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Evaluate of Antioxidant Enzymes Superoxide Dismutase, Glutathione Peroxidase and Catalase Levels in Asthma Patients

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Abstract
Asthma is a chronic airway inflammation which involves the interplay of different types of inflammatory cells and cytokines in the airway. The present study evaluates the antioxidant enzymes including superoxide dismutase, glutathione peroxidase and Catalase levels. The results showed a significant increase ($P<0.05$) in erythrocyte superoxide dismutase (SOD) activity in asthmatic patients as compared to control group. While there were a significant decrease in glutathione peroxidase (GPx) ($P<0.05$) and Catalase (CAT) ($P<0.05$) activities in patients as compared to control group, in accordance with age, severity, smoking and family history. But, there were no significant changes in the enzymes levels between males and females. The results indicated that antioxidant enzymes could play an important role in gen- environment interaction in complex lung disease such as asthma.
Keywords: MDA: Malondialdehyde, SOD: Superoxide dismutase, GPx: Glutathione peroxidase, CAT: Catalase, ROS: Reactive oxygen species.

Introduction

Asthma is a chronic inflammatory disease of the respiratory tract of the unknown etiology. Asthma is a complex disorder involving biochemical, autonomic, immunologic, infectious, endocrine and psychological factors in varying degrees in different individuals [1]. According to the world health organization (WHO), asthma is now a serious public health problem with over 100 million sufferers worldwide, death from this condition has reached over 180,000 annually [2, 3]. Asthma remains the most common chronic illness of childhood; it is regarded as the fifth cause of death in children [4]. These systems consist of antioxidant enzymes such as superoxide dismutase (SODs), glutathione peroxidase (GPx), catalase (CAT) and glutathione reductase (GRx), as well as non enzymatic antioxidants, such as vitamin C, E, glutathione and uric acid.

Superoxide dismutase (SOD) is metallo enzyme that catalyzes the disputation of the superoxide anion to molecular oxygen and hydrogen peroxide and thus form a crucial part of the cellular antioxidant defense mechanism [5]. Two superoxide dismutase are enzymes have so for been described in vertebrates i. e. Cu/ Zn- SOD (Copper/ Zinc superoxide dismutase) and Mn- SOD (Manganese superoxide dismutase).The Cu/ Zn- SOD has been demonstrated in the cytoplasm and also in the inter membrane space in mitochondria. But, Mn-SOD is found in the matrix space in mitochondria [6]. The enzyme SOD is considered the first line of defense because it firstly catalyses in the system harvesting oxygen- free radicals, therefore SOD prevents the oxidation of biological molecules. Local deficiency of SOD can lead to the formation of peroxy nitrite and other oxidizing species, while adequate SOD can scavenge superoxide anion and permit nitric oxide radical to remain active as a signaling molecule [7].

Glutathione peroxidase (GPx) are *tetra* metric enzymes consisting of four 22 KDa monomers, each of which contains a selenocysteine moiety in the active site. There are four subspecies of GPx that catalyze the reduction of hydrogen peroxide and organic hydro peroxides ROOH to water. This occurs with the oxidation of glutathione in specific tissue locations [8]. GPx1 is ubiquitous and found in the cytosol of most cells, including red blood cells (RBC). GPx2 is also cytosolic but is confined to the gastrointestinal tract. GPx3 occurs in the plasma as a glycoprotein and GPx4 interacts with complex lipids, such as cholesterol and lipoproteins damaged by free radicals, and is found in mitochondria [8].

Catalase (CAT) is an enzyme which is present mainly in the Peroxisomes of mammalian cells. It is a *tetra* metric enzyme consisting of four identical tetrahedrally arranged sub units of 60 KDa, each containing in its active center a heme group and NADPH [9]. Catalase acts catalytically remove hydrogen peroxides (H_2O_2) by forming water and oxygen. The present study was undertaken to evaluate the antioxidant enzymes: Superoxide dismutase (SOD), Glutathione peroxidase (GPx) in the erythrocyte and Catalase (CAT) concentration in the blood of asthmatic patients with several parameters (age, sex, severity, smoking and family history).

Materials and Methods

One hundred patients (55 males, 45 females) with asthma clinically diagnosed admitted to Allergic center from the first of October 2009 to the end of March 2010 in Basrah city/ Iraq whose age ranged between (7-67) years for males and females, divided into three groups (7-35) years, (36-50) years, (>50) years. The following information was recorded for asthmatic patients: age, sex, and smoking, date of admission, family history of allergy, sign and symptom and severity of the acute attack.

Those patients were classified according to the severity into three groups:
1- Mild 2- Moderate 3- Sever

This classification of severity depends on expert panel report; guidelines for the diagnosis and management of asthma, sign and symptoms were used mainly for the classification of severity [10]. Sixty healthy subjects (35 males, 25 females) were investigated as a control group from Public Health Center in Basra city aged between (18-65) years. Five mL of fresh venous blood were collected from each patient and control subjects. These samples were divided in to the following: 2.5 mL were added to heparin anticoagulant tubes. Blood samples were centrifuge at 3000 rpm for 10 minutes in order to separate the plasma. The remaining erythrocytes were washed three times with 0.9% NaCl, and lysate in 1:1 (V/V) of double – deionised water, which was lysates used for laboratories U.K..Based on [11]. Activity of glutathione peroxidase in whole blood was measured using a Kit (RANSEL) from Randox laboratories based on the method of [12]. Catalase was assayed calorimetrically at 620 nm and expressed as µmoles of H_2O_2 consumed min\mL of serum described by [13]. The reaction contain 1.0 mL of 0.01M phosphate buffer with 0.1mL of serum and 0.4mL of H_2O_2, the reaction was stopped by the addition of 2 mL of dichromate acetic acid reagent.

Instruments:
1- Spectrophotometer SP 8-100 VV pye Unicom, UK.
2- Centrifuge, Kokusan, Japan.
3- Vortex stirrer, Gallen Kamp, Germany.
4- Water bath, Gallen Kamp, Germany.
5- Stopped watch, Fisher scientific company, USA.

Materials:
The entire chemicals in this study were imported from BDH Co. and Sigma Co.

Calculations:
The concentration of Superoxide dismutase (U/mL):

$$\frac{A_1 - A_2}{3} = \Delta A/\text{min of standards or samples}$$

$$100 - \frac{(\Delta A_{Std/min} \times 100)}{(\Delta A_{\frac{S1}{min}})} = \%\ \text{inhibition}$$

$$100 - \frac{(\Delta A_{Sample/min} \times 100)}{(\Delta A_{\frac{S1}{min}})} = \%\ \text{inhibition}$$

A_1: The absorbance of the sample/30 sec.
A_2 : The absorbance of the sample/3 min.

The concentration of Glutathione peroxidase GPx (U/L):
U/L of Haemolysate = 8412 × ΔA_{340nm} / minute
GPx unit / L of whole blood = GPx U/L of Haemolysate × 41(dilution factor),
Where: 8412 = Total volume (ml) × 1000 / Sample volume (ml) × milimolar extinction coefficient (6.3) × light path (1 cm).

The concentration of Catalase CAT (U): $$K = \frac{V_t}{V_s} \times \frac{2.3}{\Delta t} \times \text{Log} \frac{A_1}{A_2} \times 60$$

K: Constant of rate.
Δt: (T2-T1) equal to 15 sec.
A_1: The absorbance/15 sec.
A_2: The absorbance/30 sec.
Vt: The volume measured (3 ml).
Vs: The volume of sample in mixed (2 ml).

Statistical Analysis:
Results were expressed as mean and standard deviation (SD). Statistical analysis was carried out using the SPSS. Program (version 15 software, SPSS Inc. Chicago, Illinois, USA). For the comparison of groups, independent student t test, ANOVA, Kruskal Wallis and Mann Whitney U test were used. Pearsorr S correlation between variable. P values of less than 0.05 were regarded as significant.

Results and Discussion:

Asthma is a chronic inflammatory airway disorder associated with recruitment of inflammatory cells. It is characterized by reversible and variable recurrent episodes of airway obstruction that resolve spontaneously or as a result of treatment. Prominent symptoms include wheezing, breathlessness, chest tightness and cough, particularly at night and or early in the morning [14]. The results of the present study show that the activity of antioxidants enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) in asthmatic patients.

SOD is an enzyme that reduces the cytoplasm and mitochondrial damage induced by superoxide radicals. The increased activity of SOD observed in the present study (table 1 and graph 1) ($P<0.05$) as compared to control subjects, may be indicative of increased superoxide anion $(O_2^{\cdot})^-$ generation. The over expression of SOD might be an adaptive response and results in increased disputation of superoxide to H_2O_2. It could be easily observed that lower limit of SOD activity in asthmatic was recorded with in the upper limit level of controls which ascertained the significant elevation of SOD in patients. The elevation recorded in the present study is in good agreement with previous studies that recorded higher RBC and SOD activity in asthmatic patients compared to control [15; 16; 17; 18; 19]. Increased concentration of SOD might be a compensatory response to the increased oxidative stress because of the increase of mitochondrial oxidation rate characterized by an over production of superoxide anion [19; 20; 21].

GPx plays a significant role in the peroxyl scavenging mechanism and in maintaining functional integration of the cell membrane. The significant decrease in GPx activity ($P<0.05$) recorded in the present study (table 2 and graph 2) as compared to control could be easily correlated to the high significant elevation of lipid peroxides. Lower GPx level in asthmatic patients could be related to the clinical presentation of the disease and could explain the presence of H_2O_2 in the breath condensate of exhaled air which is elevated in asthmatic reported in previous studies [22; 23]. Decreased GPx activity has been well documented [15; 16]. This supports the lower GPx activities observed in the present study. High SOD activity together with low GPx activity in asthmatic confirms the contribution of oxidative stress in the etiology of asthma.

CAT is responsible for detoxification of H_2O_2, that formed by SOD and other processes is scavenged by catalase that catalyzes the disputation of H_2O_2 into water and molecular oxygen [24] significantly decrease for catalase activity ($P<0.05$) in asthmatic patients compared with control as show in (table 3 and graph 3), when hydrogen peroxide could not be detoxified as a results catalase activity was decreased due to hydrogen peroxide possibly convert to hydroxyl radical by iron in asthmatic patients [25]. These significant changes in the activity of enzymes SOD, GPx and CAT increases with age and severity from mild to moderate than sever ($P<0.05$). Also there were no significant differences between male and female patients as indicated in (Tables 1, 2, 3).

These imply that patients during a cute asthmatic attack are exposed to considerable degree of lipid Peroxidation; it has a strong correlation with atopic asthma suggesting that oxidative stress [20]. Asthma severity is related to the extent of lipid Peroxidation, severity ($P<0.05$) [26]. As indicated in (Tables 1, 2, 3). On the other hand, there were a significant difference in enzymes activates (SOD, GPx and CAT) between asthmatic smokers and non smokers ($P<0.05$) as indicated in (Tables 1, 2, 3).

Cigarette smoke contains high amount of free radicals such as superoxide anion $(O_2^{\cdot})^-$ and nitric oxide ($N\dot{O}$) [9], gas phase can react chemically to from highly reactive free radical peroxy nitrite. In addition superoxide anion can react with hydrogen peroxide to form the more active hydroxyl free radical [27]. Many researchers have reported increased level of superoxide anion from circulating neutrophils and increased lipid Peroxidation products malondialdehyde (MDA) in the plasma of smokers, supporting the concept of systematic oxidative stress in these individuals [28].

Also family history seems to influence the enzymes (SOD, GPx and CAT) activities as compared to positive and negative family history of asthmatic patients, there were a significant changes ($P<0.05$) in the activities as shown in (Tables 1, 2, 3). Some genetic variant may only cause asthma when they are combined with specific environmental exposures, the genetic trait, CD_{14} single nucleotide polymorphism, and exposure to end toxin (a bacterial product). Researchers have found that the risk of asthma changes based on a person's genotype at CD_{14} and the level of end toxin exposure [28; 29]. Asthma itself may cause physiological changes in blood antioxidants burden associated with disease [30; 31].

Conclusion

The present study suggests a very high production of ROS and oxidative stress in patients with asthma and failure of antioxidants mechanisms.

Table (1): Serum level of superoxide dismutase (SOD) (U/ mL) in asthmatic patients classified according to (Age, Sex, Severity, Smoking, Family history) and healthy control.

Variable		Asthmatic patients (100)		Control (60)		P-value
		No.	Mean± SD	No.	Mean± SD	
Age/ year	7-35	43	224.511±9.502*	25	191.162±7.879	P<0.05
	36-50	37	223.541±9.916	20	191.600±8.603	
	>50	25	225.661±14.550	15	194.123±11.427	
Sex	Male	47	226.646±14.505	35	197.327±17.779	P<0.05
	Female	53	221.193±12.853	25	195.217±8.935	
Severity	Mild	32	209.876±10.427*	60	191.028±9.340	P<0.05
	Moderate	17	215.047±7.395			
	Sever	51	221.906±9.302			
Smoking	Positive	25	246.700±19.519*	33	192.541±11.916	P<0.05
	Negative	75	229.518±20.731	27	201.077±13.833	
Family history	Positive	35	226.590±18.565*	60	195.378±9.961	P<0.05
	Negative	65	221.383±16.850			

* The mean difference is significant at the 0.05 level

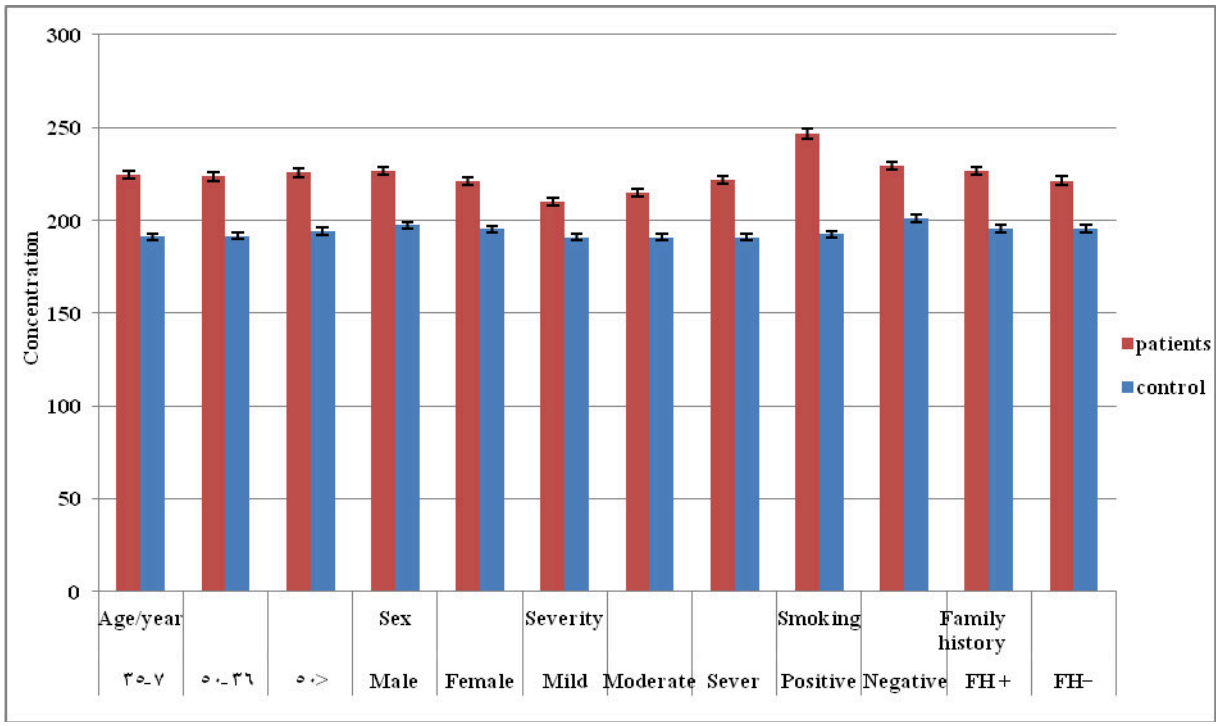

Figure 1. Serum Superoxide dismutase (U/ml) in asthmatic patients and healthy control classified according to (Age, Sex, Severity, Smoking and Family history).

Table (2): Serum level of glutathione peroxidase (GPx) (U/ L) in asthmatic patients classified according to (Age, Sex, Severity, Smoking, Family history) and healthy control.

Variable		Asthmatic patients (100)		Control (60)		P-value
		No.	Mean± SD	No.	Mean± SD	
Age/ year	7-35	43	2925.250±787.564*	25	3502.331±1386.953	P<0.05
	36-50	37	2148.736±981.368	20	3312.772±1337.986	
	>50	25	2429.260±1001.086	15	3760.584±338.175	
Sex	Male	47	2928.916±1884.176	35	3804.781±1644.137	P<0.05
	Female	53	2812.356±1655.398	25	3858.790±1974.560	
Severity	Mild	32	2709.005±731.5726*	60	3826.341±970.328	P<0.05
	Moderate	17	2426.314±632.1069			
	Sever	51	2082.321±896.7633			
Smoking	Positive	25	2148.648±787.312*	33	3767.556±1128.169	P<0.05
	Negative	75	2547.305±872.849	27	3915.384±1727.384	
Family history	Positive	35	2210.978±1395.724*	60	3870.634±1428.651	P<0.05
	Negative	65	2463.932±1735.913			

* The mean difference is significant at the 0.05 level

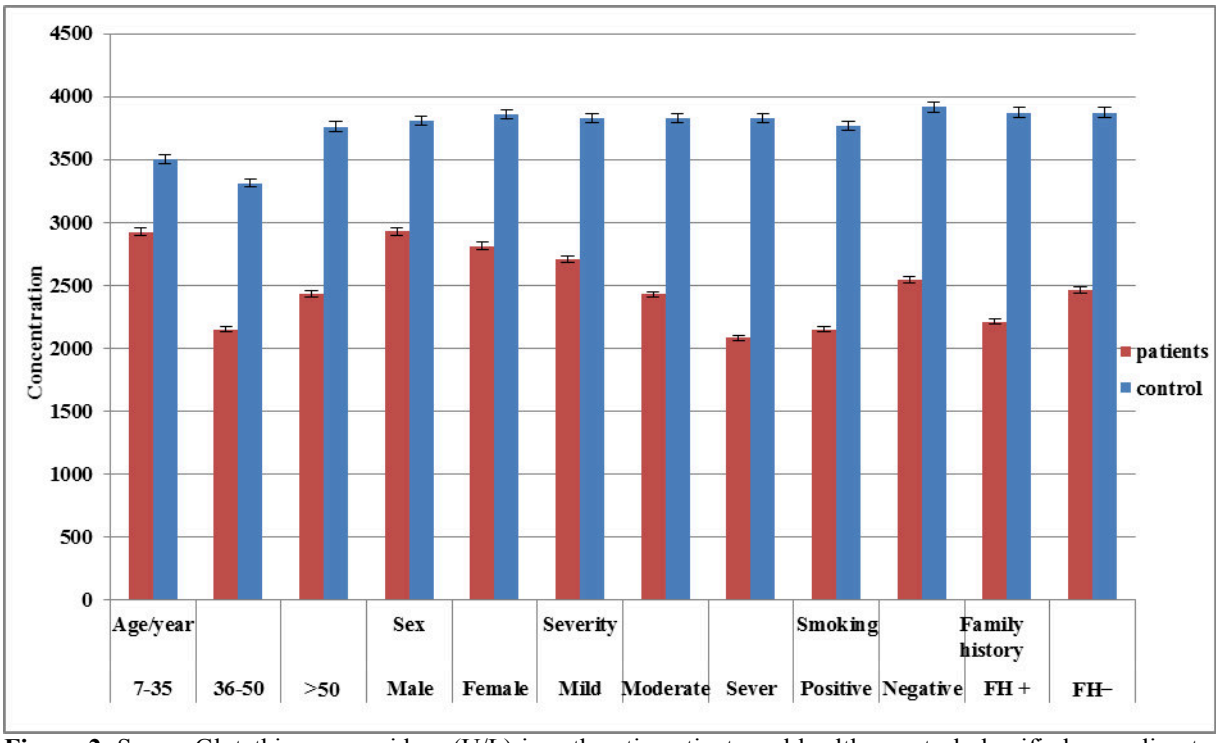

Figure 2. Serum Glutathione peroxidase (U/L) in asthmatic patients and healthy control classified according to (Age, Sex, Severity, Smoking and Family history).

Table 3. Serum level of catalase (CAT) (U) in asthmatic patients classified according to (Age, Sex, Severity, Smoking, Family history) and healthy control.

Variable		Asthmatic patients (100)		Control (60)		P-value
		No.	Mean± SD	No.	Mean± SD	
Age/ year	7-35	43	0.693±0.392*	25	1.456±0.244	P<0.05
	36-50	37	0.574±0.407	20	1.594±0.303	
	>50	25	0.485±0.351	15	1.688±0.518	
Sex	Male	47	0.820±0.386	35	1.462±0.749	P<0.05
	Female	53	0.700±0.377	25	1.527±0.930	
Severity	Mild	32	1.278±0.524*	60	1.745±0.730	P<0.05
	Moderate	17	1.027±0.777			
	Sever	51	0.712±0.447			
Smoking	Positive	25	0.703±0.674*	33	1.482±0.807	P<0.05
	Negative	75	0.484±0.164	27	1.770±0.919	
Family history	Positive	35	0.519±0.261*	60	1.654±0.429	P<0.05
	Negative	65	0.782±0.431			

* The mean difference is significant at the 0.05 level

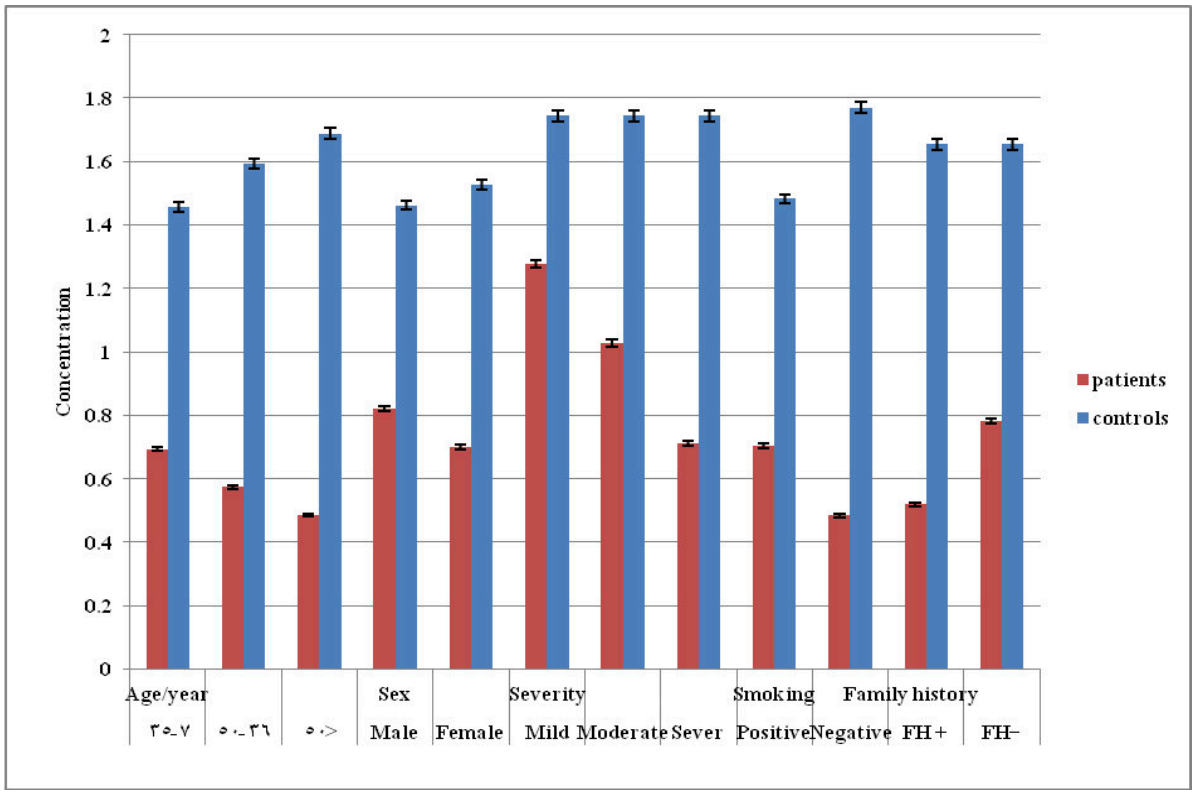

Figure 3. Serum Catalase (U) in asthmatic patients and healthy control classified according to (Age, Sex, Severity, Smoking and Family history).

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