

Correlation between Degree of Asthma Severity and Malondialdehyde in Asthmatic Children in Ismailia

Marwa A. Mohamed¹, Alaa El-Din A. Zeitoun¹, Fadia M. Attia², Nouran B. AbdAllah^{1*}

¹ Pediatric Department, Faculty of Medicine, Suez Canal University, Egypt

² Clinical Pathology Department, Faculty of Medicine, Suez Canal University, Egypt

Abstract

Background: Oxidative damage of several biomolecules is strongly involved in the pathogenesis of asthmatic inflammation. It is affecting airway functions causing an increase in the frequency of asthma attacks and deterioration. **Aim:** To determine Malondialdehyde level in both asthmatic children and the control group and to correlate its level to the degree of asthma severity. **Subjects and Methods:** Malondialdehyde (MDA) was measured in a group of Egyptian asthmatic children (55) and their matching controls (55) with an age range from 5 to 15 years who were attending the outpatient clinic in Suez Canal university hospital. **Results:** MDA level mean was higher in the studied asthmatic children than the control group being 3.32 ± 2.09 nmol/ml and 1.27 ± 0.20 nmol/ml respectively with a statistically significant difference. MDA level mean was found to correlate significantly and proportionally to the degree of asthma severity being 2.05 ± 1.21 nmol/ml in intermittent asthma, 2.75 ± 1.75 nmol/ml in mild persistent, and 4.66 ± 2.12 nmol/ml in moderate persistent (p value < 0.001). **Conclusion:** The findings of our study further strengthen the evidence that the altered oxidant-antioxidant balance is associated with airway obstruction.

Keywords: Asthma, oxidative stress, antioxidant status, free radicals, ROS

Introduction

Asthma is one of the common inflammatory diseases that affect millions of people all over the world. Among the inflammatory mediators produced in asthma, reactive oxygen species (ROS) have an important role in the pathogenesis of the disease affecting the pulmonary vessels and leading to many systemic complications⁽¹⁾. The expression of asthma is known to be a complex, interactive process depending on the interplay between two major fac-

tors, host factors, and environmental exposures occurring at a critical time affecting immune system development. These two factors elements, including innate immunity, genetics, sex, airborne allergens, food allergens, and viral respiratory infections are variable with different environments and populations. ROS produced by leukocytes plays a major role in the pathogenesis of bronchial asthma due to many of the characteristic changes in the airways that are produced by the action of ROS⁽²⁾. ROS produces tissue damage, constriction

*Corresponding Author: nouranb.abdallah@gmail.com

of the smooth muscles, increase in vascular permeability, the release of different biochemical mediators, and bronchoconstriction^(3,4). Overproduction of ROS leads to increased concentrations of MDA and total content of oxidants as well as reduced antioxidant enzyme activity in asthmatic patients^(1,5). Among the mechanisms of damage caused by ROS, lipid peroxidation is the most extensively investigated. Oxidation of cell membrane phospholipids releases unstable lipid hydroperoxides and secondary carbonyl compounds as endogenous aldehydes. Highly reactive endogenous aldehydes react directly with DNA, forming aldehyde-derived DNA that leads to DNA damage⁽⁶⁾. Some of the most reactive aldehydes are malondialdehyde, 4-hydroxy-2-nonenal, acrolein, crotonaldehyde, and methylglyoxal. The mutagenic and carcinogenic effects are attributed to the ability of these aldehydes to directly modify DNA bases or yield promutagenic exocyclic adducts⁽⁷⁾. These compounds are useful as biomarkers because they are measurable in biofluids, although complex processes might be required to prepare them for analysis⁽⁸⁾. MDA is a biomarker of lipid peroxidation in airways. Increased MDA concentrations coexist with pathological changes in respiratory epithelial cells, overproduction of mucus, smooth muscle contraction, and bronchial hyperreactivity^(9,10). Oxidative stress is prevalent in both lung and systemic circulation of asthmatic patients suggesting that lung inflammation can spill into the systemic circulation because of the proximity of pulmonary vessels with the blood capillary network. Blood contributes to the development of secondary inflammation in blood vessels and plays a role as a biomarker of oxidative stress in asthma^(11,12). Therefore, to determine the oxidant and antioxidant imbalance in children having bronchial

asthma and investigate the correlation between MDA level and degree of asthma severity, we conducted our study.

Subjects and Methods

Study population

The study was conducted on 55 children aged 5-15 years of asthmatic patients who were attending the outpatient pediatric clinic of Suez Canal university hospital while the control group was composed of 55 age-matched children seen at the outpatient department of the same hospital during the study period from March 2017 to March 2019. The patients aged 5-15 years old and diagnosed clinically as asthmatic were included in our study, diagnosis of asthma was done according to the National Asthma Education and Prevention Program (NAEPP), 2007, GINA 2017, and based on the history of recurrent or persistent wheezing with or without dyspnea, and improvement either spontaneously or with use of β_2 -agonists, While patients who had evidence of other concurrent pulmonary or systemic disease, children with acute upper or lower respiratory infections and patients taking a vitamin containing tonics were excluded. All patients were subjected to detailed clinical evaluation including history taking, clinical assessment of the severity of asthma, and physical examination was done together with pulmonary function test, skin testing with the prick method using different possible antigens, and blood sampling for Malondialdehyde (MDA), total immunoglobulin E and complete blood picture with differential for the eosinophilic count. Patients were classified according to NAEPP, 2007 into four groups: intermittent, mild, moderate, and severe persistent asthma. While the control group was composed of age-matched children seen at the outpatient department of the same hospitals during

the study period. Children were included if they have no personal or family history of atopy or signs of an atopic disorder. Malondialdehyde (MDA) was performed for them.

Malondialdehyde assay

Malondialdehyde (MDA) assay in serum is determined colorimetrically as Thiobarbituric acid (TBA) reacts with malondialdehyde (MDA) in an acidic medium at a temperature of 95°C for 30 min to form thiobarbituric acid reactive product the absorbance of the resultant pink product can be measured at 534 nm⁽⁶⁾. Total IgE is an enzyme immunoassay (EIA) for the determination of IgE antibodies in human serum. The intensity of the yellow color is proportional to the quantity of IgE-antibodies in the serum. The results are expressed in IU/ml⁽²¹⁾. Eosinophilic counts are determined from venous blood samples.

Skin Prick Testing

Skin prick testing (SPT) demonstrates an allergic response to a specific allergen. SPT is a simple, safe, and quick test, providing results within 15-20 minutes. SPT is usually carried out on the inner forearm. A wheal >3 mm in diameter was considered a positive test result for sensitization to that peculiar allergen.

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using numbers and percentages. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, and median. The significance was judged at the 5% level⁽¹³⁾. The used tests

were the Chi-square test to compare between the minisling group and the transobturator tape group. Student t-test to compare the normally distributed quantitative data between both groups. And Mann Whitney test for abnormally distributed quantitative data in both and between the two studied groups.

Results

Fifty-five asthmatic children were enrolled in the present study. They were age and sex-matched with other fifty-five healthy children as a control group with no history of atopy or any chronic disease. The age of all subjects included in the study ranged from 5 to 15 years with a mean (8.918 ± 0.4194) in asthmatic patients compared to (8.509 ± 0.4061) in the control group. Out of fifty-five asthmatic patients, 36 were males and 19 were females with a male-to-female ratio of 1.89:1, while in the control group, 31 were males and 24 were females with male to female ratio of 1.29:1. Nearly 51% of asthmatic patients lived in urban areas while 49% lived in rural ones. There was no statistically significant difference between cases and controls regarding sex ($p = 0.43$), age ($p = 0.48$), and residency denoting good matching between the two-studied groups as reported in Table (1). According to the asthma severity classification of the *National Asthma Education and Prevention Program 2007 and GINA 2017*, 27.27% had intermittent asthma, 32.73% had mild persistent asthma and 40% had moderate persistent asthma and none of the cases had severe persistent asthma. Regarding the skin prick test, 43.6% of the studied asthmatic patients were sensitive to more than two allergens. In our study immunoglobulin (Ig) E mean was 348.9 ± 341.9 IU/ml while the eosinophilic count mean was 334.8 ± 227.4 cells/mm³ and both show-

ed a statistically significant positive correlation with asthma severity ($p=0.002$; $R=0.289$ and $R=0.494$ respectively). Also, a statistically positive correlation was found between the degree of asthma severity (NAEPP, 2007) and both Ig E means and Eosinophilic count where both mean level of IgE and eosinophilic count increase with

the increase in the degree of asthma severity, with IgE mean of 117.7 ± 107.0 IU/ml in intermittent asthma, 343.9 ± 401.7 IU/ml, and 510.5 ± 311.2 IU/ml in moderate persistent while meaning eosinophilic count being 249.3 ± 177.5 cells/mm³ in intermittent asthma, 330.6 ± 239.4 cells/mm³ in mild persistent and 396.5 ± 237.4 cells/mm³ in moderate persistent.

Table 1: Basic demographic and clinical data of the patients included in the study

	Cases (n= 55)		Control (n= 55)		Test of Sig.	p
	No.	%	No.	%		
Sex						
Male	36	65.5	31	56.4	$\chi^2=$ 0.955	0.329
Female	19	34.5	24	43.6		
Age (years)						
Min. – Max.	5.0 – 15.0		5.0 – 15.0		U= 1361.50	0.364
Mean \pm SD.	8.92 ± 3.11		8.42 ± 3.04			
Median	8.0		8.0			
Residence						
Urban	28	50.9	27	49.1	$\chi^2=$ 0.036	0.849
Rural	27	49.1	28	50.9		

χ^2 =Chi square test, U=Mann Whitney test

Table 2: Comparison between the two studied groups according to MDA levels

	Cases (n = 55)	Control (n = 55)	U	p
MDA (nmol/ml)				
Min. – Max.	0.33 – 9.45	0.80 – 1.60	529.0*	<0.001*
Mean \pm SD.	3.32 ± 2.09	1.27 ± 0.20		
Median	2.90	1.30		

* Mann-Whitney U test is statistically significant at $p \leq 0.05$

The mean MDA level was higher among the asthmatic patients than the control group with a statistically significant difference as illustrated in Table (2). The MDA level mean was highest among the asthmatic patients who were sensitive to three allergens with no statistical significance. There was a non-significant correlation between MDA level

and eosinophilic count in the studied asthmatic patients as shown in Figure (1). Figure (2) showed a non-significant correlation between MDA level and Ig E in the studied asthmatic patients. Regarding oxidative stress, our study showed a highly significant positive correlation between

the degree of asthma severity and MDA level in asthmatic patients Table (3).

Discussion

Asthma is a chronic inflammatory airway disease affecting children and adults of all ages. Chronic inflammation involving the recruitment and activation of inflammatory cells has been increasingly recognized as a mechanism leading to oxidative stress in asthma⁽¹⁴⁾. The lungs are continually exposed to the oxidants generated either endogenously from mitochondria, phagocytes, and other cells, or exogenously from air pollutants and smoking. Lungs have the largest endothelial surface area which makes them the principal target for circulating oxidants^(15,16). Total antioxidant status gives information about all of the antioxidants in the organism, while MDA is a lipid peroxidation marker used to assess lipid peroxidation due to increased oxidative stress^(17,24). In our study, MDA level mean was higher in the studied asthmatic patients than in the control group being

3.32±2.09 nmol/ml and 1.27±0.20 nmol/ml respectively and was a statistically significant difference. Also, the MDA level mean was found to correlate proportionally and significantly to the degree of asthma severity which was determined using criteria defined in NAEPP, 2007 being 2.05±1.21 nmol/ml in intermittent asthma, 2.75±1.75 nmol/ml in mild persistent, 4.66±2.12 nmol/ml in moderate persistent ($p < 0.001$). The above results agree with that of EL-Alameey et al., 2017 who studied sixty asthmatic children aged 6 to 12 years in Egypt where MDA was highly significantly increased in the patients compared to controls ($p < 0.001$), and in severe asthma compared to the studied patients with moderate and mild asthma ($p < 0.001$)⁽¹⁸⁾. Also, Ahmad et al., 2012 showed that the MDA level was higher in the asthmatic patient compared to the control. It also demonstrated that the measures of oxidative stress and antioxidants differ significantly between patients according to the severity of asthma as severe persistent asthmatics had the highest levels of MDA⁽¹⁹⁾.

Table 3: Correlation between the degree of asthma severity and MDA level in the asthmatic patients (n = 55)			
	Degree of asthma severity		
MDA (nmol / ml)	Intermittent (n = 15)	Mild persistent (n = 18)	Mod. persistent (n = 22)
Min. – Max.	0.36 – 4.22	0.33 – 5.59	2.0 – 9.45
Mean ± SD	2.05 ± 1.21	2.75 ± 1.75	4.66 ± 2.12
Median	1.80	2.65	4.15
r_s(p)	0.538* (<0.001*)		

r_s: Spearman coefficient statistically significant at $p \leq 0.05$

So, all the above studies revealed that subjects with asthma have increased levels of MDA supporting the hypothesis that oxidative stress is associated with the pathophysiology of asthma and that oxidant-antioxidant imbalance which occurs in asthma plays a crucial role in disease pro-

gression and severity. An increased oxidant and/or decreased antioxidant may reverse the physiologic oxidant-antioxidant balance in favor of oxidants. Concerning the relation between eosinophilic count and degree of asthma severity, our results agree with the study done by Zeiger et al.,

2015 reported that high blood eosinophil counts were a risk factor for increased future asthma exacerbations with persistent asthma⁽²⁰⁾. While concerning the relationship between the degree of asthma severity and Ig E level, our results agree with Sherenian et al., 2015 who performed a retrospective analysis on pediatric patients aged from 3 to 26 years with the diagnosis of asthma, the inpatient cohort had a significant higher (four-fold) total IgE level than the outpatient cohort regardless of season⁽²¹⁾. This is explained by the fact that

IgE is crucial for the development of airway inflammation in patients with asthma as the activation of the allergic cascade by IgE, under allergen stimulation, leads to chronic allergic inflammation in the airways of asthmatic patients which is associated with the induction of airway remodeling. Many cells, including eosinophils, mast cells, T lymphocytes, neutrophils, and epithelial cells, infiltrate the airways to release many mediators producing inflammatory responses that produce many cytokines such as IL-4, IL-5, and IL-13⁽²²⁾.

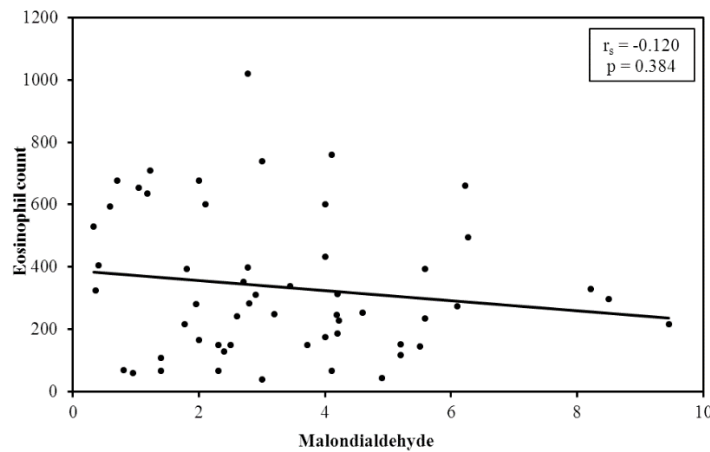


Figure 1: Correlation between MDA level and eosinophilic count in the studied asthmatic patients (r_s : Spearman coefficient)

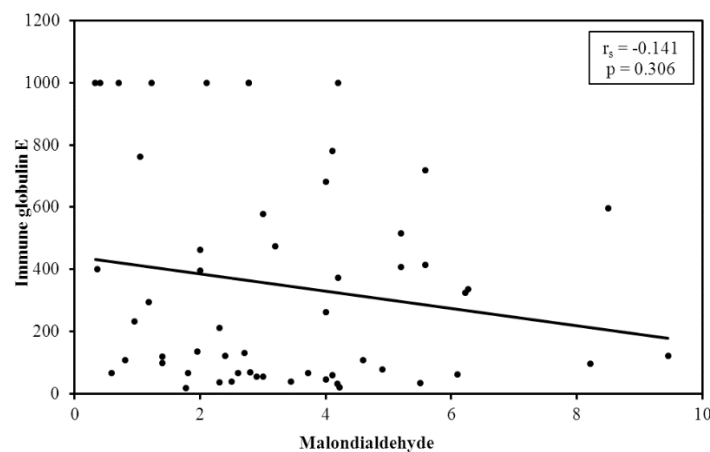


Figure 2: Correlation between MDA level and immunoglobulin E in the studied asthmatic patients (r_s : Spearman coefficient)

In our current study only, slight non-significant differences were observed in the serum MDA mean value among asthmatic subjects in relation to various clinical parameters like age, sex, residency, precipitating factors of asthma, co-morbid illness, seasonal variation, family history, and various laboratory parameters and tests as skin prick test, Ig E level and eosinophilic count. These differ from Fatani, 2014 who found noticeable differences for some sub-categories as males, females, smokers, and nonsmokers. As, in asthmatic smokers, a significantly higher level of MDA was observed as compared to healthy smokers while TAC was significantly lower in asthmatic smokers, which indicates that smoking also affects disease pathogenesis^(24,25). This difference may be related to the small sample size of our study as it is proposed that smoking either active or passive results in the production of many toxic free radicals and contributes to the occurrence of oxidative stress.

Conclusions

This work describes the role of oxidative stress in the severity of disease which provides strong evidence for oxidant-antioxidant imbalance in disease progression. The findings of this study further strengthen the evidence that the altered oxidant-antioxidant balance is associated with airway obstruction. Therefore, there is an urgent need to know how the change in oxidative stress mediators leads to the opening or closing of the airway. Furthermore, how a change in the balance between oxidants and antioxidants leads to the severity of asthmatic cases. This could lead to a new therapeutic approach and may lead to future applications in asthma management.

References

1. Al-afaleg NO, Al-Senaigy A, El-Ansary A. Oxidative stress, and anti-oxidant status in Saudi asthmatic patients. *Clin Biochem.* 2011;44(8-9):612-617.
2. Ganguly NK, Jindal SK, Biswal S, et al. *Studies on Respiratory Disorders.* Springer New York; 2014; 2:24-44.
3. Comhair SA, Ricci KS, Arroliga M, et al. Correlation of systemic superoxide dismutase deficiency to airflow obstruction in asthma. *Am J Respir Crit Care Med* 2005; 172:306-13.
4. Suski JM, Lebiezinska M, Bonora M, et al. Relation between mitochondrial membrane potential and ROS formation. *Mitochondrial Bioenergetics: Methods and Protocols.* 2012:183-205.
5. Nadeem A, Siddiqui N, Alharbi NO, et al. Airway and systemic oxidant-antioxidant dysregulation in asthma: a possible scenario of oxidants spill over from lung into the blood. *Pulmonary Pharmacol & Therapeutics.* 2014 Oct 31; 29(1):31-40.
6. Voulgaridou GP, Anestopoulos I, Franco R, et al. DNA damage induced by endogenous aldehydes: current state of knowledge. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis.* 2011 Jun 3;711(1):13-27.
7. Laher I, editor. *Systems biology of free radicals and antioxidants.* Springer Berlin Heidelberg; 2014;3:65-87.
8. Crestani B, Besnard V, Boczkowski J. Signalling pathways from NADPH oxidase-4 to idiopathic pulmonary fibrosis. *Int J Biochem & cell biol.* 2011 Aug 31; 43(8):1086-9.
9. Carroll L, Pattison DI, Fu S, et al. Reactivity of selenium-containing compounds with myeloperoxidase-derived chlorinating oxidants: second-order rate constants and implications for biological damage. *Free Radical Biology and Medicine.* 2015 Jul 31; 84:279-88.
10. Kleniewska P, Pawliczak R. The participation of oxidative stress in the pathogenesis of bronchial asthma. *Biomedicine & Pharmacotherapy.* 2017 Oct 1; 94:100-8.

11. Wood LG, Baines KJ, Fu J, et al. The neutrophilic inflammatory phenotype is associated with systemic inflammation in asthma. *Chest* 2012; 142:86-93.
12. Rodríguez-Rodríguez E, Ortega RM, González-Rodríguez LG, et al. Dietary total antioxidant capacity and current asthma in Spanish schoolchildren: a case control-control study. *Eur J Pediatr* 2013; 173:517-23.
13. Styan GP, Boyer C, Chu KL. Some comments on Latin squares and on Graeco-Latin squares, illustrated with postage stamps and old playing cards. *Statistical Papers*. 2009;50(4):917-41.
14. Mak JC, Ho SP, Ho AS, et al. Sustained elevation of systemic oxidative stress and inflammation in exacerbation and remission of asthma. *ISRN Allergy*. 2013 Aug 29; 2013:561831.
15. Reddell HK, Baclarier LB, Bateman ED et al, Global initiative for asthma (GINA) strategy 2021. Executive summary and rationale for key changes. *Eur Respir J* 2021.
16. Suski JM, Lebiezinska M, Bonora M, et al. Relation between mitochondrial membrane potential and ROS formation. *Mitochondrial Bioenergetics: Methods and Protocols*. 2012:183-205.
17. Torun AN, Kulaksizoglu S, Kulaksizoglu M, et al. Serum total antioxidant status and lipid peroxidation marker malondialdehyde levels in overt and subclinical hypothyroidism. *Clin Endocrinol*. 2009 Mar; 70(3):469-74.
18. EL-Alameey IR, Fathy GA, Shady MM, et al. Relationship of Oxidant and Antioxidant Markers to Asthma Severity in Egyptian Asthmatic Children. Open access Macedonian J Med. Sci. 2017 Aug 15;5(5):645.
19. Ahmad A, Shameem M, Husain Q. Relation of oxidant-antioxidant imbalance with disease progression in asthma. *Ann Thoracic Med*. 2012;7(4):226-32.
20. Zeiger RS, Schatz M, Li Q, et al. The association of blood eosinophil counts to future asthma exacerbations in children with persistent asthma. *J Allergy and Clin Immunol: In Practice*. 2015 Mar 1;3(2):283-7.
21. Sherenian MG, Fulkerson PC, Wang Y. Hospital Admission Associated with Higher Total IgE Level in Pediatric Patients with Asthma. *J Allergy and Clin Immunol*. 2015 Feb 1;135(2): AB151.
22. Samitas K, Delimpoura V, Zervas E, et al. Anti-IgE treatment, airway inflammation and remodeling in severe allergic asthma: current knowledge and future perspectives. *Euro Respiratory Review*. 2015 Dec 1;24(138):594-601.
23. Fatani SH. Biomarkers of oxidative stress in acute and chronic bronchial asthma. *J Asthma*. 2014 Aug 1;51(6):578-84.
24. Adaeze CA, Agozie V, Chiona LO et al. Use of global initiative for asthma (GINA) guidelines in asthma management among pediatric residents in Sub-Saharan African Country: cross sectional descriptive study. *Pan Afr Med J*. 2017;27:120.
25. Shabestari AA, Imanparast F, Mo-haghegh P, et al. The effects of asthma on oxidative stress, inflammation, and endothelial dysfunction in children with pneumonia. *BMC Pediatr* 22,534(2022).