

## **AIM OF THE STUDY**

The objective of the present study is the measurement of the serum level of 8-hydroxydeoxyguanosine (8-OHdG), as a measurement of oxidative DNA damage, Malondialdehyde (MDA), which reflects the lipid peroxidation level and Superoxide Dismutase (SOD), representing the activity of antioxidant enzymes, in patients with metabolic syndrome. The effect of Atorvastatin and Vitamin E on these parameters in different groups is also studied.

## SUBJECTS AND METHODS

### A. Subjects:

After approval by the Ethical Committee of the Medical Research Institute, Alexandria University, Egypt, the present study was conducted on 50 male subjects. They were divided into two groups:

- Group I: Included twenty non-obese, non-smokers, apparently healthy volunteers. Their mean age was (47.25 ±4.44 years) and served as control group.
- Group II: Included thirty obese patients who fulfilled the definition criteria of metabolic syndrome according to the International Diabetes Federation (IDF) definition. Their mean age was (46.86 ±5 years).

Patients were recruited from the Internal Medicine Department, Medical Research Institute, Alexandria University. All subjects provided written informed consent to participate in this study, in accordance with the Declaration of Helsinki.<sup>(212)</sup>

According to the design of the study, patients were divided randomly into two subgroups:

- Subgroup IIa (n=15): received atorvastatin tablets (10 mg/day) for three months.
- Subgroup IIb (n=15): received atorvastatin tablets (10 mg/day) and vitamin E (1000 mg/day) for three months.

All selected patients continued to the end of the study. Participants were requested to maintain their regular physical activity and lifestyle. They were followed up every two weeks during the time of the study in order to make sure that they were using the medication properly.

To all patients, general health parameters assessed included screening history, physical examination and blood tests. Patients with the following criteria were excluded from the study:

- 1) Patients who use any vitamin preparations or statins in the last three months.<sup>(213)</sup>
- 2) Patients with renal insufficiency, defined as a serum creatinine level equal to or more than 1.8 mg/dL.<sup>(213)</sup>
- 3) Patients with liver disease.<sup>(214)</sup>
- 4) Patients with chronic inflammatory diseases.<sup>(213)</sup>
- 5) Alcoholics and smokers were also excluded.<sup>(215)</sup>

Blood samples were drawn from the patients at the beginning and after 3 months of follow up between 8:30 – 10:30 am, after at least 12-14 hours of fasting. Fasting blood glucose, serum insulin, lipid profile, selected oxidative stress parameters (8-OHdG, MDA levels and SOD activity) were measured.

## B. Methods:

### Anthropometric and blood pressure measurements:

Weight and height were measured using a calibrated balance and a vertical ruler with participants wearing light clothing and no shoes. Height was recorded to the nearest 0.1 cm and weight to the nearest 0.1 kg. Body mass index (BMI) was calculated as the weight in kilograms (kg) divided by the square of the height in meters (m).

For the evaluation of fat distribution, waist and hip circumferences were measured at the mid-distance between iliac crest and last rib margin at the level of the symphysis pubis and the maximum protrusion of the buttocks respectively with a soft tape while the subject was in a standing position. Measurement was recorded to the nearest 0.1 cm. The waist to hip ratio (WHR) was then calculated.

Blood pressure was measured from the dominant arm of seated patients with a sphygmomanometer after 5 minutes of rest in sitting position.

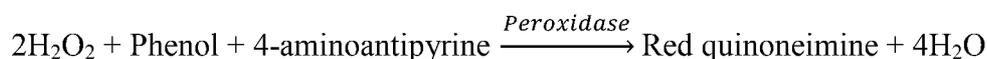
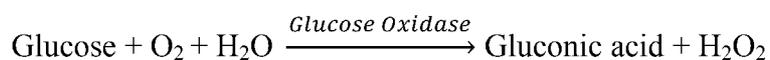
### Blood sampling:

From each individual, 10 mL of venous blood were obtained without using heparin, after an overnight fasting. The blood was placed in serum tube and left to stand for 30 minutes. The serum was prepared by centrifugation at 3000 rpm for 10 minutes. Ten  $\mu$ L of serum were immediately used for assaying fasting glucose. The remaining serum was divided into seven aliquots and stored at  $-20^{\circ}\text{C}$  for assaying insulin, lipid profile (total cholesterol, HDL, and triglycerides), 8-OHdG, MDA and SOD.

### Determination of fasting serum glucose level (FG):<sup>(216)</sup>

#### Principle:

The glucose levels were determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminoantipyrine to a red-violet quinoneimine dye as indicator.



#### Reagents:

Phosphate buffer (pH 7.5)	0.1 mol/L
4-Aminophenazone (antipyrine)	0.25 mmol/L
Phenol	0.75 mmol/L
Glucose oxidase	>15 KU/L
Peroxidase	>1.5 KU/L
Mutarotase	>2.0 KU/L

**Standard:**

Glucose standard

100 mg/dL

**Procedure:**

1- The following reagents were pipetted into three labeled test tubes:

	Blank	Standard	Sample
Working reagent	1000 µL	1000 µL	1000 µL
Distilled water	10 µL	.....	.....
Standard	.....	10 µL	.....
Sample	.....	.....	10 µL

2- The contents of the tubes were mixed thoroughly and incubated for 5 minutes at 37°C.

3- The absorbance (A) of the sample and the standard were measured against the reagent blank at 546nm within 1 hour.

**Calculations:**

The glucose concentration in the sample was calculated using the following formula:

$$\text{Glucose level(mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard (100 mg/dL)}$$

### **Estimation of fasting serum insulin concentration:<sup>(217)</sup>**

Immunoenzymatic assay for the quantitative measurement of human serum Insulin was performed using (Alpha Diagnostic International, Inc.) ELISA kit.

#### **Principle:**

The method is based on simultaneous binding of human Insulin from samples to two antibodies, one immobilized on microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and color developed. The enzymatic reaction (color) is directly proportional to the amount of Insulin present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm and the concentration of Insulin in samples and control is read off the standard curve.

#### **Reagents:**

1. Monoclonal Anti-Insulin coated microwell strip plate (96 wells).
2. Human Insulin Sample Diluent or **Std. A**, 0.00  $\mu\text{IU/mL}$ , 2 mL.
3. Human Insulin **Std. B**, 6.25  $\mu\text{IU/mL}$ , 1 mL.  
Human Insulin **Std. C**, 12.5  $\mu\text{IU/mL}$ , 0.5 mL.  
Human Insulin **Std. D**, 25  $\mu\text{IU/mL}$ , 0.5 mL.  
Human Insulin **Std. E**, 50  $\mu\text{IU/mL}$ , 0.5 mL.  
Human Insulin **Std. F**, 100  $\mu\text{IU/mL}$ , 0.5 mL.
4. Assay Diluent (14 mL).
5. Anti-Insulin-HRP **Conj Conc (20X)** 1 mL, dilute 1:20 with assay diluents.
6. **Wash Buffer Conc (20X)**; 25 mL, dilute 1:20 with distilled water.
7. **HRP Substrate Solution**, Tetramethylbenzidine (TMB), 12 mL.
8. **Stop Solution**,  $\text{H}_2\text{SO}_4$ , 12 mL.

#### **Preparation of the Reagents:**

- 1- The microtiter well strips were labeled to be used on the plate.
- 2- Wash buffer was diluted (1:20) with distilled water.

#### **Procedure:**

1. 25  $\mu\text{L}$  of standards and serum samples were pipetted into appropriate wells in duplicate.

2. One hundred  $\mu\text{L}$  of diluted Antibody-Enzyme Conjugate were dispensed into each well, the samples were gently mixed, the plate was covered and incubated at room temperature (25-35°C) for 60 minutes.
3. The plate was washed 5X with wash buffer (300  $\mu\text{L}$ /wash).
4. One hundred  $\mu\text{L}$  TMB substrate were dispensed per well. The contents of the wells were mixed gently for 1-5 seconds, the plate was covered, and incubated at room temp for 15 min. Blue color was developed in positive wells.
5. The reaction was stopped by adding 50  $\mu\text{L}$  of stop solution to all wells, mixed gently for 5-10 seconds. Blue color was turned yellow. The absorbance was measured at 450 nm using an ELISA reader within 30 min.

### Calculations:

The mean absorbance for each duplicate was calculated. The absorbance of the zero standard was subtracted from the mean absorbance values of standards and samples. The standard curve was drawn on a linear graph paper by plotting net absorbance values of standards against appropriate protein concentrations. The insulin concentrations of controls and samples were read.

### Evaluation of homeostatic model assessment of insulin resistance (HOMA-IR):

Homeostatic model assessment of insulin resistance (HOMA-IR) calculated by

$$\text{HOMA-IR} = [\text{fasting insulin } (\mu\text{IU/mL}) \times \text{fasting glucose } (\text{mg/dL})] / 405$$

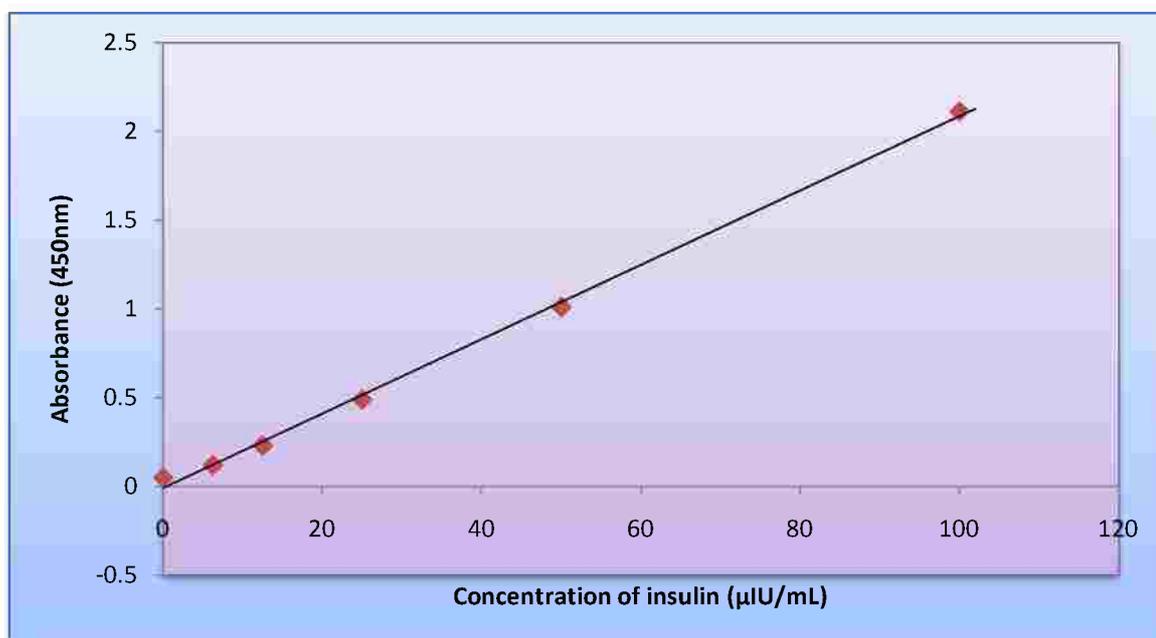
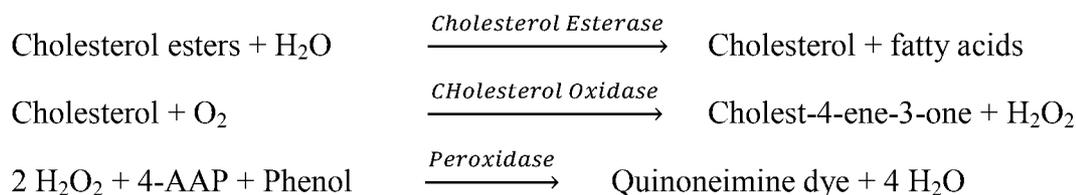


Figure (9): Standard curve of serum insulin ( $\mu\text{IU/mL}$ ).

## Estimation of serum total cholesterol (T.Cholest):<sup>(218)</sup>

### Principle:

The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine (a red-colored dye) is formed from hydrogen peroxide and 4-aminoantipyrine (4-AAP) in the presence of phenol and peroxidase.



### Reagents:

Phosphate buffer (pH 6.5)	100 mmol/L
4-Aminoantipyrine	0.3 mmol/L
Phenol	5 mmol/L
Peroxidase	>5 KU/L
Cholesterol esterase	>150 U/L
Cholesterol oxidase	>100 U/L
Sodium azide	0.05%

### Standard:

Standard cholesterol	200 mg/dL
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### Procedure:

The following volumes were pipetted into three labeled test tubes:

	Blank	Standard	Sample
Reagent	1000 $\mu$ L	1000 $\mu$ L	1000 $\mu$ L
Standard	.....	10 $\mu$ L	.....
Sample	.....	.....	10 $\mu$ L

The contents of the tubes were well mixed and incubated for 5 minutes at 37°C. Absorbance of the sample and standard against reagent blank were read at 546nm.

### Calculations:

Total cholesterol concentration (mg/dL) =  $\frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Concentration of standard (200 mg/dL)}$



The absorbance of the samples ( $A_{\text{sample}}$ ) and of the standard ( $A_{\text{standard}}$ ) was read against the blank at 546nm.

**Calculations:**

$$\text{HDL Cholesterol level (mg/dL.)} = \frac{A(\text{sample})}{A(\text{standard})} \times \text{Concentration of standard (50 mg/dL)}.$$

**Determination of serum low density lipoprotein cholesterol (LDL-C) level:<sup>(220)</sup>**

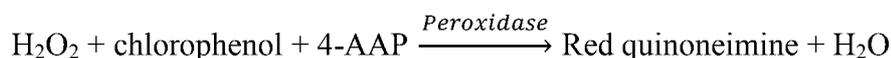
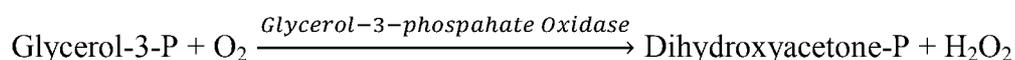
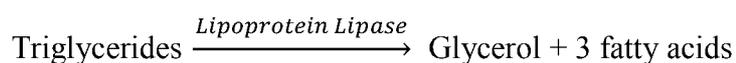
Low density lipoprotein cholesterol (LDL) was calculated by the Friedwald's equation:

$$\text{LDL-C (mg/dL)} = [\text{total cholesterol}] - [\text{HDL-C}] - [\text{triglycerides}/5]$$

## Estimation of serum triglycerides (TG):<sup>(221)</sup>

### Principle:

The triglycerides in the serum sample are hydrolyzed enzymatically by the action of lipoprotein lipase to glycerol and free fatty acids. The glycerol formed is converted to glycerol phosphate by glycerol kinase (GK). Glycerol phosphate is then oxidized to dihydroxyacetone phosphate by glycerol phosphate oxidase (GPO). The liberated hydrogen peroxide is detected by a chromogenic acceptor, 4-chlorophenol and 4-aminoantipyrine, in the presence of peroxidase (POD). The red quinoneimine formed is proportional to the amount of triglycerides present in the sample.



### Reagents:

PIPES buffer (pH 7.5)	50 mmol/L
4-chlorophenol	5 mmol/L
4-aminoantipyrine	0.25 mmol/L
Magnesium ions	4.5 mmol/L
ATP	2 mmol/L
Lipases	≥1.3 U/mL
Peroxidase	≥0.5 U/mL
Glycerol kinase	≥0.4 U/mL
Glycerol-3-phosphate oxidase	≥1.5 U/mL

### Standard:

Standard triglyceride	200 mg/dL
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**Procedure:**

The following volumes were pipette into three labeled test tubes and well mixed:

	Blank	Standard	Sample
Distilled water	10 $\mu$ L	.....	.....
Standard	.....	10 $\mu$ L	.....
Sample	.....	.....	10 $\mu$ L
Working reagent	1000 $\mu$ L	1000 $\mu$ L	1000 $\mu$ L

The tubes were incubated for 5 minutes at 37°C. Absorbance of the sample and standard against reagent blank were read at 546nm.

**Calculations:**

$$\text{Triglyceride level (mg/dL)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Concentration of standard} \left( \frac{200\text{mg}}{\text{dL}} \right)$$

## **Estimation of serum 8-OHdG:<sup>(222)</sup>**

### **Principle:**

The OxiSelect Oxidative DNA Damage ELISA kit is a competitive ELISA for the quantitative measurement of 8-OHdG. The unknown 8-OHdG samples or 8-OHdG standards are first added to an 8-OHdG/BSA (Bovine serum albumin) conjugate preabsorbed microplate. After a brief incubation, an anti-8-OHdG monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The 8-OHdG content in unknown samples is determined by comparison with predetermined 8-OHdG standard curve.

### **Kit Components:**

1. 96-well Protein Binding Plate: One strip well 96-well plate.
2. Anti-8-OHdG Antibody: One 15 µL vial of anti-8-OHdG.
3. Secondary Antibody, HRP Conjugate: One 50 µL vial.
4. Assay Diluent: One 50 mL bottle.
5. 10X Wash Buffer: One 100 mL bottle.
6. Substrate Solution: One 12 mL amber bottle.
7. Stop Solution: One 12 mL bottle.
8. 8-OHdG Standard: One 100 µL vial of 2 µg/mL 8-OHdG in 1X PBS, 0.1% BSA.
9. 8-OHdG Conjugate: One 20 µL vial of 8-OHdG-BSA conjugate at 1.0 mg/mL in PBS.

### **Preparation of the Reagents:**

- 8-OHdG Coated Plate: The proper amount of 8-OHdG Conjugate (1 mg/mL) was diluted to 10 µg/mL in 1X PBS. 100 µL of the 10 µg/mL 8-OHdG Conjugate were added to each well and incubated 2 hours at 37°C. The 8-OHdG coating solution was removed and washed once with dH<sub>2</sub>O. The plate was blotted on paper towels to remove excess fluid. 200 µL of Assay Diluent were added to each well and blocked for 1 hr at 37°C. The plate was transferred to 4°C and the Assay Diluent was removed immediately before use.
- 1X Wash Buffer: 10X Wash Buffer concentrate was diluted to 1X with deionized water, then stirred to homogeneity.
- Anti-8-OHdG Antibody and Secondary Antibody: Immediately before use the Anti-8-OHdG Antibody was diluted 1:500 and Secondary Antibody was diluted 1:1000 with Assay Diluent.
- Preparation of Standard Curve:

Dilution series of 8-OHdG standards were prepared in the concentration range of 0 ng/mL to 20 ng/mL by diluting the 8-OHdG Standard in Assay Diluent.

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<b>Standard Tubes</b>	<b>8-OHdG Standard (µL)</b>	<b>Assay Diluent (µL)</b>	<b>8-OHdG (ng/mL)</b>
1	10	990	20
2	500 of Tube #1	500	10
3	500 of Tube #2	500	5
4	500 of Tube #3	500	2.5
5	500 of Tube #3	500	1.25
6	500 of Tube #5	500	0.625
7	500 of Tube #6	500	0.313
8	500 of Tube #7	500	0.156
9	500 of Tube #8	500	0.078
10	0	500	0

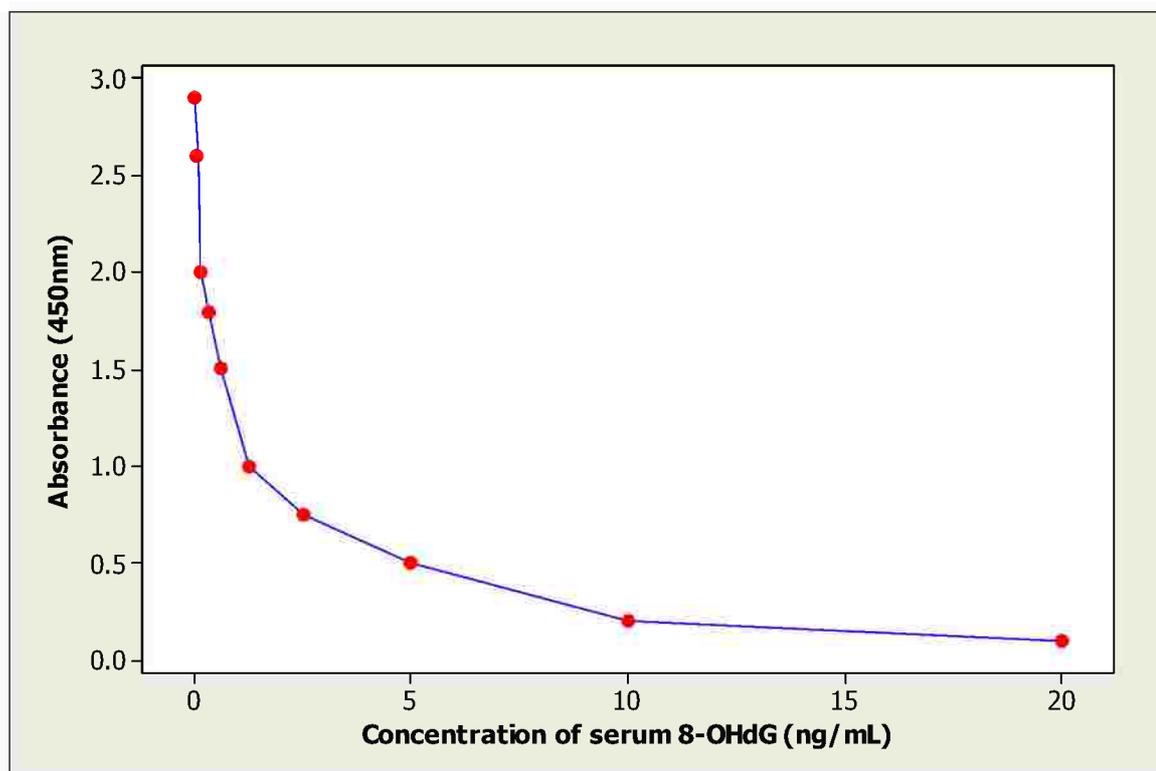
**Assay Protocol:**

1. 50 µL of unknown sample or 8-OHdG standard were added to the wells of the 8-OHdG Conjugate coated plate, and incubated at room temperature for 10 minutes on an orbital shaker.
2. 50 µL of the diluted anti-8-OHdG antibody were added to each well, and incubated at room temperature for 1 hour on an orbital shaker.
3. Microwell strips were washed 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, wells were emptied and microwell strips were tapped on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. 100 µL of the diluted Secondary Antibody-Enzyme Conjugate were added to all wells.
5. Wells were incubated at room temperature for 1 hour on an orbital shaker.
6. Microwell strips were washed 3 times according to step 4 above.
7. Substrate Solution was warmed to room temperature. 100 µL of Substrate Solution were added to each well, including the blank wells, then incubated at room temperature on an orbital shaker. Actual incubation time was 20 minutes.
8. The enzyme reaction was stopped by adding 100 µL of Stop Solution into each well, including the blank wells. Results were read immediately.
9. Absorbance of each microwell was read on a spectrophotometer using 450 nm as the primary wave length.

**Calculations:**

1. The standard curve was created by plotting the absorbance obtained from each reference standard against its concentration.

2. The absorbance value was used for each specimen to determine the corresponding concentration of 8-OHdG from the standard curve.



**Figure (10): Standard curve of serum 8-OHdG (ng/mL).**

## **Estimation of serum MDA:<sup>(223)</sup>**

### **Principle:**

Cayman's TBARS Assay Kit provides a simple, reproducible and standardized tool for assaying lipid peroxidation in serum. The Thiobarbituric Acid Reactive Substances (TBARS) Assay Kit is a tool for the direct quantitative measurement of MDA in biological samples. The unknown MDA containing samples or MDA standards are first reacted with TBA at high temperature (95°C) and acidic conditions to form the MDA-TBA adduct. After a brief incubation, the samples and standards can be read colorimetrically at 530-540nm. The MDA content in unknown samples is determined by comparison with the predetermined MDA standard curve.

### **Reagents:**

1. Thiobarbituric Acid (TBA): One vial.
2. TBA Acetic Acid: Two vials.
3. TBA Sodium Hydroxide (10X): One vial.
4. TBA Malondialdehyde Standard: One vial.
5. TBA SDS Solution: One vial.
6. 96-Well Solid Plate: One plate.
7. 96-Well Cover Sheet: Two covers.

### **Preparation of the Reagents:**

1. Thiobarbituric Acid (TBA): The vial contained 2g of TBA. It was ready to use to prepare the Color Reagent.
2. TBA Acetic Acid: Each vial contained 20 mL of concentrated acetic acid. Both vials (40mL) of TBA Acetic Acid were slowly added to 160mL of HPLC-grade water. This diluted acetic acid solution was used in preparing the Color Reagent.
3. TBA Sodium Hydroxide (10X): The vial contained a solution of sodium hydroxide (NaOH). 20 mL of TBA NaOH were diluted with 180 mL of HPLC-grade water. This diluted NaOH solution was used in preparing the Color Reagent.
4. TBA Malondialdehyde Standard: The vial contained 500 µM malondialdehyde (MDA) in water. It was ready to use to prepare the standard curve.
5. TBA SDS Solution: The vial contained a solution of Sodium dodecyl sulfate (SDS). The solution was ready to use as supplied.
6. Preparation of Color Reagent: 530 mg of TBA were weighted and added to 50 mL of diluted TBA Acetic Acid Solution. 50 mL of diluted TBA Sodium Hydroxide were added and mixed until the TBA was completely dissolved.
7. Standard preparation: 250 µL of the MDA Standard were diluted with 750 µL of water to obtain a stock solution of 125 µM. Eight clean glass test tubes were taken and labeled. The amount of 125 µM MDA stock solution and water were added to each tube as described in the next table:

Tube	MDA( $\mu\text{L}$ )	Water( $\mu\text{L}$ )	MDA Concentration( $\mu\text{M}$ )
A	0	1,000	0
B	5	995	0.625
C	10	990	1.25
D	20	980	2.5
E	40	960	5
F	80	920	10
G	200	800	25
H	400	600	50

**Assay procedure:**

1. Vial caps were labeled with standard number or sample identification number.
2. 100  $\mu\text{L}$  of sample or standard were added to appropriately labeled vial.
3. 100  $\mu\text{L}$  of SDS Solution were added to vial and swirled to mix.
4. 4 mL of the Color Reagent were added forcefully down side of each vial.
5. Vials were capped and placed in foam or some other holder to keep the tubes upright during boiling.
6. Vials were added to vigorously boiling water. Vials were boiled for one hour.
7. After one hour, the vials were removed immediately and placed in ice bath to stop reaction. Vials were incubated on ice for 10 minutes.
8. After 10 minutes, the vials were centrifuged for 10 minutes at 1,600 $\times g$  at 4°C.
9. 150  $\mu\text{L}$  were loaded from each vial to the clear plate. The absorbance was read at 530-540 nm.

**Calculations:**

1. The average absorbance of each standard and sample was calculated.
2. The absorbance value of the standard A (0  $\mu\text{M}$ ) was subtracted from itself and all other values (both standards and samples). This was the corrected absorbance.
3. The corrected absorbance values of each standard were plotted as a function of MDA concentration.
4. The value of MDA for each sample was calculated from the standard curve.

$$\text{MDA}(\mu\text{M}) = \left[ \frac{(\text{Corrected absorbance}) - (y\text{-intercept})}{\text{Slope}} \right]$$

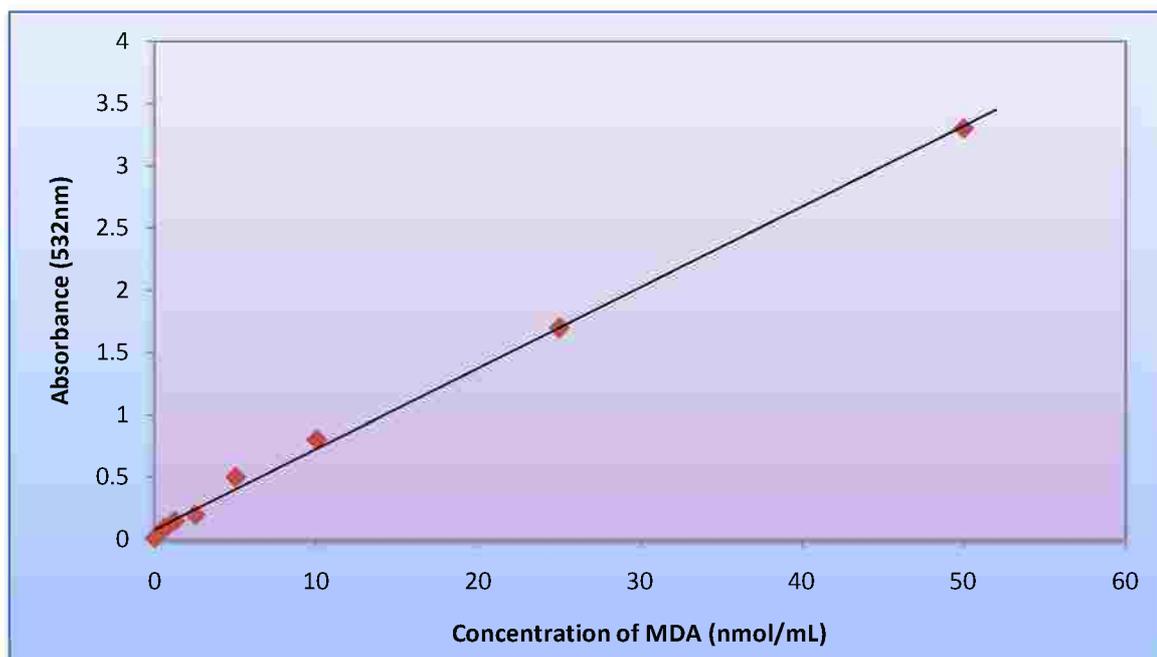


Figure (11): Standard curve of serum MDA (nmol/mL).

## **Estimation of serum SOD activity:<sup>(224)</sup>**

(By: Cayman's Superoxide Dismutase Assay Kit)

### **Principle:**

Superoxide anions ( $O_2^{\cdot -}$ ) are generated by a Xanthine/Xanthine Oxidase (XOD) system, and then detected with a Chromagen Solution (Tetrazolium salt). However, in the presence of SOD, these superoxide anion concentrations are reduced, yielding less colorimetric signal. Simply, the included chromagen produces a water-soluble formazan dye upon reduction by superoxide anions; and the activity of SOD is determined as the inhibition of chromagen reduction. The SOD assay measures all three types of SOD (Cu/Zn, Mn and FeSOD).

### **Reagents:**

1. Assay buffer (10X): One vial.
2. Sample Buffer (10X): One vial.
3. Radical Detector: One vial.
4. SOD Standard: One vial.
5. Xanthine Oxidase: Three vials.
6. 96-Well solid plate: One plate.
7. 96-Well Cover Sheet: One cover.

### **Preparation of the Reagents:**

1. **Assay buffer (10X):** 3 mL of Assay Buffer concentrate were diluted with 27 mL of HPLC-grade water for assaying 96 wells. This final Assay Buffer (50 mM Tris-HCl, pH 8.0, containing 0.1 mM diethylenetriaminepentaacetic acid (DTPA) and 0.1 mM hypoxanthine) was used to dilute the radical detector.
2. **Sample Buffer (10X):** 2 mL of Sample Buffer concentrate were diluted with 18 mL of HPLC-grade water for assaying 96 wells. This final Sample Buffer (50 mM Tris-HCl, pH 8.0) was used to prepare the SOD standards and to dilute the xanthine oxidase and SOD samples prior to assaying.
3. **Radical Detector:** The vials contained 250  $\mu$ L of a tetrazolium salt solution. Prior to use, 50  $\mu$ L of the supplied solution were transferred to another vial and diluted with 19.95 mL of diluted Assay Buffer. It was covered with tin foil and used within two hours.
4. **SOD Standard:** The vials contained 100  $\mu$ L of bovine erythrocyte SOD (Cu/Zn), were provided at (125 U/mL). The thawed enzyme was stored on ice and used for preparing the standard curve. Unit Definition: One unit was defined as the amount of enzyme needed to inhibit the rate of reduction of cytochrome c by 50% in a coupled system, using xanthine and xanthine oxidase, at pH 7.8 at 25°C in a 3.0 ml reaction volume.

- 5. Xanthine Oxidase:** These vials contained 150  $\mu\text{L}$  of Xanthine Oxidase. Prior to use, one vial was thawed and 50  $\mu\text{L}$  of the supplied enzyme were transferred to another vial and diluted with 1.95 mL of Sample Buffer (diluted).
- 6. Standard preparation:** 20  $\mu\text{L}$  of the SOD Standard were diluted with 1.98 mL of Sample Buffer (diluted) to obtain the SOD stock solution. Seven clean glass test tubes were taken and marked from A-G. The amount of SOD stock and Sample Buffer (dilute) were added to each tube as described in the next table:

<b>Tube</b>	<b>SOD Stock(<math>\mu\text{L}</math>)</b>	<b>Sample Buffer(<math>\mu\text{L}</math>)</b>	<b>Final SOD Activity(U/mL)</b>
<b>A</b>	0	1,000	0
<b>B</b>	20	980	0.025
<b>C</b>	40	960	0.05
<b>D</b>	80	920	0.1
<b>E</b>	120	880	0.15
<b>F</b>	160	840	0.2
<b>G</b>	200	800	0.25

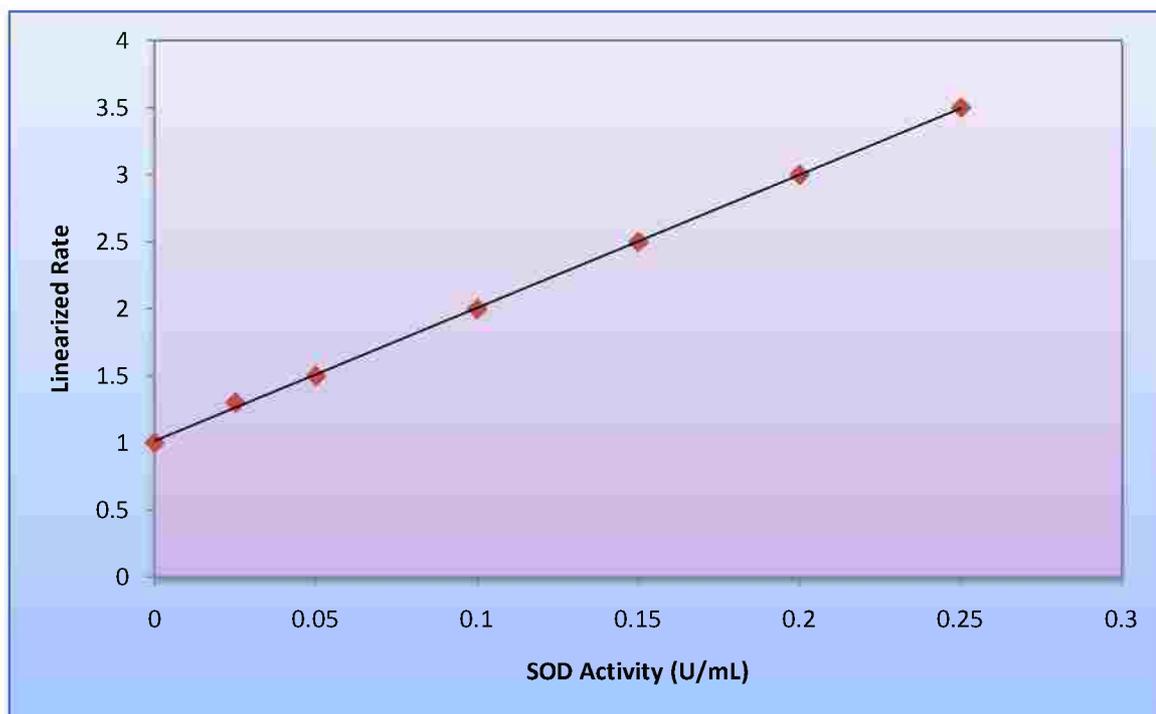
**Assay Protocol:**

- SOD Standard Wells: 200  $\mu\text{L}$  of the diluted Radical detector and 10  $\mu\text{L}$  of Standard (tubes A-G) were added to each well.
- Serum was diluted 1:5 with Sample Buffer before assaying for SOD activity.
- Sample Wells: 200  $\mu\text{L}$  of the diluted Radical Detector and 10  $\mu\text{L}$  of sample were added to the wells.
- The reaction was initiated by adding 20  $\mu\text{L}$  of diluted Xanthine Oxidase to all the using wells.
- The 96-well plate was shaken carefully for a few seconds to mix, and covered with the plate cover.
- The plate was incubated on a shaker for 20 minutes at room temperature. The absorbance was read at 440-460nm using a plate reader.

**Calculations:**

- The average absorbance of each standard and sample was calculated.
- Standard A's absorbance was divided by itself and standard A's absorbance was divided by all the other standards and samples absorbances to yield the linearized rate (LR), (i.e., LR for Std A= Abs Std A/Abs Std A; LR for Std B= Abs Std A/Abs Std B).
- The linearized SOD standard rate (LR) was plotted as a function of final SOD Activity (U/mL).
- The SOD activity of the samples was calculated using the equation obtained from the linear regression of the standard curve substituting the linearized rate (LR) for each sample.

$$\text{SOD(U/mL)} = \left[ \left( \frac{\text{sample LR} - y\text{-intercept}}{\text{slope}} \right) \times \frac{0.23\text{mL}}{0.01\text{mL}} \right] \times \text{sample dilution}$$



**Figure (12): Superoxide dismutase standard curve (U/mL).**

## Statistical Analyses of Data

All data were expressed as the mean  $\pm$  SD unless otherwise stated. Statistical analyses were done by using paired t-test, to compare the difference within the same group before and after treatment. An independent samples t-test was used to compare means from independent groups. A 2-tailed P value  $<0.05$  was considered to be statistically significant for all analyses. Pearson's correlations were also performed, and each Pearson's linear correlation coefficient was calculated, with significant relationship set at  $P < 0.05$ . All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) software, version 18.0 for Windows.

## RESULTS

### 1. Results of anthropometric parameters:

#### A. Before treatment:

Individual data, range and mean $\pm$  SD values of age, body mass index (BMI), waist circumference (WC), waist-to-hip ratio (WHR) and blood pressure (systolic and diastolic) of normal control subjects and metabolic syndrome patients were shown in tables (I) and (II) respectively in Appendix. The statistical analyses of these results were represented in table (2).

**For normal control subjects:** the mean age was  $47.25 \pm 4.45$  years (range 40 – 56), the mean BMI was  $25.02 \pm 1.09$  kg/m<sup>2</sup> (range 22.66 - 27.46), the mean WC was  $89.55 \pm 3.94$  cm (range 81 – 96), the mean WHR was  $0.87 \pm 0.02$  (range 0.81 – 0.91), the mean systolic blood pressure (s.BP) was  $122 \pm 7.14$  mmHg (range 110 – 135) and the mean diastolic blood pressure (d.BP) was  $80.25 \pm 4.99$  mmHg (range 70 – 90).

**For the metabolic syndrome (MS) patients:** the mean age was  $46.87 \pm 5$  years (range 40 – 57), the mean BMI was  $40.14 \pm 4.09$  kg/m<sup>2</sup> (range 31.74 – 48.90), the mean WC was  $118.6 \pm 5.13$  cm (range 107 – 130), the mean WHR was  $1.09 \pm .05$  (range 1.01 – 1.22), the mean systolic blood pressure was  $142.5 \pm 11.87$  mmHg (range 130 – 180) and the mean diastolic blood pressure was  $98.83 \pm 6.25$  mmHg (range 80 – 110).

Compared with control group, the metabolic syndrome patients had significant higher BMI, WC, WHR and blood pressure (systolic and diastolic), while there was no significant difference between the 2 groups in age.

**Table (2): The statistical analyses of age, body mass index (BMI), waist circumference (WC), waist/hip ratio (WHR), systolic and diastolic blood pressure in control and patients with metabolic syndrome**

	<b>Normal control group</b> (n = 20)	<b>MS group</b> (n = 30)
<b>Age (years)</b>		
Range	40 – 56	40 – 57
Mean± SD	47.25± 4.45	46.87± 5
P		P= 0.778
<b>BMI (kg/m<sup>2</sup>)</b>		
Range	22.66 – 27.46	31.74 – 48.90
Mean± SD	25.02± 1.09	40.14± 4.09
P		P<0.001*
<b>WC (cm)</b>		
Range	81 – 96	107 – 130
Mean± SD	89.55± 3.94	118.6± 5.13
P		P<0.001*
<b>WHR</b>		
Range	0.81 – 0.91	1.01 – 1.22
Mean± SD	0.87± 0.02	1.09± 0.05
P		P<0.001*
<b>Systolic BP (mmHg)</b>		
Range	110 – 135	130 – 180
Mean± SD	122± 7.14	142.5± 11.87
P		P<0.001*
<b>Diastolic BP (mmHg)</b>		
Range	70 – 90	80 – 110
Mean± SD	80.25± 4.99	98.83± 6.25
P		P<0.001*

\*: Significance was considered at P< 0.05

**B. Blood pressure in metabolic syndrome patients (subgroups IIa & IIb) before and after 3 months of treatment with either atorvastatin or atorvastatin + vitamin E:**

As shown in table (3), it was observed that systolic and diastolic blood pressure in metabolic syndrome subgroups (IIa) & (IIb) were not affected by treatment with either atorvastatin or atorvastatin + vitamin E.

**Table (3): Effect of atorvastatin and atorvastatin+ vitamin E on blood pressure in metabolic syndrome patients (subgroups IIa & IIb), after 3 months of treatment**

Parameters	MS Patients Subgroup IIa		MS Patients Subgroup IIb	
	Before treatment	After treatment with atorvastatin	Before treatment	After treatment with atorvastatin+ vitamin E
<b>n</b>	15	15	15	15
<b>Systolic BP</b>				
Range	(130 – 170)	(120 – 170)	(130 – 180)	(120 – 170)
Mean± SD	141.66± 11.44	140.66± 13.87	143.33± 12.63	141± 13.25
	P= 0.486		P= 0.11	
<b>Diastolic BP</b>				
Range	(80 – 110)	(90 – 110)	(90 – 110)	(85 – 110)
Mean± SD	98.33± 6.98	98± 6.49	99.33± 5.62	98.66± 7.89
	P= 0.751		P= 0.634	

\*: Significance was considered at  $P < 0.05$

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## 2. Biochemical results:

### A. Results of fasting serum glucose (FG mg/dL), serum insulin (SI $\mu$ IU/mL) and homeostasis model assessment-insulin resistance (HOMA-IR):

Individual data, range and mean $\pm$  SD values of FG, SI and HOMA-IR of control and metabolic syndrome subgroups (IIa and IIb), before and after treatment with either atorvastatin or atorvastatin+ vitamin E were shown in tables (IV), (V) and (VI) respectively in Appendix. The statistical analyses of these results were represented in table (4) and figures (13 – 15).

**For normal control subjects:** the mean FG was  $87.7\pm 6.48$  mg/dL (range 75 – 100), the mean SI was  $7.97\pm 1.53$   $\mu$ IU/mL (range 4.4 – 10) and the mean HOMA-IR was  $1.73\pm 0.37$  (range 0.81 – 2.42).

**For metabolic syndrome subgroup (IIa):** the mean FG was  $131.33\pm 39.03$  mg/dL (range 94 – 240), the mean SI was  $20.26\pm 5.04$   $\mu$ IU/mL (range 12 – 29) and the mean HOMA-IR was  $6.22\pm 0.78$  (range 3.91 – 7.11).

**For metabolic syndrome subgroup (IIa) treated with atorvastatin:** the mean FG was  $132.86\pm 39.44$  mg/dL (range 97 – 245), the mean SI was  $21.06\pm 5.55$   $\mu$ IU/mL (range 11.8 – 31) and the mean HOMA-IR was  $6.5\pm 0.75$  (range 5.52 – 7.73).

**For metabolic syndrome subgroup (IIb):** the mean FG was  $119.06\pm 19.59$  mg/dL (range 88 – 160), the mean SI was  $21.8\pm 4.75$   $\mu$ IU/mL (range 14 – 31) and the mean HOMA-IR was  $6.22\pm 0.57$  (range 4.62 – 6.93).

**For metabolic syndrome subgroup (IIb) treated with atorvastatin+ vitamin E:** the mean FG was  $117.4\pm 21.51$  mg/dL (range 85 – 179), the mean SI was  $22.35\pm 4.13$   $\mu$ IU/mL (range 16 – 30) and the mean HOMA-IR was  $6.32\pm 0.74$  (range 5.38 – 8.4).

The statistical analyses of these results revealed that the levels of FG, SI and HOMA-IR in the two metabolic syndrome subgroups (IIa& IIb) were nearly within the same range and were significantly higher than their corresponding values in control group. After 3 months of treatment with either atorvastatin or atorvastatin+ vitamin E, the mean values of these parameters showed insignificant differences when compared with their corresponding values before treatment and were significantly higher than those in control group. Insignificant difference was observed between the levels of FG, SI and HOMA-IR in metabolic syndrome patients treated with atorvastatin alone and those treated with atorvastatin+ vitamin E.

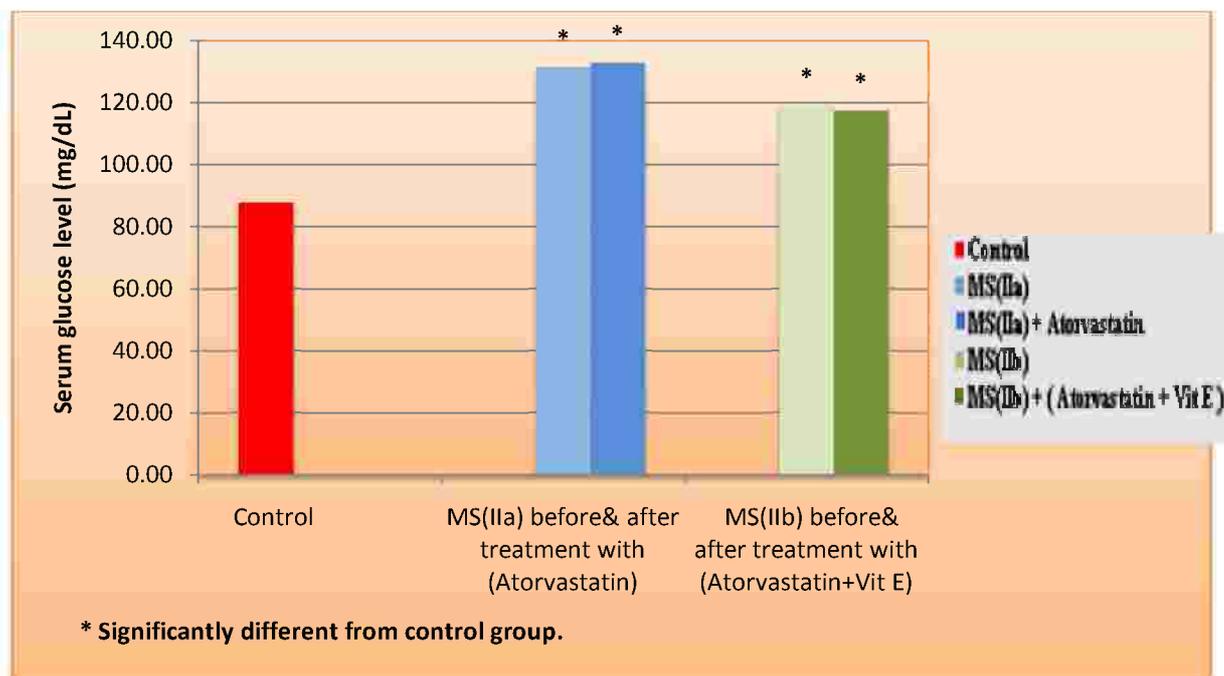
**Table (4): Statistical analyses of fasting serum glucose (FG), serum insulin (SI) and HOMA-IR in control and MS patients (subgroups IIa& IIb), before and after 3 months of treatment with either atorvastatin or atorvastatin+ vitamin E**

Parameters	Control group	MS patients Subgroup (IIa)		MS patients Subgroup (IIb)	
		Before treatment	After treatment with atorvastatin	Before treatment	After treatment with atorvastatin+ vitamin E
n	20	15	15	15	15
FG(mg/dL) Range Mean± SD	75 – 100 87.7± 6.48	94 – 240 131.33±39.03	97 – 245 132.86±39.44	88 – 160 119.06±19.59	85 – 179 117.4± 21.51
		P= 0.433		P= 0.524	
		P= 0.286		P= 0.193	
		P= 0.001*	↑	P= 0.001*	
		P< 0.001*		P= 0.001*	
		P= 0.001*		↑	
		P< 0.001*		↑	
SI(μIU/mL) Range Mean± SD	4.4 – 10 7.97± 1.53	12 – 29 20.26± 5.04	11.8 – 31 21.06± 5.55	14 – 31 21.8± 4.75	16 – 30 22.35± 4.13
		P= 0.318		P= 0.273	
		P= 0.399		P= 0.475	
		P< 0.001*	↑	P= 0.001*	
		P< 0.001*		↑	
		P< 0.001*		↑	
		P< 0.001*		↑	

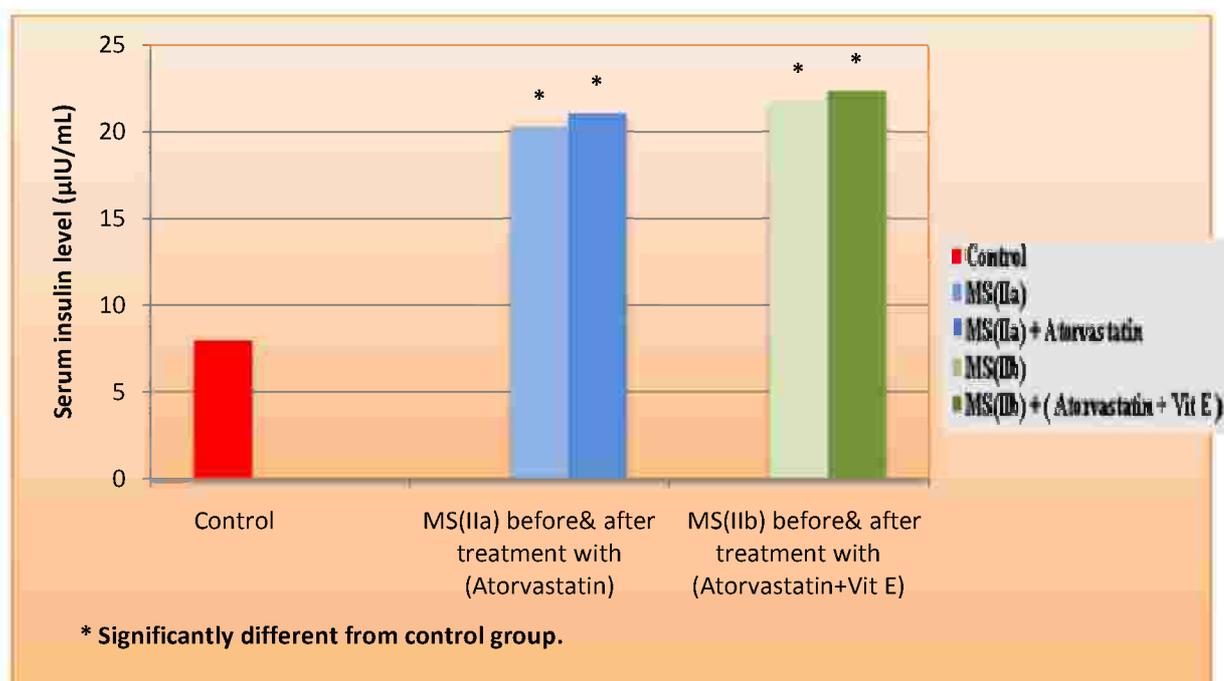
**Table (4): Statistical analyses of fasting serum glucose (FG), serum insulin (SI) and HOMA-IR in control and MS patients (subgroups IIa& IIb), before and after 3 months of treatment with either atorvastatin or atorvastatin+ vitamin E (Cont.)**

Parameters	Control group	MS patients Subgroup (IIa)		MS patients Subgroup (IIb)	
		Before treatment	After treatment with atorvastatin	Before treatment	After treatment with atorvastatin+ vitamin E
n	20	15	15	15	15
<b>HOMA-IR</b>					
Range	0.81 – 2.42	3.91 – 7.11	5.52 – 7.73	4.62 – 6.93	5.38 – 8.4
Mean± SD	1.73± 0.378	6.22± 0.78	6.5± 0.75	6.22± 0.57	6.32± 0.74
		P= 0.175		P= 0.618	
		P= 0.983			
		P< 0.001*	↑	P= 0.537	
		P< 0.001*			↑
		P< 0.001*			↑
		P< 0.001*			

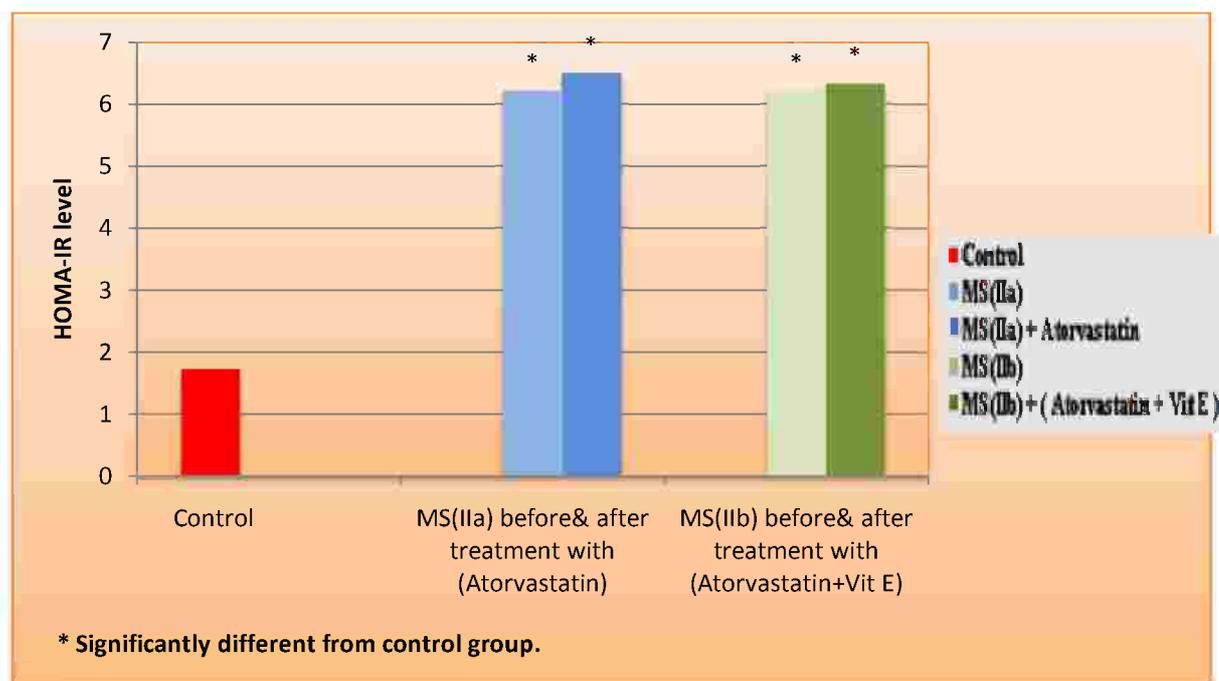
\*: Significance was considered at  $P < 0.05$



**Figure (13):** Bar chart representing the mean of fasting serum glucose levels (mg/dL) in control and both metabolic syndrome subgroups (IIa)& (IIb) before and after 3 months of treatment with atorvastatin or atorvastatin+ vitamin E.



**Figure (14):** Bar chart representing the mean of serum insulin levels (µIU/mL) in control and both metabolic syndrome subgroups (IIa)& (IIb) before and after 3 months of treatment with atorvastatin or atorvastatin+ vitamin E.



**Figure (15):** Bar chart representing the mean of HOMA-IR levels in control and both metabolic syndrome subgroups (Ia)& (Ib) before and after 3 months of treatment with atorvastatin or atorvastatin+ vitamin E.

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**B. Results of high-density lipoprotein (HDL mg/dL), triglycerides (TG mg/dL), total cholesterol (T.Cholest mg/dL) and low-density lipoprotein (LDL mg/dL):**

Individual data, range and mean $\pm$  SD values of HDL, TG, T.Cholest and LDL of normal control subjects and metabolic syndrome subgroups (IIa and IIb), before and after treatment with either atorvastatin or atorvastatin+ vitamin E were shown in tables (VII), (VIII), (IX), (X) and (XI) respectively in Appendix. The statistical analyses of these results were represented in table (5) and figures (16 – 19).

**For normal control subjects:** the mean HDL was  $51\pm 8.1$  mg/dL (range 36 – 65), the mean TG was  $136.5\pm 7.059$  mg/dL (range 121 – 149), the mean T. Cholest was  $160.95\pm 16.06$  mg/dL (range 135 – 190) and the mean LDL was  $82.65\pm 19.35$  mg/dL (range 53 – 120.4).

**For metabolic syndrome subgroup (IIa):** the mean HDL was  $33.66\pm 5.01$  mg/dL (range 24 – 46), the mean TG was  $265.13\pm 90.3$  mg/dL (range 168 – 490), the mean T. Cholest was  $275.86\pm 29.67$  mg/dL (range 235 – 333) and the mean LDL was  $189.17\pm 17.41$  mg/dL (range 159 – 211).

**For metabolic syndrome subgroup (IIa) treated with atorvastatin:** the mean HDL was  $36\pm 5.19$  mg/dL (range 26 – 48), the mean TG was  $209.73\pm 82.06$  mg/dL (range 110 – 390), the mean T. Cholest was  $189.8\pm 22.51$  mg/dL (range 161 – 233) and the mean LDL was  $111.71\pm 11.64$  mg/dL (range 91 – 129.02).

**For metabolic syndrome subgroup (IIb):** the mean HDL was  $32.73\pm 4.33$  mg/dL (range 28 – 44), the mean TG was  $243.2\pm 68.56$  mg/dL (range 150 – 408), the mean T. Cholest was  $272.06\pm 30.41$  mg/dL (range 230 – 326) and the mean LDL was  $190.69\pm 22.3$  mg/dL (range 160 – 228).

**For metabolic syndrome subgroup (IIb) treated with atorvastatin+ vitamin E:** the mean HDL was  $39.14\pm 4.14$  mg/dL (range 33.6 – 47.97), the mean TG was  $170.84\pm 45.86$  mg/dL (range 108.5 – 285.6), the mean T. Cholest was  $171.36\pm 18.03$  mg/dL (range 148.8 – 202.12) and the mean LDL was  $98.04\pm 13.31$  mg/dL (range 74.78 – 118.68).

The statistical analyses of these results revealed that the level of HDL in the two metabolic syndrome subgroups (IIa& IIb) was nearly within the same range, and was significantly lower than in control group. After 3 months of treatment with either atorvastatin or atorvastatin+ vitamin E, the level of this parameter was significantly increased when compared with its corresponding value before treatment, but still significantly lower than in control group. It was also noticed that the level of this parameter in metabolic syndrome subgroup IIb treated with atorvastatin+ vitamin E was insignificantly higher than in subgroup IIa treated with atorvastatin only.

On the other hand, the levels of TG, T. Cholest and LDL in the two metabolic syndrome subgroups (IIa& IIb) showed insignificant difference, and were higher than their corresponding control values. After 3 months of treatment with either atorvastatin or atorvastatin+ vit E, these parameters were significantly decreased when compared with their corresponding values before treatment but still higher than their corresponding control values. On the other hand, it was noticed that the levels of both T. Cholest and LDL in metabolic syndrome subgroup (IIb) treated with atorvastatin+ vitamin E were significantly lower than that in metabolic syndrome subgroup (IIa) treated with atorvastatin alone.

**Table (5): Statistical analyses of high density lipoprotein (HDL), triglycerides (TG), total cholesterol (T.Cholest) and low density lipoprotein (LDL) in control and MS patients (subgroups IIa& IIb), before and after 3 months of treatment with either atorvastatin or atorvastatin+ vitamin E**

Parameters	Control group	MS patients Subgroup (IIa)		MS patients Subgroup (IIb)	
		Before treatment	After treatment with atorvastatin	Before treatment	After treatment with atorvastatin+ vitamin E
n	20	15	15	15	15
<b>HDL(mg/dL)</b>					
Range	36 – 65	24 – 46	26 – 48	28 – 44	33.6 – 47.97
Mean± SD	51± 8.1	33.66± 5	36± 5.19	32.73± 4.33	39.14± 4.14
		$P < 0.001^*$ ↑ $P = 0.59$		$P = 0.001^*$ ↑	
		$P < 0.001^*$ ↓ $P < 0.001^*$ ↓ $P < 0.001^*$ ↓ $P < 0.001^*$ ↓		$P = 0.077$ ↓ ↓ ↓	
<b>TG(mg/dL)</b>					
Range	121 – 149	168 – 490	110 – 390	150 – 408	108.5 – 285.6
Mean± SD	136.5± 7.05	265.13± 90.3	209.73± 82.06	243.2± 68.56	170.84± 45.86
		$P < 0.001^*$ ↓ $P = 0.046$		$P < 0.001^*$ ↓	
		$P < 0.001^*$ ↑ $P < 0.001^*$ ↑ $P = 0.004^*$ ↑ $P = 0.012^*$ ↑		$P = 0.123$ ↑ ↑	

**Table (5): Statistical analyses of high density lipoprotein (HDL), triglycerides (TG), total cholesterol (T.Cholest) and low density lipoprotein (LDL) in control and MS patients (subgroups IIa& IIb), before and after 3 months of treatment with either atorvastatin or atorvastatin+ vitamin E (Cont.,)**

Parameters	Control group	MS patients Subgroup (IIa)		MS patients Subgroup (IIb)	
		Before treatment	After treatment with atorvastatin	Before treatment	After treatment with atorvastatin+ vitamin E
n	20	15	15	15	15
<b>T.Cholest(mg/dL)</b> Range Mean± SD	135 – 190 160.95± 16.06	235 – 333 275.86± 29.67	161 – 233 189.8± 22.51	230 – 326 272.06± 30.41	148.8 – 202.12 171.36± 18.03
			<p>P&lt; 0.001* ↓</p> <p>P= 0.732</p>		<p>P&lt; 0.001* ↓</p>
		<p>P&lt; 0.001* ↑</p>		<p>P= 0.02*</p>	<p>↓</p>
		<p>P&lt; 0.001*</p>		<p>↑</p>	
		<p>P&lt; 0.001*</p>	<p>↑</p>		
		<p>P= 0.087</p>			
<b>LDL(mg/dL)</b> Range Mean± SD	53 – 120.4 82.65± 19.35	159 – 211 189.17± 17.41	91 – 129.02 111.71± 11.64	160 – 228 190.69± 22.3	74.78 – 118.68 98.04± 13.31
			<p>P&lt; 0.001* ↓</p> <p>P= 0.837</p>		<p>P&lt; 0.001* ↓</p>
		<p>P&lt; 0.001* ↑</p>		<p>P= 0.006*</p>	<p>↓</p>
		<p>P&lt; 0.001*</p>		<p>↑</p>	
		<p>P&lt; 0.001*</p>	<p>↑</p>		
		<p>P= 0.012*</p>			<p>↑</p>

\*: Significance was considered at P< 0.05

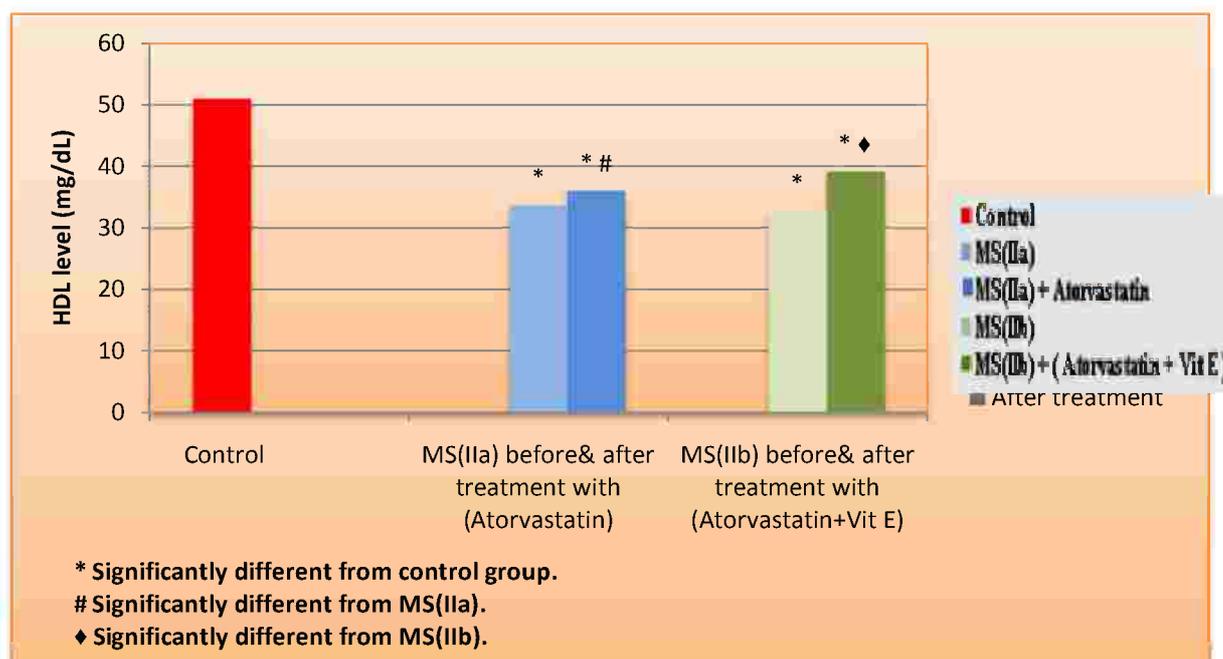


Figure (16): Bar chart representing the mean of serum HDL levels (mg/dL) in control and both metabolic syndrome subgroups (Ia)& (Ib) before and after 3 months of treatment with atorvastatin or atorvastatin+ vitamin E.

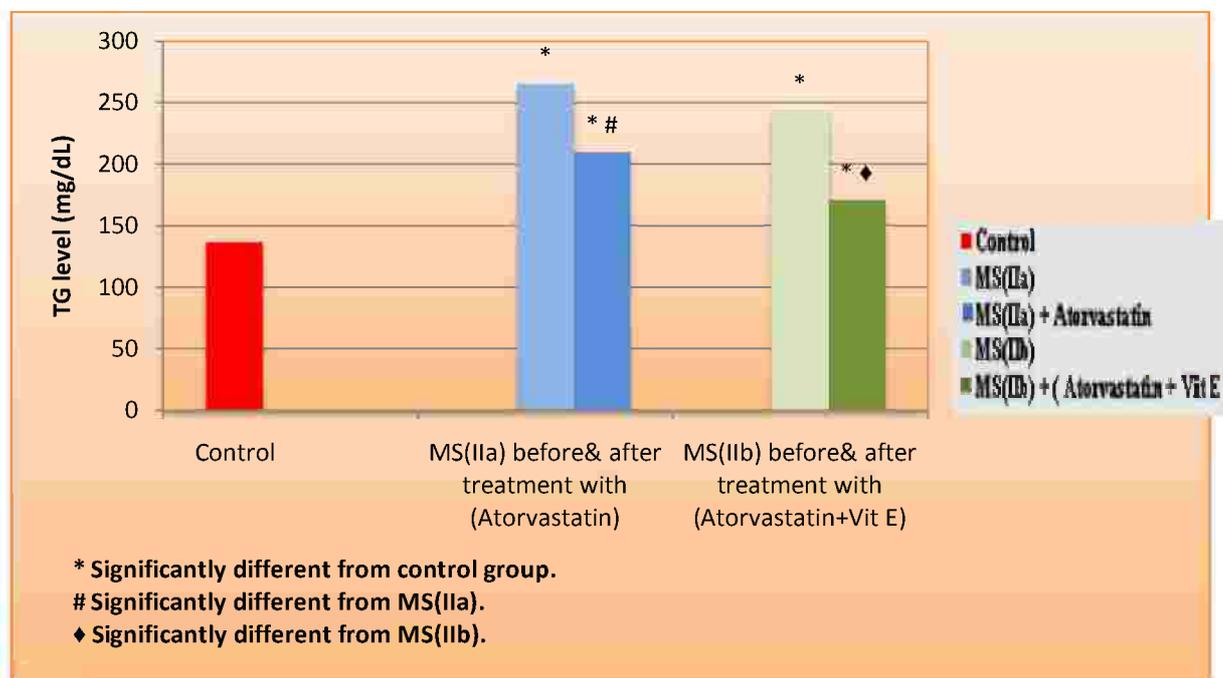


Figure (17): Bar chart representing the mean of serum triglycerides levels (mg/dL) in control and both metabolic syndrome subgroups (Ia)& (Ib) before and after 3 months of treatment with atorvastatin or atorvastatin+ vitamin E.

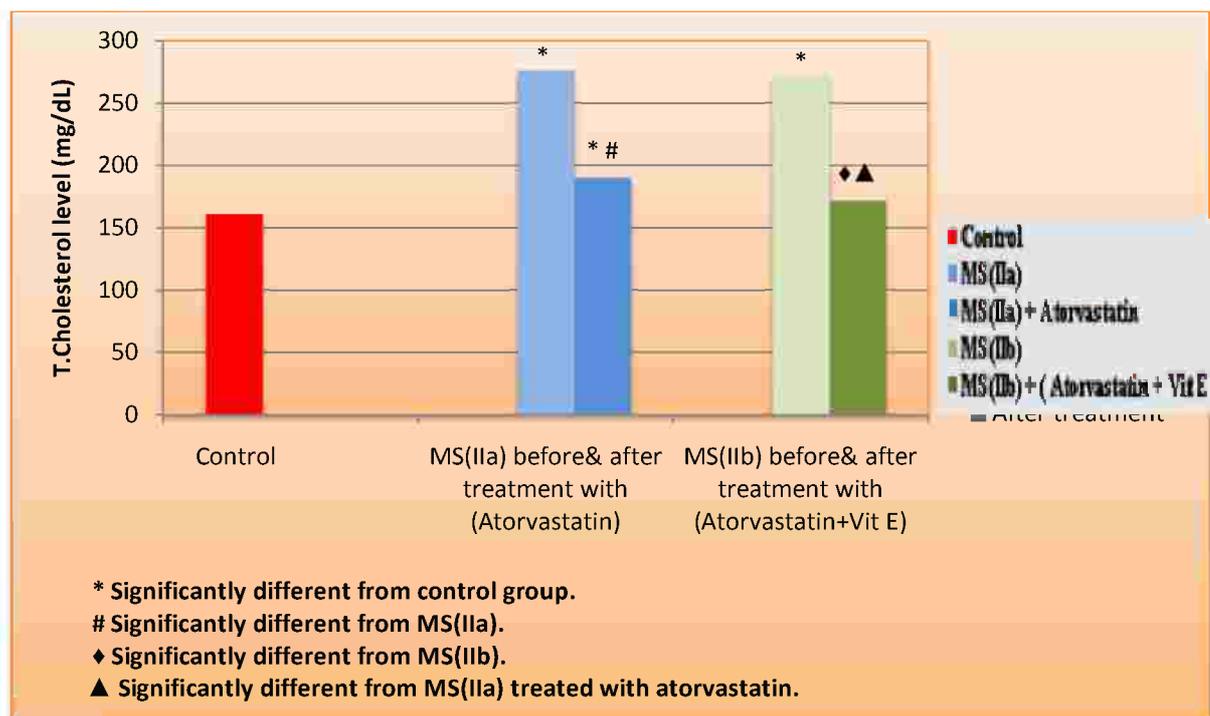


Figure (18): Bar chart representing the mean of serum total-cholesterol levels (mg/dL) in control and both metabolic syndrome subgroups (Ia)& (Ib) before and after 3 months of treatment with atorvastatin or atorvastatin+ vitamin E.

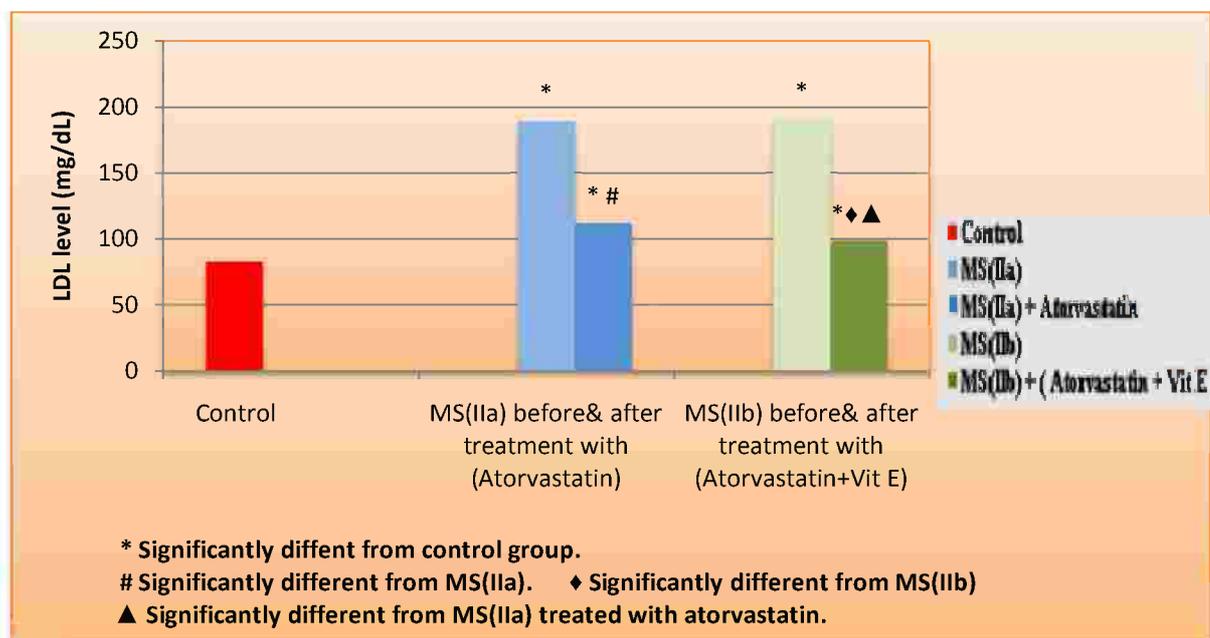


Figure (19): Bar chart representing the mean of serum LDL levels (mg/dL) in control and both metabolic syndrome subgroups (Ia)& (Ib) before and after 3 months of treatment with atorvastatin or atorvastatin+ vitamin E.

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**C. Results of serum 8-hydroxy-2'-deoxyguanosine (8-OHdG ng/mL), malondialdehyde (MDA nmol/mL) and superoxide dismutase (SOD unit/mL):**

Individual data, range and mean $\pm$  SD values of 8-OHdG, MDA and SOD of normal control subjects and metabolic syndrome patients, before and after treatment with either atorvastatin or atorvastatin+ vitamin E were shown in tables (XII), (XIII) and (XIV) respectively in Appendix. The statistical analyses of these results were represented in table (6) and figures (20 – 22).

**For normal control subjects:** the mean 8-OHdG was  $5.2\pm 1.44$  ng/mL (range 1.8 – 7.7), the mean MDA was  $3.26\pm 0.94$  nmol/mL (range 2 – 6) and the mean SOD was  $9.6\pm 1.27$  U/mL (range 7.9 – 13).

**For metabolic syndrome subgroup (IIa):** the mean 8-OHdG was  $12.66\pm 4.7$  ng/mL (range 4.4 – 19.4), the mean MDA was  $10.6\pm 1.19$  nmol/mL (range 7.7 – 12), and finally, the mean SOD was  $5.78\pm 0.54$  U/mL (range 5 – 6.9).

**For metabolic syndrome subgroup (IIa) treated with atorvastatin:** the mean 8-OHdG was  $8.87\pm 3.31$  ng/mL (range 3.2 – 13.8), the mean MDA was  $7.14\pm 0.81$  nmol/mL (range 5.4 – 8.4), and finally, the mean SOD was  $7.06\pm 0.65$  U/mL (range 6.1 – 8.4).

**For metabolic syndrome subgroup (IIb):** the mean 8-OHdG was  $12.06\pm 4.71$  ng/mL (range 4.17 – 18.87), the mean MDA was  $10.57\pm 0.96$  nmol/mL (range 8.9 – 12), and finally, the mean SOD was  $5.97\pm 0.829$  U/mL (range 4.9 – 8.06).

**For metabolic syndrome subgroup (IIb) treated with atorvastatin+ vitamin E:** the mean 8-OHdG was  $7.47\pm 3.34$  ng/mL (range 2 – 12.27), the mean MDA was  $6.1\pm 0.71$  nmol/mL (range 5.34 – 7.32), and finally, the mean SOD was  $7.5\pm 1.09$  U/mL (range 6.24 – 10.32).

The statistical analyses of these results revealed that the mean values of serum 8-OHdG and MDA in the two metabolic syndrome subgroups (IIa& IIb) showed insignificant difference, and were significantly higher than their corresponding values in control group. After 3 months of treatment with atorvastatin or with atorvastatin+ vitamin E, the levels of these parameters were significantly decreased, but still higher than their corresponding values in control group. Insignificant difference was observed between 8-OHdG values in patients treated with either atorvastatin alone or atorvastatin+ vitamin E. Furthermore, it was noticed that the level of MDA in metabolic syndrome subgroup (IIb) treated with atorvastatin+ vitamin E was significantly lower than that in patients' subgroup (IIa) treated with atorvastatin alone.

On the other hand, the level of SOD in the two metabolic syndrome subgroups (IIa and IIb) showed insignificant difference, and were significantly lower than in control group. After 3 months of treatment with either atorvastatin or atorvastatin+ vitamin E, the level of this enzyme in each subgroup (IIa or IIb) was significantly increased than its corresponding value before treatment, but still significantly lower than in control group. Insignificant difference was observed between the level of SOD in metabolic syndrome subgroup (IIa) treated with atorvastatin alone, and its level in metabolic syndrome subgroup (IIb) treated with atorvastatin+ vitamin E.

**Table (6): Statistical analyses of serum 8-hydroxy-2'-deoxyguanosine (8-OHdG), serum malondialdehyde (MDA) and superoxide dismutase (SOD) in control and MS patients (subgroups IIa& IIb), before and after 3 months of treatment with either atorvastatin or atorvastatin+ vitamin E**

Parameters	Control group	MS patients Subgroup (IIa)		MS patients Subgroup (IIb)	
		Before treatment	After treatment with atorvastatin	Before treatment	After treatment with atorvastatin+ vitamin E
n	20	15	15	15	15
<b>8-OHdG</b> (ng/mL) Range Mean± SD	1.8 – 7.7 5.2± 1.44	4.4 – 19.4 12.66± 4.7	3.2 – 13.8 8.87± 3.31	4.17 – 18.87 12.06± 4.71	2 – 12.27 7.47± 3.34
		$P < 0.001^*$ $P.C = 29.9\%$		$P < 0.001^*$ $P.C = 37.98\%$	
		$P = 0.73$			
		$P < 0.001^*$ $P.C = 143.46\%$		$P = 0.261$ $P.C = 8.08\%$	
		$P < 0.001^*$ $P.C = 131.9\%$ $P = 0.001^*$		$\uparrow$	
		$P = 0.024^*$		$\uparrow$	
<b>MDA</b> (nmol/mL) Range Mean± SD	2 – 6 3.26± 0.94	7.7 – 12 10.6± 1.19	5.4 – 8.4 7.14± 0.81	8.9 – 12 10.57± 0.96	5.34 – 7.32 6.1± 0.71
		$P < 0.001^*$ $P.C = 32.64\%$ $P = 0.947$		$P < 0.001^*$ $P.C = 42.28\%$	
		$P < 0.001^*$ $P.C = 224.65\%$		$P = 0.001^*$ $P.C = 9.64\%$	
		$P < 0.001^*$ $P.C = 223.7\%$		$\uparrow$	
		$P < 0.001^*$		$\uparrow$	
		$P < 0.001^*$		$\uparrow$	

**Table (6): Statistical analyses of serum 8-hydroxy-2'-deoxyguanosine (8-OHdG), serum malondialdehyde (MDA) and superoxide dismutase (SOD) in control and MS patients (subgroups IIa& IIb), before and after 3 months of treatment with either atorvastatin or atorvastatin+ vitamin E (Cont.,)**

<b>SOD(U/mL)</b>					
Range	7.9 – 13	5 – 6.9	6.1 – 8.4	4.9 – 8.06	6.24 – 10.32
Mean± SD	9.6± 1.27	5.78± 0.54	7.06± 0.65	5.97± 0.829	7.5± 1.09
		$P < 0.001^*$ ↑ $P.C = 22.1\%$		$P < 0.001^*$ ↑ $P.C = 25.6\%$	
		$P = 0.449$			
	$P < 0.001^*$ $P.C = 39.79\%$		$P = 0.195$ $P.C = 3.5\%$		
	$P < 0.001^*$ $P.C = 37.8\%$				
	$P < 0.001^*$ $P.C = 0.001^*$				↓
	$P < 0.001^*$				
	$P < 0.001^*$				↓

\*: Significance was considered at  $P < 0.05$

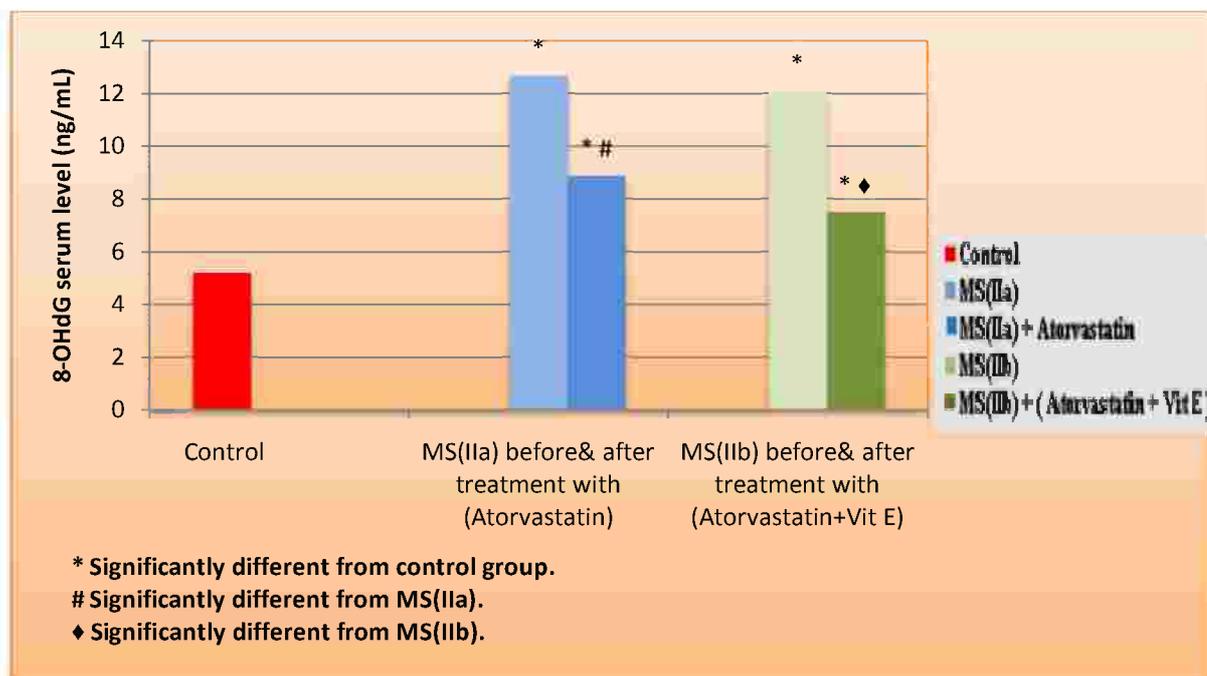


Figure (20): Bar chart representing the mean of serum 8-OHdG levels (ng/mL) in control and both metabolic syndrome subgroups (IIa)& (IIb) before and after 3 months of treatment with atorvastatin or atorvastatin+ vitamin E.

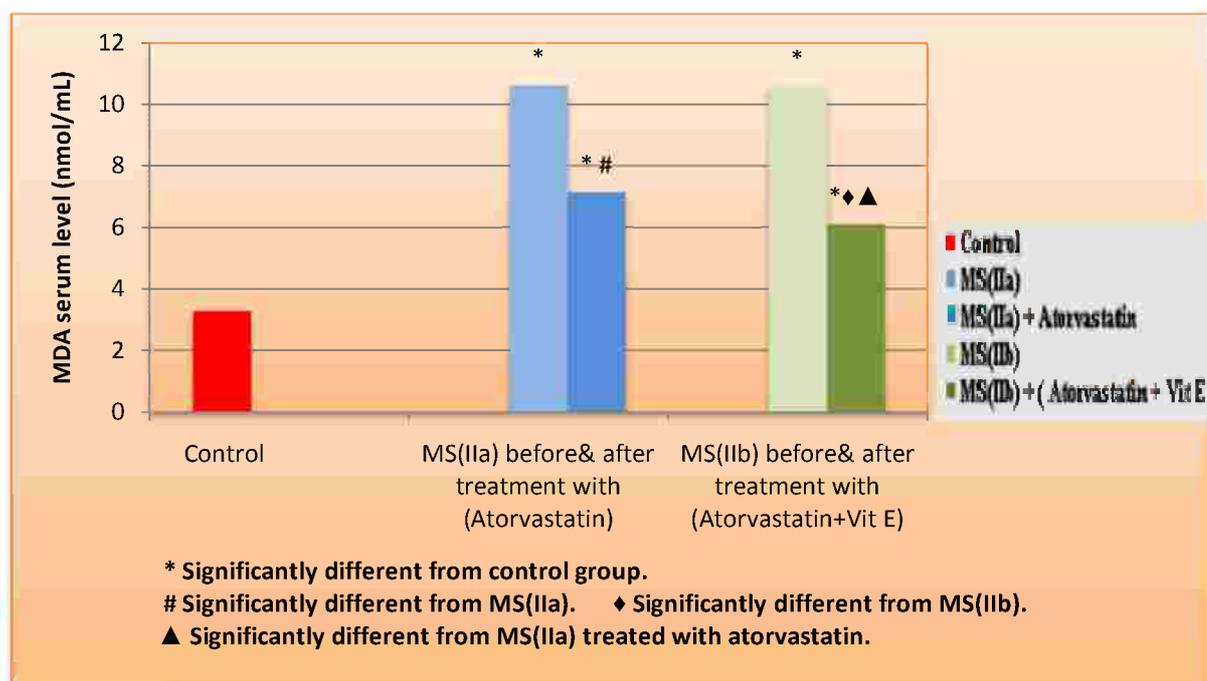
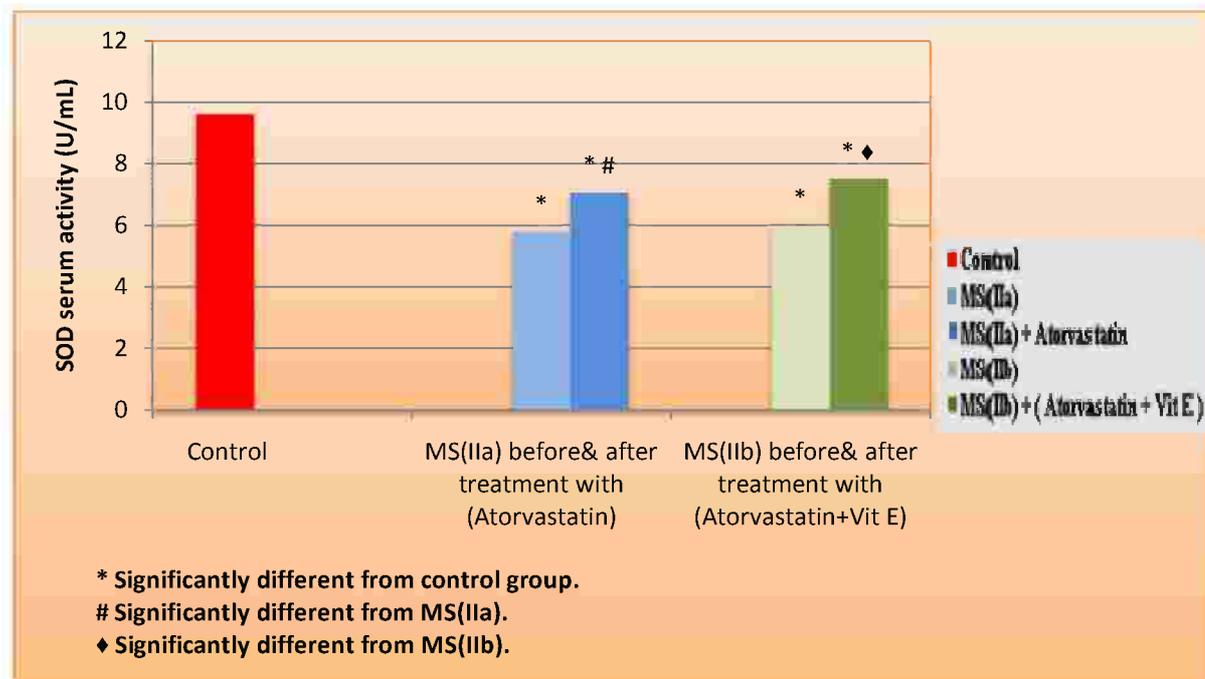


Figure (21): Bar chart representing the mean of serum MDA levels (nmol/mL) in control and both metabolic syndrome subgroups (IIa)& (IIb) before and after 3 months of treatment with atorvastatin or atorvastatin+ vitamin E.



**Figure (22):** Bar chart representing the mean of serum SOD activity (U/mL) in control and both metabolic syndrome subgroups (IIa)& (IIb) before and after 3 months of treatment with atorvastatin or atorvastatin+ vitamin E.

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**Correlations:**

From this study it was observed that serum 8-hydroxy-2'-deoxyguanosine (8-OHdG) was significantly positively correlated with waist circumference (WC) ( $r=0.744^*$ ,  $P<0.001$ ), systolic& diastolic blood pressure ( $r=0.757^*$ ,  $P<0.001$ ;  $r=0.750^*$ ,  $P<0.001$  respectively), fasting glucose (FG) ( $r=0.749^*$ ,  $P<0.001$ ), homeostasis model assessment-estimated insulin resistance (HOMA-IR) ( $r=0.408^*$ ,  $P<0.001$ ), triglycerides (TG) ( $r=0.824^*$ ,  $P<0.001$ ), total cholesterol (T. Cholest) ( $r=0.793^*$ ,  $P<0.001$ ), Low-density lipoprotein (LDL) ( $r=0.75^*$ ,  $P<0.001$ ), serum malondialdehyde (MDA) ( $r=0.790^*$ ,  $P<0.001$ ); and was significantly inversely correlated with high-density lipoprotein (HDL) ( $r= -0.715^*$ ,  $P<0.001$ ) and superoxide dismutase (SOD) ( $r= -0.780^*$ ,  $P<0.001$ ) in all studied groups; figures (23 – 33).

Similarly, MDA was significantly positively correlated with WC ( $r=0.884^*$ ,  $P<0.001$ ), systolic& diastolic blood pressure ( $r=0.625^*$ ,  $P<0.001$ ;  $r=0.730^*$ ,  $P<0.001$  respectively), FG ( $r=0.561^*$ ,  $P<0.001$ ), HOMA-IR ( $r=0.689^*$ ,  $P<0.001$ ), TG ( $r=0.716^*$ ,  $P<0.001$ ), T. Cholest ( $r=0.908^*$ ,  $P<0.001$ ), LDL ( $r=0.923^*$ ,  $P<0.001$ ); and was significantly inversely correlated with HDL ( $r= -0.775^*$ ,  $P<0.001$ ) and SOD ( $r= -0.9^*$ ,  $P<0.001$ ) in all studied groups; figures (34 – 43).

On the other hand, SOD was significantly inversely correlated with WC ( $r= -0.884^*$ ,  $P<0.001$ ), systolic& diastolic blood pressure ( $r= -0.718^*$ ,  $P<0.001$ ;  $r= -0.825^*$ ,  $P<0.001$  respectively), FG ( $r= -0.624^*$ ,  $P<0.05$ ), HOMA-IR ( $r= -0.684^*$ ,  $P<0.05$ ), TG ( $r= -0.706^*$ ,  $P<0.05$ ), T. Cholest ( $r= -0.783^*$ ,  $P<0.05$ ), LDL ( $r= -0.794^*$ ,  $P<0.001$ ); and was significantly positively correlated with HDL ( $r=0.812^*$ ,  $P<0.001$ ) in all studied groups; figures (44 – 52).

Table (7): Correlations of serum (8-OHdG), (MDA) levels and SOD activity with clinical and biochemical parameters

Parameter		WC	s.BP	d.BP	FG	HOMA-IR	HDL	TG	T.Cholest	LDL	MDA	SOD
<b>8-OHdG</b>	r	0.744	0.757	0.75	0.749	0.408	-0.715	0.824	0.793	0.75	0.79	-0.78
	P	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*
<b>MDA</b>	r	0.884	0.625	0.73	0.561	0.689	-0.775	0.716	0.908	0.923	1	-0.9
	P	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	-	0.001*
<b>SOD</b>	r	-0.884	-0.718	-0.825	-0.624	-0.684	0.812	-0.706	-0.783	-0.794	-0.9	1
	P	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	0.001*	-

r: Pearson's correlation coefficient.

\*: Significance was considered at  $P < 0.05$  (2-tailed).

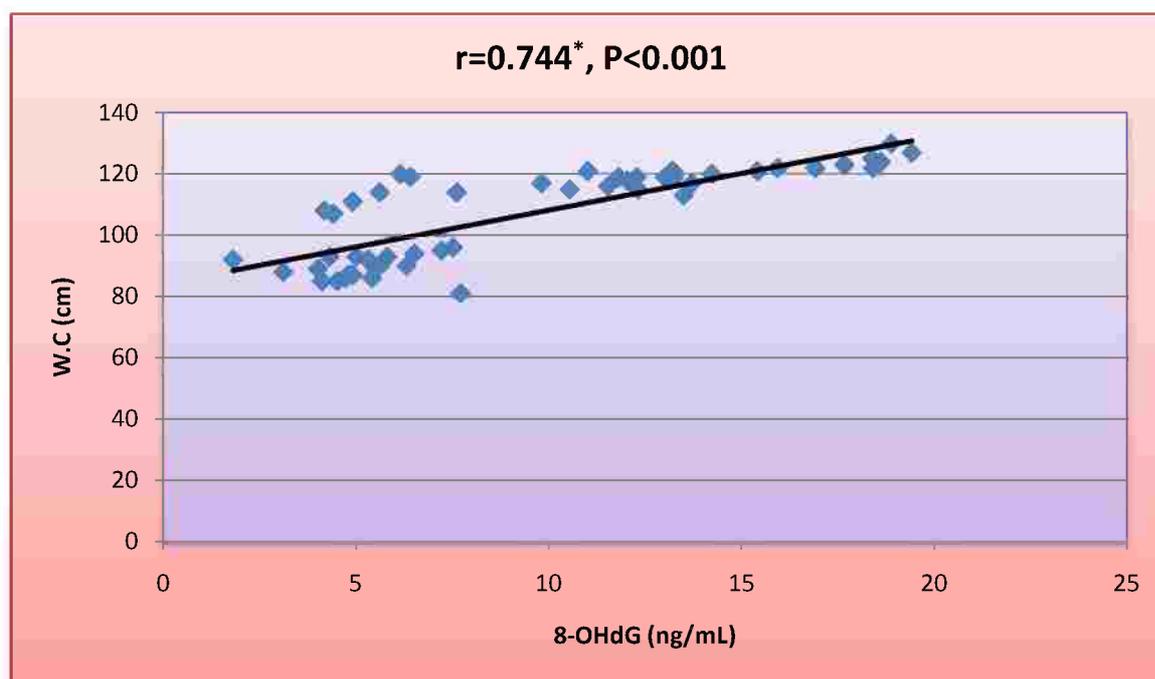


Figure (23): Correlation of 8-OHdG with waist circumference.

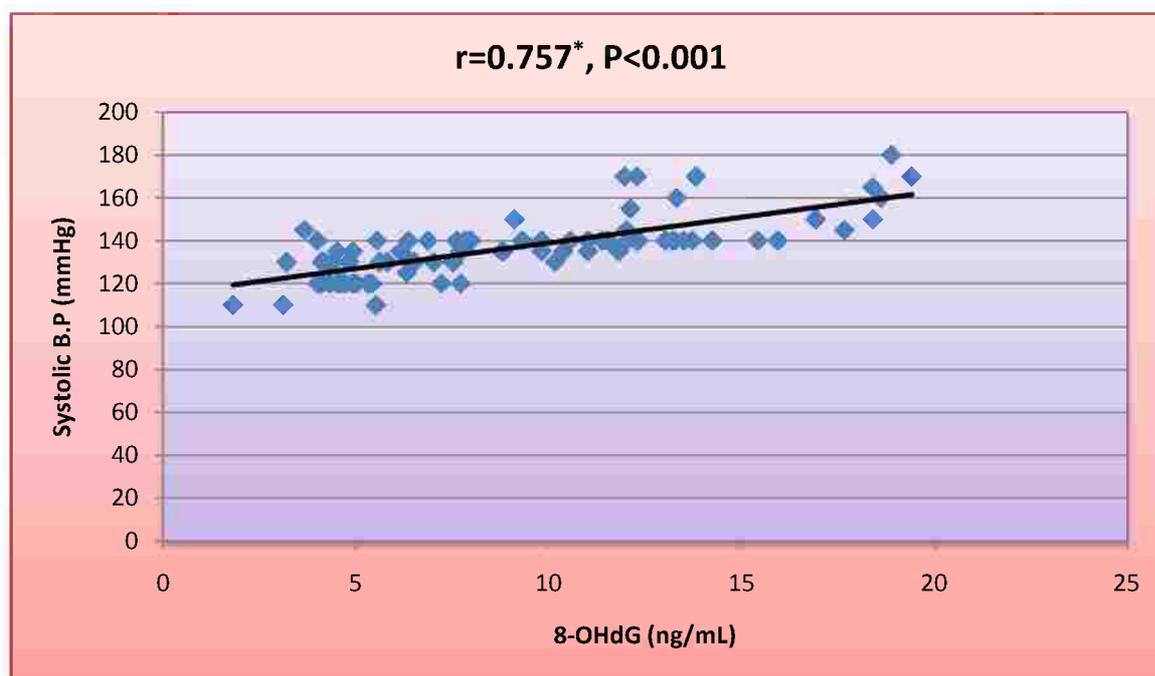


Figure (24): Correlation of 8-OHdG with systolic blood pressure.

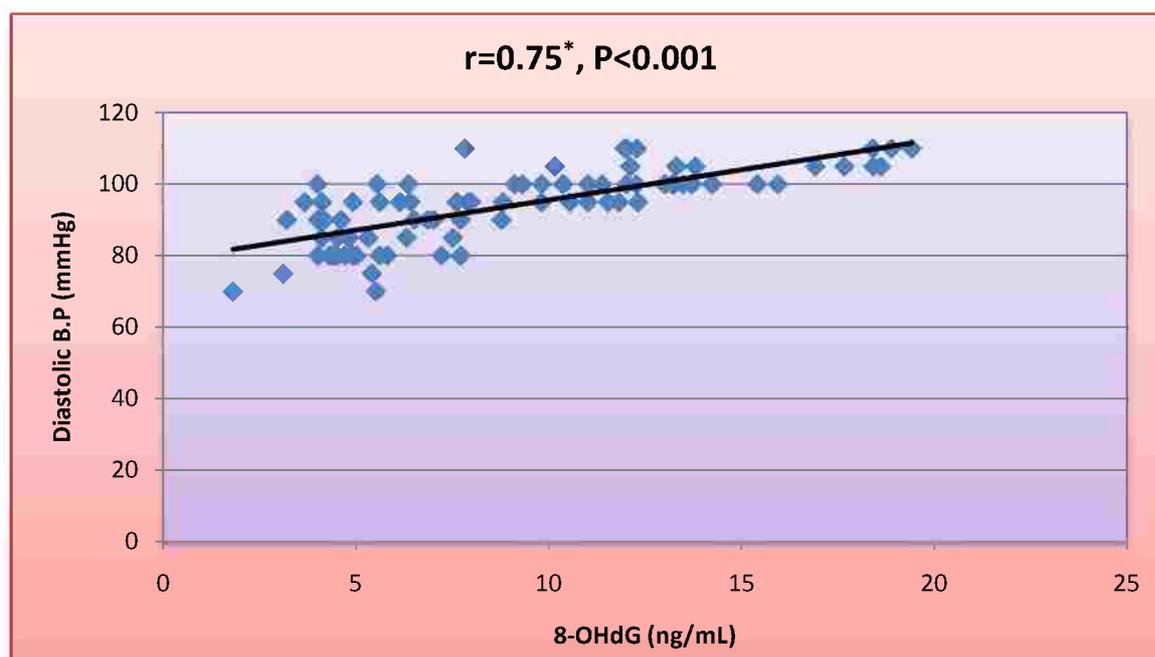


Figure (25): Correlation of 8-OHdG with diastolic blood pressure.

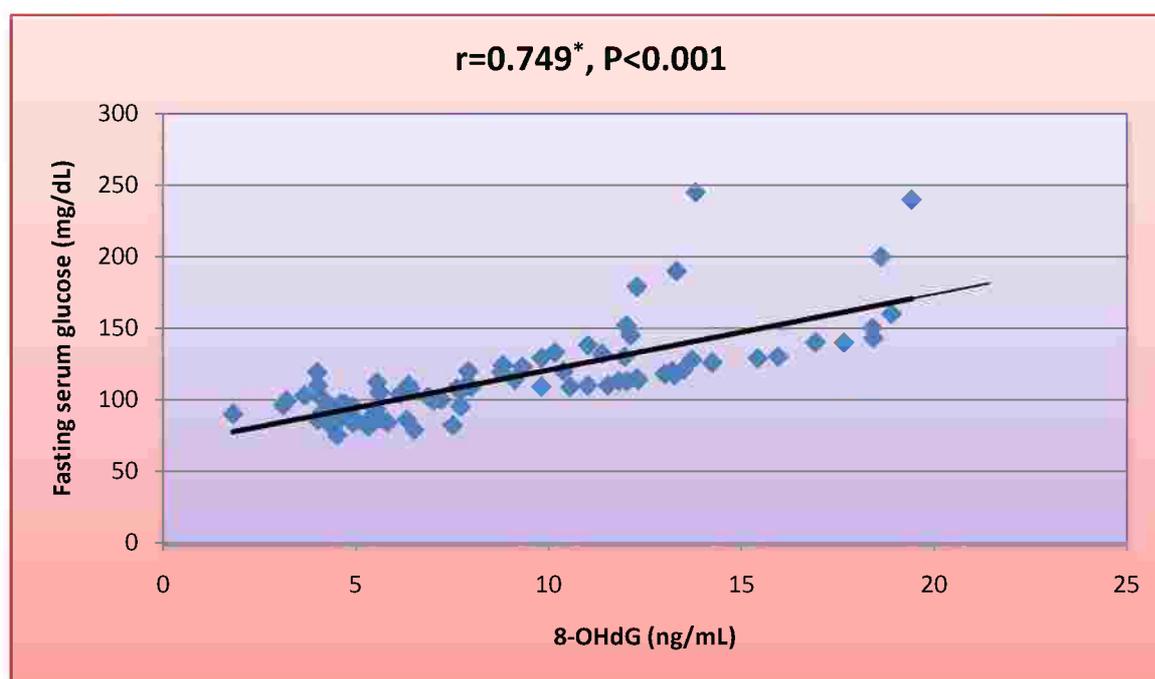


Figure (26): Correlation of 8-OHdG with fasting serum glucose.

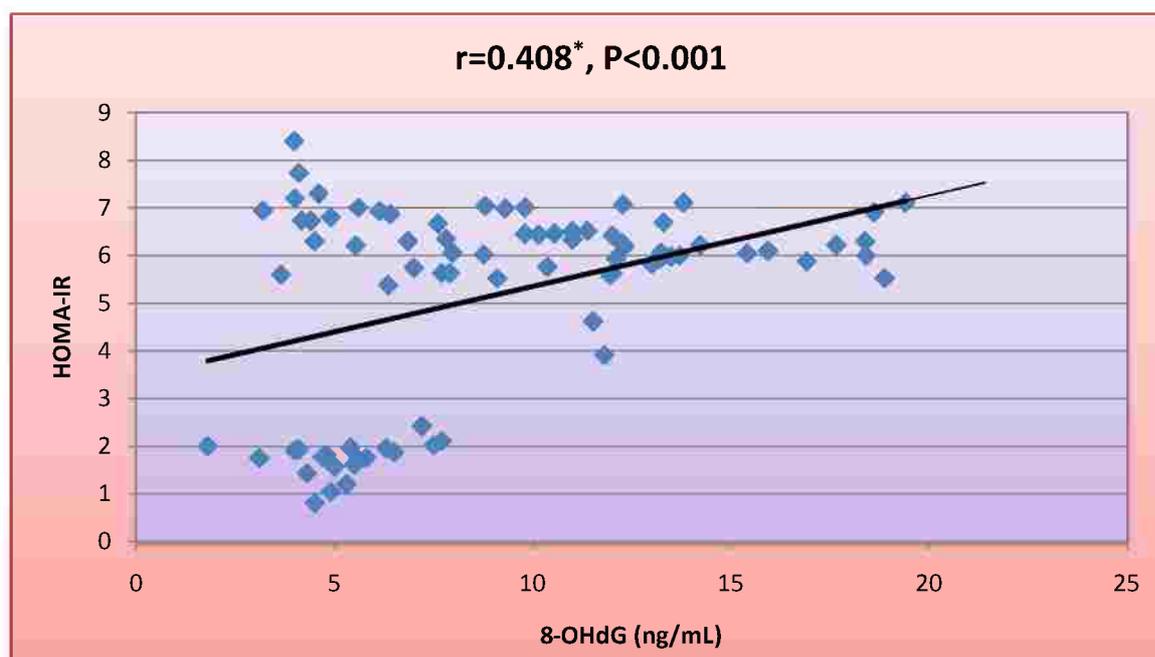


Figure (27): Correlation of 8-OHdG with HOMA-IR.

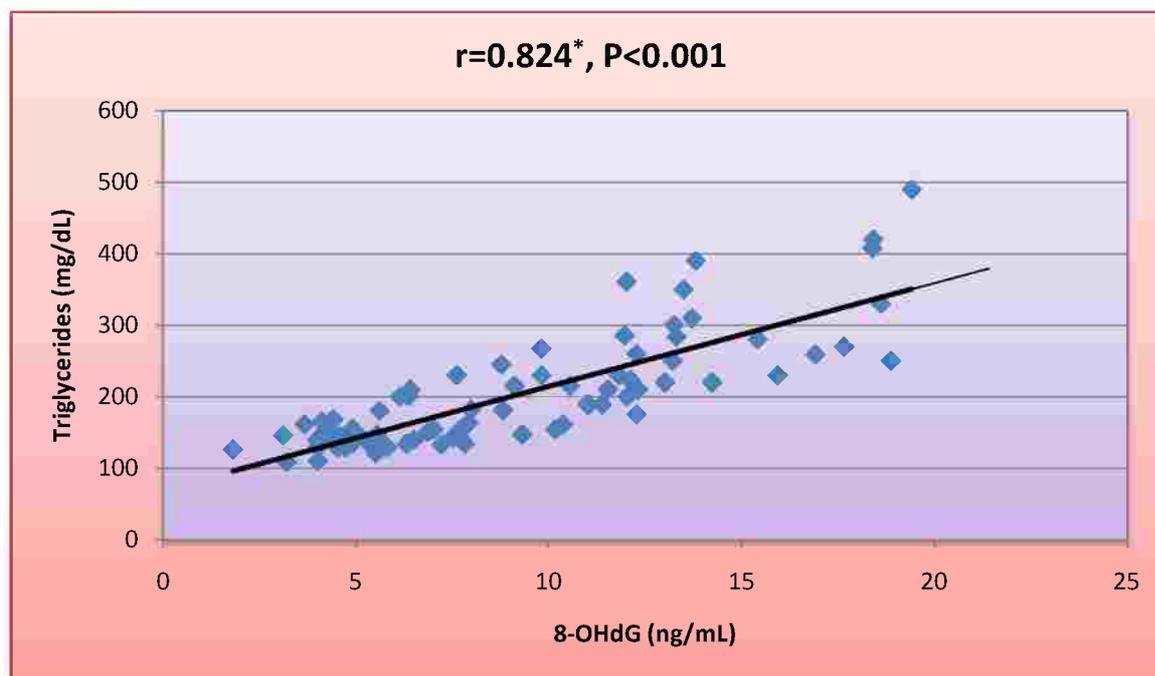


Figure (28): Correlation of 8-OHdG with triglycerides.

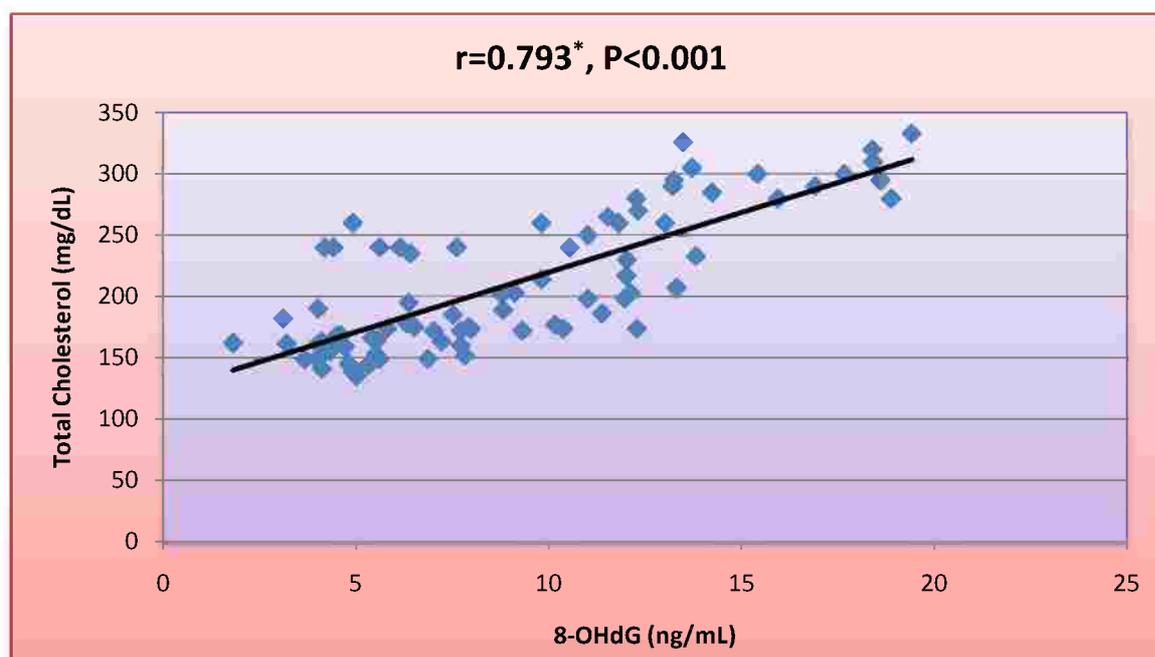


Figure (29): Correlation of 8-OHdG with total-cholesterol.

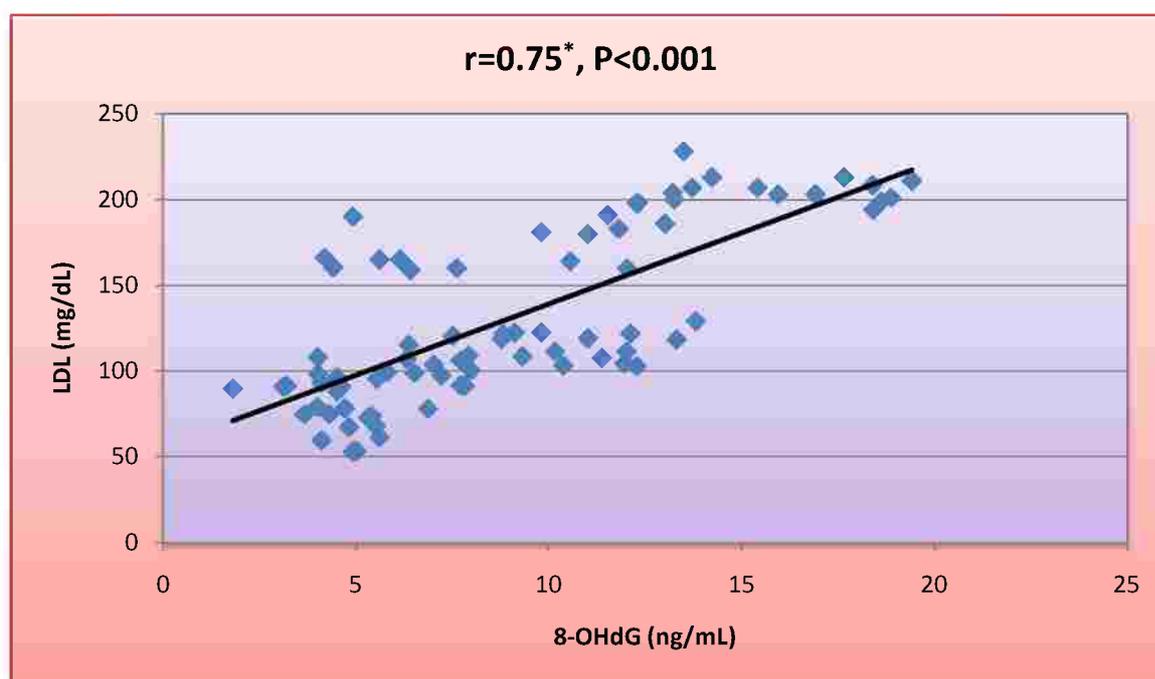


Figure (30): Correlation of 8-OHdG with LDL.

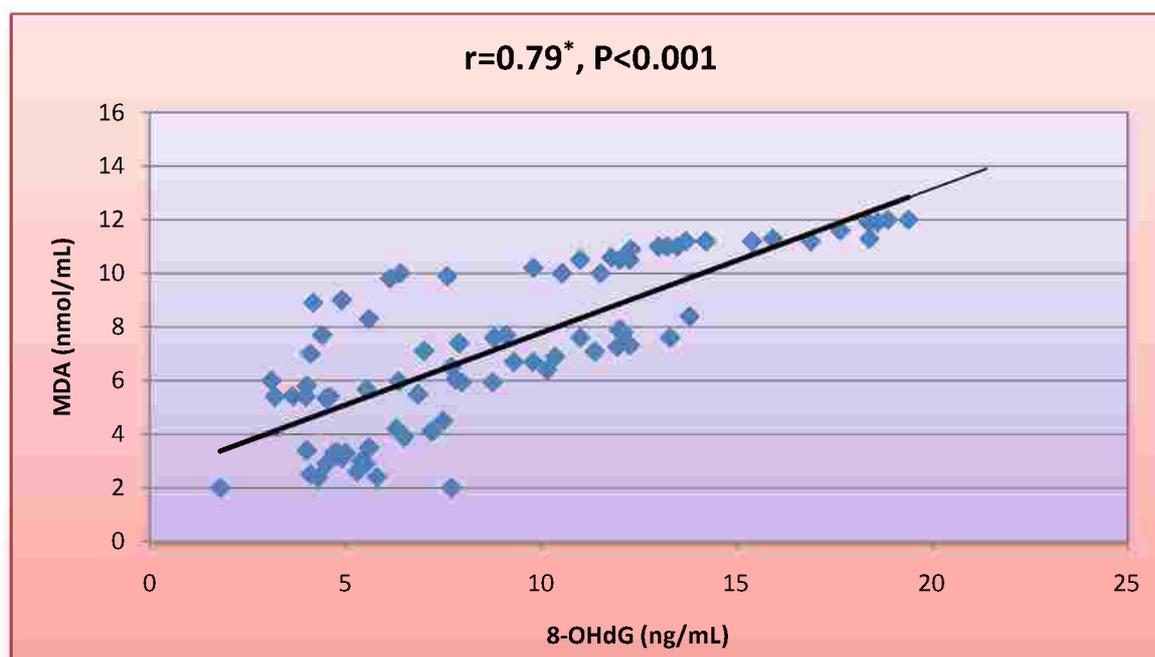


Figure (31): Correlation of 8-OHdG with malondialdehyde.

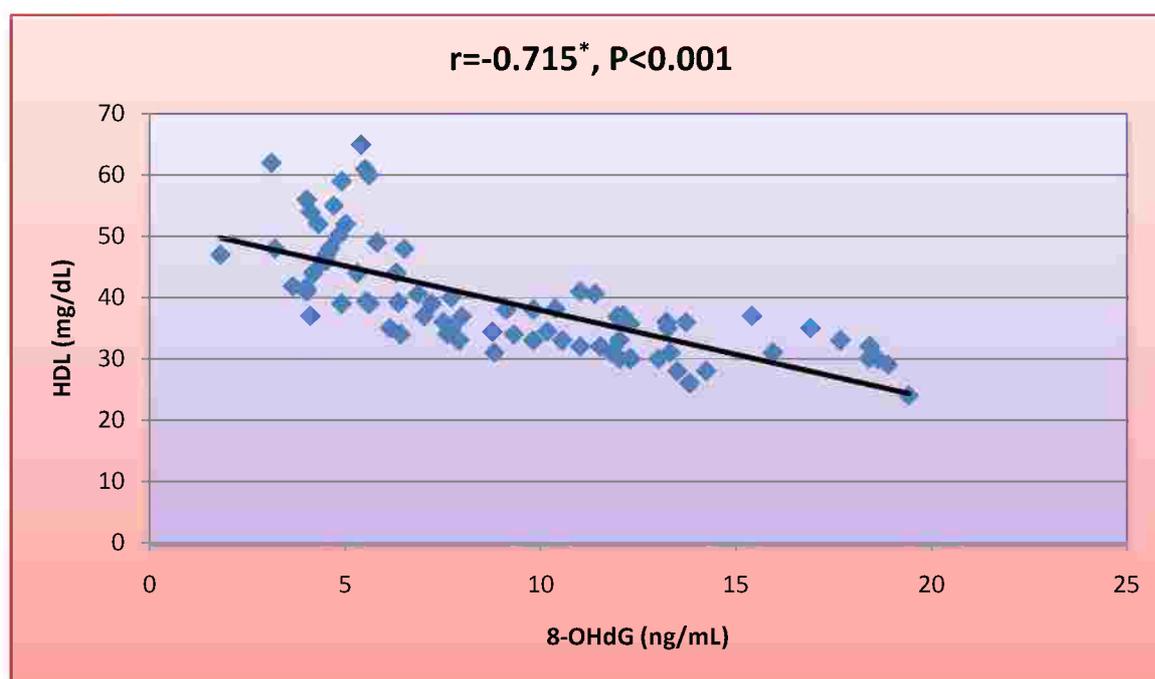


Figure (32): Correlation of 8-OHdG with HDL.

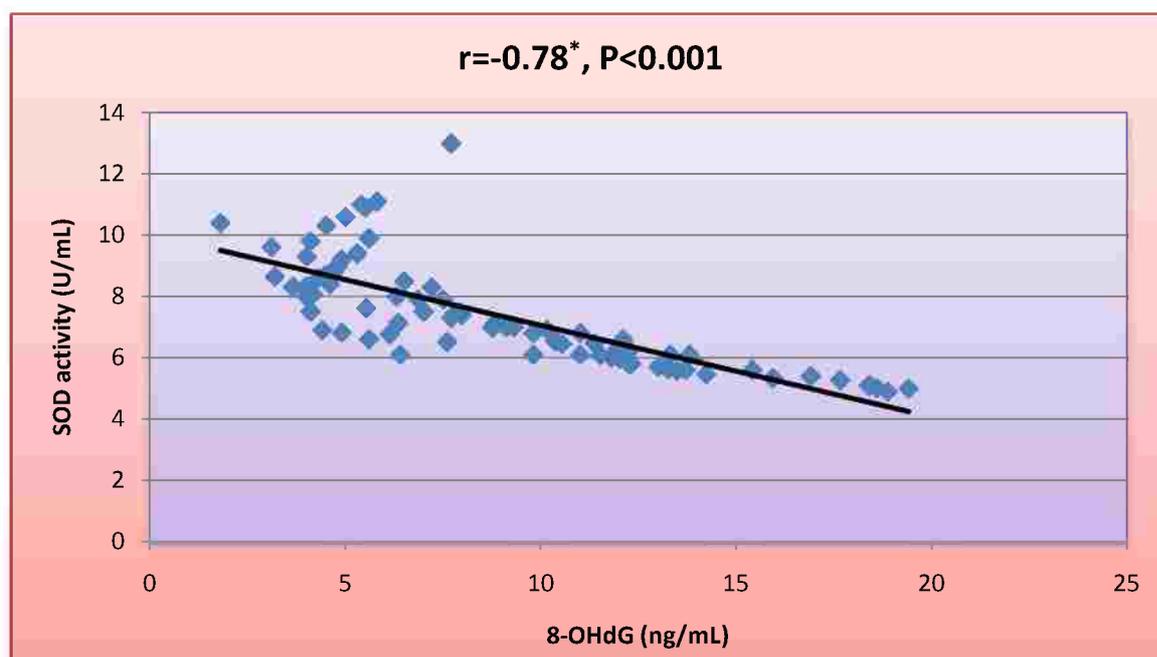


Figure (33): Correlation of 8-OHdG with SOD activity.

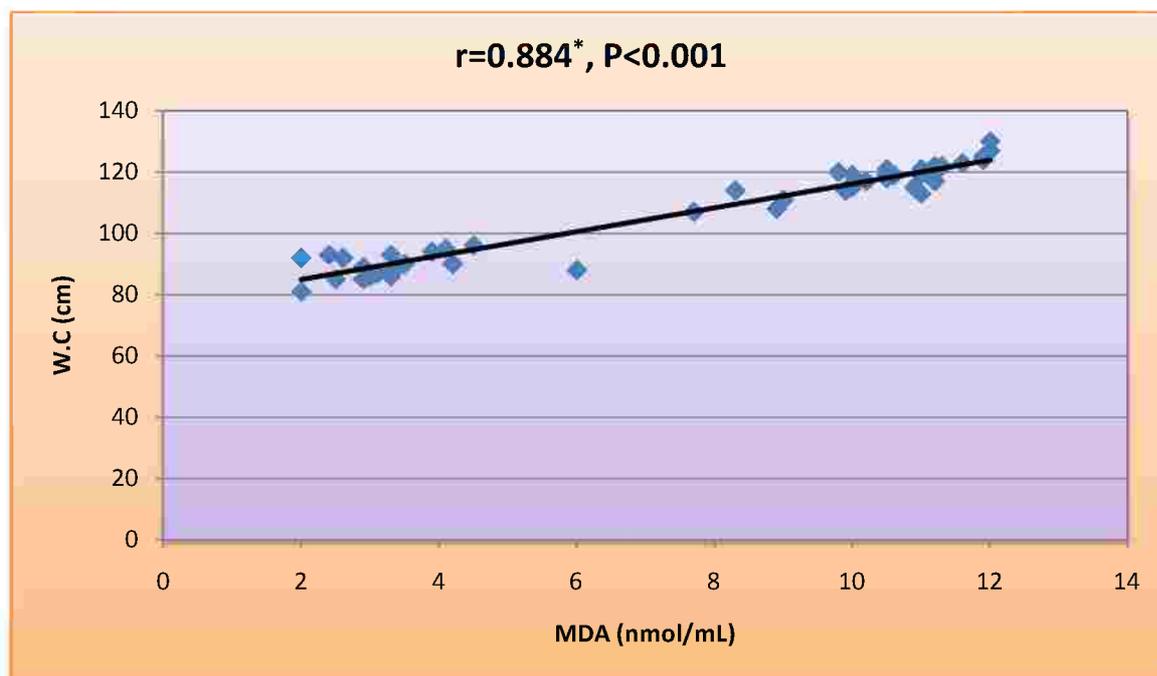


Figure (34): Correlation of malondialdehyde with waist circumference.

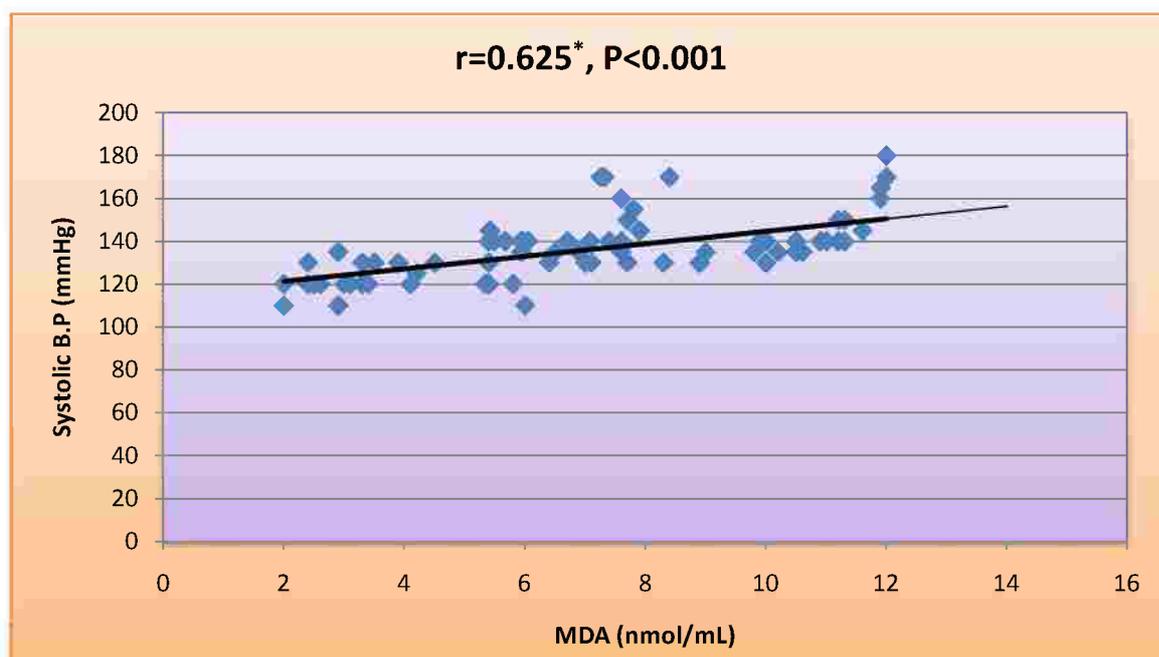


Figure (35): Correlation of malondialdehyde with systolic blood pressure.

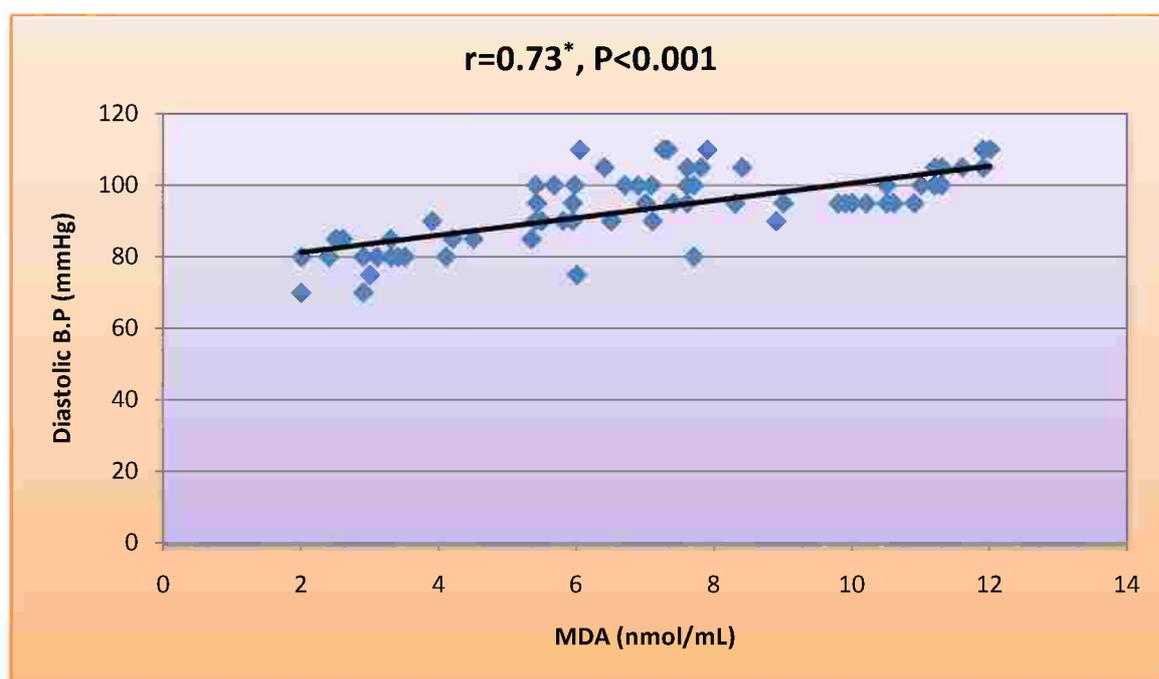


Figure (36): Correlation of malondialdehyde with diastolic blood pressure.

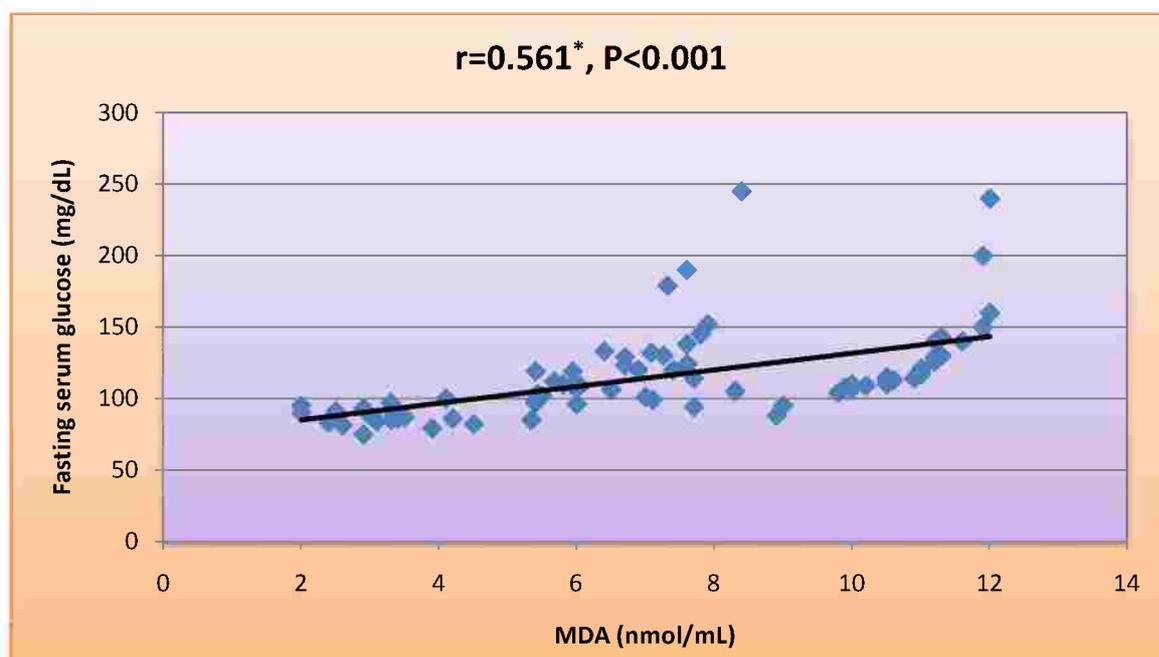


Figure (37): Correlation of malondialdehyde with fasting serum glucose.

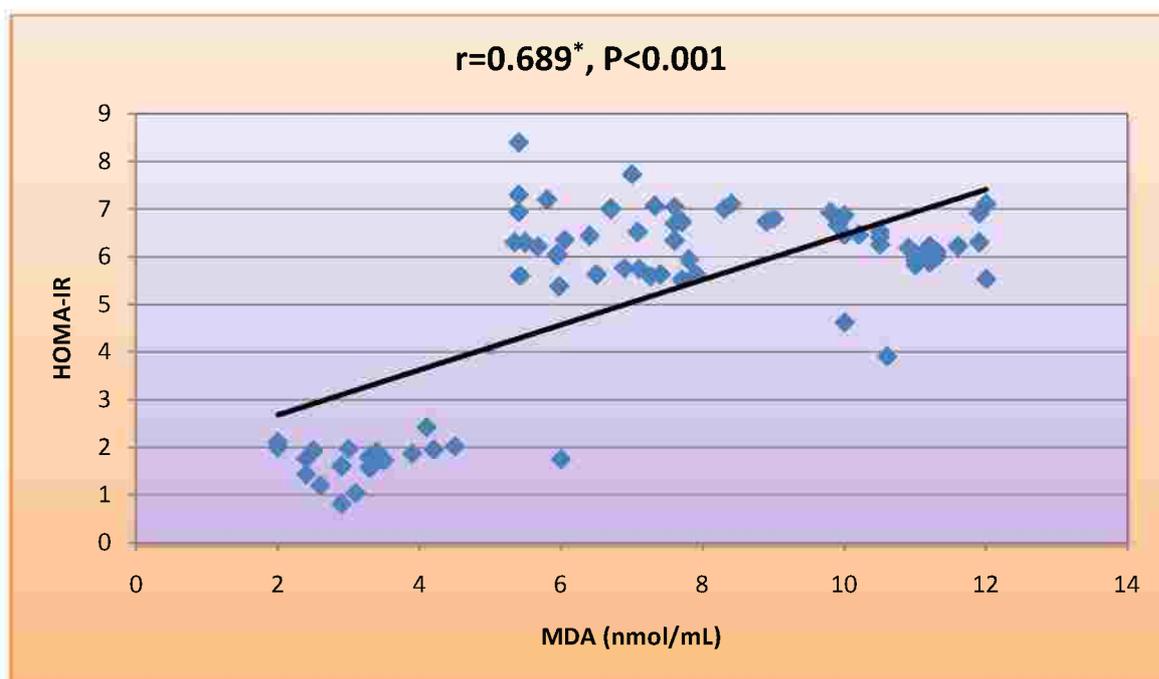


Figure (38): Correlation of malondialdehyde with HOMA-IR.

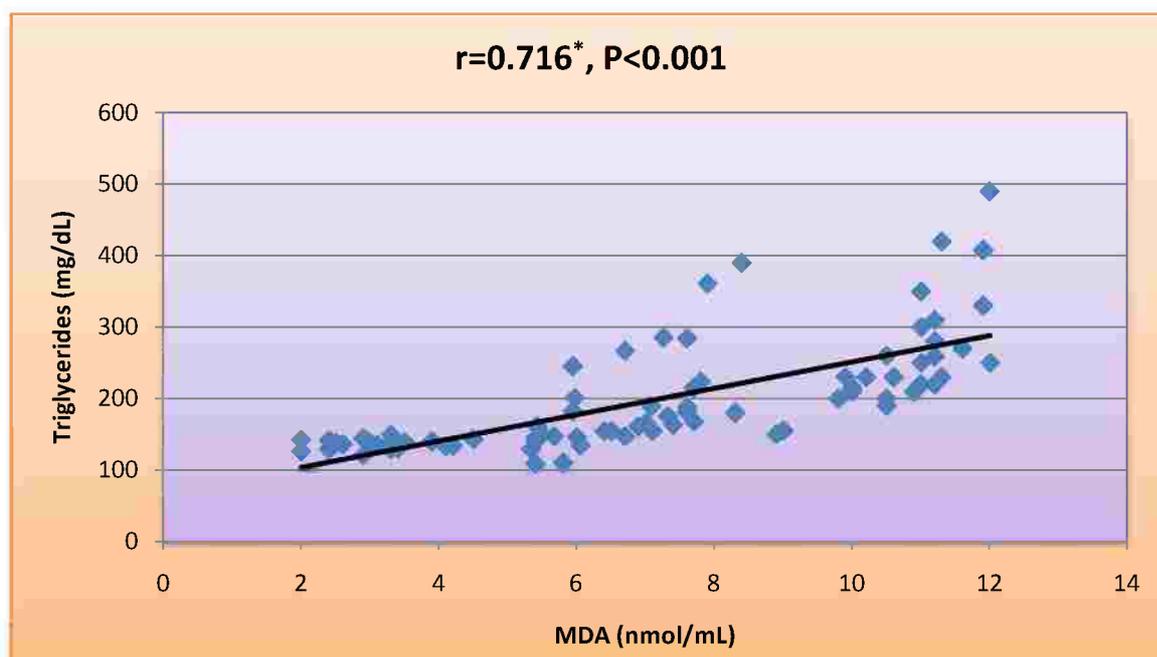


Figure (39): Correlation of malondialdehyde with triglycerides.

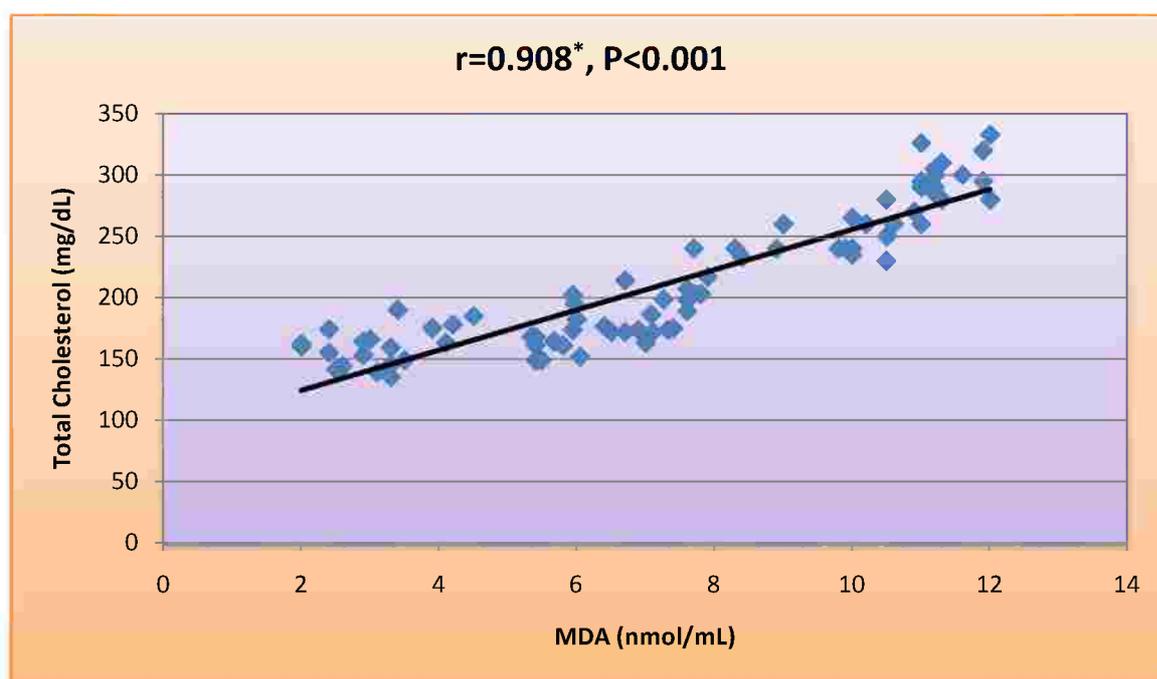


Figure (40): Correlation of malondialdehyde with total-cholesterol.

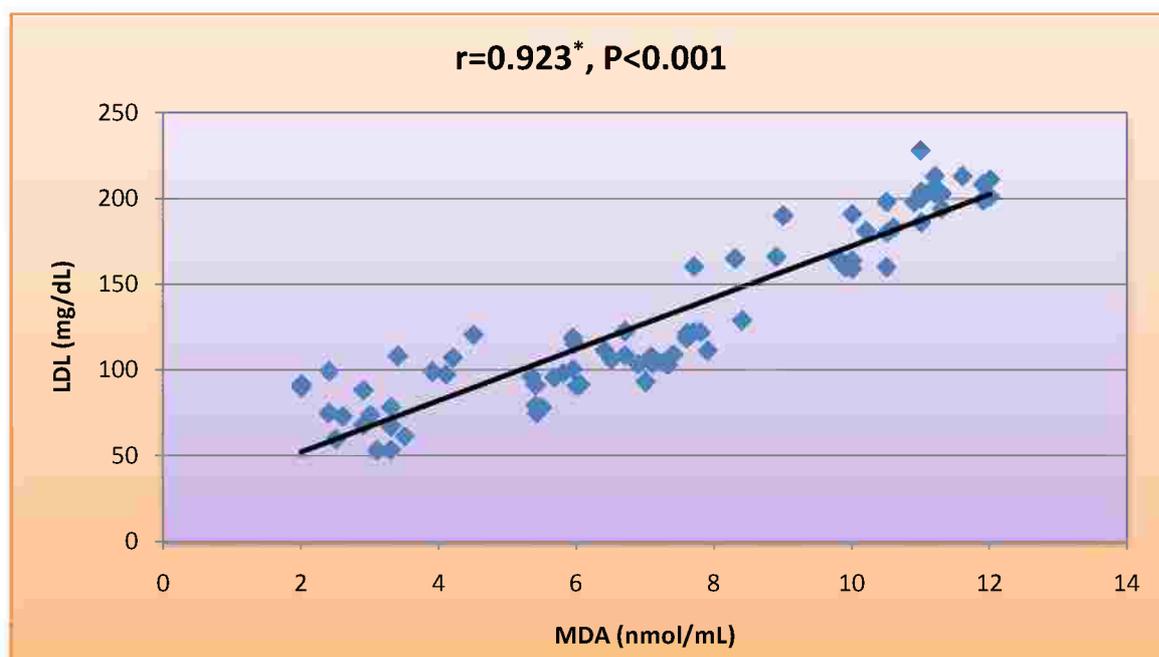


Figure (41): Correlation of malondialdehyde with LDL.

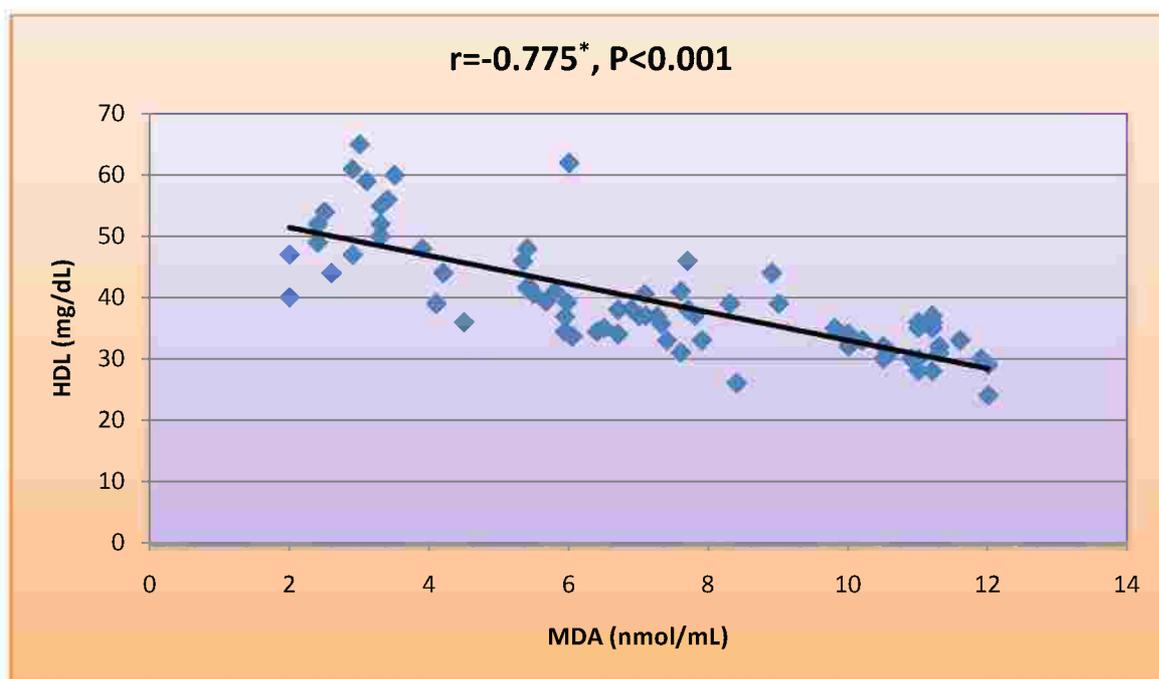


Figure (42): Correlation of malondialdehyde with HDL.

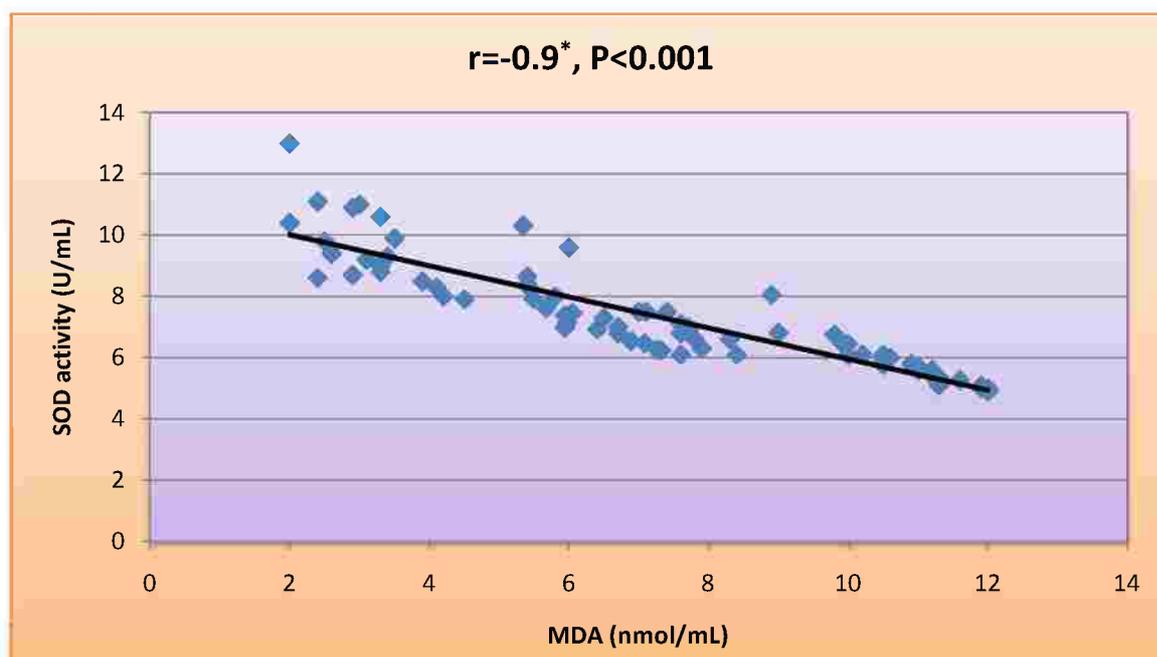


Figure (43): Correlation of malondialdehyde with SOD activity.

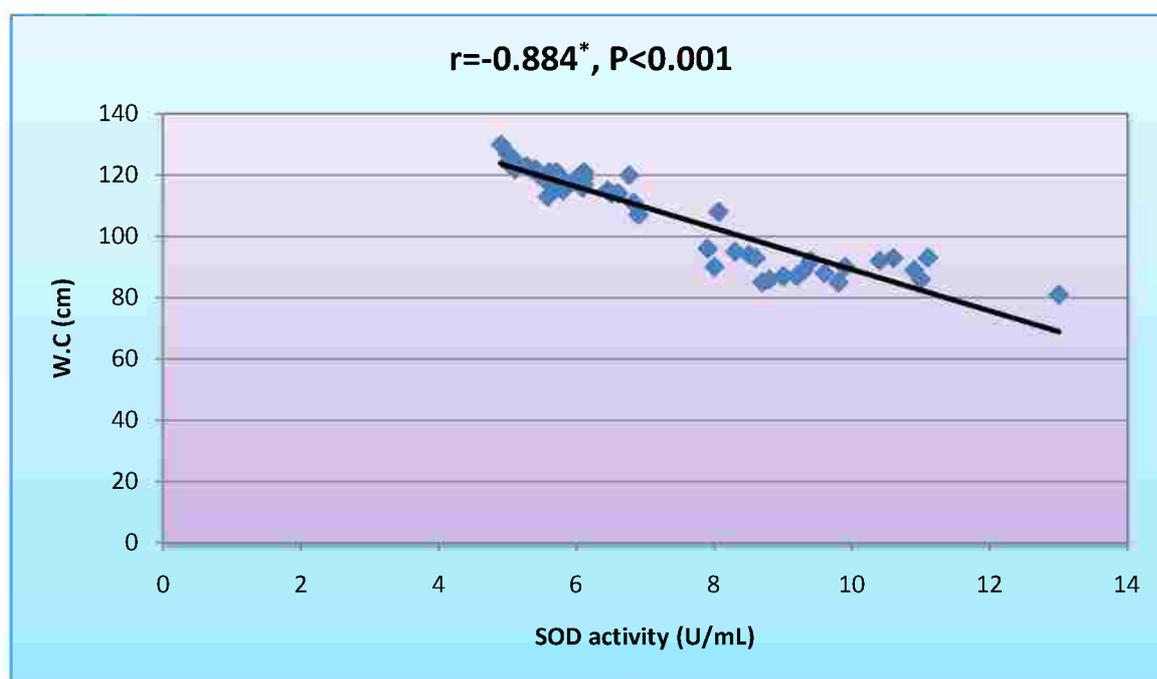


Figure (44): Correlation of SOD activity with waist circumference.

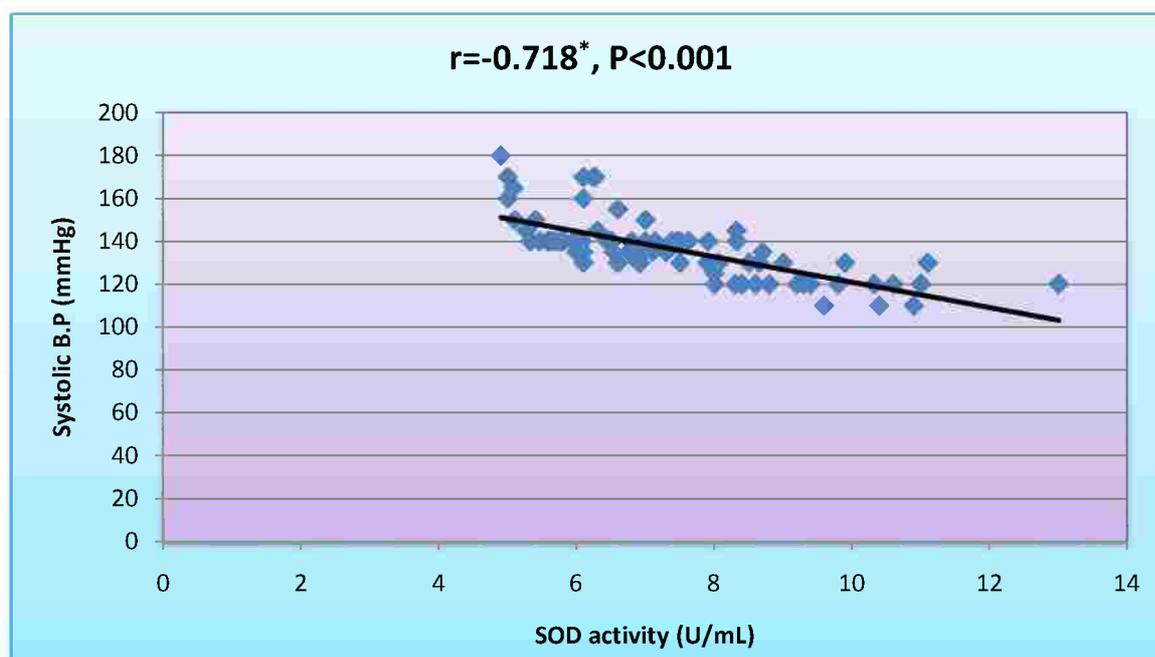


Figure (45): Correlation of SOD activity with systolic blood pressure.

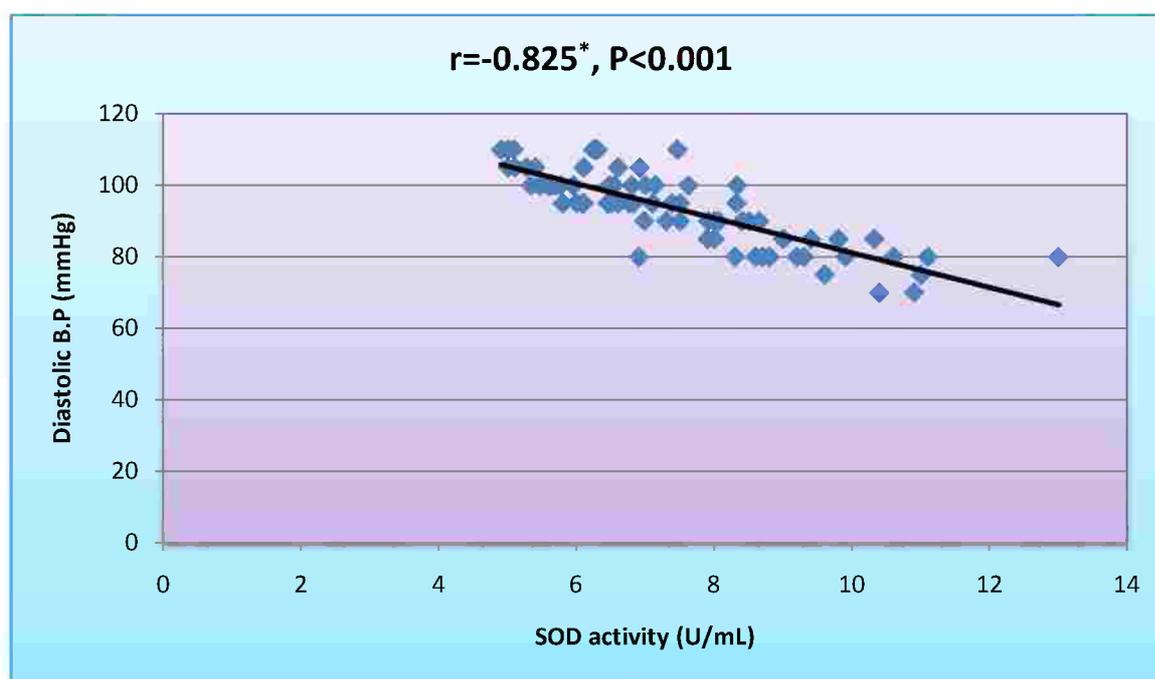


Figure (46): Correlation of SOD activity with diastolic blood pressure.

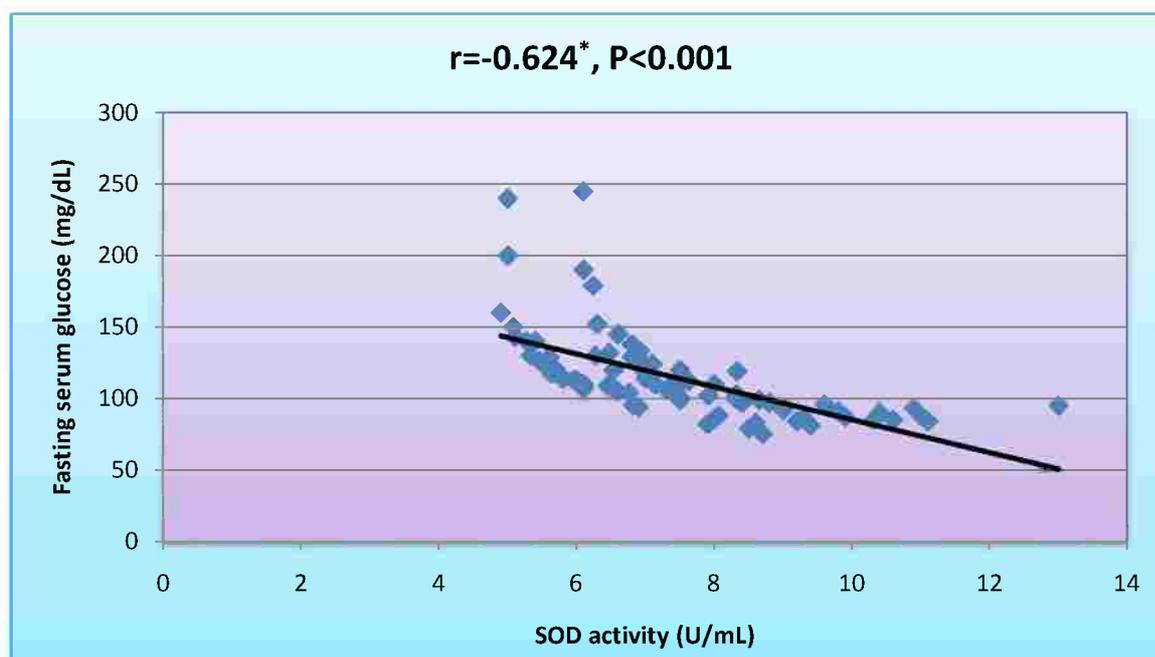


Figure (47): Correlation of SOD activity with fasting serum glucose.

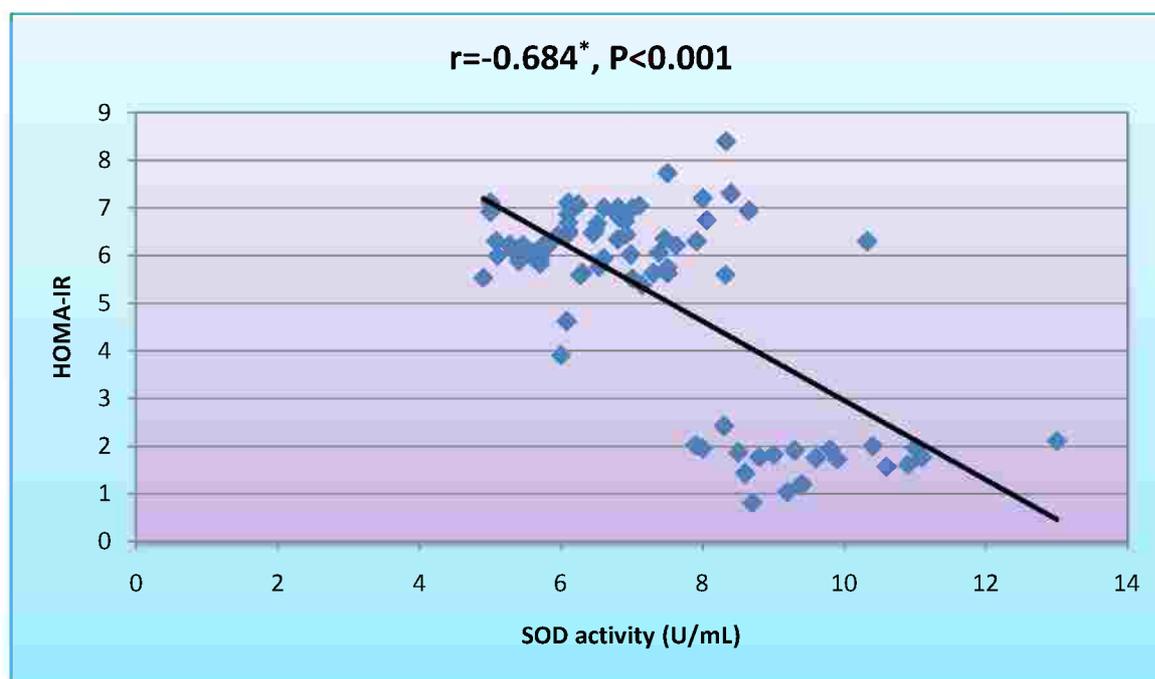


Figure (48): Correlation of SOD activity with HOMA-IR.

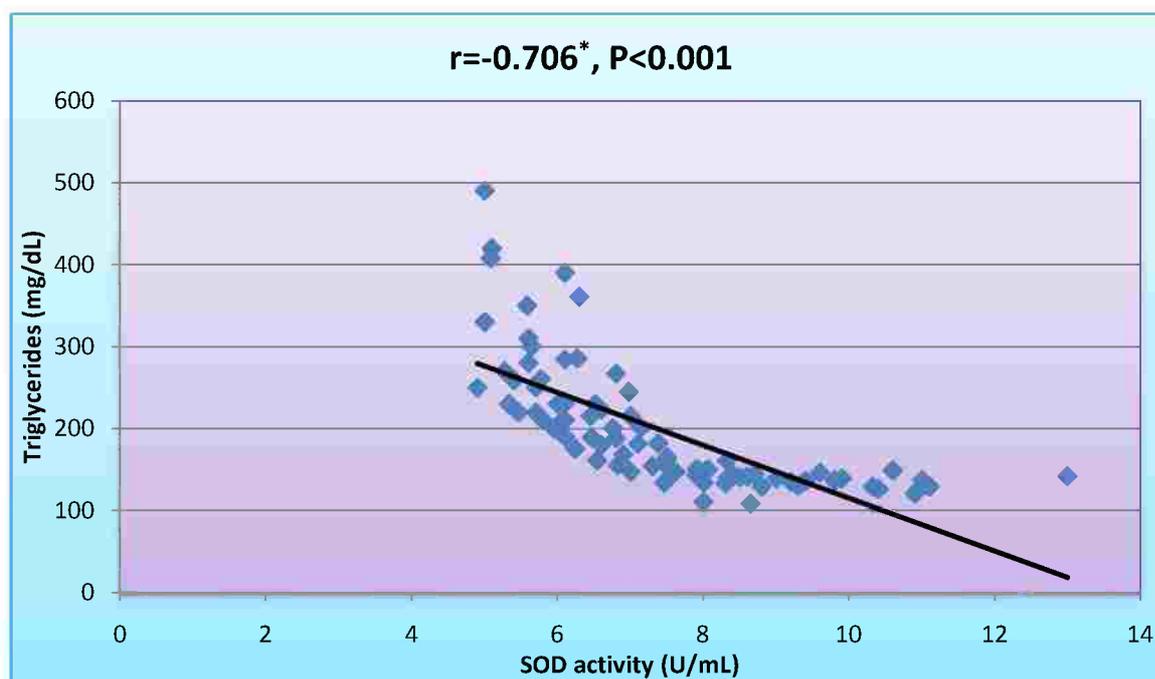


Figure (49): Correlation of SOD activity with triglycerides.

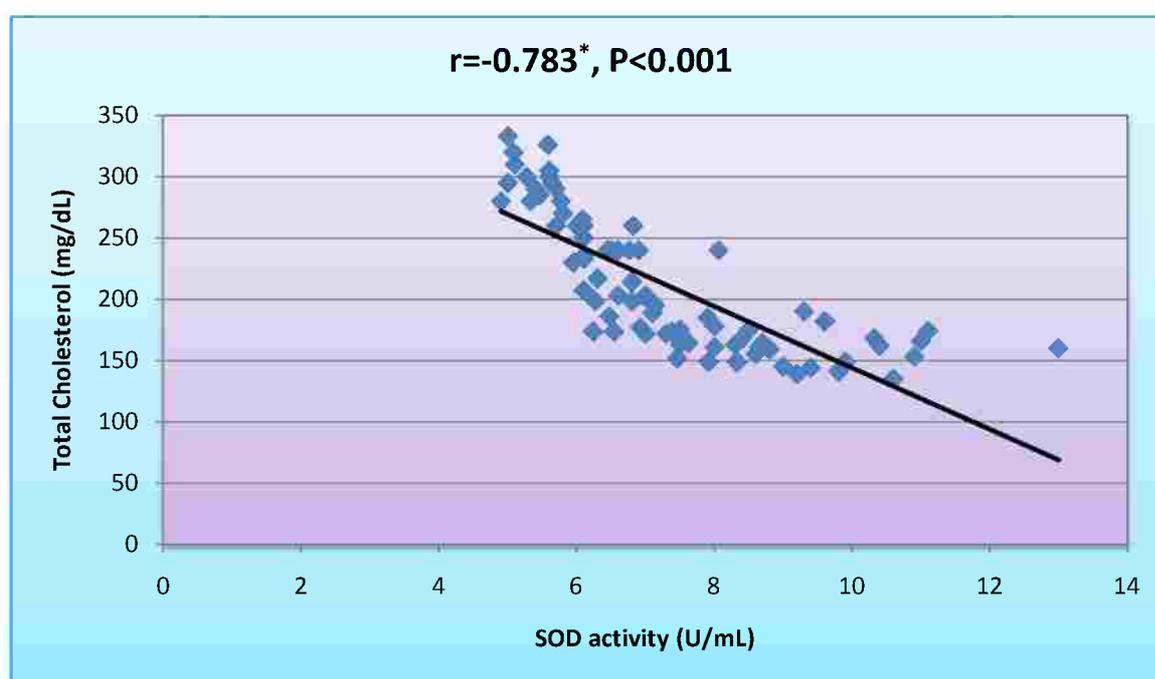


Figure (50): Correlation of SOD activity with total-cholesterol.

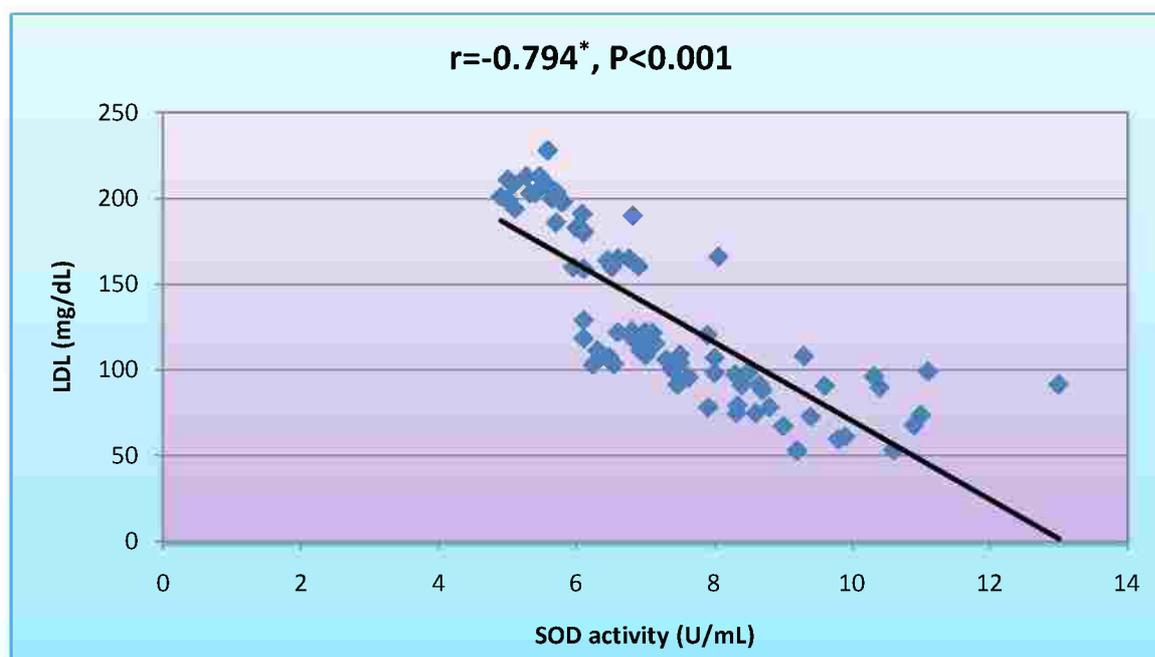


Figure (51): Correlation of SOD activity with LDL.

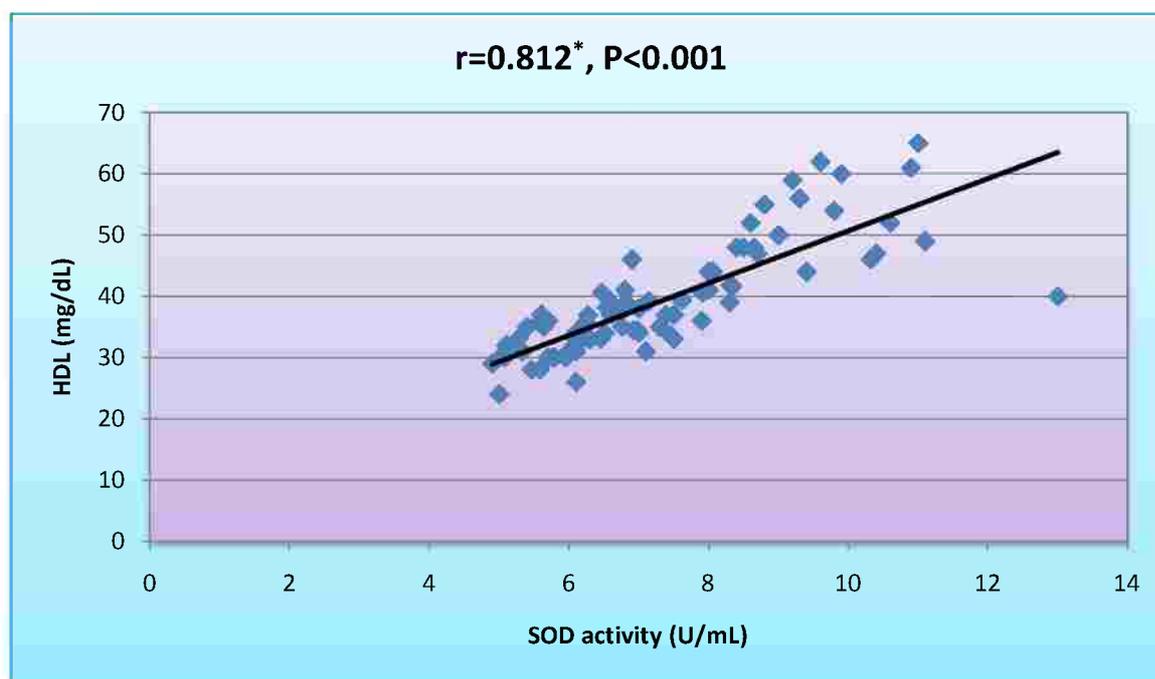


Figure (52): Correlation of SOD activity with HDL.