The Effect of Inhalation Treatment on Oxidative Stress in Asthmatic Patients

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الخلاصة

ف ي الدراس ة الحالي ة م جم ع (90)نم وذج مرض ى الرب و 30نم وذج ناصح اء مجموع ة قياس ية)لنم اذج م ن مرك ز الرب و والحساس ية ف ي مدين ة الحل ة بين ت النت ائج بشكل عام بمقارن ة مرض ى الرب و المس تخدمين ع لاج البخ اخ الس تيرويدي م ع الاص حاء (فهم مجموع ة قياس ية) ان هنال ك زي ادة معنوي ة بالم الون داي ألديهايد و انخف اض معنوي في مس توى فعالي ة س وبر اوكس يد دزميوتيز وكري اتين ك اينيز (P ≤0.05) بينما هنالك زيادة غير معنوية في مستوى فعالية كلوتوثايون اس ترانسفريز (P ≤0.05) أوض حت النت ائج مقارن ة مرض ى الرب و المس تخدمين لع لاج البخ اخ الس تيرويدي م ع المرض ى غي ر المس تخدمين للع لاج ان هنال ك زي ادة معنوي ة في مس توى فعالي ة س وبر اوكس يد دزميوتيز وكلوتوث ايون اس ترانس فريز عند الرج ال (P ≤0.05) بينم ا هنال ك زي ادة غير معنوية في مستوى كرياتين ك اينيز وكلوتوث ايون اس ترانس فريز (P ≤0.05)في نس اء في سن اليأس و انخفاض معنوي في مس توى المالون داي ألديهايد (P ≤0.05) . عن د مقارن ة مرض ى الرب و غي ر المس تخدمين الع لاج (ج ال و نس اء في س ن لي أس) م ع الاص حاء وج د ان هنالك انخفاض معنوي في مستوى فعالية سوبر سوبر اوكسيد دزميوتيز و كرياتين كاينيز (P ≤0.05) وهنال ك زي ادة غي ر معنوي ة في مس توى فعالي ة كلوتوث ايون اس ترانس فري (P ≤0.05) بينما هنالك زيادة معنوية بالم الون داي ألديهايد ,كذلك ع دم وج ود ارتب اط معن وي بين فترة اس تخدام الع لاج ولا وبر اوكس يد دزميوتيز و كلوتوث ايون اس ترانس فريز و كري اتين كاينيز والمالون داي ألديهايد في مرضى الربو.

Abstract

The present study was conducted to verify the oxidative stress status in asthma patients . To achieve this aim, ninty asthmatic patients, and thirty healthy subjects (control group) were subjected to the study *the sample were obatained from Babylon Asthma and Allergy Center in Hilla City* .. showed The results of this study in asthmatic patients (men and menopause women) with steroid inhalation treatment group when compared with control group showed a significant increase of Malondialdehyde (MDA) ,amd a significant decrease of super oxidase dismutase (SOD) and creatine kinase(CK) levels ($P < 0.05$) , wherease there was non significant increase in glutathione-S-transferase (GST), levels($P < 0.05$) .

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The results of this study for asthmatic patients (men and menopause women) with steroid inhalation treatment group when compared with asthmatic patients without treatment indicated a significant increasing levels of super oxidase dismutase (SOD) (SOD) , glutathione-S-transferase (GST) of men ($P < 0.05$) , whereas there was a non significant increase in levels of creatine kinase(CK), glutathione-S-transferase (GST) of menopause women($P<0.05$) but there was significant decrease in malondialdehyde (MDA) levels.

In asthmatic patients (men and menopause women) without treatment group when compared with those of control group estimated significant decrease of super oxidase dismutase (SOD) (SOD) and creatine kinase(CK) level ($P < 0.05$) , wherease there was a non significant increase in GST but there was a significant increase in malondialdehyde (MDA) levels ,($P < 0.05$) .

The correlation analysis indicated a non significant negative correlation in period of treatment with superoxide dismutase (SOD) and creatine kinase(CK) , as well as a non significant positive correlation for glutathione-S-transferase (GST) and malondialdehyde (MDA) .

Key Wards : Asthma ,steroid inhalation treatment

Introduction

Asthma is chronic inflammatory disorder of the airways in which many cells and cellular elements play a role. The chronic inflammation is associated with airway hyperresponsiveness that leads to recurrent episodes of wheezing, breathlessness, chest tightness and coughing, particularly at night or in the early morning. These episodes are usually associated with widespread but variable, airflow obstruction within the lung that is often reversible either spontaneously or by treatment" [1]

There is no curative therapy for asthma. Today's standard therapy consists mainly in inhaled glucocorticosteroids such as budenoside, ciclenoside and fluticasone that control airway

inflammation [2]. with beneficial effects in terms of asthma symptoms, improvement of lung function [3] decreased airway hyperresponsivness [4,5] .The conventional medical therapy of asthma involves bronchodilator and anti – inflammatory medication. Treatment broadly classified into preventers and relievers [6].

Oxidative stress" is a term introduced to illustrate the imbalance within the cells between the production of prooxidants and antioxidant defences in favour of the former. It occurs either from the increased production of reactive oxygen species (ROS) or reactive nitrogen species (RNS), or a deficiency in the antioxidant defenses systems [7, 8]

Malondialdehyde (MDA) is one of the most frequently used indicators of lipid peroxidation [9]. It is very reactive and reacts with nucleophilic amine groups such as lysine, arginine and the amino terminal of amino acids [10]. It also reacts with any ketones or aldehydes from other sources, for example, attached sugars or glycation products [11]. Antioxidants can be divided into two group enzymatic antioxidants. Such as(SOD,CAT,…..etc) and nonenzymatic antioxidants. Such as(GSH,Uric acid, Tocopherol(vitamin E) ,Ascorbic acid((vitamin C), Retinol(vitamin A) …. etc).

Superoxide dismutase is the major intracellular antioxidant enzyme, which is essential for the survival of erobic cells It catalytically scavenges the superoxide radical, which appears to be important agent for toxicity of oxygen and thus provides a defense against oxygen toxicity. Superoxide dismutase catalyzes the dismutation of the superoxide anion free radical ($O_2^{\bullet -}$) to hydrogen peroxide and molecular oxygen at a rate 10^4 times faster than spontaneous dismutation at physiological pH [12] **.** Resulting in no superoxide anion available to react with hydrogen peroxide to form hydroxyl radical through the iron catalyzed reactions. Superoxide dismutase enzyme exists in several forms and is present in mitochondrial matrix, the cytoplasm and the extracellular fluid. Superoxide dismutase is broadly classified into distinct classes depending on the metal ion content.

Cu and Zn containing SOD found in cytosol and Mn containing SOD found in mitochondria [13,14] .

The local study aimed to study the effects of asthmatic inhalation treatment on levels of Superoxide dismutase.

Glutathione S-transferases (GSTs) are a large group of multifunctional proteins that catalyse the conjugation of GSH to various electrophilic substrates [15] .GSTs appear to play an important role in protecting cells against oxidative damage by binding glutathione in such a way that the sulfur is induced to ionize more completely, and binding a second molecule close by so that a reaction can be facilitated [16,17] . This reaction is necessary to detoxify xenobiotic materials such as toxins, drugs, and other foreign compounds[129] Glutathione S-transferases are ubiquitous multifunctional enzymes. GSTs catalyses a variety of reactions and accepts endogenous and xenobiotic substrates [18]

Creatin kinase(CK) is essentinal as acatalyst in the production of energy in muscle cell[19] . Creatine kinase catalyzes the phosphorylation of creatine usingATP:[20] .

CK is widely distributed in tissues ,with highest activities found in skeletal muscle, heart muscle ,and brain tissue. Other tissue sources in which CK is present in much smaller quantities includeract, thebladder ,placenta, gastrointestinal thyroid, uterus, kindney, lung, prostate, spleen,liver,and pancreas [21].

Materials and method

Patients and Control Subjects:

A cross sectional study was conducted on the following groups in the period between October /2010 and may /2011. The samples were obtained from Babylon Asthma and Allergy center in Hilla city.

Sixty asthmatic volunteers patients with steroid inahaltion treatment (30 men and 30 menopause women) of age range (16-64 years) in men and menopause women (45-69years) were enrolled, Thirty asthmatic volunteers patients without treatment (15 men and 15 menopause women) of age range (23-42 years)

in men and (45-56 years) menopause women were enrolled . and Thirty healthy volunteers were included in this study as control . They were matched in their sex and age with patients group.

The laboratory work was carried out in the laboratory of research in the General Afak Hospital and laboratory of chemistry branch medicine college of Alqadisiya University .

Asthmatic volunteers patients and healthy control do not suffered from any diseases such as diabetes mellitus, hypertension, cardiovascular disease ,….etc

Collection of samples:

Disposable syringes and needles were used for blood collection. Blood samples were obtained from asthmatic volunteers patients and control group by vein puncture. Samples were allowed to clot at room temperature, and then centrifuged at 3000 Xg for 10 minutes (Hettich EBA 20 Germany).

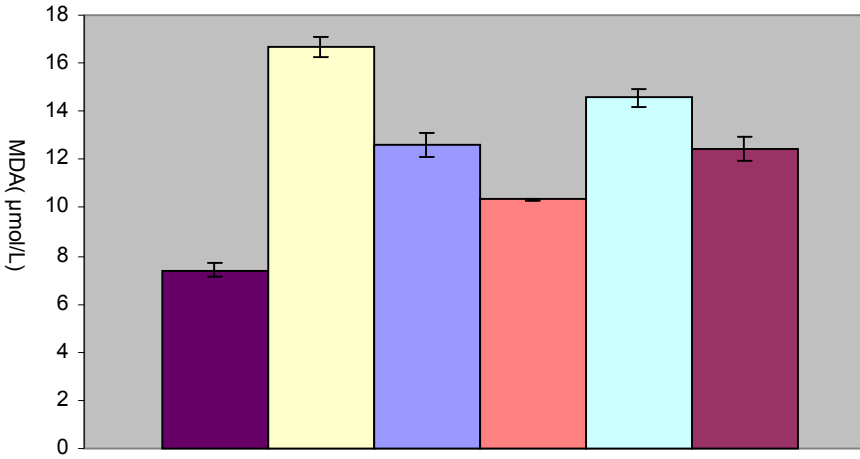
Measurement of MDA , SOD ,GST and CK by using(spectrophotometer APLE PD-303 UV Japan) at 532nm,560nm 340nm respectively [22,23,24,25,26, 27,28]

Result and Discussion

1. Lipid peroxidation **:-**

In this work , the mean ± SE of serum (MDA) levels in asthmatic men patients with steroidal inhalation treatment group was (12.59 ± 0.51 µmol/L *vs* 7.93 ± 0.29 µmol/L of healthy control group) and in asthmatic menopause women patients with steroid inhalation treatment group was (12.43 ± 0.49 µmol/L *vs* 10.33 ± 0.038 µmol/L of healthy control group) table(1), the mean ± SE of serum (MDA) levels in asthmatic men patients without treatment group was(16.66 ± 0.42 µmol/L *vs* 7.93 ± 0.29 µmol/L of healthy control group) and in asthmatic menopause women patients without treatment group was (14.57± 0.36 µmol/L *vs* 10.33 ± 0.038 µmol/L of healthy control group) table(2) The results of this study was show that using of steroidal inhalation treatment decrease the level of MDA by 24% in

asthmatic men and 14% in asthmatic menopause women patients table (3) Serum (MDA) levels in asthmatic patients with and without treatment groups (men and menopause women) was significantly higher than healthy control group level , $P \leq 0.05$ figure (3-1)



Figure(2) :level of malondialdehyde (MDA) in serum of healthy control , patients with treatment and patients without treatment(men and menopause women) . the values are the mean ± SE , $P \leq 0.05$

The results of this study indicated increased level of lipid peroxide via MDA in asthmatic patients with and without steroid inhalation treatment groups (men and menopause women) so that increased oxidative stress and this may be result from that oxidative stress in the lungs results in an influx of inflammatory cells to the lung with the subsequent generation and release of large quantities of free radicals and reactive oxygen species (ROSs) that responsible for many cytotoxic reaction , such as peroxidation,[29,230] . In the same, time the results of local study reverted that the levels of MDA in asthmatic patients without treatment group (men and menopause women)was significantly higher than the level of MDA compared with asthmatic patients with steroid inhalation treatment group(men and menopause women) this may returned to the effect of steroidal inhalation treatment that decrease ROS generation and this leading to

reduction in MDA level [31] and so that decrease the oxidative stress .

The results of this study were show non significant positive correlation under($P \leq 0.05$) between the period of treatment and level of MDA in serum of asthmatic patients table (3-5)figure (3-2)

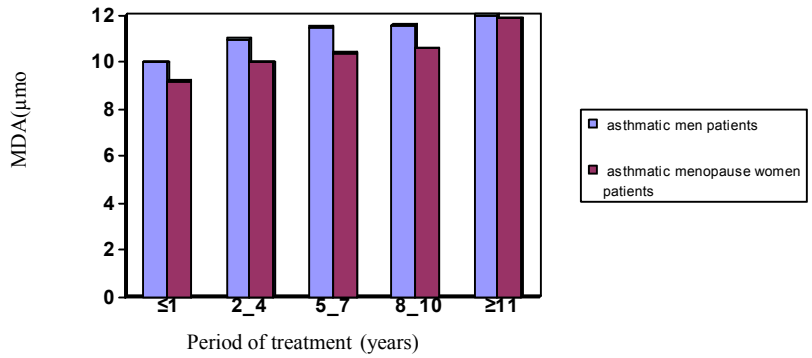


Figure (3) . Relationship between period of treatment with level of MDA in serum asthmatic patients with steroid inhalation treatment group(men and women menopause) , $P \leq 0.05$

2. **Superoxide Dismutase**

In this study , The mean ± SE of serum (SOD) activity levels levels in asthmatic men patients with steroid inhalation treatment group was (0.83 ± 0.04 U/L *vs* 1.7 ± 0.19 U/L of healthy control group) and in asthmatic menopause women patients with steroid inhalation treatment group was (0.717 ± 0.05 U/L *vs* 1.3 ± 0.097 U/L of healthy control group) table(1) and the mean ± SE of serum (SOD) levels in asthmatic men patients without treatment group was (0.55 ± 0. 045 U/L *vs* 1.7 ± 0.19 U/L of healthy control group) and in asthmatic menopause women patients without treatment group was (0.43± 0.015 U/L *vs* 1.3 ± 0.097 U/L of healthy control group) , table(2) , the results of this study that using of inhalation steroidal inhalation treatment increase the level of SOD by 33%in men and 40%in menopause women asthmatic patients table(4) , $P \leq 0.05$ figure (1)

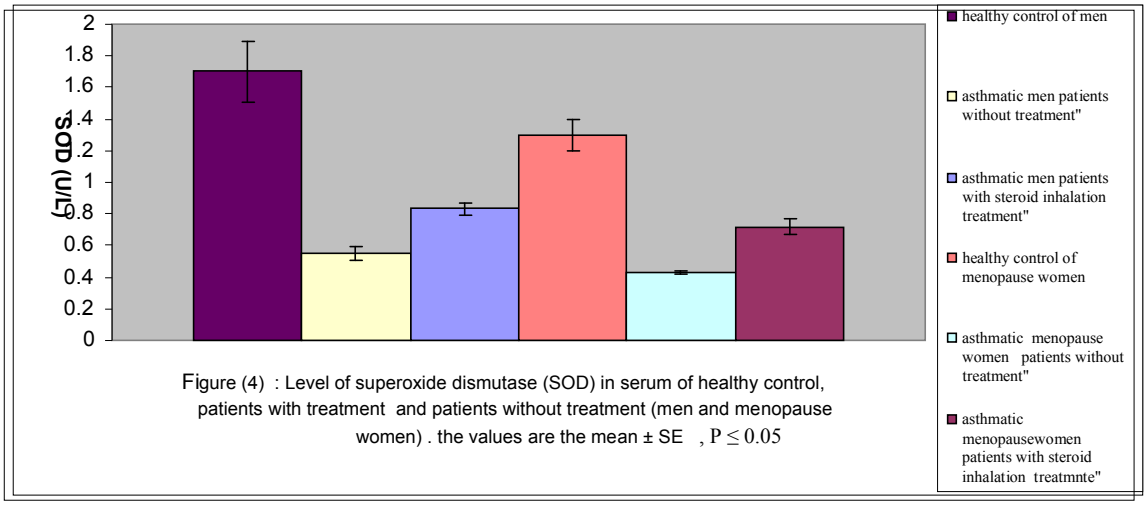


Figure (4) : Level of superoxide dismutase (SOD) in serum of healthy control, patients with treatment and patients without treatment (men and menopause women) . the values are the mean ± SE , $P \leq 0.05$

Serum (SOD) activity levels in asthmatic patients with and without treatment . (men and menopause women) was significantly lower than healthy control group level , (, $P \leq 0.05$) ,table (1,2).

Decreased In the level of serum SOD activity in asthmatic patients maybe related to airflow limitation .this was considered consistent with greater oxidant stress lead in to greater inactivation of SOD [32] . In increased oxidative stress also occur increase in produce of ROS and RNS that can react with many amino acid targets including methionine , tyrosin , histidine tryptophan , Lysin , and cysteine . All SOD enzymes are sensitive to oxidative modificantion on and inactivation [33,34 ,35]

ROS/RNS lead to Oxidative and nitrative modification Frication of tyrosione and inactivation of Mn- SOD and ES-SOD where as Cu, Zn – SOD can be Inactivited by RNS through targeting of susceptible histidine residues [34,36,37] as well as there ware significantly elevation in SOD activity level in the asthmatic patients with steroid inhalation treatment group (men and menopause women) compared with asthmatic patients without treatment group (men and menopause women) this may be attributed to the effect of inhaled steroid that reduction in the local formation of ROS this lead to increase activity of enzyme [38,39] .

The results of this study showed negative correlation but not significantly under ($P \leq 0.05$) between the period of treatment

with level of SOD in serum asthmatic patients(men and menopause women) table (5) figure(2)

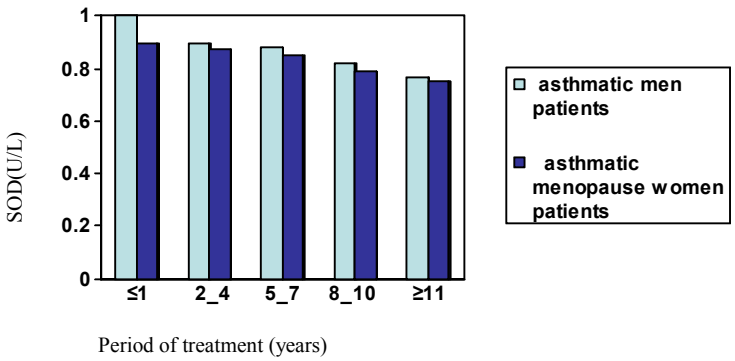


Figure (5) . Relationship between period of treatment with level of SOD activity in serum asthmatic patiens with steroid inhalation treatment(men and women menopause) , P ≤ 0.05

3. Glutathione S -transferase

In this work , The mean ± SE of serum (GST) activity levels in asthmatic men patients with steroid inhalation treatment group was (11.32 ± 0.59 U/L *vs* 8.63 ± 0.41 U/L of healthy control group) and in asthmatic menopause women patients with steroid inhalation treatment group was (10.27 ± 0.25 U/L *vs* 9.14 ± 0.34 U/L of healthy control group) table(1), the mean ± SE of serum (GST) levels in asthmatic men patients without treatment group was(9.05 ± 0. 6 U/L *vs* 8.63 ± 0.41 U/L of healthy control group) and in asthmatic menopause women patients without treatment group was (9.7± 0.25 U/L *vs* 9.14 ± 0.34 U/L of healthy control group) table(2) The results of this study showed that using of inhalation steroidal inhalation treatment increase the level of GST by 20% in asthmatic men and 5% in asthmatic menopause women patients table(5) P ≤ 0.05 figure (3) .

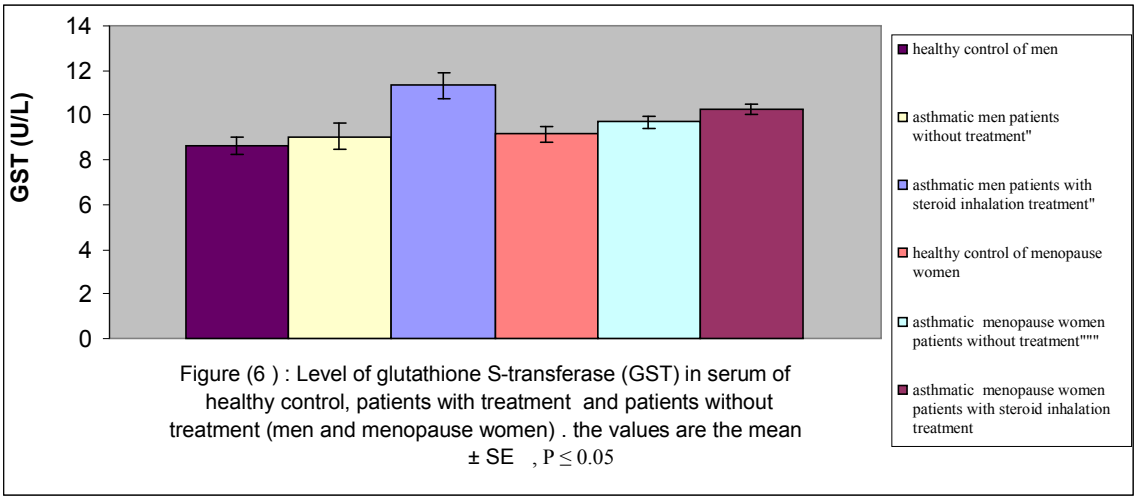


Figure (6) : Level of glutathione S-transferase (GST) in serum of healthy control, patients with treatment and patients without treatment (men and menopause women) . the values are the mean ± SE , $P \leq 0.05$

Serum(GST) activity level in patients with steroid inhalation treatment group(men and menopause women) was significantly higher than healthy control group, this increase level of serum GST activity may be due to increased oxidative stress leading to increased synthesis of Glutathione S- transferase to protect the body from toxic compound [40] elevated level of GST have been associated with tolerance of toxic compound [41, 42] GST using as detoxifed [42] GST Glutathion S – transferaes ; are involved in endogenous metabolism such as detoxification of production of oxidative stress . [39]

Serum(GST) activity level in asthmatic men with steroid inhalation treatment group was significantly higher than patients without treatment group but non significantly in menopause women; the increased level of serum GST activity may be due to effect drug using in asthmatic patients (inhalation treatment) on antioxidant enzyme activities . inhalation treatment leading to decrease level of free radical ;this leading to increase activity of enzyme [44] . Serum(GST) activity level in patients without treatment group (men and menopause women) was not significant but it was higher than healthy control group . The results of this study showed positive correlation but not significant under ($P \leq 0.05$) between the period of treatment with level of GST activity in

serum asthmatic patients (men and menopause women) table (5), figure (4)

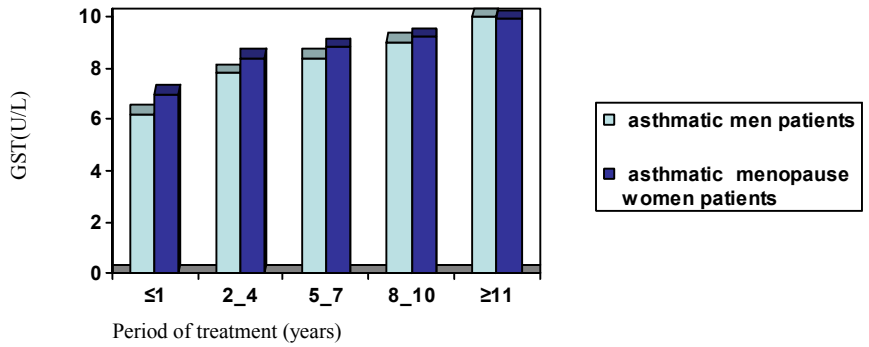


Figure (7) . Relationship between period of treatment with level of GST activity in serum asthmatic patients with steroid inhalation treatment group (men and women menopause) , P ≤ 0.05

4. Creatine Kinase

In this work , The mean ± SE of serum (CK) activity levels in asthmatic men patients with steroid inhalation treatment group was (16.24 ± 0.4 U/L *vs* 35.05 ± 0.54 U/L of healthy control group) and in asthmatic menopause women patients with steroid inhalation treatment group was (12.47 ± 0.6 U/L *vs* 18.54 ± 0.1 U/L of healthy control group), table(1) . the mean ± SE of serum (CK) levels in asthmatic men patients without treatment group was(14.83 ± 0. 4 U/L *vs* 35.05 ± 0.54 U/L of healthy control group) and in asthmatic menopause women patients without treatment group was (11.57± 0.36 U/L *vs* 18.54 ± 0.1 U/L of healthy control group) table(2)

The results of this study indicated that using of inhalation steroidal inhalation treatment increase the level of CK by 8.6% in asthmatic men and 7% in asthmatic menopause women patients table(4) P ≤ 0.05 , figure (5) .

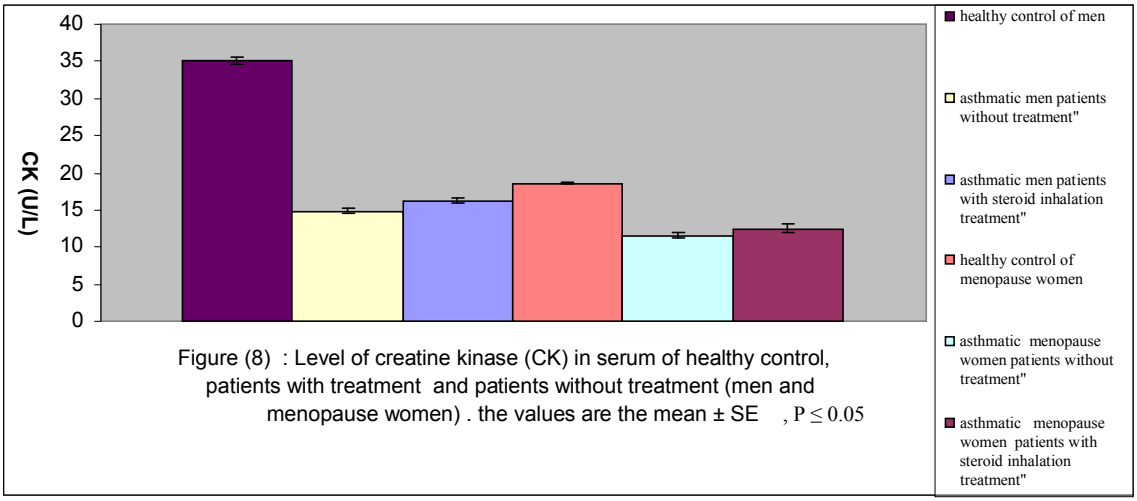


Figure (8) : Level of creatine kinase (CK) in serum of healthy control, patients with treatment and patients without treatment (men and menopause women) . the values are the mean ± SE , $P \leq 0.05$

Serum (CK) activity levels in asthmatic patients with and without treatment groups (men and menopause women) was significantly lower than healthy control group; decrease in level of serum CK activity may be related to oxidation of the cysteine residue of the enzyme [45 - 56]
and thiol group in CK enzyme severing oxidation by free radicals [53] so free radicals are interact with activity sites of enzyme and effect on the structure and function of enzyme so this lead Inactivity of CK [54, 55 , 56] as well as there was not significant difference between the serum (CK) activity levels of asthmatic patients with steroidal inhalation treatment group (men and menopause women) and asthmatic patients without treatment group (men and menopause women) , ($P \leq 0.05$) the results of this study showed negative correlation but not significant under ($P \leq 0.05$) between the period of treatment with level of CK activity in serum asthmatic patients (men and menopause women) table (5) , figure (6).

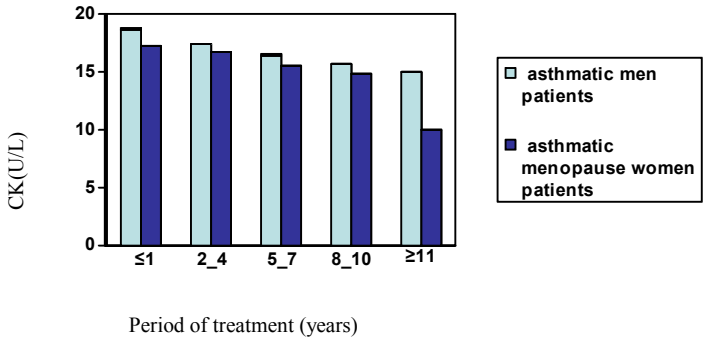


Figure (9) . Relationship between period of treatment with level of CK activity in serum asthmatic patients with steroid inhalation treatment group (men and women menopause) , (P ≤ 0.05)

Table(1): The level of several variables in serum of asthmatic men and menopause women with treatment and healthy control group

Variable	Men No.=45 Patients with treatment No.=30 Mean ±SE	Range	Control No.=15 Mean ±SE	range	p-value	menopause women No.=45 Patients with treatment No.=30 Mean ±SE	range	Control No.=15 Mean ±SE	Range	p-value
MDA(µmol/L)	12.58+0.51	10 17.5	7.93+0.29	7.1 9.6	S	12.13+0.19	9.2 16.4	10.33+0.08	9.8 10.7	S
GSI(U/L)	11.37±0.59	6.25-17.88	8.64±0.41	7-10	S	10.77±0.25	7-11.8	9.14±0.34	7.9-9.8	S
SOD(U/L)	0.87±0.01	0.49 1	1.7±0.19	0.5-2.4	S	0.71±0.03	0.49 1.2	1.7±0.097	1 1.8	S
CK(U/L)	16.24+0.1	11.1 18.76	35.05+0.51	33 37.1	S	12.17+0.6	10 17.3	18.51+0.1	18.2 19.2	S

Table(2): The level of several variables in serum of asthmatic men and women without treatment and healthy control group

Variables	Men No.=30 Patients without treatment No.=15 Mean ±SE	range	Control No.=15 Mean ±SE	range	p-value	Menopause women No.=30 Patients without treatment No.=15 Mean ±SE	range	Control No.=15 Mean ±SE	Range	p-value
MDA(µmol/L)	16.66±0.12	16 18	7.93±0.29	7.1 9.6	S	11.57±0.36	10 13	10.33±0.08	9.8 10.7	S
GSI(U/L)	9.05±0.6	7-10	8.63±0.41	7-10	NS	9.7±0.25	8.8-10.5	9.14±0.34	7.9-9.8	N S
SOD(U/L)	0.55±0.015	0.4 7	1.7±0.19	0.5-2.4	S	0.43±0.012	0.4 0.5	1.7±0.097	1 1.8	S
CK(U/L)	14.83±0.4	14-16	35.05±0.34	33-37.4	S	11.57±0.36	10-13	18.54±0.1	18.2-19.2	S

Table(3): The level of several variables in serum of asthmatic men and women with and without steroid inhalation treatment

Variables	Men		No.=45			Menopause women			No.=45	
	Patients with treatment No.=30		Patients without treatment	No.=15	P-value	Patients with treatment No.=30		Patients with out treatment	No.=15	P-value
	Mean ±SE	range	Mean ±SE	range		Mean ±SE	Range	Mean ±SE	range	
MDA(µmol/L)	12.59±0.51	10 17.3	15.66±0.12	15 18	S	12.43±0.19	9.2 15.1	14.57±0.38	13 16	S
GST(U/L)	11.32±0.59	8.23 17.88	9.03±0.6	7 10	S	10.27±0.23	7 11.8	9.7±0.25	8.8 10.3	N.S
SOD(U/L)	0.83±0.01	0.49 1	0.33±0.015	0.1 7	S	0.613±0.03	0.19 1.2	0.13±0.015	0.1 0.3	S
CK(U/L)	16.94±0.4	14.1-18.76	14.83±0.4	14-16	N.S	12.47±0.6	10-17.3	11.5±0.36	10-13	N.S

Table(4) :- Percentage of variables assessment in this study for patients(men and menopause women) using steroid inhalation treatment

Variables	Men %	Menopause women%
MDA	24% ↓	14% ↓
GST	20% ↑	5% ↑
SOD	33% ↑	40% ↑
CK	8.6% ↑	7% ↑

↑ Increase
↓ decrease

Table(5): The correlation between the period of steroid inhalation treatment and all variables assessment in this study

The correlation of Period of treatment(years)	Men No.=30		Menopause women No.=30	
	Correlation coefficition	P-value	Correlation Coefficition	P-value
Vs.MDA(µmol/L)	0.108	N.S	0.178	N.S
Vs.GST(U/L)	0.175	N.S	0.25	N.S
Vs.SOD (U/L)	-0.291	N.S	-0.204	N.S
Vs.CK (U/L)	-0.176	N.S	-0.261	N.S

****correlation is significant at the 0.01 level**
***correlation is significant at the 0.05 level**

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