

The link between diabetes mellitus and obesity

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Abstract:

Background: Diabetes mellitus is one of the most metabolic disorders associated with many complications. **Methods:** Glucose, lipid profiles, BMI, ABP were measured for 50 subjects using usual biochemical methods and sphygmomanometer for measurement of arterial blood pressure. **Results:** The mean of body mass index, total cholesterol, triacylglycerols, LDL-C and the fasting blood sugar levels, arterial blood pressure were highly significant in the diabetics as compared to those in the controls, while the mean HDL-C concentration was significantly lower in diabetics than in controls. **Conclusion:** High TC, TG, LDL, arterial blood pressure in diabetics indicates that DM is properly associated with dyslipidemia.

Key words: Diabetes mellitus, ABP, lipid profiles.

Introduction: Diabetes is one of the most costly chronic diseases of our life which has high incidence throughout the world (King *et al*, 1998). Many complications resulting from the disease which are associated with severe damage or failure to many organs including the eyes, kidneys, and nerves. Type 2 diabetic patients have also a high risk for development of coronary heart disease, peripheral vascular disease, and stroke, and they have a greater possibility to have hypertension, dyslipidemia, and obesity (Reaven 1988) & (Eastman *et al*, 1997). Diabetes may be due to either insufficient insulin production by the pancreas or improper response of the cell body to the insulin produced (Shoback *et al* 2011). Diabetes mellitus type 2 (Noninsulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes) is a metabolic disorder that is characterized by high blood glucose associated with insulin resistance and relative insulin deficiency. (Kumar *et al*, 2005). The classic symptoms are excess thirst, frequent urination, and constant hunger. Type 2 diabetes makes up about 90% of cases of diabetes with the other 10% concerning to diabetes mellitus type 1 and gestational diabetes. Obesity considers the primary cause of type 2 diabetes in people who are genetically predisposed to the disease.

Combination of lifestyle and genetic factors is concerning with the development of type 2 diabetes (Ripsin *et al*, 2009) and (Risèrus *et al*, 2009). A lack of sleep has been linked to type 2 diabetes, this may be related to the effect of sleep on metabolism (Touma and Pannian 2011). The nutrition of a mother during fetal development may have a role, through alteration of DNA methylation (Stewart and Christian 2010).

Development of type 2 diabetes depends on many lifestyle factors, including: obesity (defined by a body mass index of greater than thirty), decrease in physical activity, poor diet, stress and urbanization (Shlomo *et al*, 2011). Excess body fat is associated with 30% of cases in those of Chinese and Japanese descent, 60-80% of cases in those of European and African descent, and 100% of Pima Indians and Pacific Islanders (Shoback *et al*, 2011). Those who are not obese often have a high waist-hip ratio (Shoback *et al*, 2011).

Dietary factors also enhance the risk of developing type 2 diabetes as consumption of sugar-sweetened drinks in excess is associated with an increased risk (Malik et al 2010). Saturated fats and trans fatty acids increasing the risk while polyunsaturated and monounsaturated fat decreasing the risk (Risèrus et al, 2009). Eating lots of white rice may also increase the risk (Hu et al, 2012).

Pathophysiology

Type 2 diabetes is due to insufficient insulin production from beta cells associated with insulin resistance (Shoback et al, 2011). Insulin resistance, which is the inability of cells to respond adequately to normal levels of insulin, occurs primarily within the muscles, liver, and fat tissue (Lippincott and Wilkins 2007). In the liver, insulin normally suppresses glucose release. However with insulin resistance, the liver releases excessive glucose into the blood (Shlomo et al, 2011). The proportion of insulin resistance versus beta cell dysfunction differs among individuals, some having severe insulin resistance a minor defect in insulin secretion and others having little insulin resistance and severe deficiency of insulin secretion (Shoback et al, 2011).

Other mechanisms associated with type 2 diabetes and insulin resistance may be increasing in breakdown of lipids within fat cells, resistance to and decrease of incretins, high glucagon levels in the blood, increased retention of salt and water by the kidneys, and irregulation of metabolism by the central nervous system (Shlomo et al, 2011). It is important to know that not all people with insulin resistance develop diabetes, as an impairment of insulin secretion by pancreatic beta cells is necessary (Shoback et al, 2011).

The complications of diabetes mellitus:

The complications of diabetes mellitus are less common and less severe in people who have well-controlled blood sugar levels (Nathan et al, 2005), while associated health problems enhance the dangerous effects of diabetes including smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise.

*Chronic complications:

Hyperglycemia is not the only cause of diabetic complications as 40% of diabetics who control their blood sugar well develop neuropathy (Centofani, 1995) and also some of those who control their blood sugar still develop nephropathy (Rich 2006). Studies also suggest that type 1 diabetics, may develop retinopathy, (Kastelan et al, 2007) neuropathy, (Granberg et al, 2005) and nephropathy, (Ichinose et al, 2007).

Materials and Methods

50 persons shared in which their ages range from 40 to 70 old age with their body mass indices range from 30 to 35. People were divided into two groups: Group 1 consist of 20 subjects who are healthy individuals don't suffer from DM, hypertension or heart failure and group 2 consist of 30 patients who are diabetic patients, admitted to cardiology department of Beni Suef University Hospital (*In patients*). We took in consideration during selection of patients sharing in the study exclusion type 1 DM from the history of onset of the disease. The onset of the disease for patients shared in this study is between six to ten years. For each subject in this study the following parameters were studied:

- . Measurement of serum glucose level.
- . Measurement of serum lipid profile.
- . Measurement of HBA1c level.
- . Measurement of Arterial blood pressure.
- . Calculation of body mass index.

-Collection of blood sample and preparation of serum:

Serum is the liquid fraction of whole blood that is collected after the blood is allowed to clot. The clot is removed by centrifugation and the resulting supernatant, designated serum, is carefully removed using a Pasteur pipette.

Serum Preparation

- Blood was collected by venipuncture
- Whole blood is collected in a covered test tube.
- The blood is allowed to clot by leaving it undisturbed at room temperature about 24° C. This usually takes 15-30 minutes.
- The clot is removed by centrifuging at 1,000-2,000 x g for 10 minutes in a refrigerated centrifuge, The resulting supernatant is designated serum.

–Following centrifugation, serum was transferred into a clean polypropylene tube using a Pasteur pipette. The samples should be maintained at 2-8°C while handling.

-Measurement of serum glucose :

.Serum Glucose Estimation by method of *Trinder, 1969*-

was collected by venipuncture into test tubes. .Blood-

Serum was prepared as mentioned above.-

-Glucose was measured by glucose oxidase method using available commercially kit by Diamond Egypt (*Trinder, 1969*).

Glucose → gluconic acid +H₂O₂

2H₂O₂+ phenol +amino-4-antipyrine→ quinoneimine+4H₂O

Reagents

phosphate buffer(150 mmol/L)+phenol(10mmol/L)1 (Reagent 1-

Amino-antipyrine(0.4mmol/L) (Reagent 2-

.Enzymes: Peroxidase 300IU/L - a

-Glucose oxidase 10.000IU/L.

3-Working color reagent: Reagent (2) was mixed with Reagent (1).

4-Glucose standard: 100mg/dl.

Method: Tests tubes were labeled as Blank (B), Standard (S) and Test (T):

Table (1):Measurement of serum glucose.

	Blank	Standard	Test
Standard solution	-	10micolitre	-
Plasma	-	-	10microlitre
Working solution	1mL	1mL	1ml

The tubes were mixed and incubated for 10 minutes and 25°C and read against blank at 505 nm.

Concentration of glucose =Reading of T/Reading of S × Concentration of S

- Measurement of serum total cholesterol:

ProductDescription:

The *Cholesterol Enzymatic Assay Kit supplied by (Bio xpress U.S.A)* is a plate-based colorimetric enzymatic assay for the determination of cholesterol in serum samples.The kit uses a spectrophotometric assay to detect cholesterol directly from serum samples.

The unique features of the kit are:

-High sensitivity and low detection limit (25 mg/dl).

-A rapid (10 minutes) and robust enzyme-based assay which does not require expensive instrumentation.

-High reproducibility.

Cholesterol Enzymatic Assay Kit is a simple, direct and automation compatible method for measuring cholesterol levels. This kit uses a coupled enzymatic reaction scheme: cholesterol esters are first converted to cholesterol and fatty acids. Next, cholesterol is oxidized with O₂ to form cholesten-3-one + H₂O₂. Lastly, the hydrogen peroxide is reacted with 4-aminoantipyrine and p-HBS to yield quinoneimine (red dye) and water. The absorption measured at 520 nm, is proportional to the concentration of cholesterol in the sample. The kit also comes with a control solution containing a cholesterol standard (200 mg/dl) which can be used to calibrate the assay. This kit provides direct determination of cholesterol in serum, plasma, and other fluid samples.

In addition, the kit can be used to analyze the pharmacological effects of drugs including siRNA and miRNA on cholesterol metabolism. For example, the impact of siRNA targeting ApoB1 can be monitored using this kit. The kit contains sufficient materials to rapidly test 42 serum samples in duplicate.

Procedure Overview:

After preparing the sera, the assay was performed by adding Reagent Mix into microplate wells containing 10 µL sera. After a brief incubation, the absorbance of each well at 520 nm was then measured using a plate reader. The concentration of cholesterol in each sample was then directly determined from the 520 nm absorbance.

-Kit Contents, Storage and Shelf Life:

The *Cholesterol Assay Kit* has the capacity for 96 determinations or testing of 42 samples in duplicate (using 12 wells for standards). The kit could be stored (except for the microplate) at 4 °C.

-Kit Contents Amount Storage:

-Microtiter Plate one 20-25°C

-Reagent Mix vial 4 °C

-Cholesterol Standard (200mg/dl) tube 4 °C

-Sample preparation:

1. 20 µL of sera was carefully prepared at least using standard production procedure (if determinations are performed in singlet then 10 µL is sufficient). Avoid hemolysis as it may release erythrocyte cholesterol into the serum.

2. Cholesterol in serum is reported stable for seven days at room temperature (18-30 oC) and six months when frozen and properly protected against evaporation.

Note:

1. Samples were diluted with values above 200 mg/dl diluted 1:1 with PBS and re-tested via multiplying results by two.

2. Grossly lipemic serums require a sample blank. 5 µl of sample was added to 250 µL saline, and mixed, then reading the absorbance against water was taken. Subtract this reading value from the absorbance of each serum sample to obtain the corrected reading.

CHOLESTEROL DETERMINATION TEST PROTOCOL

Set up

Reagents were allowed to warm up to room temperature, then were turned on the plate reader and allowed lamp to warm up. The wavelength of the plate reader was adjusted to 520 nm.

Reagent Preparation

- Preparation of Reagent Mix

To reconstitute the Reagent Mix, 27 mL of deionized or distilled water was exactly added to the Reagent Mix powder. Mix was done by swirling or inverting the bottle 10 times, then contents were allowed to dissolve for 10 minutes at room temperature. The Reagent Mix is stable for 3 months after reconstitution with water.

IMPORTANT: The reconstituted Reagent Mix can be left at room temperature for short periods (30 – 60 min) prior to use. Between uses, the reconstituted Reagent.

Mix should be stored at 4 °C (for up to 3 months). So we discard the Reagent Mix 3 months after reconstitution.

Preparation of Cholesterol Control Dilutions for Standard Curve

1. 6 microfuge tubes were labeled as 1, 2, 3, 4, 5, 6.
2. The Cholesterol Standard was diluted using methanol as described in the table below.
After dilution, Each tube was mixed before performing the next dilution.

Table (2): Dilution of Cholesterol Standard.

Tube Number	Cholesterol Standard(200mg/dl)	Methanol	Total volume
1	100 μ l	0 μ l	100 μ l
2	80 μ l	20 μ l	100 μ l
3	60 μ l	40 μ l	100 μ l
4	30 μ l	70 μ l	100 μ l
5	10 μ l	90 μ l	100 μ l
6(neg)	0 μ l	100 μ l	100 μ l

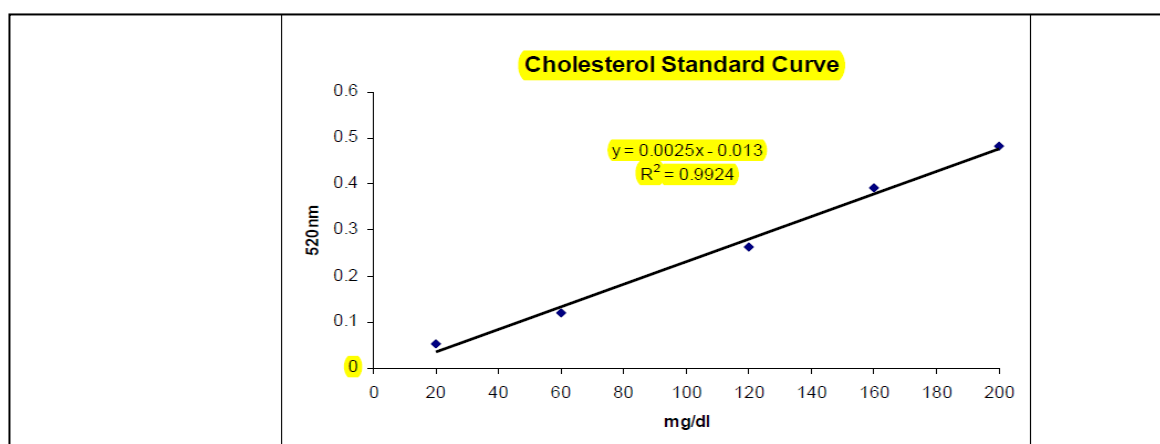
3. 5 μ L of each diluted standard is used per reaction (see below).

Sample Test Procedure

1. 5 μ L of each sample or standard (in duplicate) were diluted to the microplate wells.
2. 250 μ L of reagent mix were added to the wells.
3. Incubation at 37°C for 10 minutes was done.
4. The absorbance of each sample was measured at 520 nm.

-Cholesterol Concentration Calculation

There is a linear relationship between the concentration of cholesterol in the sample and absorbance at 520 nm. Therefore, a standard curve used to calculate the cholesterol concentration in sera samples can be constructed by plotting the mean corrected absorbance values for each of the diluted cholesterol standards as a function of cholesterol concentration.

**Figure(6) :** Standard curve for cholesterol concentration calculation.

-Measurement of serum HDL:**GENERAL INFORMATION:****Product Description:**

The *MaxDiscovery™ HDL Cholesterol Assay Kit* supplied by (*Bioo Scientific Corporation ,U.S.A 2011*) is a plate-based colorimetric assay for the determination of HDL cholesterol in serum or plasma samples. The kit uses a spectrophotometric assay to detect HDL directly from serum samples. The unique features of the kit are:

- High sensitivity and low detection limit (10 mg/dL)
- A rapid (10 minutes) and robust enzyme-based assay which does not require expensive instrumentation.
- High reproducibility

Introduction

The *MaxDiscovery™ HDL Cholesterol Assay Kit* is a simple microplate-based method for measuring HDL cholesterol levels from serum or plasma. This kit uses a specific reagent formulation to selectively stabilize non-HDL lipoprotein particles (LDL, VLDL and chylomicrons) while leaving HDL particles untouched. Next a second reagent containing a detergent and modified enzymes selectively reacts with the cholesterol present only in the HDL particles to form hydrogen peroxide. The hydrogen peroxide product then reacts with N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline to form a colored product. The resulting color change is measured at 610 nm and is proportional to the amount of HDL cholesterol originally present in the sample. The kit also comes with a HDL cholesterol standard which can be used to calibrate the assay and generate a standard curve. The kit contains sufficient materials to rapidly test 42 serum samples in duplicate.

Procedure Overview:

After preparing the sera, the assay was performed by adding HDL Cholesterol Reagent 1 into microplate wells containing 3 μ L sera to stabilize non-HDL particles. After a brief incubation, a second reagent was added to the reaction to selectively generate a colored reaction product from the HDL cholesterol in the sample. The absorbance of each well at 610 nm was then measured using a plate reader. The concentration of HDL cholesterol in each sample was then directly determined from the 610 nm absorbance.

Table(3):Contents of HDL kit.

Kit content	Amount	Storage
Microtiter plate	One	4°C
HDLCholesterol Reagent 1	24 ml	4°C
HDLcholesterol Reagent 2	8ml	4°C
Cholesterol Standard	0.8ml	4°C

-Kit Contents, Storage and Shelf Life:

The kit was stored as described in the table below. The shelf life is 6 months after receipt when the kit is properly stored.

-Sample Test Procedure:

1. 225 μ L of HDL Cholesterol Reagent 1 were added to the wells.
2. Carefully 3 μ L of each sample (in duplicate) or 15 μ L each standard (in duplicate) were added to the microplate wells.
3. Incubation at 37°C for 5 minutes was done.
4. 75 μ L of HDL Cholesterol Reagent 2 were added to the wells. Mix gently.
5. Incubation at 37°C for 5 minutes was done.
6. The absorbance of each sample was measured at 610 nm.

-HDL Cholesterol Concentration Calculation

There is a linear relationship between the concentration of HDL cholesterol in the sample and absorbance at 610 nm. Therefore, a standard curve used to calculate the HDL cholesterol concentration in sera samples can be constructed by plotting the mean corrected absorbance values for each of the diluted HDL cholesterol standards as a function of HDL cholesterol concentration.

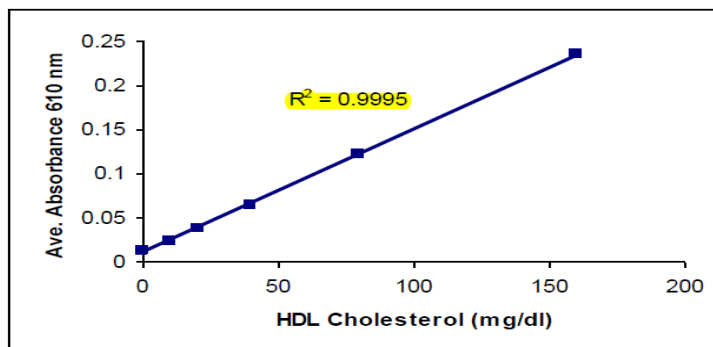


Figure (7) :Standard curve for HDL concentration calculation.

-Measurement of serum triglycerides:

Serum Triglyceride Quantification Kit supplied by *Cell Biolabs, Inc U.S.A 2014* measures triglyceride concentration in serum, plasma, and lysates by a coupled enzymatic reaction system. First, lipase hydrolyzes the triglyceride ester bond, yielding glycerol. The glycerol is then phosphorylated and oxidized, producing hydrogen peroxide which reacts with the kit's Colorimetric Probe (absorbance maxima of 570 nm).

The Serum Triglyceride Quantification Kit is a simple, colorimetric assay that quantitatively measures the amount of triglyceride in plasma, serum, and lysates in a 96-well microtiter plate format. Each kit provides sufficient reagents to perform up to 100 assays, including blanks, triglyceride standards, free glycerol controls and unknown samples. The kit contains a triglyceride standard and has a detection sensitivity limit of ~10 μ M (1 mg/dL).

Related Products

1. STA-241: Human Low Density Lipoprotein
2. STA-242: Human Very Low Density Lipoprotein
3. STA-361: Human ApoAI and ApoB Duplex ELISA Kit
4. STA-368: Human ApoB-100 ELISA Kit
5. STA-369: OxiSelect™ Human Oxidized LDL ELISA Kit (MDA-LDL Quantitation)
6. STA-375: Uric Acid/Uricase Assay Kit
7. STA-378: Creatinine Assay Kit
8. STA-390: Total Cholesterol Assay Kit
9. STA-391: HDL and LDL/VLDL Cholesterol Assay Kit
10. STA-397: Serum Triglyceride Quantification Kit (Fluorometric)
11. STA-398: Free Glycerol Assay Kit (Colorimetric)
12. STA-399: Free Glycerol Assay Kit (Fluorometric)

Kit Components

1. Triglyceride Standard (Part No. 239601): One 200 μ L vial (equivalent to 20,000 mg/dL triglyceride mixture with average MW of 873).
2. 10X Assay Buffer (Part No. 239802): One 1.5 mL vial.
3. 10X Lipase Solution (Part No. 239602): One 1 mL vial.
4. 5X Enzyme Mixture (Part No. 239803): Four 525 μ L vials.
5. 200X Colorimetric Probe (Part No. 239804): One 55 μ L amber vial.

Materials Not Supplied

1. 96-well microtiter plate
2. 10 μ L to 1000 μ L adjustable single channel micropipettes with disposable tips
3. 50 μ L to 300 μ L adjustable multichannel micropipette with disposable tips
4. Multichannel micropipette reservoir
5. Microplate reader capable of reading at 570 nm

Entire kit was stored at -80°C with avoiding of multiple freeze/thaws by aliquoting. The Colorimetric Probe is light sensitive and should be maintained in amber tubes.

Preparation of Reagents

- Triglyceride Standard, 10X Assay Buffer, 10X Lipase Solution, and 5X Enzyme Mixture should be thawed/maintained at 4°C during assay preparation. All are stable for 1 week at 4°C . For longer term storage, each should be aliquoted and frozen at -80°C to avoid multiple freeze/thaws.

- 200X Colorimetric Probe should be thawed/maintained at room temperature during assay preparation. Any unused material should be aliquoted and frozen at -80°C to avoid multiple freeze/thaws.

Preparation of Triglyceride Standard

- To prepare the triglyceride standards, A 1:100 dilution of the stock Triglyceride Standard in deionized water was performed. only enough for immediate use was prepared (e.g. By adding 10 μ L of 20,000 mg/dL Triglyceride Standard to 990 μ L deionized water). This solution has a concentration of 200 mg/dL. This 200 mg/dL triglyceride solution were used to prepare standards in the concentration range of 0 – 40 mg/dL by further diluting in deionized water (e.g. By adding 200 μ L of 200 mg/dL triglyceride solution to 800 μ L deionized water – as shown Table below). Triglyceride diluted solutions and standards should be prepared fresh.

Preparation of Samples

-Blood was collected in a tube with no anticoagulant.

The blood was allowed to clot at room temperature for 30 minutes.

-Centrifugation at 2500 x g for 20 minutes was done.

-The yellow serum supernatant was removed without disturbing the white buffy layer. -Samples should be tested immediately or frozen at -80°C for storage.

Serum must be diluted before assaying (1:5 to 1:20 in PBS). Normal triglyceride levels in human serum are considered less than 150 mg/dL; however, very high levels can exceed 500 mg/dL.

Assay Protocol

Each triglyceride standard and sample should be assayed in duplicate or triplicate. Additionally, each sample should be tested without lipase to determine free glycerol background. A freshly prepared standard curve should be used each time the assay is performed.

1. 10 μ L of the diluted triglyceride standards or samples were added to the 96-well microtiter plate.
2. All components/mixtures at 4°C were maintained. The desired volume of Reaction Mixture (based on the numbers of tests) was prepared in the following sequence:
 - a. In a sterile tube, The appropriate volume of deionized water was added .
 - b. To the water the corresponding volume of 10X Assay Buffer was added and mixed well.

- c. The corresponding volume of 5X Enzyme Mixture was added .
- d. Next,the corresponding volume of 10X Lipase Solution was added.

Note: To determine background free glycerol signal, this step should be skipped (without lipase). Deionized water should be used to make up the volume.

- e. Finally, The corresponding volume of 200X Colorimetric Probe was added and mixed well and immediately use.

3.90 μ L of the above Reaction Mixture was transferred to each well (already containing 10 μ L of triglyceride standard or sample).

4. the plate wells were covered to protect the reaction from light.
5. Incubation at room temperature for 30 minutes on an orbital shaker was done.
6. absorbance at 570 nm on a microplate reader was readen.

7. The concentration of triglyceride within samples was calculated by comparing the sample absorbance to the standard curve. Negative controls (without triglyceride) should be subtracted. Absorbance from free glycerol should also be deducted for true triglyceride values.

Example of Results

The following figures demonstrate typical Serum Triglyceride Quantification Kit results. One should use the data below for reference only. This data should not be used to interpret actual results.

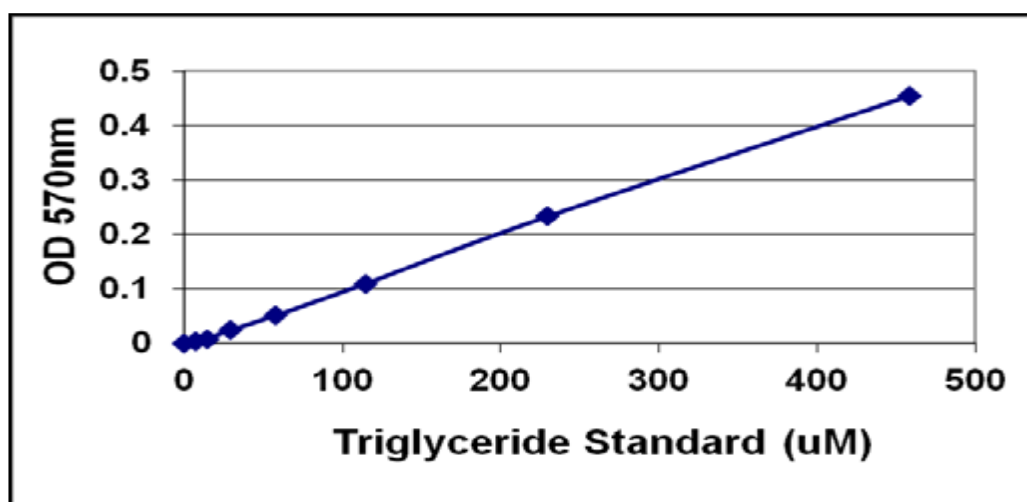
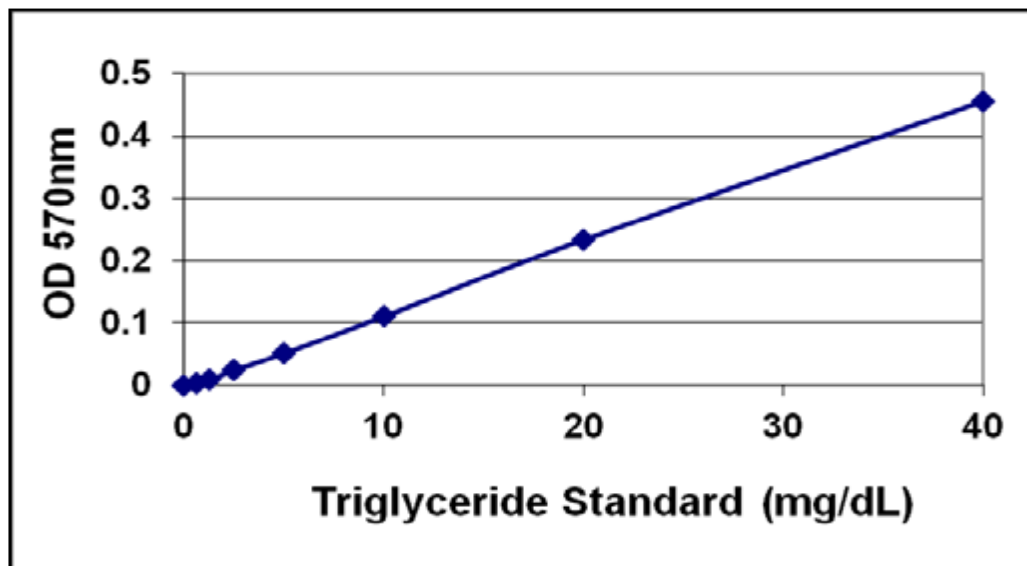


Figure 8: Triglyceride Standard Curve. Triglyceride standard curve was performed according to the Assay Protocol, data plotted in μ M (above) and mg/dL (below).

-Calculation of LDL:

By Friedewald equation:

$$\text{LDL} = \text{total cholesterol} - \text{HDL} - (\text{TG}/5)$$

- Measurement of HbA1c:**CLINICAL SIGNIFICANCE**

Measurement of hemoglobin A1c is accepted as a method to measure long-term glucose control in patients with diabetes mellitus. Determination of hemoglobin A1c provides an important diagnostic tool for monitoring the efficiency of dietary control and therapy during treatment of diabetes mellitus. The process of conversion from hemoglobin A to hemoglobin A1c depends on the blood glucose concentration. Elevated levels of %HbA1c suggest the need for more aggressive treatment of glycemia. The American Diabetes Association recommends that a primary goal of therapy should be a %HbA1c < 7%, and that physicians should re-evaluate the treatment regimen in patients with %HbA1c values consistently > 8%.⁷

METHODOLOGY

HBA1C kit supplied by *Beckman Coulter, Inc., The SYNCHRON® System(s), Irland 2010* utilizes two unique cartridges, Hb and A1c, to determine hemoglobin A1c concentration as a percentage of total hemoglobin. Hemoglobin reagent is used to measure total hemoglobin concentration by a colorimetric method. The SYNCHRON® System(s) automatically proportions the appropriate sample and reagent volumes into the cuvette. The ratio used is one part sample to 8.6 parts reagent. The System monitors the change in absorbance at 410 nanometers. This change in absorbance is directly proportional to the concentration of total hemoglobin in the sample and is used by the System to calculate and express total hemoglobin concentration. A1c reagent is used to measure the hemoglobin A1c concentration by a turbidimetric immunoinhibition method. In the reaction, hemoglobin A1c antibodies combine with hemoglobin A1c from the sample to form soluble antigen-antibody complexes. Polyhapten from the reagent then bind with the excess antibodies and the resulting agglutinated complex is measured turbidimetrically.

SPECIMEN**TYPE OF SPECIMEN**

Biological fluid samples should be collected in the same manner routinely used for any laboratory test.⁸ Freshly drawn blood treated with EDTA or heparin is the preferred specimen. Acceptable anticoagulants are listed in the PROCEDURAL

NOTES section of this chemistry information sheet.

SPECIMEN STORAGE AND STABILITY

1. Tubes of blood were kept closed at all times and in a vertical, stopper-up position.⁹
2. Whole blood samples should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, samples should be stored at +2°C to +8°C no longer than 7 days. If assays were not completed within 7 days, or the sample would be stored beyond 7 days, samples should be frozen at -15°C to -20°C. Frozen samples could be stable for 3 months and should be thawed only once. Analyte deterioration may occur in samples that were repeatedly frozen and thawed.⁹
3. Each laboratory should evaluate sample handling procedures to avoid variable results. Additional specimen storage and stability conditions as designated by this laboratory:

SAMPLE PREPARATION

1. The Hemolyzing Reagent was brought to room temperature prior to use.
2. Exactly 1000 µL Hemolyzing Reagent were pipetted into a test tube and not pipette directly from the stock bottle.
3. Whole blood sample was gently mixed to ensure a uniform distribution of erythrocytes.
4. Exactly 10 µL of whole blood sample was added to the test tube.
5. Pipette tip in Hemolyzing Reagent was rinsed by aspirating and dispensing several times.
6. The hemolysate was mixed by inverting gently, avoiding the formation of foam.

7. The hemolysate was assayed after hemolysis is complete, which is indicated by a color change from red to brown-green (approximately 1-2 minutes).

Note: The hemolysate is stable for 4 hours at room temperature, or 24 hours at +2°C to +8°C.

SAMPLE VOLUME

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, the Primary Tube Sample Template was to be referred to.

REACTIVE INGREDIENTS

REAGENT CONSTITUENTS

-Antibody Buffer (56 mL):

Anti-HbA1c Antibodies (sheep) > 0.5 mg/mL

MES (2-morpholino-ethanesulfonic acid) Buffer (pH 6.2) 0.05 mol/L

POLYHAPTEN BUFFER (12 mL):

HbA1c Polyhaptent ≥ 20 $\mu\text{g/mL}$

Hemoglobin Buffer (102 mL):

Phosphate Buffer (pH 7.4) 0.02 mol/L

Also non-reactive chemicals necessary for optimal system performance.

CALCULATIONS

Calculation of the IFCC HbA1c concentration in percent is determined using the following calculation:

$$\% \text{HbA1c} = \frac{\text{A1c(g/dl)}}{\text{Hb(gm/dl)}} \times 100$$

-Measurement of arterial blood pressure :

Systemic arterial blood pressure usually is measured using an instrument called a sphygmomanometer. This device consists of an inflatable rubber cuff connected by tubing to a compressible bulb and a glass tube containing a column of mercury. The bulb is used to pump air into the cuff, and a rise in the mercury column indicates the pressure produced. Thus, the pressure in the cuff can be expressed in millimeters of mercury. A pressure of 100 mm Hg, for example, would be enough to force the mercury column upward for a distance of 100 mm.

To measure arterial blood pressure, the cuff of the sphygmomanometer was usually wrapped around the arm so that it surrounds the brachial artery. Air was pumped into the cuff until the cuff pressure exceeds the pressure in that artery. As a result, the vessel was squeezed closed, and its blood flow stopped. At this moment, if the diaphragm of a stethoscope was placed over the brachial artery at the distal border of the cuff, no sounds can be heard from the vessel because the blood flow is interrupted.

As air was slowly released from the cuff, the air pressure inside it decreased. When the cuff pressure was approximately equal to the systolic blood pressure within the brachial artery, the artery opened enough for a small amount of blood to spurt through. This movement produced a sharp sound (Korotkoff's sound) that could be heard through the stethoscope. The height of the mercury column when this first tapping sound was heard represents the arterial systolic pressure (SP).

As the cuff pressure continues to drop, a series of increasingly louder sounds could be heard. Then, when the cuff pressure was approximately equal to that within the fully opened artery, the sounds became abruptly muffled and disappeared. The height of the mercury column when this happened represents the arterial diastolic pressure (DP).

The results of a blood pressure measurement were reported as a fraction, such as 120/80. In this notation, the upper number indicates the systolic pressure in mm Hg (SP), and the lower number indicates the diastolic pressure in mm Hg (DP).

Calculation of body mass index:

Body mass index is calculated by dividing weight in kilogram on (height)² in meters by the formula :

Weight in kg / height² in meter.

If BMI is >30 this is an indicator of obesity.

Data were coded and entered using the statistical package SPSS version 21. Data was summarized using mean \pm standard deviation. Comparisons between groups were done using unpaired T test (Chan, 2003). Correlation was done to test for linear relations between quantitative variables by Pearson correlation coefficient. P-values less than 0.05 were considered as statistically significant.

RESULTS:

Table (4): Comparison of serum glucose and HBA1c levels between diabetic and control groups:

	Control group (mean \pm SD)	Diabetic group (mean \pm SD)	P-VALUE
Serum glucose mg/dl	90.20 \pm 9.00	263.84 \pm 42.82	< 0.001
HBA1C gm/dl	3.81 \pm .43	11.34 \pm 1.86	< 0.001

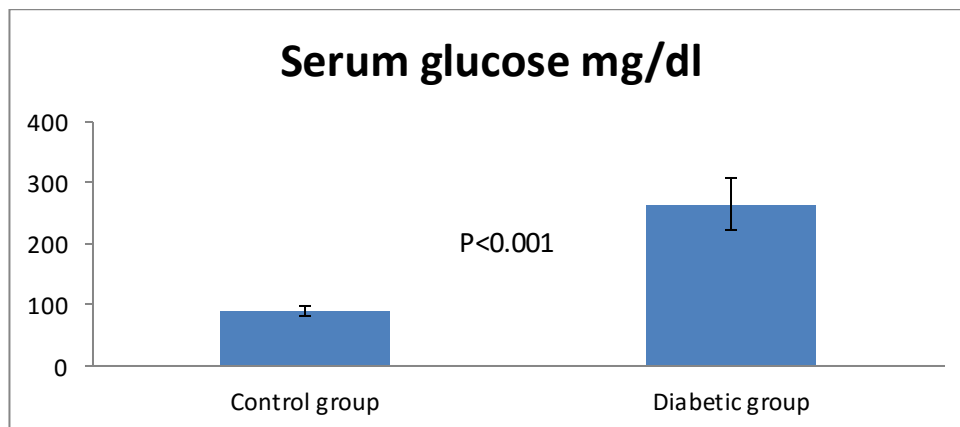


Figure (9): Comparison of serum glucose level between diabetic and control groups.

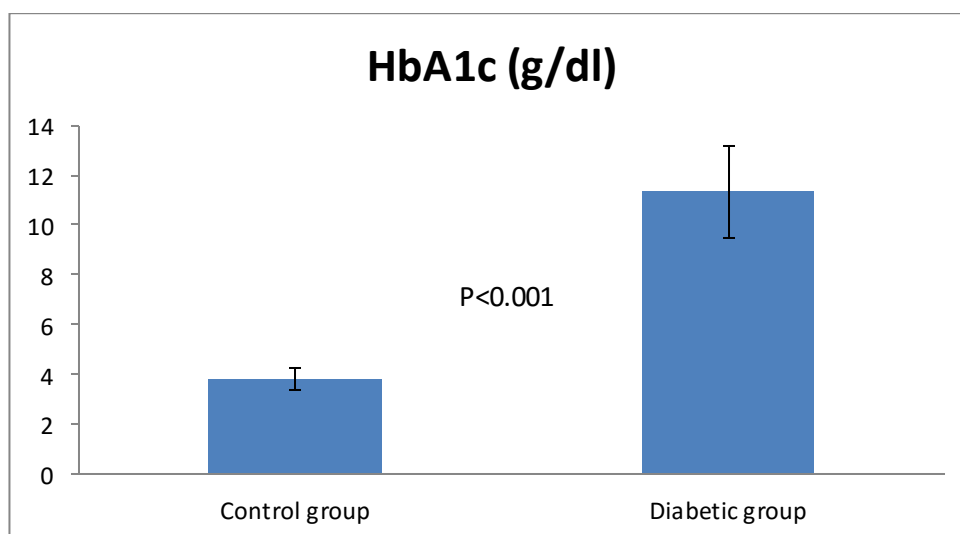
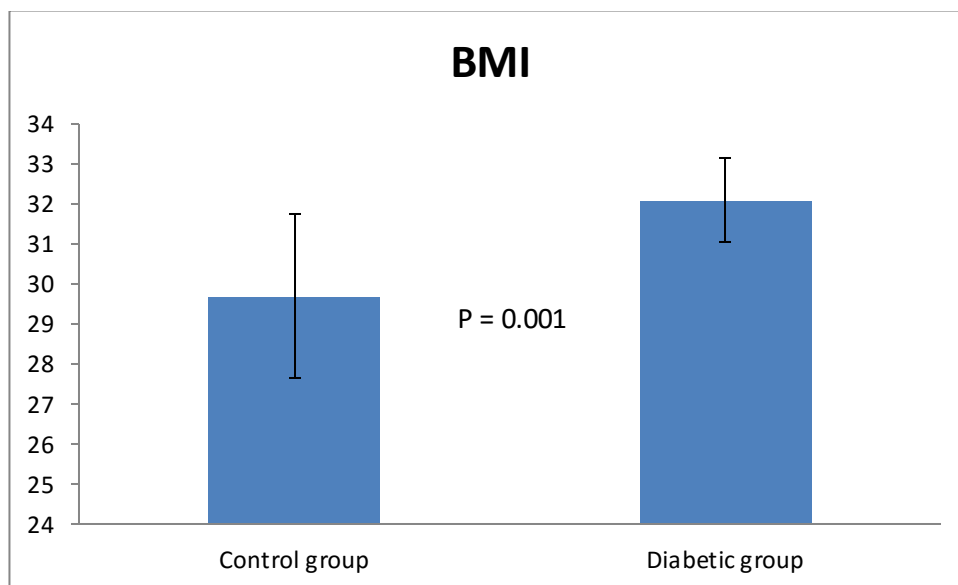


Figure (10): Comparison of HBA1C between diabetic and control groups.

As shown in table 4 and figures (9,10) there are high significant increase of both serum glucose and HBA1C levels in diabetic group (P- VALUE <0.001) with their mean values are 263.84 \pm 42.82 for glucose and 11.34 \pm 1.86 for HBA1C when compared to control group with their mean values are 90.20 \pm 9.00 for glucose and 3.81 \pm .43 for HBA1C.

Table (5): Comparison of BMI &SBP&DBP between diabetic and control groups:

	Control Group	Diabetic Group	P- value
BMI	29.69± 2.06	32.08±1.05	0.001
SBP	116.00± 8.21	127.20±21.32	0.022
DBP	74.50± 6.86	84.00±12.33	0.002



Figure(11) : Comparison of BMI between diabetic and control group .

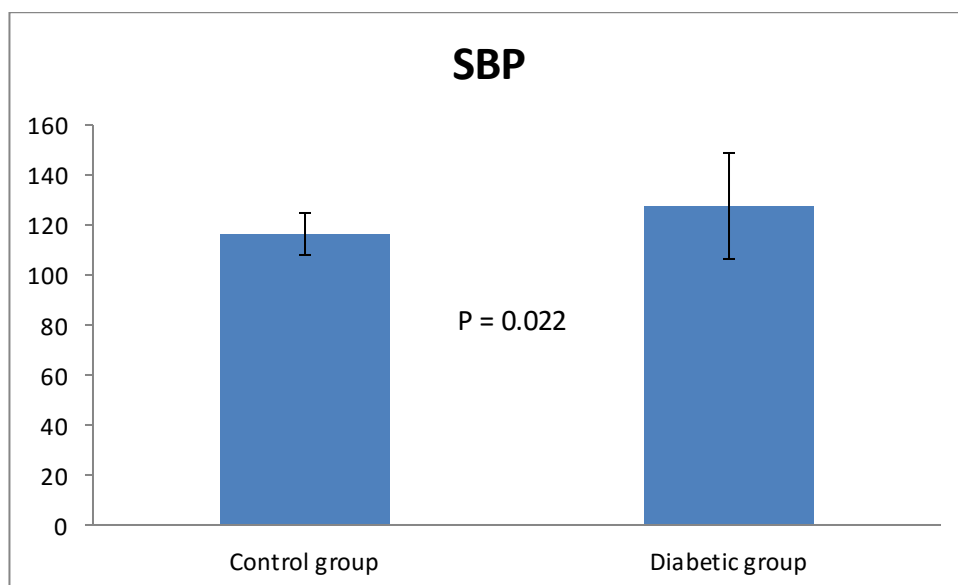
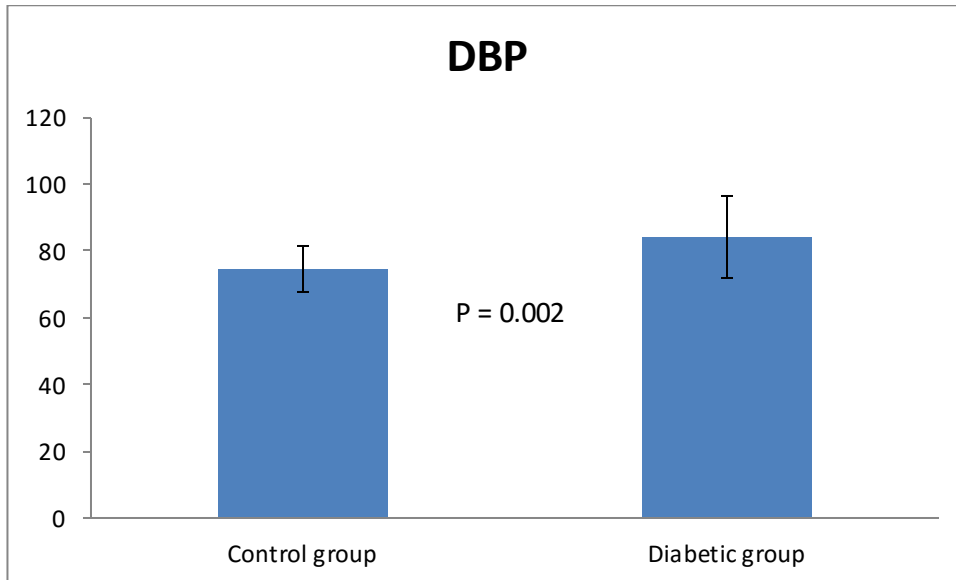


Figure (12) : Comparison between SBP between diabetic and control group .



Figure(13) : Comparison of DBP between diabetic and control groups.

As shown in table (5) and figures (11,12,13) there are significant increase of BMI &SBP and DBP in diabetic patients (p- value {0.001 &0.022&0.002}) respectively in which their mean values are. [(32.08±1.05)&(127.20±21.32)&(84.00±12.33)] respectively ,when compared to mean values of control group for the same parameters which are [(29.69±2.06)& (116.00±8.21)&(74.50±6.86)].

Table(6):Comparison of serum total cholesterol&TG&LDL AND HDL levels between diabetic and control groups:

	Control group (mean±SD)	Diabetic group (mean±SD)	p- value
Total cholesterol	141.95±27.77	247.13±51.45	< 0.001
TG	88.70±18.54	157.68±34.98	< 0.001
LDL	69.50±27.66	175.90±54.59	< 0.001
HDL	54.85±8.72	38.65±7.91	< 0.001

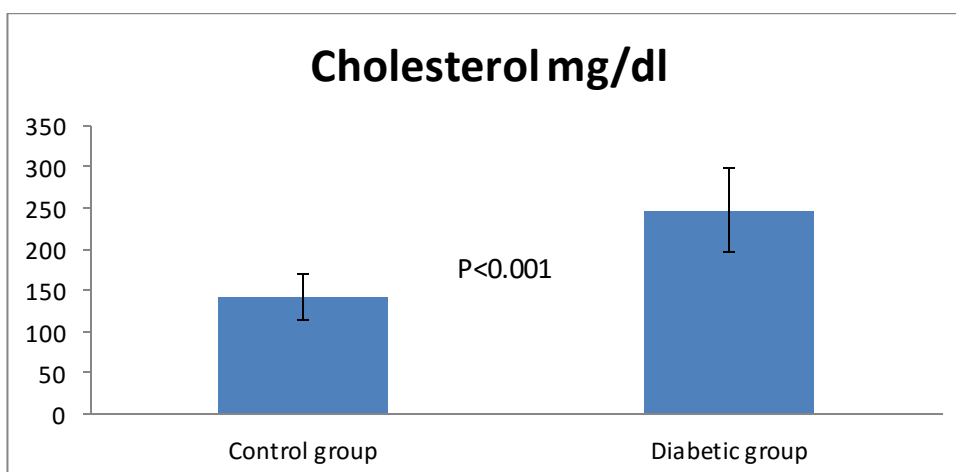


Figure (14):Comparison of serum total cholesterol level between diabetic and control groups.

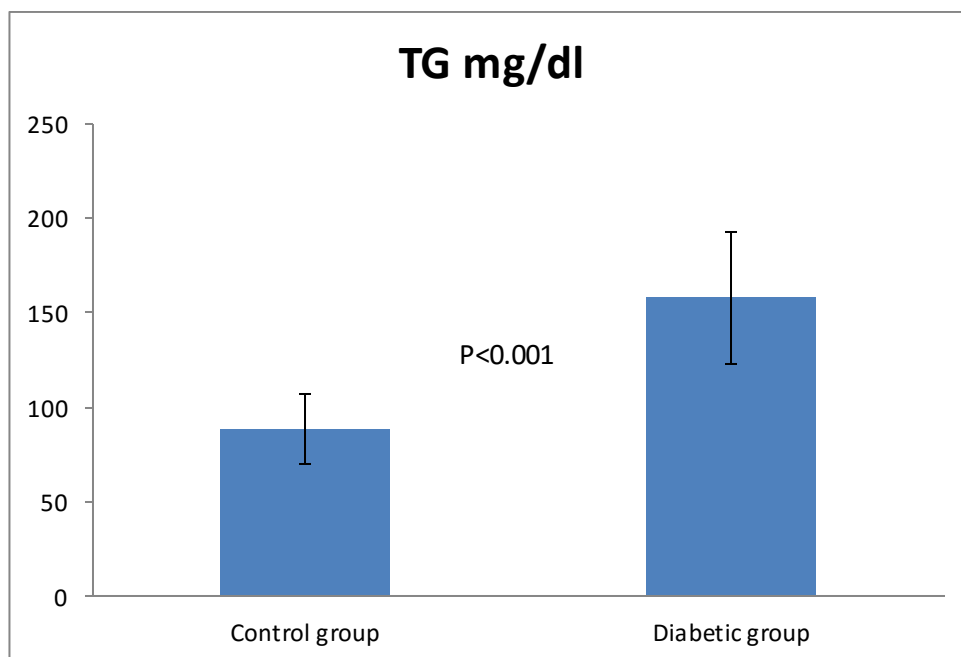


Figure (15):Comparison of serum TG level between diabetic and control groups.

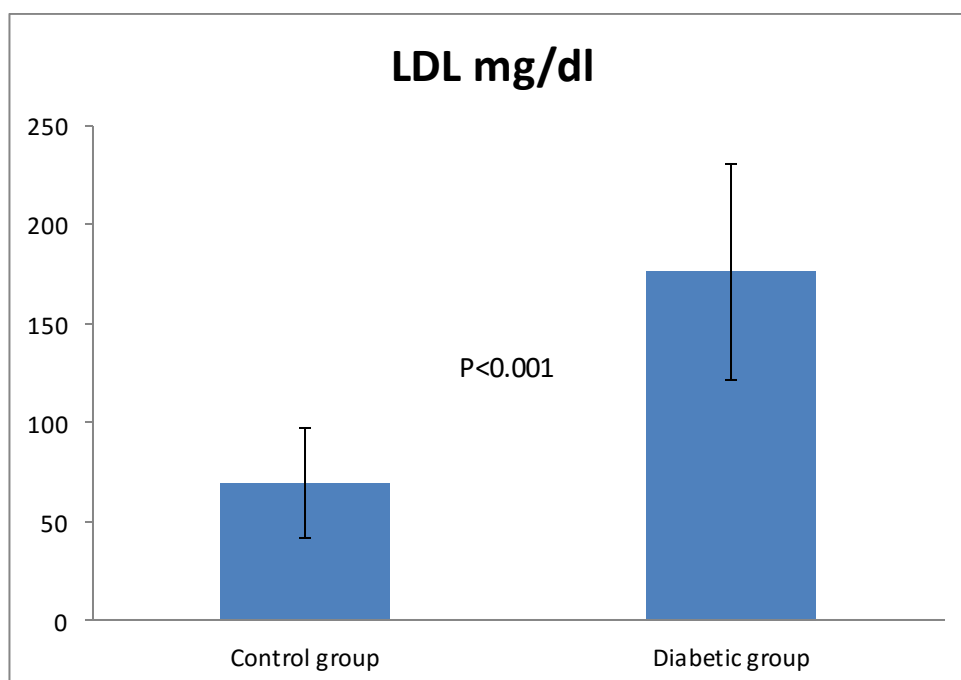


Figure (16):Comparison of serum LDL level between diabetic and control groups.

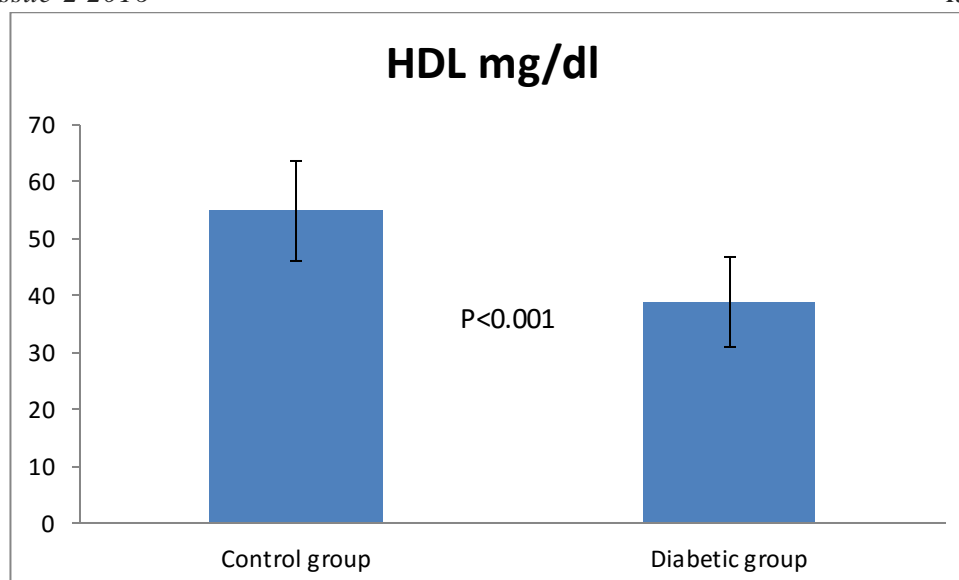


Figure (17):Comparison of serum HDL level between diabetic and control groups.

As observed in table (6) and figures(14)& (15)&(16)&(17) there are high significant increase of total cholesterol&TG and LDL level and high significant decrease of HDL in diabetic group (p -value < 0.001) with their mean values are 247.13 ± 51.45 & 157.68 ± 34.98 & 175.90 ± 54.59 and 38.65 ± 7.91 respectively in comparison to control group with their mean values are 141.95 ± 27.77 & 88.70 ± 18.54 & 69.50 ± 27.66 and 54.85 ± 8.72 respectively.

DISCUSSION

One of the important finding in this study is the combination between obesity which is estimated by BMI > 30 , diabetes mellitus and hypertension. This combination was also reported by (Giovanni et al, 2010) & (Balogun and Salako 2011). The earliest negative effects of obesity is the development of insulin resistance. The mechanism is related to overproduction of proinflammatory cytokines and hormones that directly and indirectly induce insulin resistance. (Shoelson et al 2006) & (Kahn et al 2006). Retinol-binding protein-4, secreted by the adipose tissue, induces insulin resistance by reducing phosphatidylinositol-3-OH kinase signaling in the muscle and increase expression of phosphoenolpyruvate carboxykinase in the liver, with decrease adiponectin secretion and PPAR- α activity typical in obese people (Yang et al 2005). In non diabetic individuals insulin resistance with hyperinsulinemia has a strong effect for development of type-2 diabetes (Haffner et al 2000).

Other factors, including TNF- α , IL-6, monocyte chemoattractant protein-1, and macrophage products which increase with obesity have a great role in development of insulin resistance. Izzo 2009 has demonstrated that, although insulin resistance improves with weight loss, regional adipose-specific insulin resistance and hypoperfusion does not improve, as a consequence of irreversible GLUT-4 translocation defects in the adipose tissue, and thus providing a molecular link between obesity and glucose/fat dysregulation. Another mechanism explain role of obesity in development of insulin resistance and diabetes mellitus related to induction of systemic oxidative stress by obesity through oxidative stress in the accumulated adipose tissue, leading to dearangements in adipocytokines secretion. (Furukawa et al 2004). Oxidative stress in humans has been found to play a role in the development of diabetes, hypertension, and vascular disease and may be a mechanistic link between obesity and the metabolic syndrome. (Ohara et al 1993) & (Brownlee 2001). Also a strong link with upregulation of rennin angiotensin system is found to be associated with insulin resistance, hypertension and diabetes (Richey et al, 1999) & (Brenner et al, 2001). This upregulation results in enhanced generation of ROS and may explain impaired glucose utilization as well as hypertension associated with insulin resistance and type 2 diabetes. (Sowers 2004 a) It has been proposed that increased autocrine/paracrine activity of angiotensin II results in diminished action of insulin and insulin growth factor-1 (IGF-1) signalling through the PI3K/Akt pathway, resulting in inhibition of mechanisms involved in the vasodilator and glucose transport properties of insulin and IGF-1 (Sowers 2004a) & (Sloniger et al, 2005).

Insulin activates the PI3K/Akt system in skeletal muscle, adipose, and myocardial tissues and initiates translocation of the GLUT4 glucose receptor to the cell membrane. The unregulated ANG II acts through its receptor (AT₁R) and results in formation of ROS and the activation of low-molecular-weight G proteins such as Rho A. (Sowers, 2004a) Activation of these small G proteins and consequent enhancement of the generation of ROS inhibits insulin/IGF-1 actions mediated through PI3K/Akt signalling including activation of endothelial nitric oxide (NO) synthase (eNOS) activity, Na⁺ pump activation, and Ca²⁺-myosin

light chain desensitization(Sowers2004). Other possible causes of hypertension with diabetes and insulin resistance include activation of the sympathetic nervous system, increased renal tubular sodium retention, elevated intracellular calcium concentration and vascular smooth muscle cell proliferation and atherosclerosis, and impaired NO metabolism in skeletal muscle(Rowe et al ,1981) &(Modan and Halkin ,1991) &(Sowers 2004 b).Another finding in our study is that lipid profiles are significantly increased in diabetic patients when compared to control group.This result is in agreement with Samatha et al ,2012 who found that TC level, LDL-C were high and HDL-C was low in the diabetic subjects when compared to control people.

There is high interconnection between carbohydrates and lipid metabolism, so any disorder in carbohydrate metabolism leads to disorder in lipid metabolism and vice versa(Chatterjee and Shinde 2005). In patients with uncontrolled T2DM and hyperinsulinemia, triglycerides levels are increased for many causes. As the release of stored fatty acids from adipocytes requires the conversion of stored triglycerides into FAs and monoglycerides which then transferred across the plasma membrane of the cell, the enzyme concerning with this action is hormone-sensitive lipase (HSL), which is normally inhibited by insulin. In insulin resistance, HSL becomes active, resulting in increased free fatty acids transferred from adipose tissue to the liver, leading to hypertriglyceridemia (Durrington and Sniderman 2002).Ahmed and Elinasri 2008)&(Mooradian2009)&(Smith and Lall 2008) also reported that insulin deficiency reduces the activity of hepatic lipase plus several steps in the production of biologically active lipoprotein lipase. As these two enzymes have great role in controlling lipid metabolism, so their deficiency attribute for development of dyslipidemia . Goldberg 2001 reported that hyperglycemia progressively increases the transfer of cholesterol esters from HDL-C to VLDL-C particles, so denser LDL particles take a large proportion of these HDL esters, leading to decrease in the HDL-C level .

References:

- 1- Ahmed AM ,Elinasri HA (2008):" Patterns of lipid changes among type 2 diabetes patients in Sudan." Eastern Mediter Health J, 14:2.
- 2-Balogun W.O. and Salako B.L.(2011):"Co-occurrence of diabetes and hypertension: pattern and factors associated with order of diagnosis among Nigerians " Annals OF Ibadan Postgraduate Medicine. Vol. 9 No. 2 89-93 .
- 3-Brenner B M, Cooper M E, de Zeeuw D, Keane W F, Mitch W E, Parving H-H et al(2001): "Effects of Losartan on Renal and Cardiovascular Outcomes in Patients with Type 2 Diabetes and Nephropathy" N. Engl. J. Med. ;345(12): pp. 861-869.
- 4- Brownlee M.(2005): "The pathobiology of diabetic complications: a unifying mechanism." Diabetes. ;54:1615–25.
- 5-Centofani .M(1995): "Diabetes Complications: More than Sugar?" Science News, vol. 149, no. 26/27, Dec. 23–30, p. 421.
- 6-Chan YH (2003): "Biostatistics 102: Quantitative Data – Parametric & Non-parametric Tests." Singapore Med J;44(8): 391-396.
- 7- Chatterjee MN, Shinde R(2005):" Text book of medical laboratory technology. Metabolism of carbohydrates". 6th edition. Delhi-India: Jaypee Brothers Medical publisher; :266–330.
- 8-Durrington P, Sniderman A(2002): "Secondary hyperlipidemia. In: *Hyperlipidemia*." Health Press, Oxford, UK, 71–89).
- 9-Eastman RC, Cowie CC, Harris MI(1997): "Undiagnosed diabetes or impaired glucose tolerance and cardiovascular risk". Diabetes Care 20:127–128.
- 10-Furukawa S, Fujita T, Shimabukuro M, et al (2004):" Increased oxidative stress in obesity and its impact on metabolic syndrome." J Clin Invest,114:1752–1761.
- 11-Giovanni de Simone, , Richard B. Devereux, , Marcello Chinali, , Elisa T, Lee, , James M. Galloway,, Ana Barac, , Julio A. Panza, , and Barbara V. Howard,(2010): "Diabetes and incident heart failure in hypertensive and normotensive participants of the Strong Heart Study" J Hypertens. 28(2): 353–360.
- 12- Goldberg IJ(2001):" Diabetic dyslipidemia: causes and consequences." J Clin Endo Metab 8(3):965–971.
- 13- Granberg V, Ejksjaer N, Peakman M, Sundkvist G (2005): "Autoantibodies to autonomic nerves associated with cardiac and peripheral autonomic neuropathy". *Diabetescare* 28 (8):195964.
- 14- Haffner SM, Mykkanen L, Festa A(2000): "Insulin-resistant prediabetic subjects have more atherogenic risk factors than insulin-sensitive prediabetic subjects." Circulation 101:975–980.

- 15-Hu, EA; Pan, A, Malik, V, Sun, Q (2012):** "White rice consumption and risk of type 2 diabetes: meta-analysis and systematic review". *BMJ (Clinical research ed.)* **344**: e1454.
- 16- Ichinose K, Kawasaki E, Eguchi K (2007):** "Recent advancement of understanding pathogenesis of type 1 diabetes and potential relevance to diabetic nephropathy". *Am. J. Nephrol.* **27** (6): 554–64.
- 17-Iozzo P.(2009):**" Viewpoints on the way to the consensus session. Where does insulin resistance start?" *The adipose tissue. Diabetes Care.*;32 (Suppl 2):S168–S173
- 18-Kahn SE, Hull RL, Utzschneider KM (2006):**"Mechanisms linking obesity to insulin resistance and type 2 diabetes." *Nature.* 444:840–846.
- 19- Kastelan S, Zjacić-Rotkvić V, Kastelan Z (2007):** "Could diabetic retinopathy be an autoimmune disease?". *Med. Hypotheses* **68** (5):1016–8
- 20-Kumar, Vinay; Fausto, Nelson; Abbas, Abul K.; Cotran, Ramzi S. ; Robbins, Stanley L. (2005):**" Robbins and Cotran Pathologic Basis of Disease (7th ed.). Philadelphia, Pa.: Saunders. pp. 1194–119.
- 21-Lippincott Williams & Wilkins.(2007):** "*Diabetes mellitus a guide to patient care.*.. Philadelphia: p. 15.
- 22-Malik, VS; Popkin, BM, Bray, GA, Després, JP, Willett, WC, Hu, FB (2010):** "Sugar-Sweetened Beverages and Risk of Metabolic Syndrome and Type 2 Diabetes: A meta-analysis". *Diabetes Care* **33** (11): 2477–83.
- 23-Modan M, Halkin H(1991):** "Hyperinsulinemia or increased sympathetic drive as links for obesity and hypertension", *Diabetes Care* ;14(6): pp. 470-487.
- 24- Mooradian AD(2009):** "Dyslipidemia in type 2 diabetes mellitus" *Nat Clin Pract Endocrin Metab* 5:150–159.
- 25- Nathan DM, Cleary PA, Backlund JY, et al. (2005):** "Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes". *The New England Journal of Medicine* **353**(25): 2643–53
- 26-Ohara Y, Peterson TE, Harrison DG.(1993):**" Hypercholesterolemia increases endothelial superoxide anion production. *J Clin Invest.* 91:2546–2551
- 27-Reaven GM(1988):** *Banting Lecture: role of insulin resistance in human disease. Diabetes* 37:1595–1607.
- 28-Richey J M, Ader M, Moore D, Bergman R N, (1999):**"Angiotensin II induces insulin resistance independent of changes in interstitial insulin", *Am. J. Physiol. Endocrinol. Metab.* ;277(5): pp. E920-926.
- 29- Rich SS (2006).** "Genetics of diabetes and its complications". *J. Am. Soc. Nephrol.* **17** (2): 353–60.
- 30-Ripsin CM, Kang H, Urban RJ (2009).** "Management of blood glucose in type 2 diabetes mellitus". *Am Fam Physician* **79** (1): 29–36. PMID 19145963.
- 31-Risérus U, Willett WC, Hu FB (2009).** "Dietary fats and prevention of type 2 diabetes". *Progress in Lipid Research* **48** (1): 44–51.
- 32-Rowe J W, Young J B, Minaker K L, Stevens A L, Pallotta J, Landsberg L,(1981):** "Effect of insulin and glucose infusions on sympathetic nervous system activity in normal man", *Diabetes* ;30(3): pp. 219-225.
- 34-Shlomo Melmed, Kenneth S. Polonsky, P. Reed Larsen, Henry M. Kronenberg (2011):** *Williams textbook of endocrinology.* (12th ed.). Philadelphia: Elsevier/Saunders. pp. 1371–1435.
- 35-Shoback, edited by David G. Gardner, Dolores (2011).** *Greenspan's basic & clinical endocrinology* (9th ed.). New York: McGraw-Hill Medical. pp. Chapter 17. ISBN 0-07-162243-8.

- 36-Shoelson SE, Lee J, Goldfine AB(2006).** Inflammation and insulin resistance. *J Clin Invest.* 116:1793–1801.
- 37-Sloniger J A, Saengsirisuwan V, Diehl C J, Dokken B B, Lailerd N, Lemieux A M et al.,(2005):** "Defective insulin signaling in skeletal muscle of the hypertensive TG(mREN2)27 rat", *Am. J. Physiol. Endocrinol. Metab.* 288(6): pp. E1,074-1,081.
- 38- Smith S, Lall AM(2008):** A Study on lipid profile levels of diabetics and non-diabetics among Naini region of Allahabad. *India Turkish J Biochem* 33(4):138–141.
- 39-Sowers J R,(2004a):** Insulin resistance and hypertension, *Am. J. Physiol. Heart Circ. Physiol.* ;286(5):H pp. 1,597-1,602.
- 40-Sowers J R(2004 b):,** Treatment of hypertension in patients with diabetes, *Arch. Intern. Med.*164(17): pp. 1,850-1,857.
- 41-Stewart, CP, Christian, P; (2010).** "Maternal micronutrient deficiency, fetal development, and the risk of chronic disease". *The Journal of nutrition* 140 (3): 437–45
- 42-Touma, C; Pannain, S (2011).** "Does lack of sleep cause diabetes?". *Cleveland Clinic journal of medicine* 78 (8): 549–58.
- 43-Trinder P(1969):** Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. *J Clin Pathol.* 1969 Mar;22(2):158–161.
- 44-Yang Q, Graham TE, Mody N, et al.(2005)** Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature.* 436:356–362.



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