (expressed as nmol MDA/mg of protein); SOD, Superoxide Dismutase (nmol $O2^{\bullet-}$ consumed/min/mg of protein).
[a]Shows significant difference between healthy controls and asthmatic patients.
[b]Shows significant difference between controlled asthma and uncontrolled asthma patients.
[c]Shows significant difference between healthy controls and controlled asthma patients.
[d]Shows significant difference between healthy controls and uncontrolled asthma patients.

2.6 | Advanced oxidation of protein products level assay

As an index of protein oxidation, the AOPP level was measured according to the method described by Kayali et al.[20] The concentration of AOPP (determined at 340 nm) was expressed in nmol AOPP/mg of protein using a molar extinction coefficient of 261 $mM^{-1}cm^{-1}$.

2.7 | Reduced glutathione level assay

The oxidation of the GSH by the sulfhydryl reagent 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) generates the yellow derivative 5′-thio-2-nitrobenzoic acid (TNB).[21] The result was measured at 412 nm and expressed as nmol GSH/mg of protein.

2.8 | Determination of glutathione peroxidase activity

The activity of GPx was performed by the method of Flohe and Günzler.[22] The principle of this method consists of the GSH oxidation by GPx in the presence of DTNB. The optical density was determined at 412 nm. GPx activity was expressed as nmol of GSH consumed/min/mg of protein.

2.9 | Determination of superoxide dismutase activity

The activity of SOD was evaluated by the method of Beauchamp and Fridovich.[23] The latter is based on the capacity to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) by superoxide anion ($O_2^{\bullet-}$). The blue-colored formazan was followed at 580 nm. One unit of SOD activity caused 50% inhibition of the rate of NBT photoreduction. SOD activity was expressed as nmol $O_2^{\bullet-}$ consumed/min/mg of protein.

2.10 | Statistical analysis

The statistical tests were performed by the SPSS program, version 20. Data presented as mean ± SD, and all statistical comparisons were performed using the nonparametric Mann–Whitney U test, ANOVA, or ANCOVA on ranks.

Logistic regression was used to establish the associated factors with oxidative stress and asthma control level, all controlled for the effect of age at diagnosis, sex, age of onset, BMI, spirometry, skin prick test positivity, and duration of illness as covariates. Multicollinearity in the regression model was assessed using the variance inflation factor (VIF) that identifies a correlation between independent variables and the strength of that correlation. A value of VIF = 1 indicates that multicollinearity does not affect it, and we can trust this coefficient and *p*-value with no further action.

The statistical significance of the correlation coefficient was determined using the Pearson correlation coefficient. A value of $p < 0.05$ was considered to be statistically significant.

3 | RESULTS

3.1 | Characteristics of the participants

A total of 60 asthmatic patients (34 M/26 F; mean of age 52 ± 12.9 years) and 48 healthy controls (26 M/22 F; mean of age 46 ± 9.6 years) participated in our study. Patients were represented according to their asthma control level (Table 1). In short, no significant differences were observed for basic characteristics between the two asthmatic patient subgroups ($p > 0.05$) (Table 1).

3.2 | Oxidative stress profile and asthma

The comparison of oxidative profiles between healthy controls and asthmatic patients is presented in Table 2. Plasmatic MDA levels of the asthmatic patients were significantly higher than those of the healthy controls ($p < 0.001$; Figure 1A). Additionally, plasmatic AOPP levels were high in asthmatic patients compared to controls ($p < 0.001$; Figure 1B). Interestingly, we observed markedly low levels of plasma GSH ($p < 0.001$; Figure 2A) and GPx activity ($p < 0.001$; Figure 2B) in asthmatic patients compared to controls. In contrast, the SOD activity was higher in asthmatic patients' plasma compared to the healthy control group ($p < 0.001$; Figure 2C).

3.3 | Oxidative stress profile and control asthma level

We also compared the oxidative and antioxidant biomarkers separately between the patient subgroups and healthy controls (Table 2). This comparison showed significantly increased MDA and AOPP levels in uncontrolled asthma patients than controlled asthma patients ($p < 0.001$, $p = 0.001$; Figure 1A,B, respectively). Besides, SOD activity was higher in uncontrolled asthma patients compared to controlled asthma patients ($p < 0.001$; Figure 2C). However, we found lower GPx activity and GSH level in uncontrolled asthma patients than those of controlled asthma patients ($p = 0.019$, $p = 0.004$; Figure 2A,B, respectively). We found significant differences in MDA and GSH levels and GPx and SOD activities in both patient subgroups compared to healthy control subjects ($p < 0.05$) (Table 2).

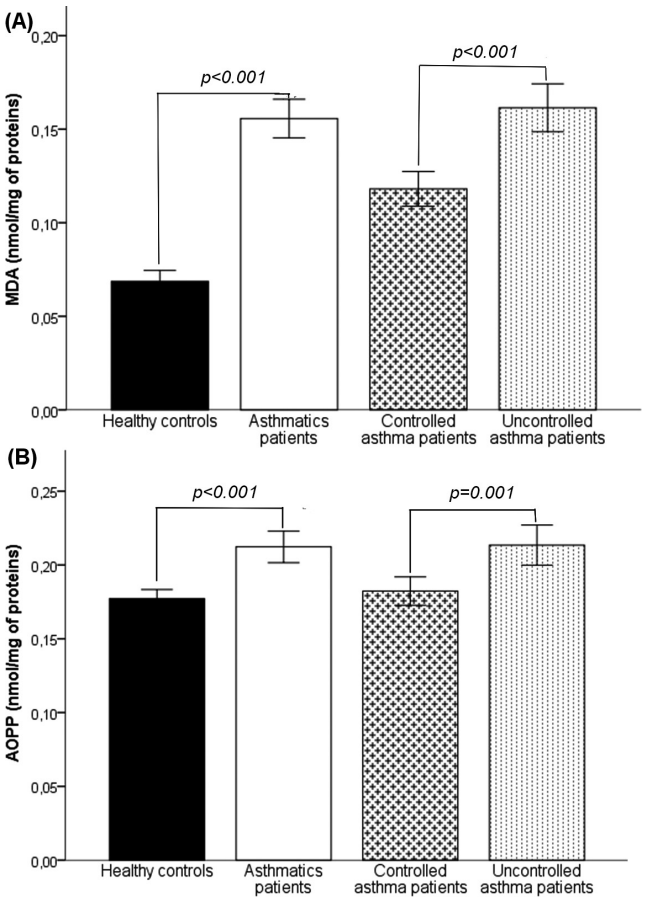

FIGURE 1 Oxidant plasma levels in asthmatic patients versus healthy controls and in controlled asthma versus uncontrolled asthma. Each column represents mean ± SD

The multiple regression model showed that the potential risk factor of uncontrolled asthma was significantly associated with increased MDA level (OR = 1.6; 95% IC = 1.5–1.8; $p = 0.017$), reduced GPx activity (OR = 0.08; 95% IC = 0.07–0.1; $p = 0.016$), and increased SOD activity (OR = 1.03; 95% IC = 1.01–1.06; $p = 0.004$).

To investigate the link between oxidative stress status and airflow rate, correlation analyses were performed with spirometry (Figure 3). Spirometry level was significantly correlated with SOD activity ($r = 0.447$; $p = 0.010$) (Figure 3E), whereas no significant correlations were found with the other parameters (MDA, AOPP, GSH, and GPx) (Figure 3A–D, respectively).

4 | DISCUSSION

In the present study, we chose to investigate the possible impact of oxidative damage in asthma development and the asthma control level for several reasons. First, oxidative stress plays a crucial part in asthma pathogenesis. Indeed, the excess ROS production

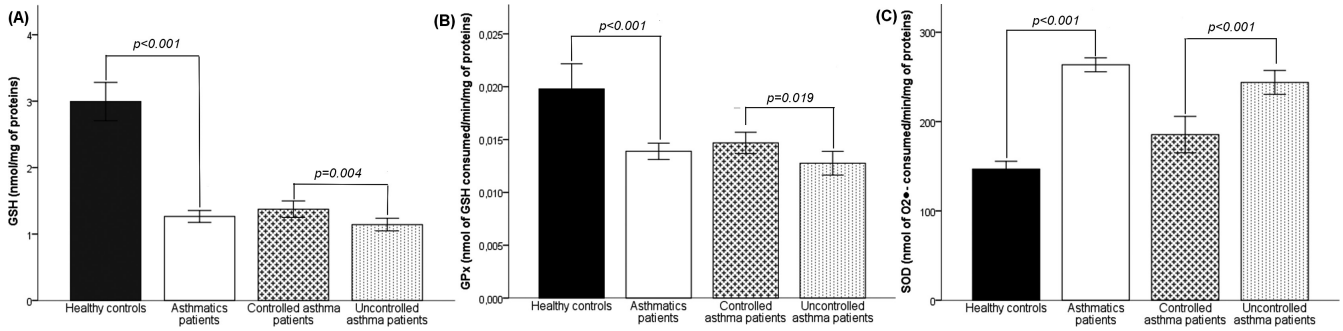

FIGURE 2 Antioxidant enzymatic and non-enzymatic defenses in asthmatic patients versus healthy controls and in controlled asthma versus uncontrolled asthma. Each column represents mean ± SD

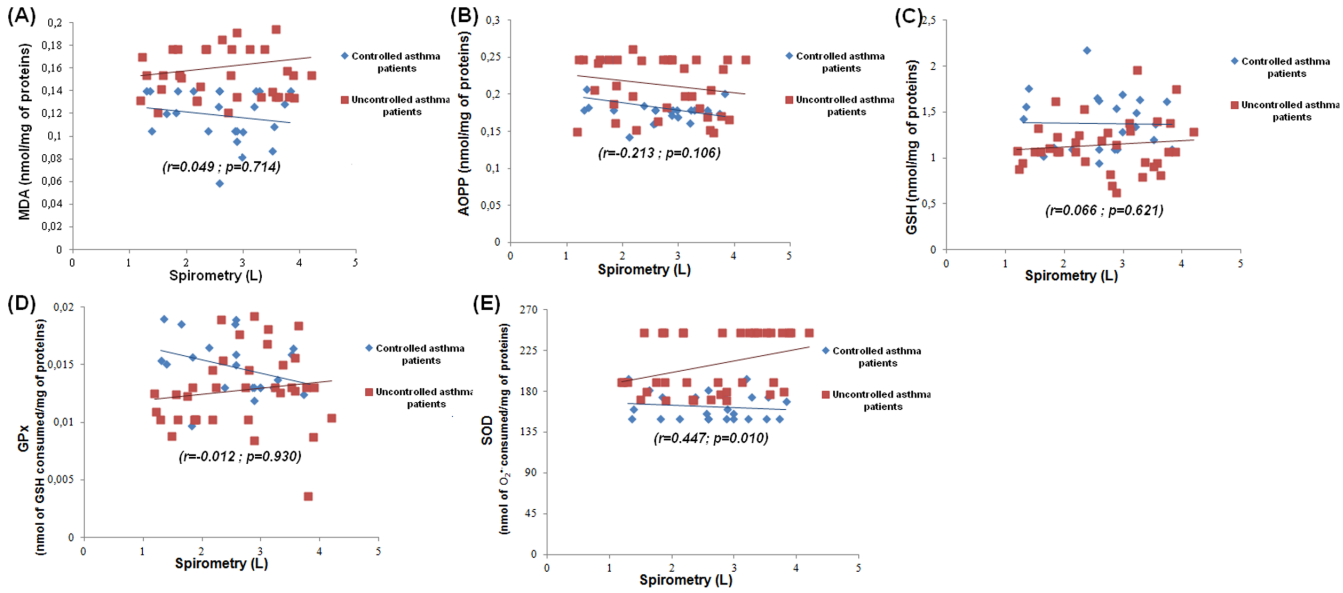

FIGURE 3 (A) Correlation between MDA level and spirometry in controlled asthma and uncontrolled asthma patients. (B) Correlation between AOPP level and spirometry in controlled asthma and uncontrolled asthma patients. (C) Correlation between GSH level and spirometry in controlled asthma and uncontrolled asthma patients. (D) Correlation between GPx activity and spirometry in controlled asthma and uncontrolled asthma patients. (E) Correlation between SOD activity and spirometry in controlled asthma and uncontrolled asthma patients

increases infiltration of the inflammatory cell into the lungs and, at the same time, stimulates the production of extracellular matrix protein and the elevation of pro-inflammatory cytokine in airway ducts.[14,24] Second, the outcomes reported on systemic antioxidant defenses in asthmatic patients have been inconsistent and/or controversial.[14–17] Third, the impact of oxidative stress biomarkers on the level of asthma control is not well studied.[18] To the best of our knowledge, this is the first Tunisian study to compare the oxidant/antioxidant status in asthmatic patients according to their ACT scores.

Our study shows remarkable elevations in MDA and AOPP levels accompanied by a decreased GSH level and GPx activity and increased SOD activity in asthmatics patients compared to those of healthy controls, in agreement with previous reports.[15,18,25–27] However, for AOPP and SOD, contrasting outcomes with no significant variation in AOPP level and decreased activity of SOD between asthmatic patients and control subjects were reported.[17] The inconsistency of data can be due to the effect of lifestyle variations, the impact of dietary factors, and other factors.

Interestingly among asthmatic patients, we found a significant increase in MDA and AOPP levels in uncontrolled asthma compared to the controlled asthma group. This enhancement of the MDA rate is consistent with a study reported by B Karadogan et al.[18] Furthermore, some studies reveal the presence of oxidative stress in asthma disease even in asymptomatic phases and after therapy.[28,29] Likewise, Fernando et al. observed significantly higher nitric oxide metabolites in children with asthma regardless of disease control level than healthy controls. Our result is in line with these studies since the MDA level is significantly higher in the controlled asthma subgroup compared to the control group. So, we inferred that conditions of uncontrolled asthma strongly evoke oxidative damages. In addition, according to the multivariate logistic

regression analysis results, we concluded that plasma MDA level is a potential and predictive biomarker of asthma control level in patients with asthma. This ascertainment was consistent with a study reported by El-Alameey et al. which found that serum concentration of MDA is the most predictive biomarker of asthma severity.[31]

The antioxidant defense systems might be high due to a defense response or low due to neutralization by oxidants, whereas there might be no change if the reserves are satisfactory.[15] Herein, we observed significant decreases in the GSH level and GPx activity and an increase in SOD activity, for the first time, in uncontrolled asthma compared to controlled asthma patients.

Glutathione peroxidase catalyzes the oxidation of GSH to glutathione disulfide (GSSG) during the detoxification of hydrogen peroxide and toxic lipid peroxidation products, continuously generated as a consequence of sequestration and infiltration of inflammatory leukocytes.[32] Thus, the decrease in GPx activity in our patients was probably due to the depleted levels of GSH, enhanced hydrogen peroxide, as well as MDA levels, or deficiency of selenium.[33,34] Likewise, the depletion in GSH levels may be due to increased consumption of GSH or a lack of glycine and glutamic acid found in the structure of glutathione.[33,35]

Superoxide dismutase is one of the first-line defense antioxidants that act as a good therapeutic agent against ROS-mediated diseases.[36] Conflicting results have been reported about the SOD activity level in asthma patients. Ben Anes et al.[17] found a decreased activity of SOD between asthmatic patients and healthy controls, whereas Mak et al.[16] revealed an increased expression of this antioxidant enzyme in asthma. The contrasts in SOD activity in literature can be partially attributed to the analytical approach used to assay this marker and to the fact that some studies were carried out in different populations, and there may be interindividual variations, especially in antioxidant capacity.[37] In our study, SOD activity increase in the plasma of subjects suffering from asthma, especially in the case of uncontrolled asthma, might be a compensatory mechanism to overcome oxidative damage induced by catalyzing the dismutation of $O_2^{\bullet -}$. This hypothesis was confirmed by the significantly increased plasmatic SOD activity in controlled asthma patients compared to healthy controls and the positive correlation between the spirometry level and this activity. Interestingly, the multivariate logistic regression analysis suggests that GPx and SOD activities are potential predictive biomarkers of asthma control level in our patients.

In general, our statistical comparison between the patient subgroups found that asthma is never completely controlled even after treatment. The significantly increased MDA level and SOD activity and decreased GSH level and GPx activity in the controlled asthma subgroup compared to the control group support this idea. Additionally, we showed that the potential risk factor of uncontrolled asthma is significantly associated with an increased MDA level and SOD activity and a reduced GPx activity. However, these findings need to be validated with clinical analyses in larger cohorts.

5 | CONCLUSION

In summary, our results have clearly shown a significant increase in oxidative stress and decreased antioxidant status in asthmatic patients, even under controlled conditions. These observations support the data found by the afore-mentioned investigations. Besides, our data indicate that the assessment of the plasmatic MDA level and GPx and SOD activities have the potential to be used as practice predictors of disease severity given the fact that they are easy to assess to confirm good asthma control levels. However, these findings need to be verified with further large-scale prospective investigations with accurate recording of clinical exacerbations which may enable the control of multiple confounding factors.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All available data can be obtained by contacting the corresponding author.

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