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RESEARCH ARTICLE

Studying the Relationship Between Oxidative Stress Malondialdehyde and Heamatological Parameters in patients With Asthma in AL-Muthanna Province- Iraq

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Asthma , malondialdehyde **(MDA)**, Red blood cells **(RBCs)**, Heamoglobin **(Hb)**, Heamatocrit **(Hct)** and Erythrocyte sedimentation rate **(ESR).**

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Abstract

Bronchial asthma is one of very common diseases in iraq. Exposing the body to high oxidizing agent daily during the work will cause a failure of the defense system (immunity system) to protect of the body, which increase the number of oxidizing agent in the body which known as vintage (oxidative stress) which consider as mean factor risk for many diseases one of them is Bronchial asthma. This work aims to investigate oxidative status in asthmatic patients as a measure of oxidative stress that may affect hematological parameters. Oxidant malondialdehyde (MDA) parameter was estimated in blood samples. The results of the malodialdehyde parameter of patients was compared to that of the normal control group. The results showed that samples of asthmatics group had high rates of oxidative stress. The results also showed a significant decline in most the blood indices as well as blood cell count as compared to the control group. Whereas, erythrocyte sedimentation rate was higher in male and female asthmatic patients as compared to the control group. **In conclusion**, the study reveals a critical increase in oxidant status accompanied by a decline in hematological parameters that may lead to permanent inflammation if not diagnosed early and treated.

Introduction

Asthma is a disease in which the airways become blocked or narrowed. These effects are usually temporary, but they cause shortness of breath, breathing trouble and other symptoms **[1].** Asthma prevalence has increased dramatically in many countries over recent decades, demonstrating that environmental exposures play a dominant role in the etiology of this disease **[2].** Worldwide, 130 million people have asthma. In 1990, the management of asthma among USA population already accounted for more than $6 billion in medical expenditures **[3].** When an asthma attack occurs, the respiratory passageways thicken and constrict, making it very difficult for patients to breath **[4].** This often leads to respiratory distress, which can become grave in matter of minutes. The lungs may also begin to discharge mucus into the airways, leading to fits of coughing and wheezing **[5, 6].** Several mechanisms operate in cellular damage and death, lipid peroxidation caused by free radicals being one of the most important mechanisms **[7].** A free radical is an atom or molecule that has one or more unpaired electrons, they are two types; a reactive oxygen species (ROS), such as super oxide anion radical $(O^{\cdot}_2)^{-}$, hydrogen peroxide (H2O2), hydroxyl radical (OH^{-}) and singlet oxygen ($O2^1$). And reactive nitrogen species (RNS), such as nitrous oxide ($NO^{\cdot}$) nitric oxide ($NO2^{-}$), peroxynitrite ($OONO^{\cdot}$) Because ROS are so reactive, they can inflict considerable damage on living cells if formed in significant amounts. These damage results primarily form enzyme inactivation, polysaccharide depolimerization, DNA breakage and membrane destruction **[8, 9].** Oxidative stress doesn't only cause direct injurious effects in the lungs but also activates molecular mechanisms that initiate lung inflammation **[10].** Thus, an imbalance between oxidants and antioxidants is considered to play an important role in the pathogenesis of **asthma**. Hemopoietin, it is produced by interstitial fibroblasts in the kidney in close association

with peritubular capillary and tubular epithelial tubule. It is also produced in perisinusoidal cells in the liver **[11].** Under hypoxic conditions, the kidney will produce and secrete erythropoietin to increase the production of red blood cells by targeting colony-forming unit-erythroid CFU-E, proerythroblast and basophilic erythroblast subsets in the differentiation **[12].** The routine laboratory tests done to the patients, referred to the pediatrician for evaluation of allergy are: hemoglobin concentration, leukocyte count, differential count, ESR **[13, 14].** An increased ESR is associated with some condition such as: anemia, macrocytosis, tilted ESR tube, infection, inflammation, malignancy, etc. **[15].** Degranulation of mast cell is triggered by IgE-antigen binding. Then, some mediator such as histamine, neutrophil chemo tactic factor, and eosinophil chemo tactic factor are released and synthesis of leukotriens and prostaglandin occurred. All of these events increase vascular permeability so interstitial exudation and chronic inflammatory reaction occurred **[16].**

The aim of this study is evaluate different of the haematological parameters in the mentioned disease by measurement **(**Red blood cells **(RBCs)** count, Heamoglobin **(Hb)** , Heamatocrite **(Hct)**, and Erythrocyte sedimentation rate **(ESR))** in males and females asthmatic patients and compared with control group, To shed a light on the possible correlation relationships **(r)** between (**MDA** and studied parameters), and to evaluate the effect of asthma disease on all these haematological parameters.

MATERIAL AND METHOD

Selection of Subjects:

This study was conducted at AL-Hussein teaching Hospital in AL-muthanna governorate. The study includes (100) subjects , Fifty patients with asthma [25 males and 25 females] with age range [15- 65] years and Fifty supposed healthy subjects (control) [25 males and 25 females] with age range [15-65] years.

Sample with draw:

From the patients with asthma and control,(6mL) blood sample was taken, then divided into two parts, the first (4mL) of blood samples were drawn into a tube that contains ethylene diamine tetra acetic acid (EDTA) as anticoagulant for performing the complete blood cells count (CBC). All tubes were mixed immediately after collection and analyzed within 2 hours. Other (2mL) of blood samples were put in to a plain tubes without anticoagulant. Samples were allowed to clot at room temperature for 30 minutes for clot formation, and then centrifuged at 3000 rotor per minute (rpm) for 10 min to obtain sera, serum malondialdehyde were tested within 24 hours.

Methods:

Lipid peroxidation Marker in Asthma (MDA)

Lipid peroxidation is determined using the thiobarbituric acid method. In this method, MDA level of the serum was measured by the following procedure according to a modified method of Fong et al.,(1973) **[17].** It concentrations were calculated using the extinction coefficient of MDA (εMDA) equal to 1.56 x10 mol -1 . cm **[18].**

Determination of Heamatological Parameters (RBC, Hb and Hct).

The haematological Parameters measured by using **Cell-DYN Ruby® System operator's manual** (is a multi-parameter automated haematology analyzer designed for in vitro diagnosis) according to the flow cytometric techniques **[19].** (2 mL) blood samples were drawn into a test tube containing EDTA and then mixed gently on the blood mixer. The assay was performed according to the instructions provided by the manufacturer.

Determination of Erythrocytes Sedimentation Rate (ESR): [20].

The sedimentation rate was calculated red blood cells (mm/h) by westergren method. (2 mL) of whole blood was added to test tube containing 0.5mL (tri-sodium citrate solution) and mixed well. The mixture was added to westergren tube graduated (0-200) and fixed carefully in westergren rack vertically for one hour at room temperature away from sunlight direct, then ESR read (mm/h).

Statistical Analysis:

Statistical analysis was done using the software **SPSS** version 15.0,the results were expressed as mean ± standard deviations (mean ± SD). One way ANOVA-test was used to compare parameters in different studied groups. P-values ($p \leq 0.05$) were considered statistically significant. Person correlation coefficient (r) was used to test the correlation relationship among the different parameters in each patients group**[21].**

RESULTS AND DISCUSSION

Asthma is defined as a common chronic inflammatory disease of the airways characterized by variable and recurring symptoms, reversible airflow obstruction, and broncho spasm **[22].** Symptoms usually include wheezing,

coughing, chest tightness, and shortness of breath and it can be prevented by avoiding triggers, such as allergens and irritants **[23].**

Symptoms of bronchial asthma has been proved biochemically in asthmatic group by the statistical significant increase ($p \leq 0.05$) in malondialdehyde levels among all males and females asthmatic patients studied as compared to their normal counter parts as shown in (Table **1**), indicating thereby the relation of level of serum MDA with that of the underlying inflammatory process in bronchial asthma. These imply that patients during asthmatic attack are exposed to considerable degree of lipid peroxidation. This finding is consistent with observations of others **[24, 25].** MDA is a marker of lipid peroxidation, it has a strong correlation with atopic asthma suggesting that oxidative stress occurs simultaneously on lipid peroxidation. Oxidative stress can have many effects on airway function, including airway smooth muscle contraction, induction of airway hyper responsiveness, mucus hyper secretion, epithelial shedding and vascular exudation. Furthermore, ROS can induce cytokin and chemokin, the production through the induction of oxidative stress-sensitive transcription on nuclear factor in bronchial epithelial cells involves the uptake of oxygen and subsequent release of ROS into surrounding cells **[26].** Thus, the excess quantities of ROS that are produced by asthmatics may overcome the host antioxidant defenses and cause oxidative stress **[26, 27].**

Table 1: Serum level of malondialdehyde in asthmatic patients classified according to Sex and healthy control.

Groups	n	MDA(nmol/mL) Mean ± SE	
		Male	Female
Control	25	13.72 ± 0.49^{b}	14.63 ± 0.35^{b}
Patients	25	405.50 ± 9.14^{a}	338.00 ± 10.38^{a}
LSD		18.53	20.78

- Each value represents mean± SE values with non-identical superscript (a, b or c… etc.) were considered significantly differences ($p \leq 0.05$) .

- The Hematological Parameters

RBCs, Hb and Hct Count:

The statistical data were reported in table **(2)** , indicated to the present of non-significant decrease in RBCs count in all males and females asthmatic patients in comparison with control group CTR, although mean RBCs values for males were higher than females.

This revealed that both males and females might have signs of iron deficiency anemia. The non-significant differences in RBCs count in this study is in agreement with the results of other studies **[28, 29].** Similarity, table **(2)** showed there were non-significant decrease in Hb and Hct levels in all females asthmatic patients in comparison with control group CTR. Furthermore, in another study by Nadi et al.,(2013) and Mohamed, (2008) they revealed that haemoglobin and heamatocrit levels were not significantly ($p \leq 0.05$) different in the females asthmatic patients in comparison the control group **[28, 30].** On the other hand, this study showed there were a significant decrease in Hb and Hct levels in males asthmatic patients in comparison with the control group, although mean Hb values for males were higher than females. The results of this study is in agreement with the results of other studies **[31, 32].**

More recently, Jessica et al. (2006) found lower hemoglobin concentrations are among children with asthma when compared with non asthma children **[33].** Results and findings of the current study emphasize that patients with asthma might suffer from anemia. Therefore, there were a significant decrease in these heamatological parameters. In addition, John et al. (2006) found that the overall prevalence of anemia in 7737 COPD patients was 23.1% and concluded that the high prevalence of anemia in hospitalized COPD patients gives evidence that anemia is also a comorbidity in COPD and may contribute to exercise limitation and dyspnea **[34].**

Table 2: RBCs, Hb and Hct count in asthmatic patients classified according to Sex and healthy control.

- Each value represents mean± SE values with non-identical superscript (a, b or c… etc.) were considered significantly differences ($p \leq 0.05$) .

Groups	n	Mean ± SE					
		Male			Female		
		RBC (10e6/uL)	Hb (g/dL)	Hct %	RBC (10e6/uL)	Hb (g/dL)	Hct %
Control	25	5.19 ± 0.11 [a]	14.02 ± 0.29 [a]	45.03 ± 0.90 [a]	4.94 ± 0.08 [a]	12.51 ± 0.09 [a]	40.86 ± 0.45 [a]
Patients	25	5.00 ± 0.13 [a]	13.36 ± 0.28 [b]	41.33 ± 0.98 [b]	4.69 ± 0.09 [a]	13.07 ± 0.36 [a]	39.88 ± 1.16 [a]
LSD		0.24	0.59	1.93	0.26	0.77	2.59

Erythrocyte Sedimintation Rate Levels (ESR):

Table (**3**) illustrates a significant elevation ($p \leq 0.05$) in ESR count in all males asthmatic patients in comparison with the control group. On the other hand, ESR count showed non-significant increase in all males asthmatic patients in comparison with the control group. This finding is consistent with observations of others **[35, 36].** Increased ESR was found in all asthmatic patients indicating that in the allergic patients, the ESR could be increased. As mention before it may be due to the mediator IL-1 and tumor necrosis factor TNF produced by mast cell, basophile and macrophage. Then, these mediators stimulate the liver to produce acute phase proteins, such as fibrinogen. Increasing level of fibrinogen will increase positive charge of dielectric plasma protein and rouleaux formation will be found **[37, 38].**

Table 3: Erythrocyte sedimentation rate count in asthmatic patients classified according to Sex and healthy control.

Groups	n	ESR(mm/hour) Mean ± SE	ESR(mm/hour) Mean ± SE
		Male	Female
Control	25	12.81±1.02 [b]	18.25 ±1.39 [a]
Patients	25	28.42 ±3.78 [a]	20.28 ±1.89 [a]
LSD		5.65	4.87

- Each value represents mean± SE values with non-identical superscript (a, b or c… etc.) were considered significantly differences ($p \leq 0.05$) .

Correlation between MDA and Heamatological Parameters:

Correlation coefficient **(r)** between MDA and heamatological parameters were studied in both male and female patients group; The results of this study illustrate presence of non-significant positive correlation between MDA and RBCs in both male and female asthmatic patient (r = 0.07 and r = 0.04 respectively) as shown in (Figure **1** and Figure **2** respectively). However, there was non-significant negative correlation between MDA and Hb in male asthmatic patients (r = -0.11) as shown in (Figure **3**). The result of Correlation coefficient of this study is matched with the results of Al-Khalaf, (2014) **[32].** Whereas the results showed non-significant positive correlation between MDA and Hb in female asthmatic patients (r = 0.01) as shown in (Figure **4**). Furthermore, The results of this study illustrate presence of non-significant negative correlation between MDA and Hct level in both male and female asthmatic patient (r = - 0.47 and r = - 0.05 respectively) as shown in (Figure **5** and Figure **6** respectively). The result of Correlation coefficient of this study is matched with the results of Al-Khalaf, (2014) **[32].** On the other hand, The

results of this study illustrate presence of non-significant positive correlation between MDA and ESR in male asthmatic patients (r = 0.01) as shown in (Figure **7**), While the results showed non-significant negative correlation between MDA and ESR in female asthmatic patients (r = - 0.06) as shown in (Figure **8**).

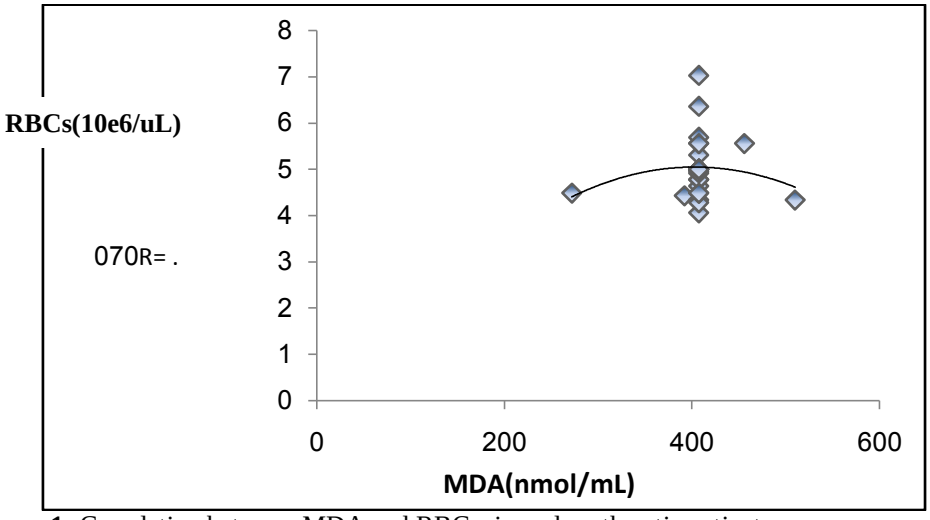

Figure 1: Correlation between MDA and RBCs in male asthmatic patients.

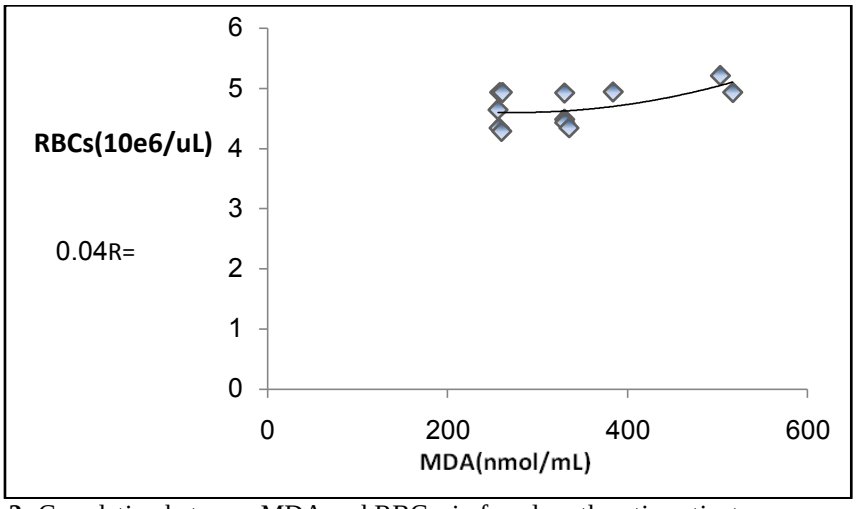

Figure 2: Correlation between MDA and RBCs in female asthmatic patients.

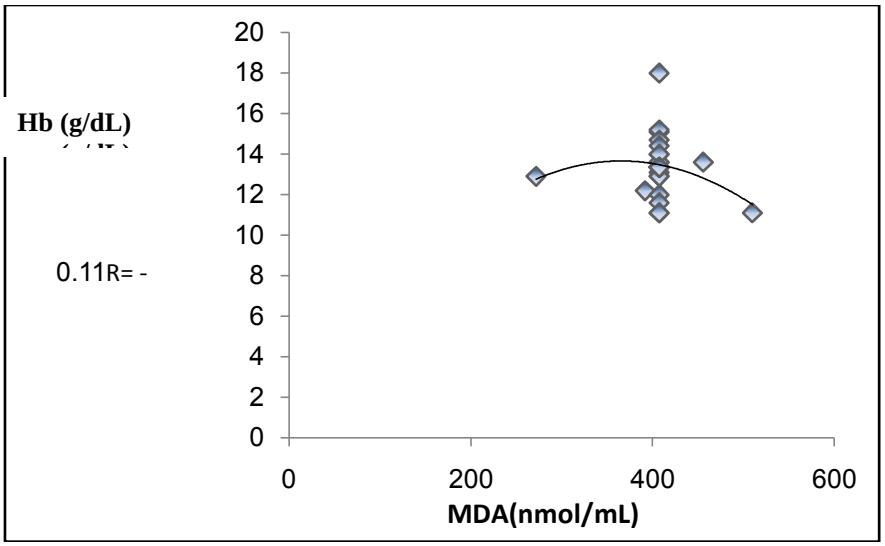

Figure 3: Correlation between MDA and Hb in male asthmatic patients.

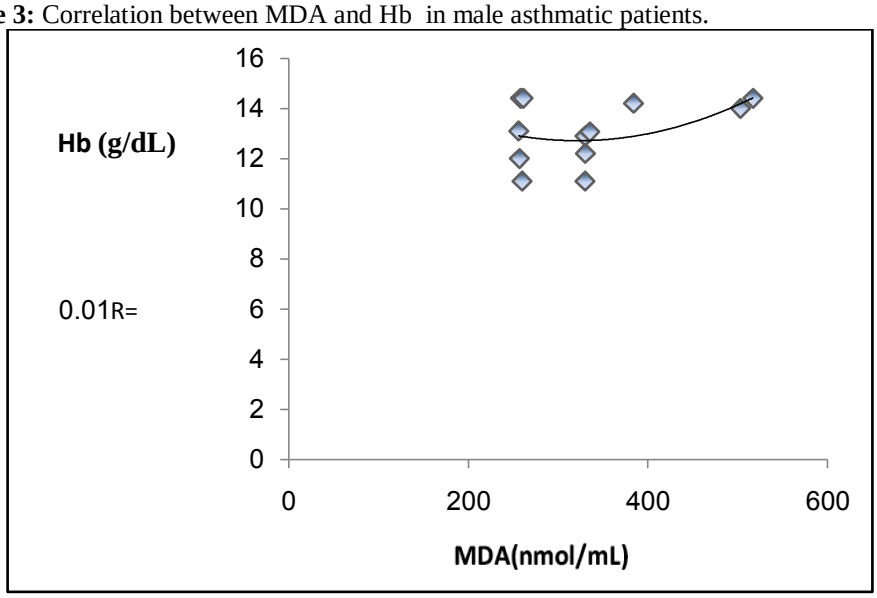

Figure 4: Correlation between MDA and Hb in female asthmatic patients.

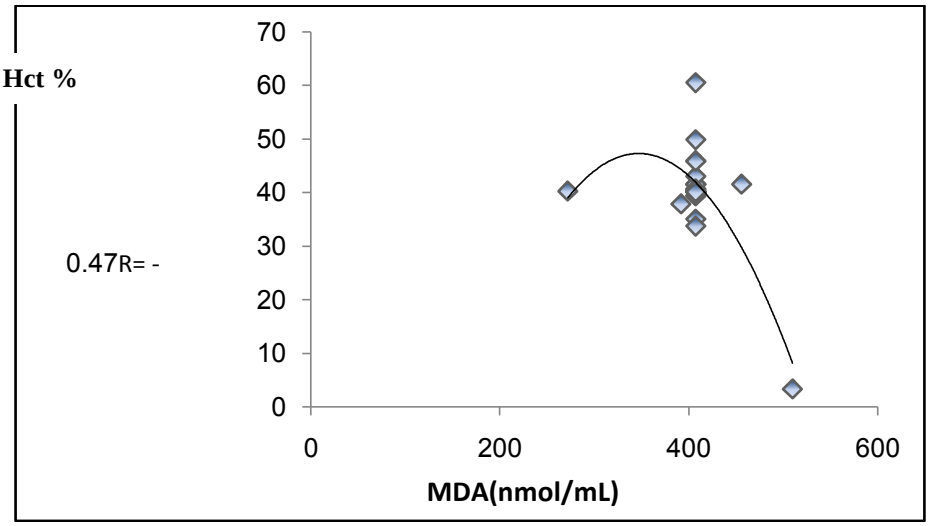

Figure 5: Correlation between MDA and Hct in male asthmatic patients.

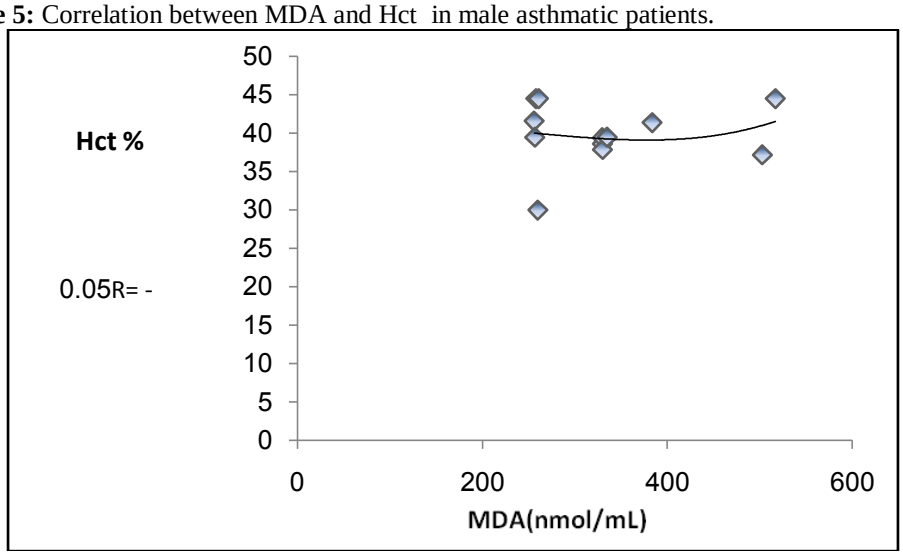

Figure 6: Correlation between MDA and Hct in female asthmatic patients.

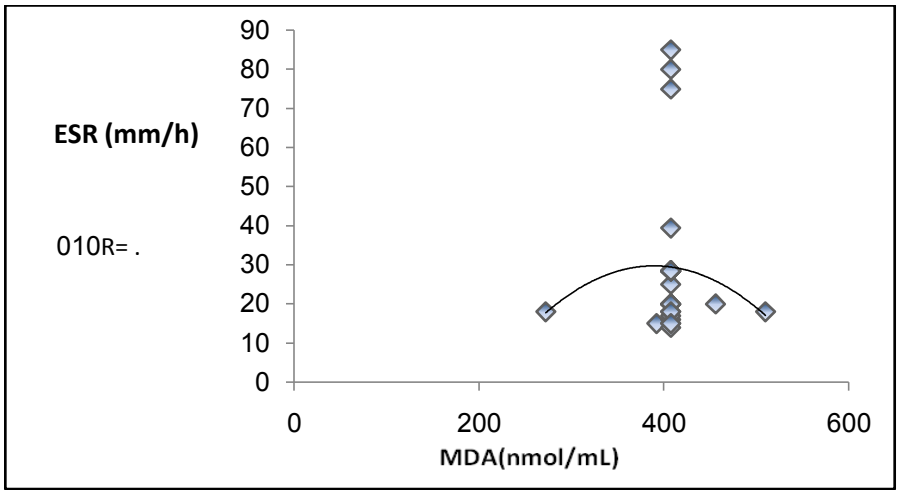

Figure 7: Correlation between MDA and ESR in male asthmatic patients.

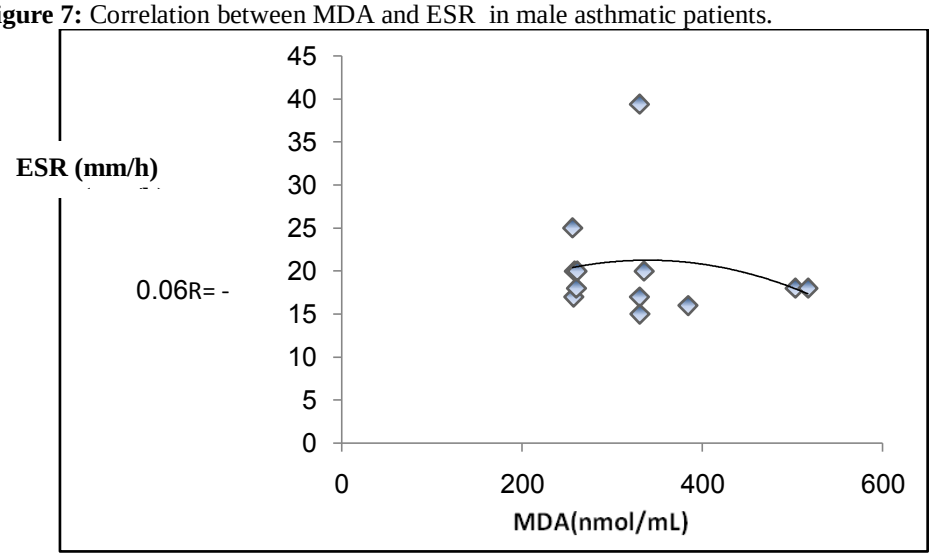

Figure 8: Correlation between MDA and ESR in female asthmatic patients.

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