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Original Research Article

Association of Adiponectin , Oxidative Stress, and Obesity in Asthmatics children

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Abstract

There is an increasing evidence of a positive correlation between asthma and obesity in children and adults. Adipokines regulate several metabolic and inflammatory functions. Several studies have shown that reactive oxygen species (ROS) play a key role in initiation as well as amplification of inflammation in asthmatic airways. This study is to highlight the oxidant-antioxidant imbalance in relation to adiponectin level in asthmatic children in Hilla province of Babylon.

Adiponectin , glutathione , total antioxidant and malondialdehyde were measured in 100 children; 60 newly diagnosed with asthma and 40 non asthmatic children with the comparable age and sex were enrolled in this study. Asthmatic children subdivided in two groups 30 patients in each group(obese and non-obese). Ages of patients and control ranged between (2years -12 years). The study was carried out in Babylon Teaching Hospital for Gynecology & Pediatrics in Babylon Province.

Adiponectin and total antioxidant were estimated by ELISA technique, glutathione by HPLC and malondialdeheyde by spectrophotometer. There was a negative significant correlation between adiponectin with glutathione and positive significant correlation between total antioxidant and malondialdehyde.

The results revealed the existence of an oxidant-antioxidant imbalance among obese asthmatic children, in parallel to changes in adepokines level.

Key Words: Adiponectin, glutathione, total antioxidant and malondialdehyde.

الخلاصة

هناك أدلة متزايدة على وجود علاقة إيجابية بين الربو والبدانة لدى الأطفال والبالغين. أديبوكينس تنظيم العديد من وظائف التمثيل الغذائي والالتهابات. وقد أظهرت العديد من الدراسات أن أنواع الأوكسجين التفاعلية (ROS) تلعب دورا رئيسيا في بدء وكذلك تضخيم الالتهابات في الشعب الهوائية الربو. هذه الدراسة هي لتسليط الضوء على اختلال أكسدة ومضادات الأكسدة فيما يتعلق بمستوى أديبونيكتين في الأطفال الربو في محافظة الحلة بابل.

تم قياس أديبونيكتين، الجلوتاثيون، ومضادات الأكسدة الكلية والملونديالديهايد في ١٠٠ طفل؛ تم تسجيل ٦٠ مريضا حديثا بالربو و ٤٠ طفلا غير مصابين بالربو مع العمر والجنس المقارنين في هذه الدراسة. الأطفال الربو تقسيمها إلى مجموعتين ٣٠ مريضا في كل مجموعة (يعانون من السمنة وذوي الوزن الطبيعي). تراوحت أعمار المرضى والسيطرة بين (٢ سنة –١٢ سنة). أجريت الدراسة في مستشفى بابل التعليمي لأمراض النساء وطب الأطفال في محافظة بابل.

تم تقدير أديبونكتين ومضادات الأكسدة الكلية بواسطة تقنية إليسا، الجلوتاثيون بواسطة هبلك و مالونديالدهيد بواسطة الطيف الضوئي.

كان هناك ارتباط معنوي سلبي بين الأديبونيكتين مع الجلوتاثيونوإرتباط معنوي إيجابي بين مجموع مضادات الأكسدة والمالونديالدهيد.

وأظهرت النتائج وجود اختلال أكسدة–مضادات الأكسدة بين الأطفال المصابين بالربو يعانون من السمنة المفرطة، بالتوازي مع التغيرات في مستوى أديبوكينس.أديبونيكتين، الجلوتاثيون، ومضادات الأكسدة الكلية و مالونديالدهيد.

Introduction

Asthma is the most common chronic lower respiratory disease in childhood throughout the world. Asthma most often starts early in life and has variable courses and unstable phenotypes which may progress or remit over time. The impact of asthma on the quality of life of patients, as well as its cost are very high [1]. Currently, primary prevention is not possible. However, in established disease, control can be achieved and maintained with appropriate treatment, education and monitoring in most children [2].

Causes of asthma; All phenotypes of asthma are multifactorial disorders which are the result of a complex interplay between genetic and environmental factors. These factors are thought to lead to inflammatory and structural changes which cause asthma symptoms [3].

In addition to storing energy, adipose tissue exerts an extremely active endocrine function and produces a variety of factors which circulate and regulate systemic metabolism and inflammation [4,5]. Among these factors, adipokines are defined as those cytokines secreted by adipose tissue. Leptin, adiponectin, resistin, chemerin, apelin, visfatin, plasminogen activator inhibitor 1 (PAI1), monocyte chemo-attractant protein 1 (MCP1), tumour necrosis factor alpha (TNFα) and interleukin 6 (IL6) [6].

Adiponectin, a collagen-like plasma protein produced specifically by adipose tissue, is abundantly present in the circulation that is involved in the homeostatic control of circulating glucose and lipid levels [6]. Some but not all studies demonstrate that low serum total adiponectin concentrations are associated with a greater risk for asthma among women and peripubertal girls [7]. In children, the clinical studies about this issue are limited. Actually oxygen is an essential molecule for aerobic metabolism, but it also has adverse properties that induce cell damage and cell toxicity. Through redox reaction oxygen and nitrogen atoms are candidates for the production of free radical species during electron transfer in living systems [8]. Free radicals are chemical species that process an unpaired electron which give them an extremely high chemical reactivity [9]. In biological systems, there are two types of free radical: reactive oxygen species (ROS) and nitrogen species (RNS) which include not only reactive molecules with an unpaired electrons; such as, hydroxyl radical ($OH\cdot$), superoxideanion radical ($O_2\cdot^-$), nitric oxide ($NO\cdot$), and nitric dioxide ($NO_2\cdot$) but alsoreactive molecules that do not contain unpaired electrons, suchas hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and peroxynitriteanion ($ONOO^-$) [10]. ROS vary in their reactivity and toxicity, whereas H_2O_2 is less reactive but more toxic than ($O_2\cdot^-$) because it has ability for penetrating biological membrane. Reactive nitrogen species are produced by oxidation of one of the terminal amino nitrogen atoms of L-arginine which is catalyzed by enzyme nitric oxide synthase [11]. Oxidative stress represents an imbalance between ROS production and the cellular antioxidant defense system. ROS production can rise when the breakdown of metabolites in the tricarboxylic acid (TCA) cycle exceeds the capacity of the electron transport chain (ETC) to assimilate the resulting electrons .

In stress conditions, ROS levels increase and, because of their high reactivity, participate in a variety of chemical reactions. they were involved in cell damage, necrosis, and apoptosis via oxidation of lipids, proteins, and DNA and provoke also endothelial dysfunction, in filtration, and activation of inflammatory cells [12].

Malondialdehyde is the organic compound with the formula $CH_2(CHO)_2$. It is the end product of lipid oxidation and is a marker for oxidative stress [13]. In cell membranes, lipid peroxidation

begins when electrons from lipids are kidnapped by unstable free radicals promoting a chain reaction with successive oxidations that results in lipid instability and formation of by products such as malondialdehyde (MDA) [14]. All biological membranes are characterized by the large amounts of polyunsaturated fatty acid (PUFAs) associated with amphipathic lipid and a variety of membrane proteins. Both isolated polyunsaturated fatty acid (PUFAs) and those incorporated into lipid are readily attacked by free radicals, becoming oxidized into lipid peroxidation. Therefore peroxidized membrane lipid occurs as a result of oxidative stress in intact cells [13]. Peroxidation of these labile unsaturated fatty acids can damage both protein and lipids as well as disrupt the structure and function of the membrane in most body cells [15]. It is very reactive and reacts with nucleophilic amine groups such as lysine, arginine and the amino termini of amino acids [16]. Antioxidants are molecules, capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent [17]. Oxidation reactions can produce free radicals which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves [18]. Antioxidant molecules can be divided into two categories : Endogenous antioxidants exogenous antioxidants**.** Glutathione reductase is a homodimeric enzyme that is a member of the flavor protein disulfide oxidoreductase [19]. It has an indirect impact in the prevention of oxidative damage in cells by helping to maintain intracellular reduced glutathione (GSH). Thus, measuring the activity of the enzyme is an indicator of oxidative stress. It is a ubiquitous enzyme that catalyzes the NADPH dependent reduction reaction of oxidized glutathione (GSSG) to reduced glutathione (GSH) [20]. Oxidized glutathione is reduced through a multi-step reaction in which glutathione reductase is reduced by NADPH, which in turn reacts with a GSSG molecule. This creates a disulfide interchange reaction that creates two GSH molecules and restores glutathione reductase to its oxidized form. Regenerated GSH is available to detoxify hydrogen peroxide. Maintenance of GSH is vital in oxidation-reduction processes as well as detoxification of hydrogen peroxide and organic peroxides brought on by inflammation in cells [21]. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction involving the loss of electrons or an increase in oxidation state. Oxidation reactions can produce free radicals. In turn, these radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell [22]. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid (vitamin C), or polyphenols [23]. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness [24]. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials of antioxidant supplements including beta-carotene, vitamin A, and vitamin E singly or in different combinations suggest that supplementation has no effect on mortality or possibly increases it [25-27].

Materials and Methods

Ethical issues: This study was approved by the local ethics committee, all patients' parents take part in the study

were already informed about the aim of the study, agreed, and signed a consent.

Date and duration: The study was carried out in Babylon Teaching Hospital for Gynecology & Pediatrics in Babylon Province, Hilla city, from February 2016 to May 2016.The practical side of the study was performed at the laboratory of Biochemistry Department at College of Medicine/ University of Babylon.

Study design: This study design was a case control study.

Patients and control: Sample size was calculated according to Daniel sample size formula equation. This study included 100 samples (60 patients divided into two groups (obese and nonobese), 40 apparently healthy control) the age of them from 2 years to 12 year.

Chemicals and methods: Chemicals that have been used in the present study obtained from thoughtful international companies were total antioxidant and adiponectin (Sandwich) ELISA kit (Elabscience/China) .[28] Gutathione by HPLCmethanol for HPLCacetonitrile for HPLCortho-phthalaldehyde (OPA)[29-31]**.** Malondialdeheyde by spectrophoto-meterthiobarbituric acid (TBA) trichlor-oacetic acid (TCA) [32]. Total anti-oxidant by ELISA kit [33].

Results

The total number of study groups were 100 childe describe on 3 groups

1.Obese asthmatics children with asthma (n = 30)

2.Nonobeseasthmatics children with asthma (n = 30)

3. Non obese children without asthma as a control (n = 40**)**

Age: There was no significant difference in age (as mean) between control and asthmatics children (obese and non-obese) as demonstrated in figure (1) .

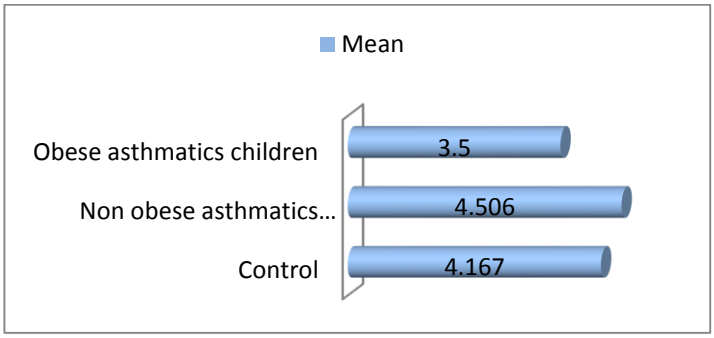

Figure 1: Age studied groups

Gender: Distribution of asthmatic children according to gender that show in figure (2). The present study demonstrated that the incidence of asthma in male (58.33%) is greater than females (41.66 %).

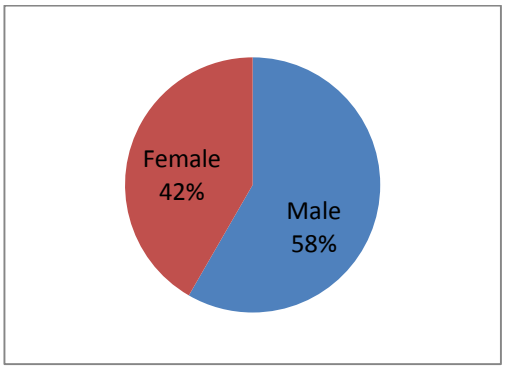

Figure 2 : Distribution of study groups according to gender

Body Mass Index: Distribution of asthmatic children according to body mass index (BMI) that show in figure (3). The (mean ±SE) of BMI for control, non-obese and obese patients were (15.912 ± 0.331) ,(15.946 ± 0.433) and (20.078 ± 0.349) restrictively. present result revealed that BMI was significantly (P < 0.01) greater in obese group when compared with non-obese patient or control, as shown in figure (3).

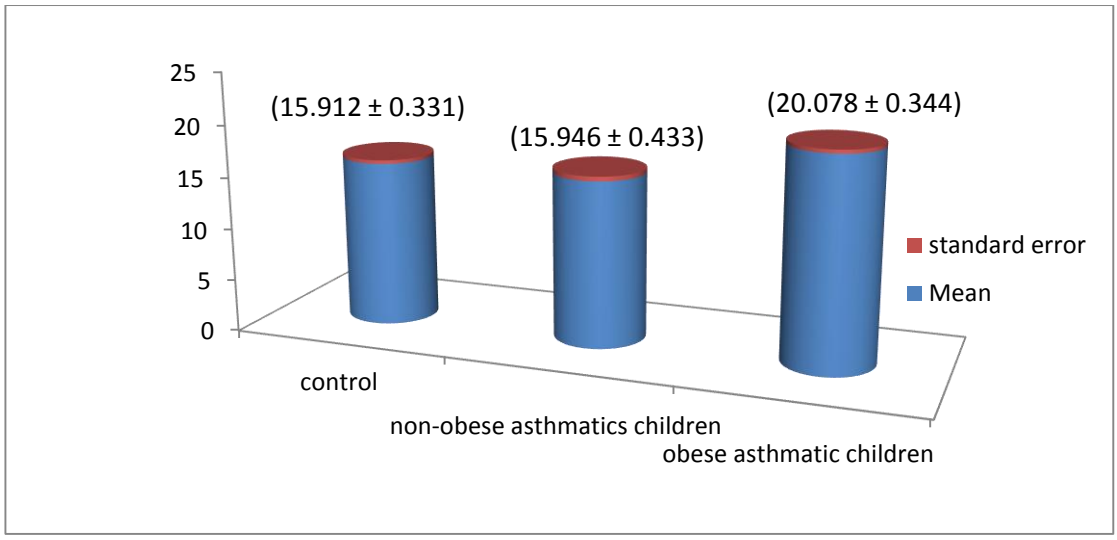

Figure 3 : Distribution of study groups according to body mass index**.**

Serum Adiponectin Concentration as (mean ± SE) of asthmatics children and control.

In the present study, serum concentrations of adiponectin mean ±SD were significantly decrease (p<0.01) in asthmatics (obese) children when compared with asthmatics (non-obese) and control group, as shown in table (1).

Table 1 : Adiponectin concentration in asthmatic children and control

	Asthmatics children			
Parameters	**Obese Mean ± SE N = 30**	**Non- obese Mean ± SE N = 30**	**Control Mean ± SE N = 40**	**P value**
Adiponectin	**46.884 ± 2.552**	----------	**52.840 ± 0.827**	**< 0.01***
Adiponectin	----------	**52.145 ± 0.706**	**52.840 ± 0.827**	**>0.05****

SE- Stander error.
N-number of study group .
*- P value between obese asthmatic children and control, and between(non-obese and obese) asthmatic children.
** – p value between non-obese asthmatic children and control

(157.21 ± 10.637) respectively. There were significant (p< 0.01) differences between asthmatics children when compared GSH and TAO with control groups. Otherwise present result did not show any differences between asthmatics children obese and non-obese for are antioxidant level that were determined in these studied.

Serum Antioxidant Level Glutathione Reduced (GSH) and Total Antioxidant (TAO)) of Study Groups

In table (3) shows there (mean ± SE) of GSH and TAO in asthmatics children (obese and non-obese), and control, where (20.313 ± 1.097), (16.802 ± 1.149) and (50.322±1.211), (85.144±8.937) ,(93.849±7.920) and

Table 2: Mean ± SE values of Glutathione and Total Antioxidant in asthmatics children groups (obese and non-obese) compared to control group.

	Asthmatics children			
Parameters	**Obese Mean ± SE N = 30**	**Non-obese Mean ± SE N = 30**	**Control Mean ± SE N = 40**	**P value**
GSH (µg/ml)	**20.813 ± 1.097**	**----------**	**50.322 ± 1.211**	**<0.01**
	20.813 ± 1.097	**16.802 ± 1.149**	**----------**	**<0.01**
TAO (ng/ml)	**85.144 ± 8.937**	**93.849 ± 7.920**	**157.21 ± 10.637**	**< 0.01**
	85.144 ± 8.937	**93.849 ± 7.920**	**----------**	**<0.01**

SE- Stander error.N-number of study group

Serum malondialdehyde (MDA) Concent. (mean ± SE) of study groups

The concentration of serum malondi-aldehyde (MDA) was significantly lower (p<0.01) in control children than in those of asthmatics children groups (obese and non-obese), as shown in table (3).

Table 3 : Mean ± SD values of malondialdehyde (MDA) in asthmatics children.

	Asthmatics children			
Parameters	**Obese Mean ± SE N = 30**	**Non-obese Mean ± SE N = 30**	**Control Mean ± SE N = 40**	**P value**
MDA (µmol/l)	**1.613 ± 0.1271**	**---------**	**1.277 ± 0.038**	**< 0.01**
	1.613 ± 0.1271	**1.701 ± 0.102**	**----------**	**< 0.01**

SE-Stander error.
N-number of study group.

Correlation Between Serum Adiponectin and Antioxidants in Asthmatics children:

In the present study, correlation between adiponectin with antioxidant (GSH) were shown negative significant correlation, while adiponectin shown insignificant correlation with (TAO) in asthmatic children, as shown in table (4) and figure (4).

Table 4 : Correlation between adiponectin with antioxidant in asthmatics children.

Antioxidant	Adiponectin (ng/ml)	
	r	P
GSH(µmol/ml)	- 0.278	0.032
TAO(µmol/ml)	- 0.237	0.069

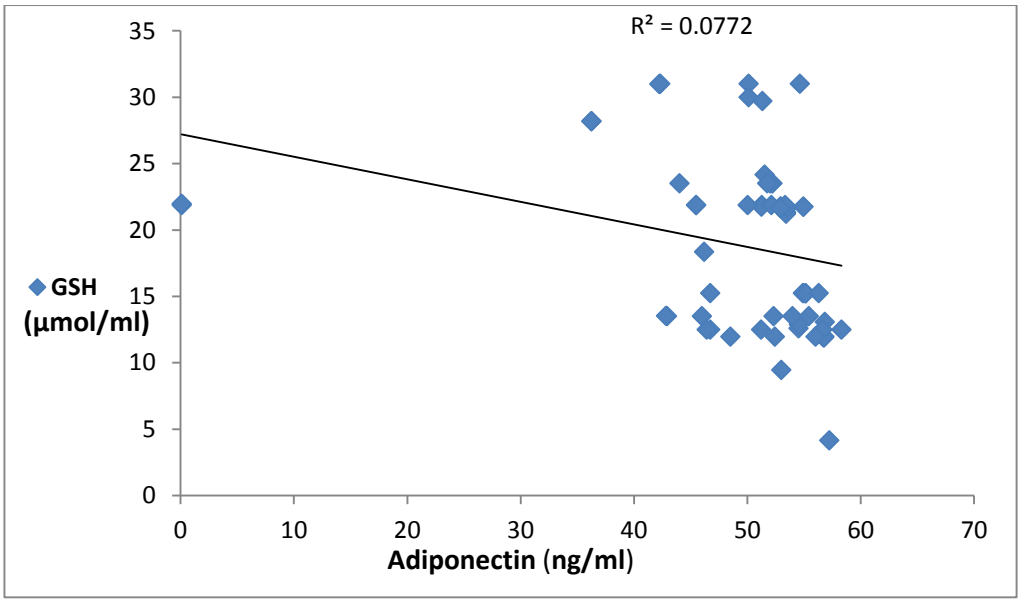

Figure 4: Correlation between adpokines (adiponectin) with GSH in asthmatics children.

Correlation between serum MDA and Antioxidant in Asthmatics Children:

Current results revealed that no-significant correlation observed between antioxidants (GSH) and MDA, on the other hand a significant (p= 0.04) positive correlation indicated only between TAO and MDA, as shown in table (5) and figure (5).

Table 5 : Correlation of CAT ,GSH and TAO in asthmatics children with MDA.

Antioxidant	MDA (µmol/ml)	
	r	P
GSH (µmol/ml)	0.061	0.642
TAO (µmol/ml)	0.266	0.040

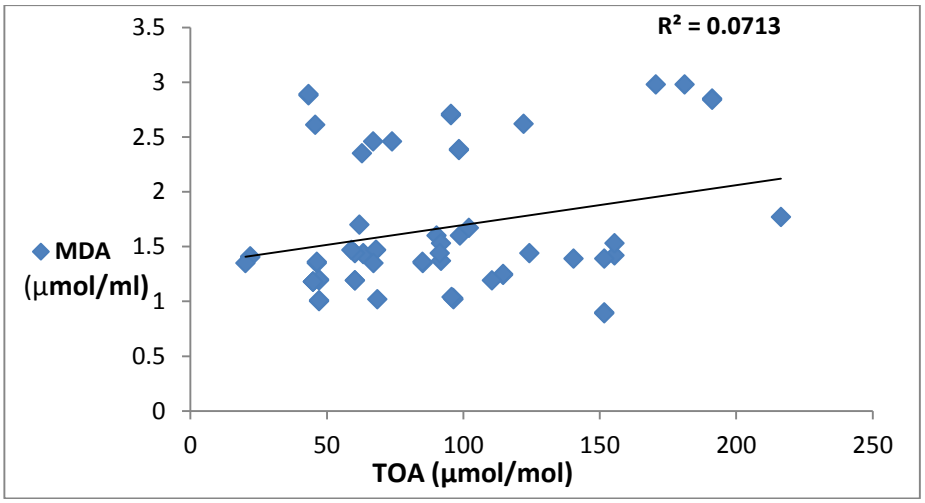

Figure 5 : Correlation of TAO in asthmatic children with MDA children.

Discussion

Asthma represents as a chronic inflammatory disorder of the airways and is associated with airway hyper-responsiveness that leads to recurrent episodes of often reversible, widespread airflow obstruction within the lung [34, 35]. In the past decades, important insights have been gained into the pathogenesis of asthma. While recently, hormones and cytokines released from adipose tissue, especially adipokines and ghrelin, have been the focus of research regarding this association [35-37]. According to the statistics of this study, the mean age of patients who were diagnosed as asthmatic children (obese with non-obese) and control were (3.656, 4.506 and 4.167) years respectively. There was no significant difference in age (as mean) between controls and patients as shown in figure (1). This age matching helps to eliminate differences in parameters' results that may originate due to the big variation in age. Many studies [38], Mahmood *et al* [39] and Hazim *et al* [40] are consistent with the fact that the majority of asthmatic children report disease onset prior to 6 year of age and depend on the same principle of age matching. On the other hand the present study demonstrates a slight male predominance with an 1.4:1 ratio. This finding is going with those of Tiran *et al* [41] and Mahmood *et al* [39]. The adipose tissue in obese subjects leads to a systemic inflammatory state which produces a rise in the serum concentrations of proinflammatory adipokines, such as adiponectin as anti-inflammatory. Adiponectin is negatively associated with obesity because its

concentration increases with weight loss [43]. The decrease of adiponectin in obesity may be related to the association between obesity and asthma, [42-44] as allergen challenge leads to less airway responsiveness and inflammation in animal models with higher adiponectin levels [42]. Moreover, adiponectin inhibits the proliferation of cultured vascular smooth muscle cells [45]. If adiponectin were to have a similar influence on airway smooth muscle, the decrease in adiponectin in obese individuals could contribute to increased smooth muscle mass in asthmatic individuals [46]. also, the lower of adiponectin levels in obese compared with non-obese children with asthma and the lower adiponectin levels in non-obese children with asthma compared with controls in the present study support an anti-inflammatory role for this adipokine, as demonstrated in table (1). These results agreed with [42-46]. Asthma itself may cause physiological changes in serum antioxidant status, perhaps because of increased oxidant burden associated with the disease. Numerous disturbances of antioxidant defense mechanisms have been described in asthma, as that of the epithelial lining fluid of the lung which contains high a concentration of antioxidants providing a first line of defense against inhaled and endogenously oxidant agents [47]. This study revealed a significant increase of MDA among all asthmatic children studied compared to non-asthmatic children. This implies that children during acute asthmatic attack are exposed to a considerable degree of lipid peroxidation. This finding is consistent with other observations Narula *et al* [48] and Nada *et al* [49]. This review studied the total antioxidant status of asthmatic patients compared to controls. It could be observed that asthmatic patients have significantly low total antioxidant status **[50].**Glutathione has antioxidant properties due to the thiol group in its cysteine moiety, which is a reducing agent and can be reversibly oxidized and reduced. GSH functions as an antioxidant by acting as a sacrificial target for ROS and other products of lipid peroxidation, such as reactive carbonyls. In doing so, GSH becomes oxidized to its dimeric form (GSSG) or forms adducts with reactive carbonyls. Furthermore, enzymes such as glutathione peroxidase and glutathione transferase can facilitate this process. Oxidized glutathione can itself be reduced back to GSH by glutathione reductase using NADPH. In cells, glutathione is maintained in the reduced form by the enzyme glutathione reductase and in turn reduces other metabolites and enzyme systems as well as reacting directly with oxidants. We examined the extent of reduced glutathione, it shows that asthmatic patients have significantly lower level of glutathione compared to control subjects. It goes with the results of other studies Noaf *et al* [50] and Oberholzer *et al* [51]. Host antioxidant systems are generally activated in response to the oxidant attack, but individuals have different capacities of antioxidant defense, which are in part genetically determined [52].

Although there are conflicting data regarding the role of antioxidant and adipokines in the pathogenesis of asthma in children, there is still limited studies about the association between these two factors explaining some scientific rationale for potential effects of these mediators in the asthma pathogenesis. The current study found a significant relationship among adiponectin and GSH**.** Again the small number of our patients and control group might be the cause of this result, this can be explored and validated in another larger study.

Asthma is associated with increased exhaled breath condensate levels of malondialdehyde (MDA) and reduced antioxidant, both demonstrated in children [53,54].

Antioxidant, in its reduced form, protects airway epithelial cells from free radicals while MDA is formed due to the action

of reactive oxygen species on membrane phospholipids and is a marker of oxidative stress. Plasma MDA levels are increased and antioxidant levels are decreased in children with asthma, with the highest levels of oxidant stress occurring in children with more severe disease [55]. Oxidant–antioxidant imbalance plays an important role in asthma; however, the effects of obesity [56].

Conclusion

The results revealed the existence of an oxidant-antioxidant imbalance among obese asthmatic children, in parallel to changes in adepokines level.

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