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Antioxidant status of atorvastatin in hypercholesterolemic patients

MOHAMMAD A. NASAR[1], ABDALLA JARRARI[1], MOHAMMAD A. NASEER[2], TARANNUM F. SUBHANI[3], BEENA V. SHETTY[3] and FAIYAZ SHAKEEL[4]*

[1]*Department of Biochemistry, Faculty of Medicine, Al-Arab Medical University, Benghazi, Libya,* [2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India,* [3]*Department of Biochemistry, Kasturba Medical College, Manipal University, Mangalore, India and* [4]*Department of Pharmaceutics, Faculty of Pharmacy, Al-Arab Medical University, Benghazi, Libya*

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Abstract: This study was an attempt to establish the extent of increased oxidative stress in hypercholesterolemic patients and to evaluate the effect of atorvastatin on the oxidative stress and antioxidant status. Blood samples of 15 subjects (age and sex matched) from three groups: group I (healthy subjects), group II (hypercholesterolemic patients with atorvastatin treatment) and group III (hypercholesterolemic patients without any hypolipidemic drug) were taken and centrifuged to separate the plasma, which was used for the determination of vitamin E. The separated cells were washed thrice with 0.90 % w/v cold normal saline and used for the assay of the percentage hemolysis of the RBCs, and the determination of malondialdehyde, superoxide dismutase and hemoglobin. The levels of oxidative stress were higher in the hypercholesterolemic in comparison to the control and atorvastatin group. The levels of antioxidants were higher in the atorvastatin group than in the hypercholesterolemic one but were lower than the controls. From these findings, it was concluded that there is an increase in oxidative stress in hypercholesterolemia but it decreased significantly after 2 months of atorvastatin therapy and antioxidant status also improves in patients taking atorvastatin.

Keywords: atorvastatin; oxidative stress; antioxidant effects; hypercholesterolemic patients.

INTRODUCTION

Statins, such as atorvastatin, significantly reduce cholesterol synthesis through inhibition of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and are widely prescribed for hyperlipidemia to reduce the risk of atherosclerotic com-

* Corresponding author. E-mail: faiyazs@fastmail.fm
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plications.[1] Their efficacy in reducing cardiovascular morbidity and mortality was demonstrated in large intervention trials.[1] However, debate continues as to whether the beneficial effects of statins can be ascribed purely to their ability to reduce cholesterol or whether additional actions, independent of lowering cholesterol, play a significant role.[1–3] Studies showed that oxidized low density lipoprotein (LDL) is a major correlate of oxidative stress in hypercholesterolemic patients and that statins may reduce oxidative stress by reducing enhanced plasma levels of LDL, which are more susceptible to peroxidation in hypercholesterolemia, and change the LDL structure, making them more resistant to peroxidation.[1,4,5] Some studies further showed that statins may also inhibit NAD(P)H oxidase, thus decreasing the generation of reactive oxygen species (ROS), thereby adding or synergizing the biological effects of antioxidants.[4,6] Some studies also showed that statins or their metabolites may act as antioxidants, directly or indirectly by removing "aged LDL", which is more prone to oxidation, from the circulation.[7] Based on these findings, it is evident that among their properties, statins also possess antioxidant activities.[8,9] Therefore, the aim of the present investigation was to evaluate the scientific evidence of atorvastatin having such an effect and its possible clinical relevance. The antioxidant effects of statins contribute to the inhibition of atherogenesis, stabilization of atherosclerotic plaque, inhibition of myocardial hypertrophy and remodeling and modulation of vascular tone.[6] Based on these arguments, which formed the backbone of this study, an attempt was made to determine whether there really is increased oxidative stress in hypercholesterolemics and if it is relatively decreased following atorvastatin therapy when compared to normal individuals.

In this study, the levels of malondialdehyde (MDA), percentage hemolysis and superoxide dismutase (SOD) in red blood cells and vitamin E in plasma were measured. Red blood cells were chosen as they are well known to be subject to increased hazards of free radical damage. Moreover, these cells have a finite life span in circulation and their sequestration and disposal by macrophages may be related to the extent of peroxidative damage of their membrane lipids, cytoskeletal proteins and enzymes.

EXPERIMENTAL

Study population

This study was conducted on 3 groups each of 15 subjects in the age group 40–70 years. Both male and female subjects were taken in all groups.

Group I consisted of 15 healthy subjects (8 males and 7 females) in the age range 40–70 years having a normal lipid profile (control group).

Group II consisted of 15 patients (8 males and 7 females) who had already been diagnosed as hypercholesterolemic and who had been treated with HMG–CoA reductase inhibitors (atorvastatin) for a minimum of 2 months at a minimum dosage of 10 mg/day of atorvastatin. Treatment with atorvastatin was given only in this group in order to compare the results with control and hypercholesterolemic group without administration of any hypolipidemic drug.

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Group III consisted of 15 (8 males and 7 females) recently diagnosed hypercholesterolemic patients who were not taking any lipid lowering agent (hypercholesterolemic group).

Selection of cases

The test groups II and III for this study, consisting of 15 individuals in each group, were taken from the following hospitals: 1) the K. M. C. Hospital, Ambedkar Circle; 2) the K. M. C. Hospital, Attavar; 3) the Yenepoya Hospital, Kodialbail; 4) the A. J. Institute of Medical Sciences, Kuntikana; 5) the City Hospital, Kadri; 6) the Ullal General Hospital, Kudroli and 7) the Dr. Uday Nayak Clinic, Bejai, all in Mangalore, India.

While choosing the subjects for the test and control groups, care was taken to eliminate those with habits such as smoking, tobacco chewing, and alcohol consumption, as well as those with a history of chronic inflammatory disease such as tuberculosis, rheumatoid arthritis, diabetes mellitus and malignancy, all of which play a vital role in contributing to oxidative stress injury. Approval to perform these studies on human subjects was obtained from the Institutional Clinical Ethics Committee of the Kasturba Medical College, Mangalore, India, and their guidelines were followed throughout the studies.

Sample collection

Venous blood (5.0 ml) was collected in ethylenediaminetetraacetic acid (EDTA) containers from the median cubital vein or basilic vein of each study subject under strictly aseptic conditions. The blood samples were centrifuged at 3000 rpm for 10 min within 3 h of collection. The plasma was separated and used for the determination of vitamin E. The separated cells were washed thrice with 0.90 % w/v cold normal saline, after which they were suspended in an equal volume of the same saline solution. This was then stored as a 50 % cell suspension in a refrigerator (4–5 °C) until used for the assay of:

– percentage hemolysis of RBCs at 0 and 2 h (which represented before and after incubation with hydrogen peroxide, respectively);
– malondialdehyde (MDA);
– superoxide dismutase (SOD);
– hemoglobin (Hb).

Lipid peroxidation (MDA)

Red cell lipid peroxidation was studied as thiobarbituric acid (TBA) reaction products. The method of Stocks and Dormandy was followed with certain modifications.[10] The sample under test was heated with TBA at a low pH and the resulting pink chromogen, allegedly a $(TBA)_2$–MDA adduct, was measured spectrophotometrically at a wavelength of 535 nm.[11] An erythrocyte suspension (1.0 ml) was added to 8.5 ml of 0.90 % w/v normal saline and mixed well. Then 0.50 ml of 0.44 M H_2O_2 was added.

From this mixture, a 2.5 ml of aliquot was immediately taken, to which 1.0 ml of 28 % trichloroacetic acid (TCA) in 0.10 M sodium metaarsenite was added. This was mixed well and allowed to stand for 10 min, after which it was centrifuged. An aliquot (3.0 ml) of the supernatant was then taken, to which 1.0 ml of 1.0 % TBA in 50 mM NaOH was added. This was then kept in a boiling water bath for 15 min and then immediately cooled under tap water. The pink chromogen was determined spectrophotometrically at a wavelength of 535 nm. The values are expressed as nanomoles of MDA formed per dl of RBC, taking the molar extinction coefficient of the chromogen as 1.56×10^5 l/mol/cm.[10]

MDA (10^{-9} mol/100 ml of RBC) was determined using the equation:

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$$MDA = \frac{10^{11} A_T DFV}{\varepsilon}$$

where A_T is the absorbance of the test sample, DF is the dilution factor, V is the volume of the RBC suspension and ε is the extinction coefficient.

Oxidative hemolysis of RBCs or percentage hemolysis of RBCs

Oxidative hemolysis of the erythrocytes was measured by the method of Kartha and Krishnamurthy[12] at 0 and 2 h, *i.e.*, before and after 2 h incubation with H_2O_2. The principle of this method is based on the fact that an accelerated form of non-enzymatic breakdown can be induced in red blood cells by exposure to H_2O_2.[10]

RBC suspension (1.0 ml) was added to 8.5 ml of 0.90 % w/v of normal saline and mixed well. Then 0.50 ml of 0.44 M H_2O_2 was added and the mixture incubated at 37 °C.

Immediately, 0.50 ml aliquots each were withdrawn and put into 2 different centrifuge tubes labeled as "saline" and "water".

To the centrifuge tube labeled "saline", 4.5 ml of 0.90 % w/v of normal saline was added and centrifuged. The supernatant was then separated and its absorbance (optical density) was determined at 520 nm in a colorimeter. This represented non-hemolysed RBCs (NH) at 0 h or before incubation with H_2O_2.

To the centrifuge tube labeled "water", 4.5 ml of distilled H_2O was added and centrifuged. The supernatant was then separated and its optical density was determined at 520 nm in a colorimeter. This represented complete hemolysis of RBCs (CH) at 0 h or before incubation with H_2O_2.

The above procedure was again repeated after 2 h incubation with H_2O_2 at 37 °C. The centrifuge tubes labeled "saline" and "water" now represented non-hemolysed (NH) and completely hemolysed (CH) RBCs, respectively, at 2 h or after incubation with H_2O_2.

The percentage hemolysis of RBCs at 0 and 2 h was determined using the equation:

$$\text{Hemolysis}\,(\%) = 100\frac{O.D.\text{ of NH (saline)}}{O.D.\text{ of CH (water)}}$$

Vitamin E (α-tocopherol)

Plasma vitamin E was measured using the Emmorie Engel reaction, which is based on the reduction of ferric to ferrous ions by tocopherols, which then form a red complex with α,α'-dipyridyl. Tocopherol and carotenes were extracted into petroleum ether and the extinction at 450 nm was measured. A correction was made for carotenoids after adding $FeCl_3$. The reading was taken at 520 nm after 90 s.[13,14]

Plasma (1.0 ml) was thoroughly mixed with 1.0 ml of redistilled 95 % ethanol in a 15 ml stoppered tube. Petroleum ether (3.0 ml) was then added and the tube shaken vigorously for 3 min. This was then centrifuged and 2.0 ml of the clear supernatant was transferred to a clear dry cuvette. The optical density was measured at 450 nm in a colorimeter for carotenes; petroleum ether served as the blank.

The petroleum ether was then evaporated off at room temperature and the residue dissolved in 1.0 ml of chloroform; 1.0 ml of 95 % ethanol was then added followed by 1.0 ml of 0.20 % α,α'-dipyridyl and 0.10 ml of 0.10 % $FeCl_3$. After 1.5 min, the absorbance at 520 nm was measured in a colorimeter.

The concentration of vitamin E, mg/l of plasma, was determined using the equation:

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$$c=\frac{A_T-A_C}{A_S}$$

where A_T, A_C and A_S are absorbance of test, carotene and standard sample, respectively.

Superoxide dismutase (SOD)

The method of Beauchamp and Fridovich was followed for the measurement of SOD. The enzyme superoxide dismutase catalyses the reaction:

$$2O_2^{\bullet-} + 2H^+ \xrightarrow{\text{SOD}} H_2O_2 + O_2$$

Inhibition of the reduction of nitroblue tetrazolium (NBT) by superoxide radicals, generated by the illumination of riboflavin in the presence of oxygen was the principle of SOD determination. An electron donor, methionine, was used for the assay of superoxide dismutase.[15]

The hemolysate was prepared by the method of McCord and Fridovich.[16] To 1.0 ml of erythrocytes (washed with 0.90 % w/v normal saline), 1.0 ml of deionized water was added to lyse the cells. To this, 0.50 ml of distilled ethanol followed by 0.30 ml of chloroform were added, mixed well and allowed to stand for 15 min. Then 0.20 ml of H_2O was added and then the mixture was centrifuged at 4 °C.

The supernatant contained the SOD activity and was used for the assay of SOD after dilution with potassium phosphate buffer (pH 7.8, 0.050 M); 0.10 ml of hemolysate was diluted with 1.9 ml of potassium phosphate buffer. This was the final diluted hemolysate that was used in the procedure given below.

Four test tubes were taken and labeled as "test", "control", "test blank" and "control blank".

To the "test", 2.9 ml of reaction mixture with NBT containing 149 mg of methionine, 4.93 ml of NBT (1 mg/ml), 0.63 ml of riboflavin (1 mg/ml) was diluted to 100 ml with potassium phosphate buffer (pH 7.8, 0.050 M) and 0.10 ml of diluted hemolysate was added. To the "test blank", 2.9 ml of same reaction mixture without NBT and 0.10 ml of diluted hemolysate was added. To the "Control", 2.9 ml of same reaction mixture with NBT and 0.10 ml of potassium phosphate buffer (pH 7.8, 0.050 M) was added. To the "control blank", 2.9 ml of the same reaction mixture without NBT and 0.10 ml of potassium phosphate buffer (pH 7.8, 0.050 M) was added. Each of the above solutions was poured into a 10 ml beaker. The beakers were kept in an aluminum foil lined box fitted with a 15 W fluorescent lamp for 10 min.

The absorbance of the solutions after irradiation was measured at wavelength of 560 nm in a spectrophotometer.

One unit of SOD activity was taken as that producing a 50 % inhibition of NBT (nitroblue tetrazolium) reduction. The obtained values, expressed as units /g Hb, were calculated using the equation:

$$\text{Unit/dl SOD } (x) = 2.4\times10^5\,\frac{C-T}{C}$$

$$\text{SOD activity (units/g Hb)} = \frac{x}{\text{Hb}}\ \text{(Units/g Hb of SOD)}$$

where C and T are the absorbance of the control and test, respectively.

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Estimation of hemoglobin

The hemoglobin content of the erythrocytes was determined by the cyanmethemoglobin method.[17]

Hemoglobin was treated with a reagent containing potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate (Drabkins Reagent). The ferricyanide oxidizes hemoglobin to methemoglobin, which is converted to cyanmethemoglobin by the cyanide. The absorbance was measured at 540 nm in a colorimeter.

RBC suspension (20 µl) was added to 4.0 ml of ferricyanide reagent and allowed to stand for 4 min. The absorbance was measured at 540 nm in a colorimeter against a reagent blank. The absorbance of the standard solution was measured by directly taking 4.0 ml of a standard cyanmethaemoglobin solution (60 mg/dl).[18]

Hemoglobin (g/dl) was determined using the equation:

$$\text{Hemoglobin (g/dl)} = \frac{A_{\text{T}} \times \text{Dilution factor} \times \text{Conc. of std. (mg/dl)}}{1000 A_{\text{S}}}$$

where Conc. of std. is 60 mg/dl.

Statistical analysis

All the biochemical parameters were compared using the Fishers *F*-test for analysis of variance (ANOVA), except for hemolysis. The student's paired *t*-test was used for hemolysis (0 and 2 h). The statistical software SPSS (statistical package for social sciences), version 11, was used for this purpose.

RESULTS AND DISCUSSION

In this study, the products of lipid peroxidation, hemolysis of RBCs and antioxidant levels were compared between those having a high cholesterol level, those who had been on treatment with atorvastatin for a minimum of 2 months (minimum dosage of 10 mg/day) and those individuals of the same age group having a normal lipid profile, who were taken as controls.

Free oxygen radicals and insufficient antioxidant enzymes have been implicated in the pathogenesis of hypercholesterolemia.[2,4,19,20] Statins have been used effectively for the treatment of hypercholesterolemia.[2,20,21] These facts formed the basis of this study, *i.e.*, to investigate the antioxidant system and oxidative stress in hypercholesterolemic patients, as well as in patients who had been treated with atorvastatin for a minimum of 2 months.

The mean total cholesterol levels of the 3 groups are given in Table I. In patients taking atorvastatin, the mean total cholesterol was reduced after 2 months of treatment (246.66±8.54 mg/dl) as compared to the hypercholesterolemic patients (325.33±11.23 mg/dl).

RBC malondialdehyde (MDA)

As a measure of oxidative stress, MDA, the end-product of lipid peroxidation, was estimated by the TBA method. RBC MDA levels were highest in the hypercholesterolemic group (742.67±74.10 µmol/l) and lowest in control group (545.63±48.03 µmol/l). These values are statistically highly significant ($p = 0.001$)

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when the hypercholesterolemic group was compared with the control group and statistically significant (p = 0.034) when the atorvastatin group was compared with the control group, as indicated in Table II.

TABLE I. Mean total cholesterol levels of the 3 groups, *i.e.*, control, atorvastatin and hypercholesterolemic

Group	No. of subjects	Mean total cholesterol level, mg/dl[a]
Control	15	161.25±5.43
After a minimum of 2-months--atorvastatin therapy	15	246.66±8.54
Hypercholesterolemic	15	325.33±11.23

[a]Mean±*SD*

TABLE II. Mean RBC MDA levels of the 3 groups (control, atorvastatin and hypercholesterolemic) determined by Fisher's *F*-test

Group	No. of subjects	Mean, μmol/l	*SD*	*p*	Remarks
Control	15	545.63	48.03	–	–
Atorvastatin	15	590.23	39.63	0.034	S[a]
Hypercholesterolemic	15	742.67	74.10	0.001	HS[b]

[a]Significant; [b]highly significant

The high level of MDA in the hypercholesterolemic group suggests an increase in oxidative stress in patients with hypercholesterolemia. Its relatively lower level in the atorvastatin group suggests a decrease in oxidative stress. These findings further confirm the antioxidant properties of statins such as atorvastatin.[2]

Percentage hemolysis of RBCs and vitamin E

The percentage hemolysis of RBCs was measured as an indicator of damage to RBC membranes as a result of oxidative stress. Amongst the antioxidants, vitamin E was chosen because, despite its low molar concentration in membranes, it effectively serves as the major lipid-soluble, chain-breaking antioxidant.[22] This study showed an increased hemolysis of RBCs in the hypercholesterolemic group (4.52±1.06 %) as compared to the atorvastatin (2.48±0.67 %) and control groups (1.69±1.04 %), but in the atorvastatin group it was more than in the control but less than the hypercholesterolemic group, both before (2.48±0.67 %) and after incubation (3.52±0.81 %) with hydrogen peroxide (Table III). The values are statistically highly significant both before and after incubation with hydrogen peroxide ($p < 0.05$). This could be explained based on increased oxidative stress in the hypercholesterolemia patients and the effect of atorvastatin in reducing oxidative stress in the hypercholesterolemic patients after 2 months of treatment. The mean vitamin E levels were lower in the atorvastatin group (7.74±1.05 mg/dl) and the lowest in hypercholesterolemic group (6.28±0.68 mg/dl) as compared to the control group (9.31±1.36 mg/dl) as shown in Table IV. The values are statistically highly significant ($p < 0.05$). Studies showed that vitamin E plays a critical role

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in protecting the polyunsaturated fatty acids of cell membranes against lipid peroxidation through its free-radical quenching activity at an early stage of free-radical attack, thus suppressing hemolysis.[22] This has made vitamin E one of the important factors determining the susceptibility of red cells to auto-oxidation hemolysis.[22] Vitamin E appeared to be highly efficient as an antioxidant and is accepted as the first line of defense against lipid peroxidation.[22–25]

TABLE III. Percentage hemolysis of RBCs before and after incubation with H_2O_2 in the 3 groups (control, atorvastatin and hypercholesterolemic). Name of the test used: Fisher's *F*-test

Group	No. of subjects	Mean	*SD*	*p*	Remarks
Hemolysis before incubation					
Control	15	1.69	1.04	–	–
Atorvastatin	15	2.48	0.67	0.001	HS[a]
Hypercholesterolemic	15	4.52	1.06	0.001	HS
Hemolysis after incubation					
Control	15	2.78	0.88	–	–
Atorvastatin	15	3.52	0.81	0.001	HS
Hypercholesterolemic	15	6.01	1.12	0.001	HS

[a]Highly significant

The popular finding that vitamin E is inversely related to the respective tissue MDA level fits here as the MDA level in this study was found to be the highest in the hypercholesterolemic group (742.67±74.10 μmol/l) and the lowest in control group (545.63±48.03 μmol/l). The increased hemolysis of RBCs in the hypercholesterolemic group could be further documented by the decreased levels of vitamin E, which is the first line of defense against membrane damaging lipid peroxidation.[22–25]

In the atorvastatin group, the level of vitamin E was more than in the hypercholesterolemic group but less than in the control group (Table IV). The results of percentage hemolysis of RBCs were in the same order; hemolysis was the lowest in the control group, higher in the atorvastatin group and the highest in the hypercholesterolemic group. The controls had the highest level of vitamin E as they are considered to be the group with the least oxidative stress due to the normal lipid profile of their blood. It may be hypothesized that due to increased oxidative stress in the hypercholesterolemic group, the utilization of vitamin E, which is an antioxidant, might have increased. This agrees with the work of Moriel *et al.*[19]

TABLE IV. Plasma vitamin E levels of the 3 groups (control, atorvastatin and hypercholesterolemic). Name of the test used: Fisher's *F*-test

Group	No. of subjects	Mean, mg/dl	*SD*	*p*	Remarks
Control	15	9.31	1.36	–	–
Atorvastatin	15	7.74	1.05	0.001	HS[a]
Hypercholesterolemic	15	6.28	0.68	0.001	HS

[a]Highly significant

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Superoxide dismutase (SOD)

Superoxide dismutase was chosen in this study as it plays an important role in the removal of superoxide radicals ($O_2^{\bullet -}$) formed in red cells and because hemoglobin and SOD were shown to be in close association with red cells. In addition to this, some studies also suggested that SOD is one of the most important enzymes in the front line of defense against oxidative stress and is the most effective in protecting RBCs against damage by exogenous superoxide radicals ($O_2^{\bullet -}$), especially at higher concentrations.[26–28] This study showed low levels of SOD in the hypercholesterolemic group (6.28±0.69 units/mg), as shown in Table V. In the atorvastatin group, the SOD level (7.74±1.05 units/mg) was more than in the hypercholesterolemic but less than in the controls. The values were statistically highly significant ($p < 0.05$). Low levels of SOD in the cellular and extracellular fluids reduce their oxygen-derived, free radical (ODFR) scavenging capacity, making the tissues more vulnerable to ODFR damage.[28] The low level of SOD found in hypercholesterolemic patients is indicative of increased oxidative stress in patients with hypercholesterolemia (Table V). Studies that showed that there is a reduced activity of SOD in hypercholesterolemia, which improved after 3 months of treatment with fluvastatin, further strengthens the idea of increased oxidative stress in hypercholesterolemics and reduction of oxidative stress after the use of atorvastatin, which inhibits superoxide anion production, preserves intracellular SOD and prevents ROS (reactive oxygen species) permeation into lipoproteins.[2,26,27] Thus, it can be concluded that the trends seen in this study definitely suggest that there is an increased occurrence of oxidative stress as a result of hypercholesterolemia and that after the use of atorvastatin, the oxidative stress decreased, for which there may be two reasons. The first reason may be due to the decreased cholesterol levels and the second, due to the anti-oxidant effect of atorvastatin, as shown in many studies.

TABLE V. RBC superoxide dismutase (SOD) levels in the 3 groups (control, atorvastatin and hypercholesterolemic). Name of the test used: Fisher's *F*-test

Group	No. of subjects	Mean, units/mg	*SD*	*p*	Remarks
Control	15	8078.63	762.50	–	–
Atorvastatin	15	7432.69	657.92	0.001	HS[a]
Hypercholesterolemic	15	5281.79	525.19	0.001	HS

[a]Highly significant

CONCLUSIONS

This study was an attempt to establish the extent of increased oxidative stress in hypercholesterolemic patients and to evaluate the effect of atorvastatin on the oxidative stress and antioxidant status after 2 months of treatment.

The levels of oxidative stress were higher in the hypercholesterolemic patients than in the control and atorvastatin groups. The levels of oxidative stress in

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the atorvastatin group were lower than in the hypercholesterolemic group but higher than in the control group. The levels of antioxidants were higher in the atorvastatin group than hypercholesterolemic group but were lower than in the controls. From these findings, it can be concluded that there is an increase in oxidative stress in hypercholesterolemia but it decreases significantly after 2 months of atorvastatin therapy and there is simultaneously an improvement in the antioxidant status in patients taking atorvastatin.

И З В О Д

АНТИОКСИДАТИВНИ СТАТУС КОД ПАЦИЈЕНАТА СА ХИПЕРХОЛЕСТЕРОЛЕМИЈОМ НА ТЕРАПИЈИ АТОРВАСТАТИНОМ

MOHAMMAD A. NASAR[1], ABDALLA JARRARI[1], MOHAMMAD A. NASEER[2], TARANNUM F. SUBHANI[3], BEENA V. SHETTY[3] и FAIYAZ SHAKEEL[4]

[1]*Department of Biochemistry, Faculty of Medicine, Al-Arab Medical University, Benghazi, Libya,* [2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Jamia Hamdard, New Delhi, India,* [3]*Department of Biochemistry, Kasturba Medical College, Manipal University, Mangalore, India u* [4]*Department of Pharmaceutics, Faculty of Pharmacy, Al-Arab Medical University, Benghazi, Libya*

У овој студији је проучавано повећање оксидативног стреса код пацијената са хиперхолестеролемијом и праћен је ефекат аторвастатина на оксидативни стрес и антиоксидативни статус. Анализирана је плазма људи из три групе (по 15 узорака): здрави људи, пацијенти са хиперхолестеролемијом на терапији аторвастатином и пацијенти са хиперхолестеролемијом без терапије. У плазми је одређиван витамин Е. Изоловане ћелије су испране три пута хладним физиолошким раствором и коришћене су за одређивање степена хемолизе еритроцита и концентрације малондиалдехида, супероксид-дисмутазе и хемоглобина. Степен оксидативног стреса је био већи код пацијената без терапије него код здравих особа и пацијената на третману аторвастатином. Ниво антиоксиданаса је био већи код пацијената под терапијом, али ипак мањи него у контролној групи. На основу резултата је закључено да је оксидативни стрес повећан код пацијената са хиперхолестеролемијом, али да се значајно смањује после двомесечне терапије аторвастатином, уз побољшање антиоксидативног статуса.

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