Abstract

Background:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both, the chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels that impose a tremendous burden on the individual with diabetes and on the health care system.

Objective:

To study the relationship between retinol binding protein -4 (RBP-4) / retinol ratio with insulin resistance in patients with type 2 diabetes mellitus.

Subjects and methods:

A case- control study was performed at Chemistry and Biochemistry Department, College of Medicine, Al-Nahrain University, Baghdad, Iraq from March 2012 to June 2012. Forty five patients and thirty four healthy controls were enrolled in this study. The patients and controls were taken with cluster random sampling method. The present study includes measurements of hbA1c levels by ion-exchange HPLC method. Fasting blood glucose, lipid profile, blood urea and serum creatinine levels were determined by enzymatic colorimetric methods. Fasting serum insulin, retinol binding protein -4 (RBP-4), retinol levels were measured by enzyme-linked immune sorbent assay in 45 patients with type 2 diabetes mellitus (mean ± SE,MEAN AGE = 52.14 ± 1.01 years).

The results were compared with 34 aged matched apparently healthy control subjects (mean age 48.79 ± 1.17 years) divided according to body mass index (BMI) into two groups:

1. Controls with body mass index (BMI) < 25 kg/m^2: normal control group (n = 9, mean age = 49.22 ± 1.88 years).

2. Controls with body mass index (BMI) ≥ 30 kg/m^2: obese control group (n = 25, mean age = 48.38 ± 1.45 years) .

Results:

The results showed significant elevation in blood glucose, glycated hemoglobin HbA1c, serum triglyceride TG, serum very low density lipoprotein VLDL, atherogenic index of plasma AIP, fasting insulin resistance index, fasting glucose insulin ratio, Homeostatic model assessment of β-cells (β-cells function) HOMA-β and serum retinol binding protein 4 in the diabetic patients as compared with both control subjects.

High density lipoprotein HDL, insulin and Homeostatic model assessment of insulin resistance HOMA-IR presented significant increase in T2DM when compared to normal control group but not significant difference when compared to obese

Insulin levels and insulin resistance expressed in HOMA –IR showed significant elevation in T2DM as compared to normal control group but not significant when compared to obese control group.

Serum cholesterol, low density lipoprotein showed no significant difference between patients and both control groups.

Insulin sensitivity index ISI was significantly decreased in T2DM patients as compared to both control groups.

Conclusion:

It was concluded that serum retinol / retinol binding protein 4 ratio may be considered as indication of type 2 diabetes mellitus rather than RBP 4 itself.

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List of Abbreviation

ARAT	Acyltransferase
ADH	Alcohol dehdrogenase
ADA	American Diabetes Association
BMI	Body mass index
BW	Body weight
CRBP-I	Cellular retinol binding protein type I
CRBP-I KO	Cellular retinol binding protein type I- knock-out
CRBP-II	Cellular retinol binding protein type II
CRBP-III	Cellular retinol binding protein type III
CRBP-III KO	Cellular retinol binding protein type III- knock-out
CRBP-IIII	Cellular retinol binding protein type IIII
CNS	Central nervous system
Da	Dalton
DCCT	Diabetes Control and Complications Trial
DCCT	Diabetes Control and Complications Trial
DM	Diabetes mellitus
FPG	Fasting plasma glucose
FI	Food intake
GDM	Gestational Diabetes Mellitus

HbA1c	Glycated hemoglobin
HDLc	High density lipoprotein cholesterol
HPLC	High Performance Liquid Chromatography
IGT	Impaired glucose tolerance
IDDM	Insulin dependent diabetes mellitus
IR	Insulin resistance
ICD	International Classification of Diseases
IND	International Nomenclature of Diseases
LRAT	Lecithin: retinol acyltransferase
LDLc	Low density lipoprotein cholesterol
MRDM	Malnutrition–related Diabetes Mellitus
MODY	Maturity-onset diabetes of the young
NGSP	National Glycohemoglobin Standardization Program
NIDDM	Non-insulin dependent diabetes mellitus
PCOS	Polycystic Ovary Syndrome
RAL	Retinaldehyde
RALDH	Retinaldehyde dehydrogenase
RA	Retinoic acid
RAR	Retinoic acid receptors
RXR	Retinoid x receptors
ROH	Retinol

RBP4	Retinol-binding protein 4
REs	Retinyl esters
rpm	Revolution per minute
STRA6	Stimulated by Retinoic Acid 6
TTR	Transthyretin
TG	Triglyceride
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
VLDL	Very low density lipoprotein
WC	Waist circumference
WHR	Waist-to-hip ratio
WAT	White adipose tissue
WT	Wild-type
WHO	World Health Organization

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Questionnaire

patient's name: *case no*

Age: *years*

Weight: *kg*

Height: *cm*

BMI:

Waist circumference: *cm*

Duration:

Drugs:

Genetic factor:

Smoking: *yes* ☐ *No* ☐

Marital status: single *married*

Complications:

Retinopathy ☐

Nephropathy ☐

Neuropathy ☐

Examining committee certification

We , the examining committee ,certify that we have read this thesis and have examined the student Anas Hashim Sadek in its context and in our opinion , it is adequate with standing: excellent as a thesis for the degree of Master of science in clinical Biochemistry.

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Chapter one

Introduction

1.1. Regulation of glucose homeostasis:

Homeostasis (the maintenance of optimal internal conditions) is achieved through a complex set of physiological and behavioral responses to external and internal stimuli.(Grayson et al, 2013) . The concept of energy homeostasis to the combined processes that manage energy intake, storage and usage to maintain stable levels of stored fuel in the form of adiposity and to enable adequate access to stored energy during times of limited food access, enhanced energy intake and storage when food is available, and appropriate metering of fuels to tissues at all times. The term glucose homeostasis refers to the hormonal and neural regulatory elements that specifically control glucose production and use. Glucose homeostasis maintains plasma glucose levels within a relatively small range (70-110 mg per dl), even in the face of physiological challenges, including meal ingestion, fasting and intense exercise (Grayson et al, 2013).

Obesity is a progressive metabolic disorder of energy homeostasis, and type 2 diabetes mellitus (T2DM) is a progressive metabolic disorder of glucose homeostasis. They are physiologically linked and are respectively associated with increased levels of adiposity and glucose that are actively maintained and defended. This link is illustrated by the facts that the incidences of both conditions are increasing in parallel and that

weight loss lowers plasma glucose (Diabetes Prevention Program Research Group, 2009).

For example, the brain, which has a very large demand for glucose (120 g/day) would suffer adverse effects (functional impairments, coma and even death) if there was a decrease in plasma glucose to below 4.0 mmol/l . Hyperglycemia , sustained elevation of fasting plasma glucose above 7 mmol/l , may result in organ damage or ketosis in chronic or acute cases , respectively . Plasma glucose comes from the dietary intake of sugars and endogenous production in the liver and kidney . there can also be a rise in plasma glucose when some tissues reduce their uptake; some tissues achieve this by producing glucose for their own consumption , although this is a minor mechanism predominantly effective in starvation . Glucose is the primary source of energy for most tissues; therefore there is a constant drain of glucose from the blood . A balance between supply and demand must be maintained to prevent the complications mentioned above, but more importantly to keep the body functioning as a whole (Yeo and Sawdon, 2013).

1.2. Diabetes Mellitus (DM)

1.2.1. Definition of diabetes mellitus :-

Diabetes mellitus is a common endocrine and metabolic disease (Hallikerimath et al., 2011) which causes deaths worldwide (third greatest cause of death) (Yadav et al., 2012; Samatha et al., 2012). Hyperglycemia caused by complete or relative insulin deficiency (Lyer and Desai, 2010; Al-maskari et al., 2011) leading to impaired metabolism of carbohydrates, lipids and proteins (Prasad et al., 2010) . The two most common forms of diabetes are type 1 diabetes and type 2 diabetes both

lead to hyperglycemia, excessive urine production , compensatory thirst , increased fluid intake, blurred vision, unexplained weight loss, lethargy, and change in energy metabolism (Lin and Sun, 2010).

Depending on the etiology of DM , factors contributing to hyperglycemia may include reduced insulin secretion, decreased glucose usage and increased glucose production(Mohmad et al., 2011).

Diabetes mellitus is a serious chronic condition associated with devastating complications (Becker and Hux, 2011). Diabetic complications are two type , they are short term complications and long term complications. Short term complications are like diabetic ketoacidosis, hyperosmolar non ketotic coma and hypoglycemia (Goodman and Gilman., 2006; Charles et al., 2010). Long – term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure ,peripheral neuropathy with risk of foot ulcers, amputation, and charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary , and cardiovascular symptoms and sexual dysfunction. Patient with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial, and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes (American diabetes association, 2012).

The number of adults with diabetes in 2010 was estimated to be 285 million, with prevalence of 6.4 % . By 2030, the estimated number will increase to 439 million with prevalence of 7.7 %. Number of deaths in adults due to diabetes among all age groups is 6.8 % at global level (Panchbhai, 2012; Madhikarmi et al., 2013).

The five countries with the greatest number of people with diabetes as of 2000 are India having 31.7 million, China 20.8 million, the United States 17.7 million, Indonesia 8.4 million, and Japan 6.8 million (Wild et al., 2004).

According to American diabetes association (American diabetes association, 2012) the current diagnostic criteria for diabetes are :

1. HA1C ≥ 6.5% the lest should be performed in a laboratory using a method that is National Glycohemoglobin Standardization Program (NGSP) certified and standardized to the Diabetes Control and Complications Trial (DCCT) assay.

OR

2. FPG ≥ 126 mg/dl (7.0 mmol/l). fasting is defined as no caloric intake for at least 8 h.

OR

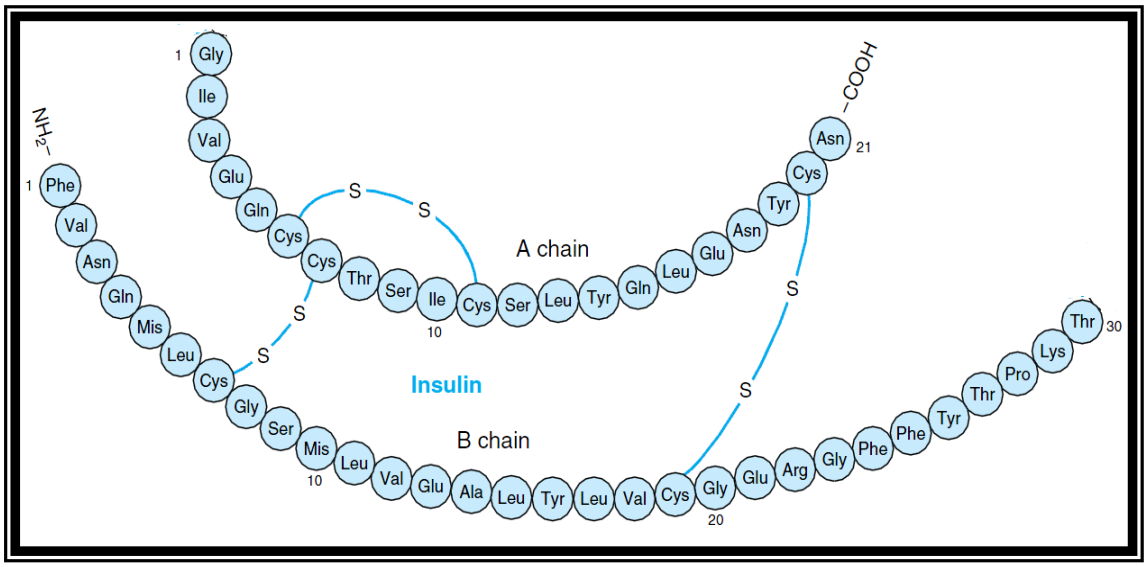
3. 2 h plasma glucose ≥ 200 mg/dl(11.1 mmol/l) during an OGTT. The test should be performed as described by the world health organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.

OR

4. In patients with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥ 200 mg/dl(11.1 mmol/l).

Insulin is a peptide hormone composed of 51 amino acid residues and has a molecular weight of 5808 Da. It is produced in the Islets of Langerhans in the pancreas. (Tippavajhala and Yamsani, 2010). Insulin consists of two polypeptide chains, the A- and B- chains which are linked together by two disulfide bonds (Priya et al., 2013) figure (1-1).

Insulin affects a wide range of physiological processes, although it is best known for its important regulatory role in glucose homeostasis. In response to elevation in plasma glucose, insulin secretion is increased and it stimulates glucose uptake and glycogen synthesis and inhibits glycogenolysis and gluconeogenesis , thus maintaining normoglycaemia. (Lin and Sun, 2010).



Figure(1-1): The chemical structure of human insulin (Murray et al., 2000)

1.2.2. Classification of diabetes Mellitus :-

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 and, in modified form, in 1985. The 1980 Expert Committee proposed two major classes of diabetes mellitus and named them, IDDM or Type 1, and NIDDM or Type 2. In the 1985 Study Group Report the terms Type 1 and Type 2 were omitted, but the classes IDDM and NIDDM were retained, and a class of Malnutrition–related diabetes Mellitus (MRDM) was introduced. In both the 1980 and 1985 reports other classes of diabetes were included other Types and Impaired Glucose Tolerance (IGT) as well as Gestational Diabetes Mellitus (GDM). These were reflected in the subsequent International Nomenclature of Diseases (IND) in 1991, and the tenth revision of the International Classification of Diseases (ICD–10) in 1992. The World Health Organization (WHO) in 1999 published the criteria that proposed four major classes of diabetes, and named them: type1, type 2, other specific types, and gestational diabetes(World Health Organization, 1999).

Expert committee on the diagnosis and classification of diabetes mellitus and American diabetes association made up the classification associated with gene defects of the pancreatic β cell function and insulin resistance; other syndrome associated with diabetes; disease of the exocrine pancreas; and endocrinopathies and diabetes induced by drugs, chemicals or infective agents (Expert committee on the diagnosis and classification of diabetes mellitus, 2003; American diabetes association, 2011).

Classification of diabetes mellitus (American diabetes association, 2011)

i. Type 1 diabetes *(β-cell destruction, usually leading to absolute insulin deficiency)
   A. Immune mediated
   B. Idiopathic

ii. Type 2 diabetes * (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)

iii. Other specific types
   A. Genetic defects of β-cell function
      1. Chromosome 12, HNF-1- (MODY3)
      2. Chromosome 7, glucokinase (MODY 2)
      3. Chromosome 20,HNF -4- (MODY1)
      4. Mitochondrial DNA
      5. Others
   B. Genetic defects in insulin action
      1. Type A insulin resistance
      2. Leprechaunism
      3. Rabson-Mendenhall
      4. Lipoatrophic diabetes
      5. Others
   C. Disease of the exocrine pancreas
      1. Pancreatitis
      2. Trauma/pancreatectomy
      3. Others
   D. Endocrinopathies
      1. Acromegaly
      2. Cushing's syndrome

3. Glucagonoma
4. Pheochromocytoma
5. Hyperthyroidism
6. Somatostatinoma
7. Aldosteronoma
8. others

E. Drug- or chemical- induced

1. Vacor
2. Pentamidine
3. Nicotinic acid
4. Glucocorticoids
5. Thyroid hormone
6. Diazoxide
7. β-a drenergic agonists
8. Thiazides
9. Dilantin
10. α- Interferon
11. Others

F. Infections

1. Congenital rubella
2. Cytomegalovirus
3. Others

G. Uncommon forms of immune-mediated diabetes

1. "Stiff-man"syndrome
2. Anti-insulin receptor antibodies
3. Others

H. Other genetic syndromes sometimes associated with diabetes

1. Down's syndrome
2. Klinefelter's syndrome
3. Turner's syndrome
4. Wolfram's syndrome
5. Friedreich's ataxia
6. Huntington's chorea
7. Laurence-Moon-Biedl syndrome
8. Myotonic dystrophy
9. Porphyria
10. Prader-Willi syndrome
11. Others

iv. Gestational diabetes mellitus (GDM)

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* Patients with any form of diabetes may require insulin treatment at some stage of their disease. Such use of insulin does not, of itself, classify the patient

1.2.2.1. Type 1 diabetes Mellitus (Insulin dependent diabetes or Juvenile diabetes):-

Type 1 diabetes mellitus (T1DM) is sometimes called juveline diabetes mellitus because most patients are children or adolescents at the time of diagnosis (Gorus et al,. 2004)

T1DM is usually due to autoimmune distruction of the pancreatic beta cells (Al-Saadi et al., 2011; Raha et al., 2013).

The subsequent lack of insulin leads to increased blood and urine glucose. The classical symptoms are polyuria (frequent urination), polydipsia (increased thirst), polyphagia (increased hunger), and weight loss (Cooke and Plotnick., 2008). Type 1 diabetes is fatal unless treated with insulin (Graveling and McIntyre., 2009).The first recipient of insulin was Leonard Thomopson a 14 years old on January 11, 1922 at Toronto general hospital (Khardori and Pauza,. 2003). The pathophysiology in diabetes type 1 is basically a destruction of beta cells in the pancreas, regardless of which risk factors or causative entities have been present. Individual risk factors can have separate pathophysiological processes to, in turn, cause this beta cell destruction. Still, a process that appears to be common to most risk factors is an autoimmune response towards beta cells, involving an expansion of autoreactive CD4+ T helper cells and CD8+ T cells, autoantibody-producing B cells and activation of the innate immune system (Bluestone et al., 2010; Chatzigeorgiou et al., 2010).

T1DM is determined by genetic factors (one of these genetic factors is the HLA system), lifestyle factors and environmental factors, in other words T1DM is a multifactorial disease (Gorus et al., 2004, Daneman, 2006, Cerna 2008, , Bluestone et al., 2010).

Beside the genetic predisposition, there is also environmental trigger required to develop T1DM. To date, a lot of environmental triggers have been suggested. However, Rubella is the only one that has been documented thoroughly. Other evidence for the important environmental role comes from the observation that migrants obtain similar incidence of the country they have been migrated (Daneman., 2006).

1.2.2.2. Type2 diabetes Mellitus (Noninsulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes):-

The most common form of diabetes is type 2 diabetes (T2DM). About 90- 95 percent of people with diabetes have type 2. This form of diabetes is most often associated with older age, obesity, family history of diabetes, previous history of gestational diabetes, physical inactivity, and certain ethnicities. About 80 percent of people with type 2 diabetes are overweight. When T2DM is diagnosed, the pancreas is usually producing enough insulin, but for unknown reasons the body cannot use the insulin effectively a condition called insulin resistance but after several years, insulin production decrease (diabetes overview, 2008).

Approximately 80 % of patients with type 2 diabetes are obese both of these condition are associated with insulin resistance(Thawnashom et al., 2011).

T2DM is commonly associated with other features of the insulin resistance syndrome: hyperlipidemia, hypertension, acanthosis nigricans, ovarian hyperandrogenism, non-alcoholic fatty liver disease(Criag, 2009).

Management of type 2 diabetes can be established by medication or surgery but it focuses on lifestyle interventions, lowering cardiovascular risk factors , such as hypertension, high cholesterol, and microalbuminuria, and maintaining blood glucose levels in the normal range (Ripsin et al., 2009). Intensive blood pressure management (less than 130/80 mmHg) as opposed to standard blood pressure management (less than 140–160/85–100 mmHg) results in a slight decrease in stroke risk but no effect on overall risk of death (McBrien et al., 2012).

There are several classes of anti-diabetic medications available. Metformin is generally recommended as a first line treatment as there is some evidence that it decreases mortality. Injections of insulin may either be added to oral medication or used alone (Ripsin et al., 2009). A second oral agent of another class may be used if metformin is not sufficient (Qaseem et al., 2012). Other classes of medications include: sulfonylureas, nonsulfonylurea secretagogues, alpha glucosidase inhibitors, thiazolidinediones, glucagon-like peptide-1 analog, and dipeptidyl peptidase-4 inhibitors (Ripsin et al., 2009, American Diabetes Association, 2012).

Weight loss surgery in those who are obese is an effective measure to treat diabetes (Picot et al., 2009). Many are able to maintain normal blood sugar levels with little or no medications following surgery (Frachetti and Goldfine, 2009) and long-term mortality is decreased (Schulman et al., 2009). There however is some short-term mortality risk of less than 1% from the surgery (Colucci, 2011). The body mass index cutoffs when surgery is appropriate are not yet clear (Schulman et al., 2009). It is recommended that this option be considered in those who are unable to get both their weight and blood sugar under control (Dixon et al .,2012).

Multiple defects in physiology have been identified in T2DM, including insulin resistance in muscle and adipose tissue, increased hepatic glucose production, impaired insulin secretion, decreased secretion and action of intestinal incretin hormones, and altered balance of CNS (central nervous system) pathways controlling food intake and energy expenditure. Therefore, when considering genetic and environmental factors in diabetes risk, it is critical to assess the individual

and collective impact of these defects on tissue-specific metabolism and net whole-body physiology (Figure1- 2)(Jin and Patti, 2009).

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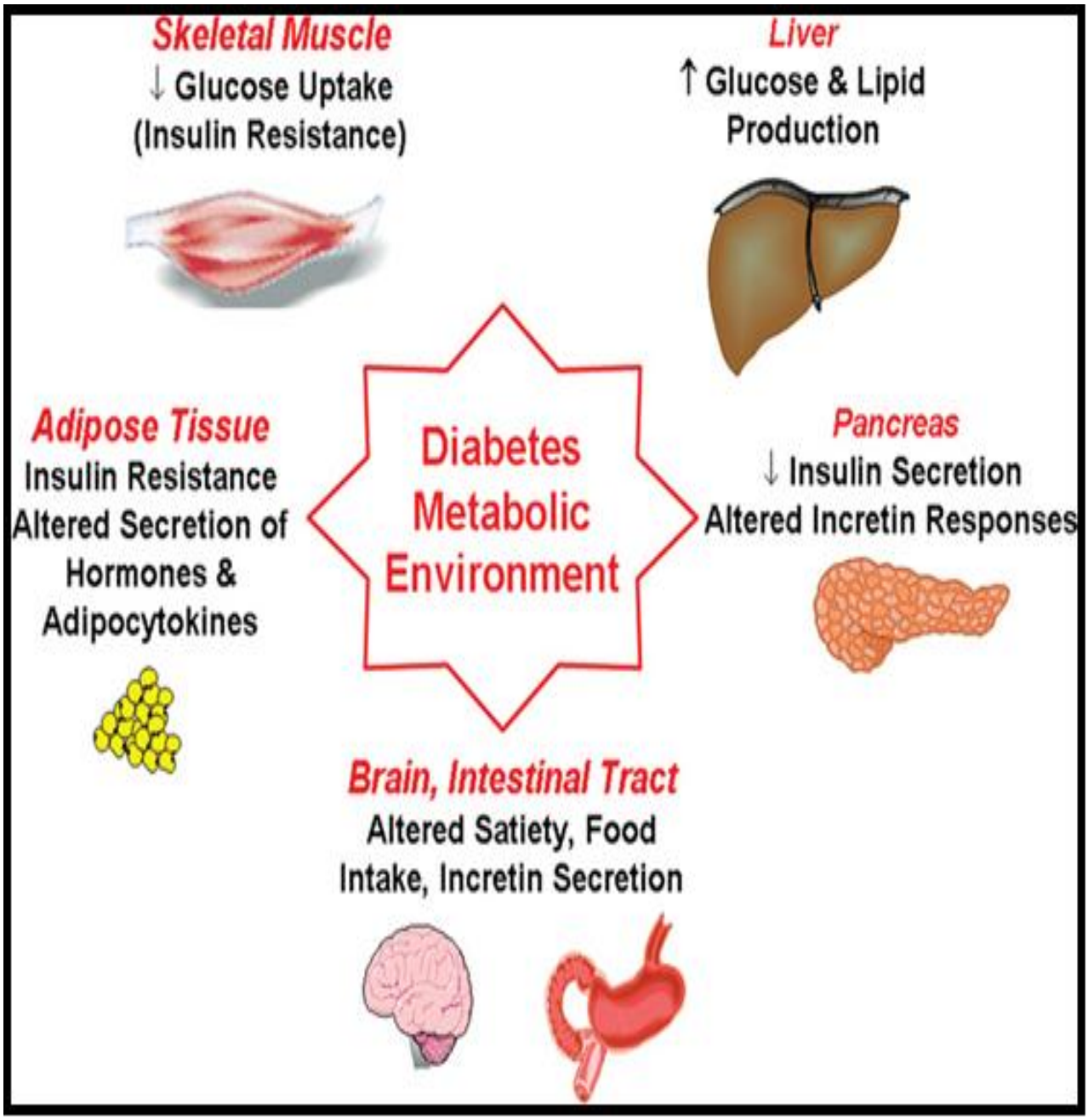


Figure(1-2): Multi-tissue contributions to the pathogenesis of T2DM (Jin and Patti, 2009).

1.2.2.3. Gestational diabetes mellitus (GDM):

Gestational Diabetes Mellitus (GDM) is a common disorder of pregnancy that can impact the health of both mother and baby. It is defined as an intolerance to carbohydrate that begins during pregnancy (Hedderson et al., 2010). This condition occurs in 7% of all pregnancies and prevalence rates are on the rise (The American College of Obstetricians and Gynecologists, 2011). Prevalence rates are increasing in all ethnic groups. However, there is an increased risk and prevalence of GDM in Hispanic and Asian women (Hedderson et al., 2010). In addition to ethnicity, other factors associated with increased risk for GDM are advanced maternal age, abnormally high body mass index (BMI) prior to pregnancy, and family history of diabetes. (The American College of Obstetricians and Gynecologists, 2011).

Gestational diabetes mellitus is characterized with both insulin resistance and impaired insulin secretion (Aris et al., 2011). It has well defined that GDM is an important risk factor for developing type 2 diabetes mellitus (T2DM) after pregnancy (Maghbooli et al., 2010).

Classical risk factors for developing gestational diabetes are (Ross, 2006):

- Polycystic Ovary Syndrome (PSO)
- A previous diagnosis of gestational diabetes or prediabetes, impaired glucose tolerance, or impaired fasting glycaemia
- A family history revealing a first-degree relative with type 2 diabetes

- Maternal age - a woman's risk factor increases as she gets older (especially for women over 35 years of age).
- Ethnic background (those with higher risk factors include African-Americans, Afro-Caribbeans, Native Americans, Hispanics, Pacific Islanders, and people originating from South Asia)
- Being overweight, obese or severely obese increases the risk by a factor 2.1, 3.6 and 8.6, respectively (Chu et al., 2007).
- A previous pregnancy which resulted in a child with a macrosomia (high birth weight: >90th centile or >4000 g (8 lbs 12.8 oz))
- Previous poor obstetric history
- Other genetic risk factors: There are at least 10 genes where certain polymorphism are associated with an increased risk of gestational diabetes (Zhang et al., 2013).

In addition to this, statistics show a double risk of GDM in smokers (Bjorge et al., 2004) although relevant evidence remains controversial (Toulis et al., 2009). Some studies have looked at more controversial potential risk factors, such as short stature (Ma et al., 2007).

1.2.2.4. Other specific types of diabetes:

Maturity-onset diabetes of youth is characterized by impaired insulin secretion with minimal or no insulin resistance (Froguel, 1993). Patients typically exhibit mild hyperglycemia at an early age. The disease is inherited in an autosomal dominant pattern with at least six different loci identified to date. Genetic inability to convert proinsulin to insulin

results in mild hyperglycemia and is inherited in an autosomal dominant pattern. Similarly, the production of mutant insulin molecules has been identified in a few families and results in mild glucose intolerance. Several genetic mutations have been described in the insulin receptor and are associated with insulin resistance. Type A insulin resistance refers to the clinical syndrome of acanthosis nigricans, virilization in women, polycystic ovaries, and hyperinsulinemia. In contrast, type β insulin resistance is caused by autoantibodies to the insulin receptor. Leprechaunism is a pediatric syndrome with specific facial features and severe insulin resistance because of a defect in the insulin receptor gene. Lipoatrophic diabetes probably results from postreceptor defects in insulin signaling (Froguel, 1993).

1.2.3. Markers of insulin sensitivity and resistance:

Various insulin sensitivity & resistance measures as reported by different authors were listed in table (1-1).

Table (1-1): Various insulin sensitivity & resistance measures

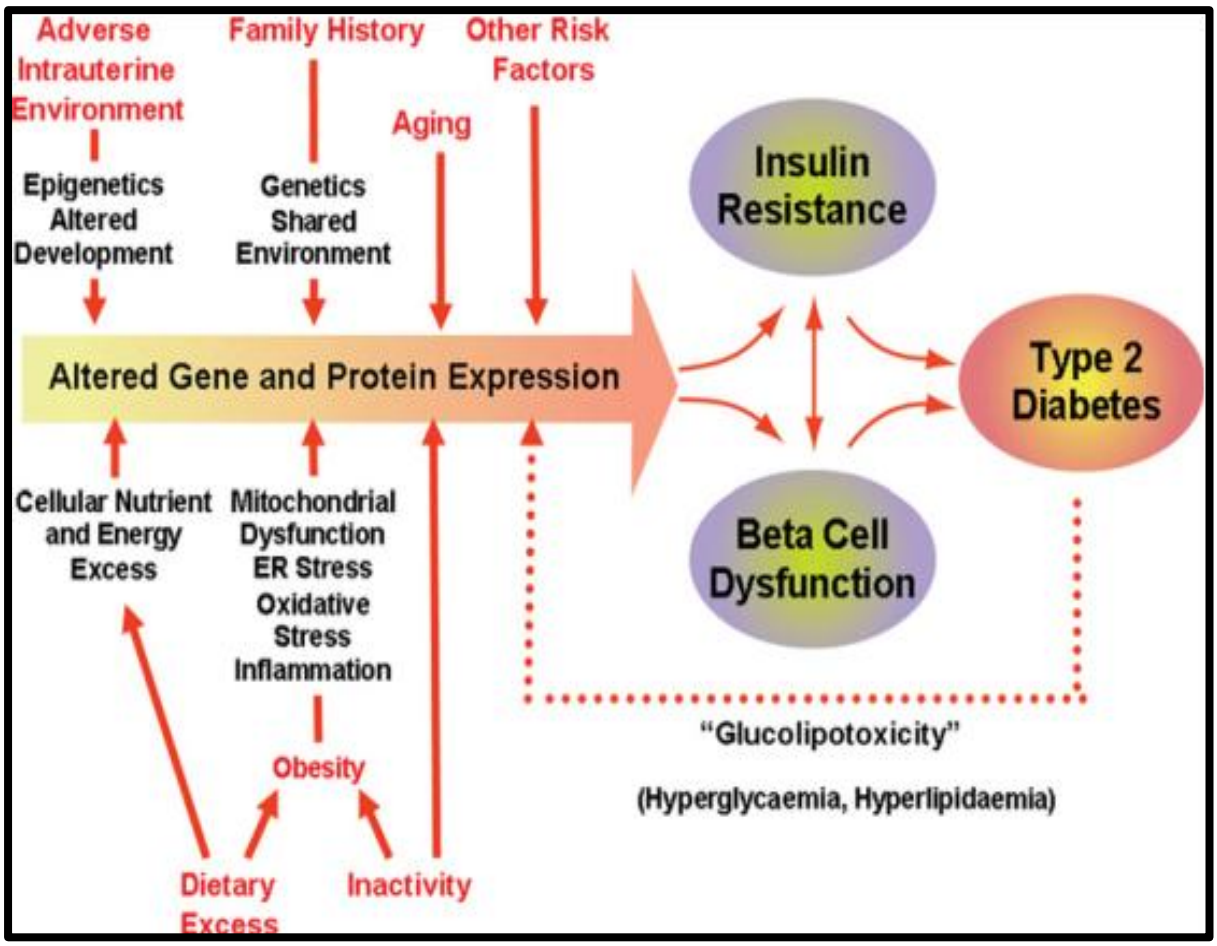
1. Homeostatic model assessment of insulin resistance
   HOMA-IR = FI * FG /405 (Esteghamati et al., 2010)
2. Insulin sensitivity index
   ISI = 10^4 /FI*FG (Gupta and Jain, 2004)
3. Fasting insulin resistance index
   FIRI = FI * FG /25(Singh and Saxena, 2010)
4. Fasting glucose insulin ratio
   FGIR = FG /FI mg/10^{-4}U (Gupta and Jain, 2004)
5. Homeostatic model assessment of β-cell function
   HOMA-β = 20* FI (μU/dl) / (FG (Mm)-3.5) (Mills et al., 2009)

1.3. Risk factors for diabetes and progression to T2DM

Risk factors for the development and/or progression of T2DM include

1. genetics/family history, resulting in alterations in DNA sequence (Uusitupa et al., 2011; Park, 2011).
2. Obesity & Physical inactivity: Insulin resistance is believed to be associated with decreased physical activity (Midhet et al., 2010; Sethi et al., 2011).
3. Lipid: many studies have suggested that the consumption of specific dietary fats, particularly low omega-6 polyunsaturated fatty acids and high trans unsaturated fatty acids, increases the risk of T2DM and suggest that omega-3 fatty acids could lower the risk of T2DM (Kaushik et al., 2009).
4. gestational diabetes (Kwak et al., 2013).
5. Smoking: Several prospective studies reported that current smoking is a risk factor for developing type 2 diabetes (wang et al., 2013).

Previous factors and another factors contribute to the development of T2DM as shown in Figure (1-3).



Figure(1-3): Multiple risk factors are involved in the pathogenesis of T2DM(Jin and Patti, 2009).

1.3.1. Definition and classification of obesity:

Obesity refers to excess body fat. It is due to greater energy intake compared with energy expenditure (Mukhopadhyay, 2012). The predisposing factors to obesity may be physical, nutritional, pharmacological, maturational, socioeconomic and environmental factors (Osfor et al., 2013).

The degree of obesity is most commonly classified according to the BMI [defined as the weight divided by the square of the height (kg/m2)] (Table 1-2).

Table (1-2): The world health organization's (WHO)classification of obesity

	BMI (kg/m^2)	**Risk of comorbidity**
Underweight	<18.5	Low
Normal	18.5-24.9	Average
Overweight	≥25.0	
Pre obese	25.0-29.9	increased
Obese class I	30.0-34.9	Moderate
Obese class II	35.0-39.9	Severe
Obese class III*	≥40.0	Very sever

*extreme obesity

In addition, other anthropometric measures, and especially measures of abdominal obesity, have been used to identify subjects at risk of obesity related metabolic conditions. Due to different patterns of fat accumulation in men and women sex-specific cut-off values exist. In Caucasians, waist-to-hip ratio (WHR) > 1.0 in men and > 0.85 in women indicates abdominal fat accumulation (James, 1996). Furthermore, cut-off point for waist circumference (WC), which may provide a more practical

correlate of abdominal fat distribution than WHR, has been suggested (Table 1-3)(world health organization, 2010).

Table (1-3): Sex specific waist circumference and risk of metabolic complications associated with obesity in caucasians.

	Waist circumference (cm)	
risk of metabolic complications	men	women
increased	≥94	≥80
Substantially increased	≥102	≥88

Obesity is increasingly prevalent worldwide and has reached epidemic proportions. It is tightly associated with type 2 diabetes (T2DM) (Grill, 2006), cardiovascular disease (Haffner, 2006), and various cancers (Calle, 2004).

Normally, human body is equipped with physiological and biochemical responses to counteract day-to-day fluctuations in food intake (FI). For instance, acute elevations in insulin and leptin induced by nutrients (Rossetti, 2000)) decrease FI and increase energy expenditure (Lopez et al., 2007). Conversely, the fasting state shifts the energy balance such that energy stores are maintained by an increase in FI (Lelliott and Vidal-puig, 2004). As a result of these energy status feedback signals, the caloric storage and body weight (BW) are generally stable for most humans over long periods of time despite the wide variations in day-to-day FI patterns. Unfortunately, chronic exposure to

high caloric diets combined with reduced physical activity lead to a disruption in this precise energy homeostatic control (Lopez et al., 2007).

1.3.2. Obesity as a risk factor of type 2 diabetes:

Obesity is associated with increased risk of developing insulin resistance and type 2 diabetes. In obese individuals adipose tissue releases increased amounts of non-esterified fatty acids, glycerol, hormones، pro-inflammatory cytokines and other factors involved in the development of insulin resistance. When insulin resistance is accompanied by dysfunction of the beta cells, the following fall in insulin secretion results in failure to control blood glucose level leading to type 2 diabetes. Many genes interact with the environment leading to obesity and in some also to diabetes. Many genes have been shown to be involved in determining the whole range of BMI in a population, with each gene only explaining a few hundred grams difference in body weight (Hebebrand and Hinney, 2009). Genes responsible for obesity and insulin resistance interact with environmental factors such as increased fat/ calorie intake and decreased physical activity resulting in the development of obesity and insulin resistance followed ultimately by the development of type 2 diabetes (Kahn et al 2006, O'Rahilly and Farooqi 2006).

1.4. vitamin A:

The retinoids, a family of molecules, that are related to dietary retinol (vitamin A), are essential for vision, reproduction, growth and maintance of epithelial tissues. They also play a role in immune function. Retinoic acid derived from oxidation of retinol, mediates most of the actions of the retinoids, except for vision, which depend on retinal, the aldehydes derivative of retinol (Denise and Ferrier,2014).

1.4.1. Vitamin A metabolism:

Vitamin A belongs to the group of fat-soluble vitamins and exists in several chemical structures such as retinol (ROH), retinyl esters (REs), retinaldehyde (RAL) and the biologically most active form retinoic acid (RA) (Gottesman *et al.* 2001). In particular, REs are the storage form of ROH and the biologic active form RA may act in the nucleus as a transcription factor. Since mammals cannot synthesize vitamin A, it is essential, that the diet contains adequate amounts of the vitamin (Goodman 1984; Sporn *et al.* 1994). Dietary vitamin A sources of preformed vitamin A are animal products such as liver, milk and eggs whereas dietary sources of the provitamin A carotenoids such as β-carotene are vegetables and fruits (Goodman 1984).

The retinoids, ROH and its metabolites, are involved in the process of vision, the immune function, reproduction and embryogenesis. The major end-point of vitamin A deficiency is blindness which is still very common in the developing countries (Desvergne 2007). Vitamin A is also essential for cell proliferation and differentiation (Blaner 2007): For example, after experimental induced myocardial infarction, hepatic ROH

is mobilised and delivered to the damaged tissue (Palace *et al.* 1999). The action of vitamin A is accomplished by RA which performs its functions through the binding to nuclear hormone receptors by activating the transcription of certain genes (Chambon 2005; Piantedosi *et al.* 2005; Isken *et al.* 2008).

1.4.2. Absorption, transport and storage:

After the digestion of REs or carotenoid containing foods ROH is absorbed from the intestine and is bound to a specific retinol-binding protein (RBP), the cellular retinol binding protein 2 (CRBP2), in the enterocyte. The binding is important, since ROH is hydrophobic and can easily be incorporated into membranes and disturb normal cell activities. Therefore RBPs protect the cells against ROH toxicity (Piantedosi et al. 2005). CRBP2 facilitates the incorporation of ROH into chylomicrons. Within the chylomicrons ROH is transported to the liver (Ghyselinck et al. 1999) where these are taken up by the liver cells. Within the hepatocytes ROH is bound to cellular retinol binding protein type I (CRBP-I) which transports ROH to the hepatic stellate cells. There it is esterified with long chain fatty acids to its main RE, retinyl palmitate, and stored. This process is mainly catalysed by the enzyme lecithin: retinol acyltransferase (LRAT) in the liver and the eye and by the enzymatic activity called acyl-CoA: retinol acyltransferase (ARAT) (Ghyselinck et al. 1999; O'Byrne et al. 2005). To that date the molecular identification and characterization of the enzyme carrying out the ARAT reaction in the liver and extra-hepatic tissues remains unclear (O'Byrne et al. 2005). The liver is the central organ in ROH metabolism, storing and releasing ROH in dietary excess and deficiency, respectively (Ghyselinck et al. 1999).

The release of ROH from the liver is triggered by the binding of retinol-binding protein 4 (RBP4) to ROH and the additional binding of this complex to transthyretin (TTR). TTR is therefore an indirect ROH transport protein and protects the RPB4-ROH complex of being filtered by the renal glomerulus. Within the complex ROH is transported to its target tissues e.g. the eye where RBP4 binds to its receptor (Stimulated by Retinoic Acid 6, STRA6) and ROH is transferred into the target cell (Kawaguchi et al. 2007). The remaining RBP4, which is then called apo-RBP4, is rapidly catabolised in the kidneys (Gerlach and Zile 1991). Through the reabsorption of ROH and RBP4 in the proximal tubular system, the kidneys are known to maintain whole body ROH homeostasis (Raila et al. 2007). REs – in contrast to ROH, which is bound to RBP4 – are transported via lipoproteins in the serum which account for 5% of total plasma ROH (Mills et al. 2008).

1.4.3. Cellular actions of vitamin A and its metabolites:

Within the cytoplasm of target cells ROH is bound to its cellular retinol-binding proteins (CRBPs), namely CRBP-I, CRBP-II and CRBP-III1 (Noy 2000; Zizola et al. 2008). CRBPs bind ROH and mediate esterification and facilitate the oxidation to the intermediate RAL and to the major biologically active end-product RA (Zizola et al. 2008). RA regulates gene expression through its binding to the nuclear hormone receptors retinoic acid receptor (RAR) and the retinoid X receptors (RXR) (Blaner 2007). The enzymes alcohol dehydrogenase (ADH) and retinaldehyde dehydrogenase (RALDH) catalyse the oxidation of ROH to RA (Figure 1). Next to the liver, the white adipose tissue (WAT) may store about 20% of body retinoids as REs and the per cell retinoid content

is twice as much as in hepatic stellate cells (Tsutsumi et al. 1992; Yang et al. 2005; Yoshida et al. 2006).

However, adipose tissue is also a target for retinoids as these may modulate adipose structure and its metabolic function through their binding to the nuclear hormone receptors RAR and RXR. In fact, by binding to the ligand binding domain of RAR or RXR RA can inhibit or enhance adipogenesis, respectively (Ziouzenkova and Plutzky 2008). However, to that date, metabolism of ROH concerning the storage and action in adipose tissue remains unclear. The intracellular metabolism of vitamin A and its metabolites is exemplified in Figure (1-4).

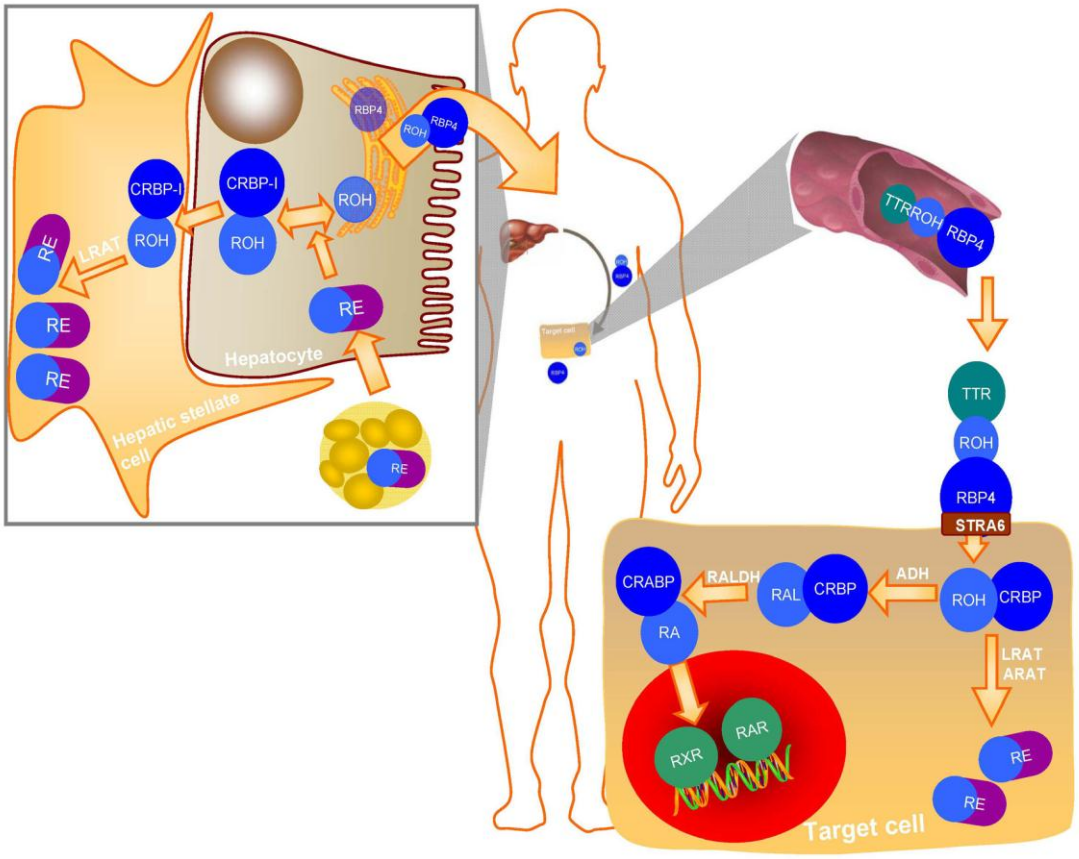


Figure (1-4) Intracellular metabolism of retinol **(ROH).**ROH is transported in a complex with RBP4 and TTR to the target cells where it binds to the receptor Stimulated by Retinoic Acid 6 (STRA6). Within the cell ROH is bound to cellular retinol binding proteins (CRBP) and may be oxidised to retinaldehyde (RAL) and retinoic acid (RA). The reactions are catalysed by alcohol dehydrogenase (ADH) and RA dehydrogenase (RALDH). RA may activate the nuclear receptors RXR and RAR and induce the transcription of certain genes

1.5. Retinol binding proteins:

1.5.1. Intracellular retinol-binding protein type I (CRBP-I)

CRBP-I has been discovered in 1973 and since that date its localization, expression and structure have been studied (Ghyselinck et al. 1999). CRBP-I is highly expressed in the liver, kidney, heart, muscle and brain (Noy 2000; Piantedosi et al. 2005) and also in WAT (Zizola et al.

2008). The main function of CRBP-I is to transport ROH to its metabolizing enzymes for esterification to RE or oxidation to RA (Ghyselinck et al. 1999). Therefore, CRBP-I knock-out (CRBP-I KO) mice show reduced hepatic retinyl palmitate (main ester of ROH) stores but do not show signs of ROH deficiency if maintained on a vitamin A containing diet (Ghyselinck et al.1999). In contrast, if maintained on a retinoid-insufficient diet CRBP-I KO mice show signs of hypovitaminosis A earlier compared to wild-type (WT) mice (Piantedosi et al. 2005). CRBP-I expression increases when intracellular levels of ROH are low thereby increasing the uptake of ROH into the cell (Noy 2000). The main ROH esterifying enzyme in the liver is lecithin:retinol acyltransferase (LRAT). Although CRBP-I is also synthesised in extra-hepatic tissues such as the adipose tissue, the function of CRBP-I in these tissues remains unclear. In fact, the process of esterification of ROH in extra-hepatic tissues is known to be mediated by the enzymatic activity ARAT but the enzyme behind the process is unclear (Ghyselinck et al. 1999; Piantedosi et al. 2005). In addition, by its binding to ROH it protects the cells of the membranolytic effects of ROH (Noy 2000). The concentration of ROH is always lower than that of CRBP-I, as free ROH is toxic for cells (Ghyselinck et al. 1999).

1.5.2. Intracellular retinol-binding protein type II (CRBP-II)

CRBP-II is mainly expressed in the small intestine and is involved in retinoid incorporation into chylomicrons (Ghyselinck et al. 1999; Noy 2000; Piantedosi et al. 2005).

1.5.3. Intracellular retinol-binding protein type III (CRBP-III)

CRBP-III was discovered in 2001 and belongs – like CRBP-I and CRBP-II – to the family of fatty acid binding proteins (Vogel et al. 2001). CRBP-III is highly expressed in tissues with little or no CRBP-I expression. In adipose and mammary tissue both proteins are expressed, whereas no CRBP-III is found in the liver. CRBP-III binds ROH, but not RAL, in the cytosol (Piantedosi et al. 2005). This is in contrast to CRBP-I and CRBP-II which both also bind RAL. To that date it is believed that the main function of CRBP-III is to facilitate ROH esterification in the mammary gland during lactation (Piantedosi et al. 2005). Mice lacking CRBP-III (CRBP-III KO) are viable and healthy but show a decreased amount of retinoids in the milk (Piantedosi et al. 2005). Apart from this, its physiologic role is still under consideration. In fact, it has been

shown that CRBP-III KO mice maintained on a high-fat diet (HFD) remain more glucose tolerant and accumulate less triglycerides in the liver compared to wildtype (WT) mice. This is partly due to a lower release of free fatty acids from the adipose tissue in the circulation (Zizola et al. 2008). Moreover, CRBP-III is expressed in both adipocyte cell types, adipocytes and stromal vascular cells. Since CRBP-I is entirely expressed in stromal vascular cells one might assume that the proteins have different physiological roles within adipose tissue (Vogel et al .2001).

In summary, both, extra- and intracellular retinol-binding proteins, solubilize and stabilize their hydrophobic ligand ROH in the aqueous space and are important for the regulation of ROH transport, metabolism and action (Noy 2000).

1.5.4. Extracellular retinol binding protein type IIII:

Retinol-binding protein (RBP4), a21 KD protein synthesized in the liver and adipose tissue (Koch et al, 2010) belongs to a lipocalin protein family and functions as a carrier protein for vitamin A in serum. It was first isolated from human sera (Kanai et al., 1968) and subsequently from the sera of other vertebrates-birds, amphibians, reptile, and fish (Zanotti and Berni, 2004). Human retinol-binding protein circulating in blood consists of 183 amino acid residues. In blood RBP4 carries retinol (vitamin A) which is bound to RBP4 in equimolar ratio (Jaconi S., 1995).

Unlike other adipokines, RBP4 is predominantly synthesized in hepatocytes and secreted into the circulation bound to transthyretin (TTR). Binding to TTR increase the molecular weight of RBP4 from approximately 21 KDa to approximately 76 KDa, preventing its loss through filtration by the renal glomeruli (Thawnashom et al, 2011) and according to Jaconi et al. only small fraction of free RBP4 can be found in serum (Jaconi S., 1995).

RBP4 has been studied since 1960s mainly as a transporter of retinol, but recent data suggest that RBP4 may contribute to pathogenesis of type 2 diabetes. Yang et al. demonstrated that serum RBP4 levels are elevated in patients with obesity and type 2 diabetes. Studies in mice showed that serum RBP4 may cause insulin resistance (Yang et al, 2005). So, on the one hand, there is growing body of evidence demonstrating that RBP4 is a promising marker of the risk of type 2 diabetes, but on the other hand there is conflicting situation in the literature regarding RBP4 clinical utility in predicting insulin resistance and type 2 diabetes (Qi et al, 2007). Some authors show strict correlation between circulating RBP4 and magnitude of insulin resistance in subjects with obesity and type 2

diabetes and non-obese subjects with family history of type 2 diabetes (Graham et al, 2006). On the contrary, others (Lewis et al, 2008, Promintzer et al, 2007) had not found any correlation between those variables. This confusing situation could be at least partially explained by the heterogeneity of the RBP4 in serum and by methodological short-comings in determining level of circulating RBP4 (Graham et al, 2007).

Experimental studies have shown that increased RBP 4 expression impairs insulin signaling in muscle and increase gluconeogenesis in animals, suggesting its involvement in the pathogenesis of insulin resistance. However, data in humans are less conclusive: serum RBP 4 levels have been described elevated in obesity, type 2 diabetes mellitus, and polycystic ovary syndrome, but other authors have failed to confirm these observation (Soloni et al, 2009).

1.6. Aims of the study:

1. Evaluate the levels of retinol and retinol binding protein 4 as well as RBP4 / retinol ratio in patients and healthy controls with type 2 diabetes mellitus.
2. Study the correlation between RBP 4 and retinol in healthy controls and patients with type 2 diabetes mellitus.
3. Study the correlation between RBP 4 levels & insulin resistance

Chapter two

Subjects and Methods

2.1. Subjects and study protocol:-

The study was carried out during the period from March 2012 to June 2012. The study group comprised 45 patients (mean ± SE, 7 men and 38 women) with obese type 2 diabetes mellitus (mean age 52.14 ± 1.01 years) diagnosed according to American diabetes association(American diabetes association, 2012) and 34 controls (mean ± SE, 6 men and 28 women, mean age 48.79 ± 1.17 years). The controls were divided into two groups: normal (group 1, n=9) and obese (group 2, n= 25).

All patients were recruited from the International Center of Diabetes and outpatient Diabetes clinic of the AL-Kadhmia Teaching Hospital, under supervision of Dr. Mahmood Sh. Khudhair, A senior physician at AL-kadhmia Teaching Hospital .

The main exclusion criteria were as follows:- any recent illness, impaired thyroid or renal disease, pancreatic, liver diseases, diabetic patients treated with insulin, pregnant women.

Clinical characteristic of the groups studied:

Patient group contains 45 oral diabetic patients. Control group (n=34) were divided according to body mass index (BMI) into:

1. Group 1: normal control group with BMI < 25 (n = 9) .

2. Group 2: obese control group with BMI ≥ 30 (n = 25) .

The basic anthropometric and clinical parameter of the groups studied are presented in table (2-1). In this study, there were no statistical significant differences between age groups ($p > 0.05$). The standard error of mean age (Mean ± SE) of patients group (group 1) was 52.14 ± 1.01 years, it was 49.22 ± 1.88 years in normal controls group (group 2) while, in obese controls group(group 3) was 48.38 ± 1.45 years. The standard error of mean(Mean ± SE) for BMI 36.62 ± 0.8 Kg/m^2 in group (1) while it was (22.53 ± 0.73 & 35.2 ± 1.00 Kg/m^2) in group (2&3) respectively. The standard error of mean(Mean ± SE) for W.C. 114.37 ± 1.76 cm in group (1) while it was (88.86 ±2.89 & 108.9 ± 2.13 cm) in group (2&3) respectively.

Table (2-1): Basic anthropometric and clinical parameter of the groups studied.

Characteristic	**patient**	**Group 1**	**Group 2**	**P-value**
Number	45	9	25	
Male/female	7/38	4/5	2/23	
Age (year)	52.14 ± 1.01	49.22 ± 1.88	48.38 ± 1.45	N.S
BMI (Kg/m^2)	36.62 ± 0.8	22.53 ± 0.73	35.2 ± 1.00	N.S
W.C. (cm)	114.37± 1.76	88.86 ± 2.89	108.9 ± 2.13	N.S
Duration (years)	4.06 ± 0.67			

2.2. Blood collection and sample preparation;-

About ten milliliters of venous blood were collected from each subject in the study after a 12- hour fast. The blood was divided into two parts; one part (about two milliliters) was collected into EDTA containing tubes and sent to the hospital laboratory for Glycated HbA1c assay (was measured using Ion-Exchange High Performance liquid Chromatography (HPLC)) . The remaining part of the blood was centrifuged at 3000 rpm for 7 min in a centrifuge after about 30 minute from the time of blood collection.

The serum formed was divided into small aliquots for:-

a- Immediate measurements of serum glucose, lipid profile (total cholesterol, HDLc, TG), serum urea and serum creatinine was done using appropriate enzymatic colorimetric method .
b- The rest was stored at -20 until assayed for insulin, Retinol binding protein 4 and Retinol. All of these were measured using enzyme immunoassay using enzyme-linked immunosorbent assay (ELISA) kits.

2.3. Instruments:-

The instruments used in this study are listed in the following:-

Table (2-2): The instruments and their companies:

INSTRUMENT	**COMPANY**
Centrifuge	Hettich universal
(UV/VIS)Spectrophotometer	Shimadzu-1601
ELISA reader & washer	Bio-teck ELX800
Water bath	w- Germany

*2.4. **Chemicals:***

The biochemical kits used in this study were obtained from the following companies:-

Table (2-3): The kits and their companies:

CHEMICALS	**COMPANY**
Glucose Kit	Biolabo-France
Total Cholesterol Kit	Biolabo-France
Triglyceride Kit	Biolabo-France
High Density Lipoprotein Kit	Biolabo-France
Urea Kit	Linear Chemicals-Spain
Creatinine Kit	Linear Chemicals-Spain
CRP-hs Kit	demeditec -Germany

Insulin Kit	demeditec-Germany
Retinol binding protein 4 Kit	Cusabio-China
Retinol Kit	Cusabio-China

2.5 Methods:-

2.5.1. Determination of HbA1c:-

Glycated hemoglobin (HbA1c) was measured by Variant ^{TM} HbA1c program, which is intended for the determination of HbA1c in human whole blood using Ion-Exchange High Performance Liquid Chromatography (HPLC) (Rohfing, 2000; ADA position statement,1997).

Principle:-

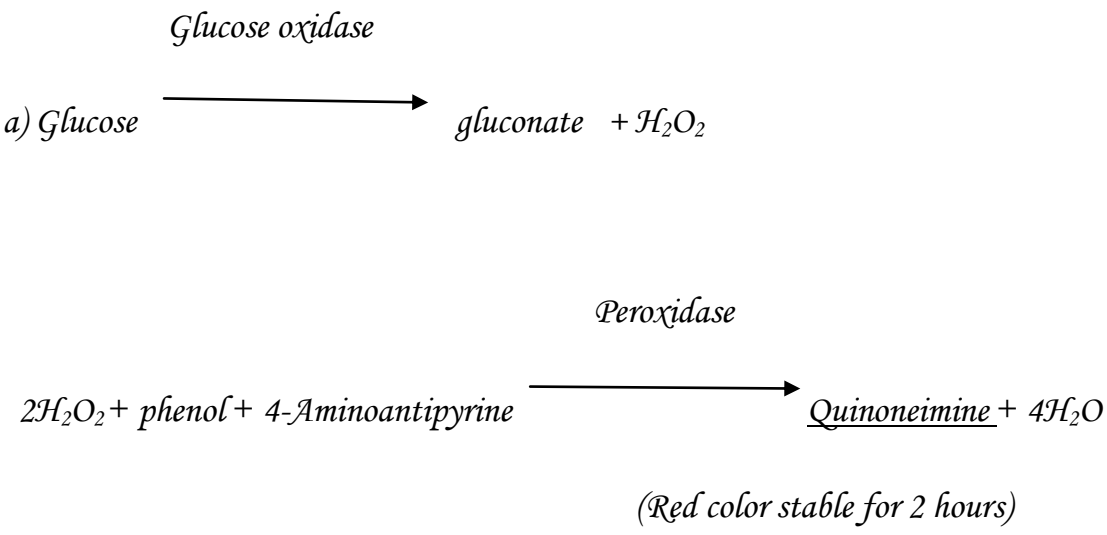
The Variant HbA1c program utilizes the principles of Ion-Exchange HPLC for the automatic and accurate separation of HbA1c. The separation of HbA1c is performed rapidly and precisely without interference from Schiff base, lipemia or temperature fluctuations.

The Variant dual-piston delivers a programmed buffer gradient of increasing ionic strength to the system. Prepared samples are automatically injected into the analytical flow path and applied to the cation exchange column, where the column Hemoglobin (Hb) is separated based upon the attraction of the Hb to the column material. The separated Hb then passes through a flow cell of the filter photometer,

where changes in absorbance (415nm) are measured. Background variations are corrected by an additional filter at (690nm). Preceding analysis, a simple preparation of the patient's sample is required to hemolyse the blood and then incubated at (18-28°c) for a minimum of (15 min)

2.5.2. Determination of serum glucose:-

Serum glucose was measured by the glucose kit PAP 100(Biolabo, France), using the enzymatic method (Burtis and Ashwood, 1999; Bernard, 1989) based on the following reaction:-



The absorbance was read at 500 nm after 5 min at 37 °C.

2.5.3. Determination of serum lipid profile:

2.5.3.1. Determination of serum total cholesterol (T.C):

Serum total cholesterol (T.C) was measured by the cholesterol kit CHOD PAP 100 (Biolabo, France), using the enzymatic method (Allian, 1974) based on the following reaction:-

a) Cholesterol ester $\xrightarrow{\text{Cholesterol esterase}}$ Cholesterol + free fatty acids

b) Cholesterol + O_2 $\xrightarrow{\text{Cholesterol oxidase}}$ Cholestene 4 one 3+ $2H_2O_2$

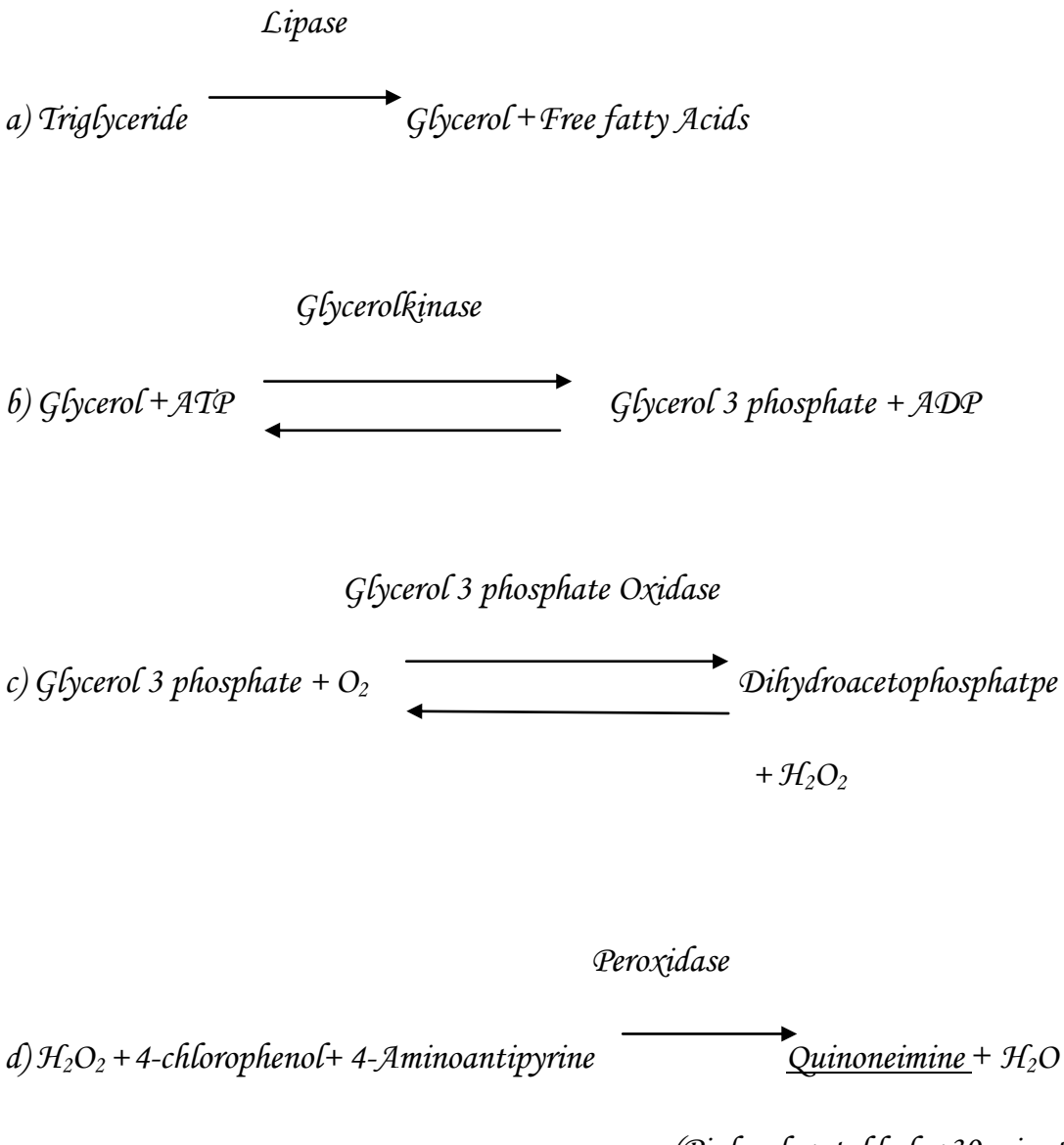
c) $2H_2O_2$ + phenol + 4-Aminoantipyrine $\xrightarrow{\text{Peroxidase}}$ Quinoneimine + $4H_2O$

(Pink color stable for 30 minute)

The absorbance was read at 500 nm after 5 min at 37 °C

2.5.3.2. Determination of serum triglycerides (T.G):-

Serum triglyceride was measured by the triglyceride kit PAP 150 (Biolabo, France), using the enzymatic method (Trinder, 1969; fossati, 1982)



The absorbance was read at 500 nm after 5 min at 37 °C.

2.5.3.3. Determination of serum high density lipoprotein cholesterol (HDLc):-

Serum high density lipoprotein cholesterol was measured by the HDLC kit PAP 150(Biolabo, France), using the enzymatic method (Tietz, 2006) which is based on the following:

The chylomicron and very low density lipoprotein (VLDL) and low density lipoprotein (LDL) contained in the sample are precipitated by the addition of phosphotungestic acid in the presence of magnesium ions. The supernatant obtained after centrifugation contains HDL in which the cholesterol can be determined using cholesterol enzymatic reagents.

2.5.3.4. Determination of serum low density lipoprotein cholesterol (LDLc):-

Serum LDL can be determined indirectly using Friedewald equation (Friedewald &Levy, 1972):

Total cholesterol = HDLc + (T.G/5) +LDLc or

LDLc = Total cholesterol - HDLc - (T.G/5)

*This formula is only valid when T.G. concentration not exceeds 3.5 mmol/L (400 mg/dl)

2.5.4. Determination of serum urea:-

Serum urea was measured by the urea kit 150(Linear, Spain), using the enzymatic method (Chaney and Marbach, 1962; Searcy, 1967) based on the following reaction:-

$$a)\ Urea + H_2O \xrightarrow{Urease} 2NH_3 + CO_2$$

$$b)\ NH_4^+ + salycilate + NaClO + OH^- + nitroprusside \rightarrow \underline{indophenol} + Nacl$$

green color stable for 2 hour

The absorbance was read at 600 nm after 5 min at 37 °C

2.5.5. Determination of serum creatinine:-

This procedure is based upon a modification of the original picrate reaction. Creatinine under alkaline conditions reacts with picrate ions forming a reddish complex. The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time which is proportional to the concentration of creatinine in the sample (Rartels and Bohmer 1971, Larsen 1972, Heinegaard and Tinderstrom 1973).

Blood urea & serum creatinine were measured for all subjects to exclude any renal problem.

2.5.6. Determination of serum C-Reactive Protein high sensitive (hsCRP):-

a- principle

The hsCRP determination is based on Enzyme Linked Immunosorbent assay(ELISA). The assay system utilizes microtiter strips coated with anti-CRP antibody are incubated with diluted standard sera and patient samples. During the incubation step CRP is bound specifically to the wells. After removal of the unbound serum proteins by a washing procedure, the antigen-antibody complex in each well is detected with specific peroxidase-conjugated antibodies.

After removal of the unbound conjugated, the strips are incubated with a chromogen solution containing tetramethylbenzidin and hydrogen peroxide: a blue color develops in proportion to the amount of immunocomplex bound to the wells of the strips. The enzymatic reaction is stopped by the addition of 1N acidic solution and the absorbance values at 450 nm are determined.

A standard curve is obtained by plotting the absorbance values versus the corresponding standard values. The concentration of CRP in patient samples is determined by interpolation from the standard curve.

CRP high sensitive was measured for all subjects to exclude any inflammatory problem.

b-assay procedure

1. The standard sera are diluted 1:100 as follows :10 µl of each calibrator was pipette into separate glass dilution tubes, 990 µl of diluted Specimen Dilution Buffer was added and mixed carefully.
2. The patient samples are diluted 1:1000 in two consecutive steps: 10 µl of each patient sample was pipette into separate glass dilution tubes and 990 µl of diluted Specimen Dilution Buffer was added. Mixed thoroughly, 450 µl of diluted Specimen Dilution Buffer was added to 50 µl of these 100 × prediluted samples. Mix thoroughly.
3. 100 µl of the diluted calibrators and samples were pipette into each of a pair of adjacent wells.
4. The covered microtiter strips were incubated for 30 ± 2 min at room temperature.
5. The microtiter strips were washed three times with Washing Solution. This can either be performed with a suitable microtiter plate washer by briskly shaking out the contents of the strips and immersing them in washing solution. Washing solution was changed for each cycle. Finally the microtite rstrips was empty and excess fluid was removed by blotting the inverted strips on adsorbent paper.
6. 100 µl of conjugate solution was added and the covered microtiter strips was incubated for 30 ± 2 min at room temperature.
7. The washing procedure was repeated as described in 5.
8. 100 µl of Chromogen solution was added to each well.
9. Incubated for 10 ± 2 min at room temperature. Exposure to light was avoided during this step.
10. 50 µl of stopping solution was added to each well.

11. The absorbance of each well was determined at 450 nm within 30 min following the addition of acid.

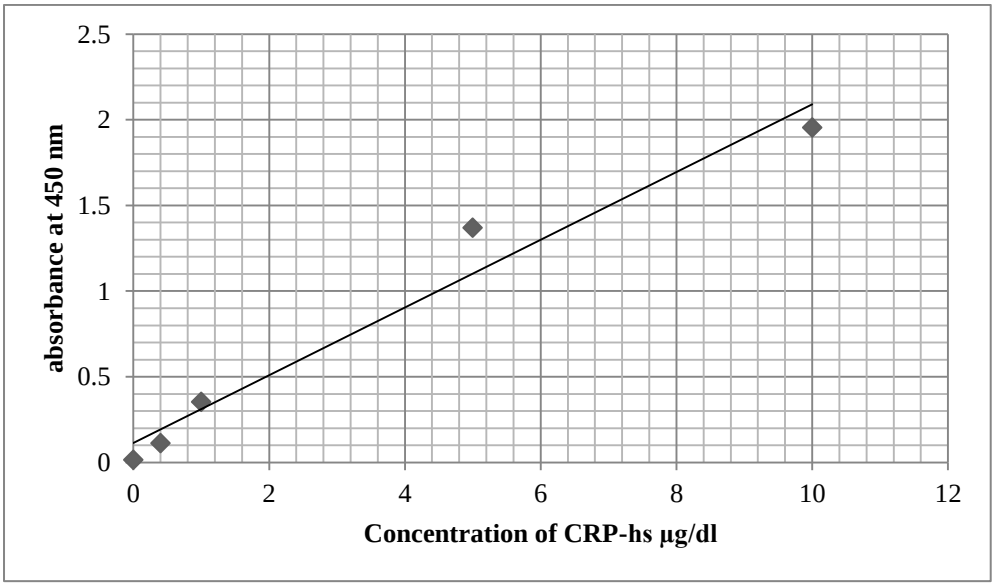


Figure (2-1): Standard curve of CRP-hs.

2.5.7. Determination of serum insulin:-

a- principle

The insulin determination is a solid phase Enzyme Linked Immunosorbent assay(ELISA) based on the sandwich principle.

The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on the insulin molecule.

An aliquot of patient sample containing endogenous insulin is incubated in the coated well with enzyme conjugate, which is an anti-insulin antibody conjugated with Biotin. After incubation the unbound conjugate is washed off.

During the second incubation step Streptavidin Peroxidase Enzyme Complex binds to the biotin-anti insulin antibody. The amount of bound HRP complex is proportional to the concentration of insulin in the sample.

Having added the substrate solution, the intensity of color developed is proportional to the concentration of insulin in the patient sample.

b-assay procedure

1. The desired number of microtiter wells was secure in the frame holder.
2. 25 µl of each standard, control and samples were dispense with new disposable tips into appropriate wells.
3. 25 µl of enzyme conjugate was dispense into each well. Thoroughly mixed for 10 seconds.
4. Incubated for 30 min at room temperature.
5. The continent of the wells was briskly shaked out.
6. 50 µl of enzyme complex was added to each well.
7. Incubated for 30 min at room temperature.
8. The content of the wells was briskly shaked out.
   The wells was rinse 3 times with diluted wash solution (400 µl per well). The wells was striked sharply on absorbent paper to remove residual droplets.
9. 50 µl of substrate solution was added to each well.
10. Incubated for 15 min at room temperature.
11. The enzymatic reaction was Stoped by adding 50 µl of stop solution to each well.

12. The absorbance (OD) of each well was determined at 450 ± 10 nm with a microtiter plate reader. It is recommended that the wells be read within 10 after adding the stop solution.

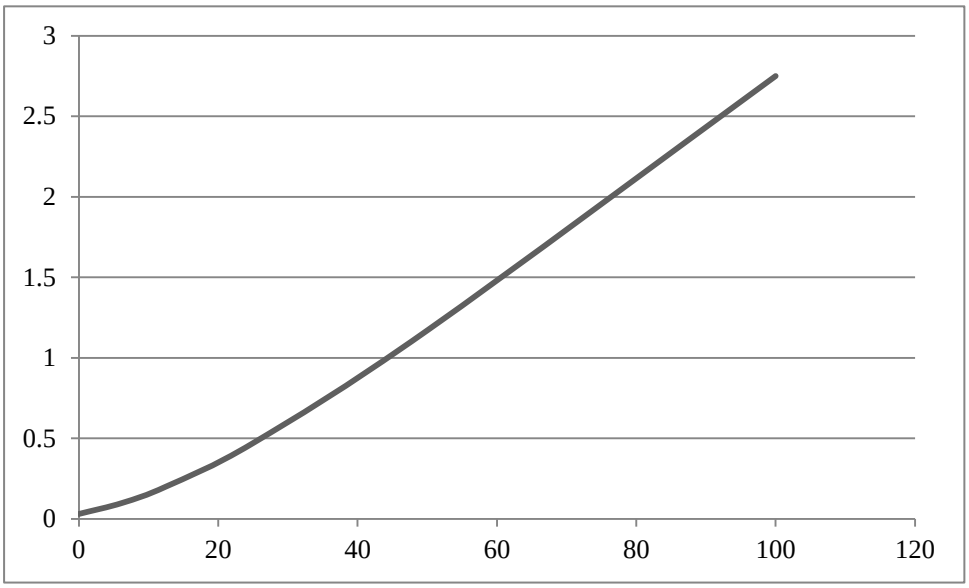


Figure (2-2): standard curve of insulin

2.5.8. Determination of serum Retinol binding protein 4(RBP-4):-

a- principle

This assay employs the competitive inhibition enzyme immunoassay technique. An antibody specific to RBP-4 has been pre-coated onto a microplate. Standards or samples are added to the appropriate microtiter plate wells with HRP-conjugated RBP-4 with the pre-coated antibody specific for RBP-4. The more amount of RBP-4 in samples, the less antibody bound by HRP-conjugated RBP-4. Then the substrate solution are added to the wells, respectively. And the color develops in opposite to the amount of RBP-4 in the intensity of the color is measured.

b-assay procedure

1. A blank well was set without any solution. 50 µl of standard or sample per well. Standard need test in duplicate.
2. 50 µl of HRP-conjugate was added to each well (not to blank well), mixed well and then incubated for 1 hour at 37º C.
3. Each well was filled with wash buffer (about 200 µl), stay for 10 seconds and spinning. The process was repeated for a total of three washes. The removal of liquid was completed at each step is essential to good performance. After the last wash, any remaining wash buffer was removed by aspirating or decanting. The plate was inverted and blotted it against clean paper towels.
4. 50 µl of substrate A and substrate B to each well, mixed well. incubated for 15 min at 37º C. keeping the plate away from drafts and other temperature fluctuations in the dark.
5. 50 µl of stop solution was added to each well when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
6. The optical density of each well was determined within 10 min, using a micro plate reader set to 450 nm.

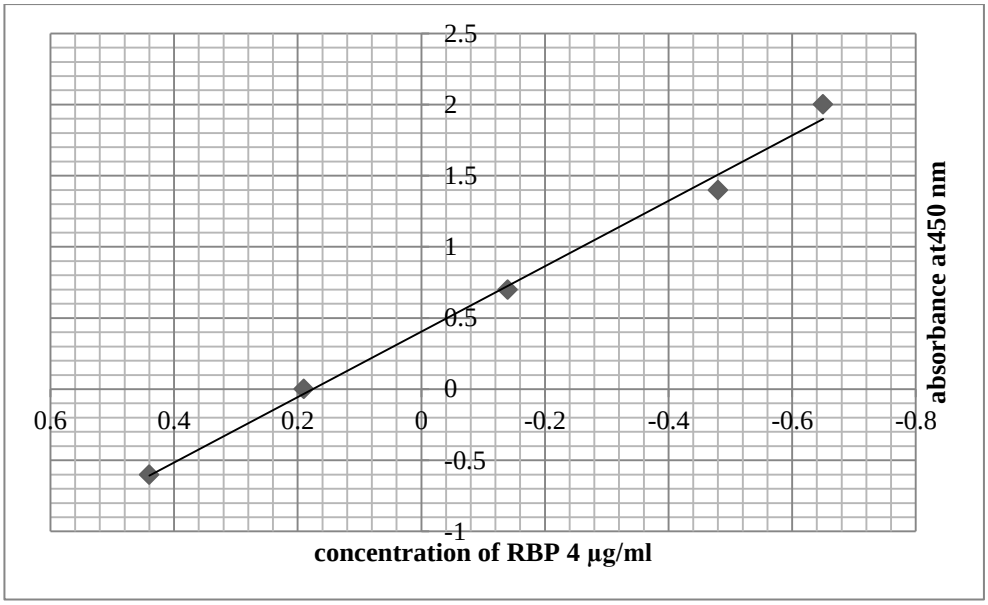


Figure (2-3): standard curve of RBP 4.

2.5.9. Determination of serum Retinol:-

a- principle

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with an antibody specific to vitamin A (VA). The competitive inhibition reaction is launched between with HRP-conjugated VA and VA in samples. A substrate solution is added to the wells and the color develops in opposite to the amount of VA in the sample. The color development is stopped and the intensity of the color is measured.

b-assay procedure

1. All reagents, working standard, and samples were prepared as directed.
2. A blank well set without any solution.
3. 50 µl of standard and sample was added per well.
4. 50 µl HRP conjugate was added to each well immediately (not to blank well). The plate was shaked gently for 60 seconds.
5. Covered with the adhesive strip provided. Incubated for 60 min at 37º C.
6. Each well was aspirated and washed, The process was repeated four time for a total of five washes. Wash by filling each well with wash buffer (200 µl) and let it stand for 2 min. The removal of liquid was completed at each step is essential to good performance. . After the last wash, any remaining wash buffer was removed by aspirating or decanting. The plate was inverted and bloted it against clean paper towels.
7. 90 µl of TMB substrate was added to each well. Incubated for 20 min at 37º C. protected from light.
8. 50 µl of stop solution was added to each well, the plate was gently tapped to ensure thorough mixing.
9. The optical density of each well was determined within 5 min, using a micro plate reader set to 450 nm .

2.6. Statistical analysis:

Data were analyzed using SPSS version 16 and Microsoft excel 2007. Numeric data were expressed as mean ± SE. ANOVA (F-test) was used for compare mean of more than two groups.

Student's t test was used to calculate individual p-value. Pearson correlation coefficient was used to determine correlation coefficient. P value < 0.05 was considered significant.

Chapter Three

Results

3.1. Glycated hemoglobin (HbA_1c):

The diabetic group showed significant elevation of the glycated hemoglobin (HbA1c) level in comparison with the normal control group ($p<0.001$). The diabetic group also had higher significant glycated hemoglobin (HbA1c) level in comparison to the obese control group ($p<0.001$) but no significant difference had been observed between the two control groups when compared with each other ($p = 0.736$). Moreover, a significant difference had been observed among all group ($p< 0.001$)as it clear from table (3-1).

3.2. Serum glucose:

The diabetic group showed significant elevation of the serum glucose in comparison to both control groups (normal & obese) ($p<0.001$) but no significant difference had been observed between the two control groups ($p = 0.558$).

Moreover, p-value by F test showed a significant difference among patient, normal control and obese control groups. Table (3-1) .

Table (3-1): Glycated hemoglobin and fasting blood sugar levels.

Parameters	PATEINT	CONTROL		F test
	T2DM	Group1	Group 2	
HbA_1C	9.12±0.33*	5.45±0.17	5.69±0.15	<0.001
F.B.S(mg/dl)	188.33±8.98*	84.33±3.99	96.28±3.39	<0.001

Patient vs control subjects: *p<0.001.

3.3. Serum lipid profile:

Serum total cholesterol (TC), triglyceride (TG), high density lipoprotein cholesterol (HDL c), low density lipoprotein cholesterol (LDL c), atherogenic index plasma (expressed as AIP= LOG (TG/HDL c)) and LDL size index (expressed as TG/HDL c) were measured in all groups studied. Table (3-2) shows the mean serum lipid profile in diabetic and two control groups.

3.3.1. Serum total cholesterol:

Total cholesterol (TC) was not significantly elevated in the diabetic patients group compared with those normal control group (p = 0.225) and obese control group (p = 0.207). TC were also not significantly elevated in the normal controls group compared with obese control group (p=0.741) and there was no significant difference among all groups (F test p=0.441) as seen in table (3-2).

3.3.2. Serum triglyceride:

Diabetic group had a significantly higher serum triglyceride when compared to normal control group and obese control group ($p<0.001$). Furthermore, there was not significantly elevated in the normal controls group compared with obese controls group ($p=0.303$) As shown in table (3-2)..

3.3.3. Serum HDLc:

Normal control group showed higher insignificant elevation of the HDLc in comparison to the patient group ($p=0.057$) . Obese control group showed no significant difference of SHDLc level when comprise with T2DM group ($p=0.851$) and there was no significant difference between the two control groups ($p=0.094$).

F test showed no significant difference among all group ($p =0.269$) table (3-2).

3.3.4. Serum LDLc:

In both normal and obese control groups LDL were not significantly change in comparison with diabetic group, p value respectively are (0.723, 0.721).

There is no significant difference between the two control groups and no significant change among all groups ($p=0.916$) as seen in table (3-2).

Table (3-2): Serum lipid profile (mean ± SE) in diabetic and control groups

Parameters	PATEINT	CONTROL		F test
	T2DM	Group1	Group 2	
T.C (mg/dl)	203.60±9.0	180.78±12.62	187.36±7.19	0.441
TG (mg/dl)	183.71±14.13*	64.67±16.72	95.68±7.56	<0.001
HDLc(mg/dl)	44.16±1.64	51.44±3.52	44.64±2.01	0.269
LDLc(mg/dl)	122.44±8.01	116.33±11.62	118.24±7.73	0.916

Patient vs control subjects: *p<0.001.

3.4. Serum insulin:

Patient group shows significant increase in serum insulin when compared with normal control group (p <0.05) whereas no significant different between diabetic group and obese control group (p=0.35).

There is no significant elevation in insulin level in obese control group when compared with normal control group (p=0.19) also one way ANOVA showed no significant different among the control and patient groups (p=0.086) as shown in table (3-3).

3.5.Markers of insulin sensitivity and resistance:

3.5.1. Homeostatic model assessment of insulin resistance HOMA- IR:

There was no significant increase in HOMA-IR levels in T2DM group in compared to obese control group p value > 0.05. but significant increase was observed when compared to normal control group. Also

HOMA-IR show not significant elevation in obese control group in compared to normal control group (p> 0.05).

F test show no significant difference between all groups as seen in table (3-3).

3.5.2. Insulin sensitivity index ISI:

In T2DM group insulin sensitivity index was significantly reduced in comparison with each control groups and when compared with the two control groups (normal & obese) the p value was <0.001.

F test also showed significant difference among all groups (p<0.001). table (3-3).

3.5.3. Fasting insulin resistance index FIRI:

Fasting insulin resistance index were significantly elevated in the diabetic patients compared with those normal controls (p=0.003) and obese controls (p=0.002) while there is no significant difference between the control groups (p=0.42).

FIRI also were significantly different among all groups (p<0.001). As shown in table (3-3).

3.5.4. Fasting glucose insulin ratio FGIR:

In T2DM group FGIR was significantly higher in comparison with the obese control groups (p=0.021) and normal control group (p<0.05). also no significant difference between the two control groups (p=0.409), as seen in table (3-3).

3.5.5. Homeostatic model assessment of β-cell function HOMA-β:

HOMA-β shows a significant decrease in T2DM in compared to each of control group ($p<0.001$). but there was no significant difference between controls groups ($p=0.521$). as seen in table (3-3).

Table (3-3): Different marker of insulin sensitivity and resistance index

Parameters	PATIENT	CONTROL		F test
	T2DM	Group 1	Group 2	
Insulin	12.9 ± 1.6	5.44 ± 0.36	10.06 ± 1.14	0.086
HOMA-IR	5.82 ± 0.66	1.11 ± 0.05	2.44 ± 0.32	< 0.05
ISI	6.65 ± 0.75*	22.62 ± 1.19	13.62 ± 1.33	< 0.001
FIRI	94.23 ± 10.73*	18.03 ± 0.81	39.49 ± 5.2	< 0.001
FGIR	20.11 ± 1.82*	6.38± 1.09	11.94±1.05	0.03
HOMA-β	42.5 ± 4.58*	148.92±28.35	173.46± 58.22	< 0.001

Patient vs control subjects: *$p<0.001$.

3.6. Serum retinol binding protein 4:

Serum retinol binding protein 4 was significantly elevated in diabetic group compared with the normal control and obese control groups ($p <0.001$) but no significant different between two control groups ($p = 0.4$) as shown in table (3-4).

3.7. Serum retinol:

Type 2 diabetes mellitus group showed significant elevation of the serum retinol level in comparison to the control group p value = 0.016 table (3-4).

3.8. Retinol / RBP 4:

There was a significant decreased in retinol / RBP 4 ratio in T2DM in comparison to control group (p = 0.025) as seen in table (3-4)

Table (3-4): Retinol, retinol binding protein 4 and retinol/RBP 4 ratio levels.

Parameters	PATIENT	CONTROL		F test
	T2DM	Group 1	Group 2	
Retinol	3.16 ± 0.25*	1.86± 0.55	2.32± 0.27	0.073
RBP 4	427.28±23.2*	202.82±23.60	244.19±16.63	< 0.001
Retinol/RBP4	0.007±0.001*	0.0086± 0.0012	0.009± 0.0011	< 0.05

Patient vs control subjects: *p<0.001.

3.9. correlation between serum retinol binding protein 4 and serum retinol:

There was a significant positive correlation between serum retinol binding protein 4 and serum retinol in diabetic patients (r=0.767, p=0.000) figure (3-1) , normal control (r=0.798, p=0.031) figure (3-2) and obese control (r=784, p=0.000) figure (3-3)

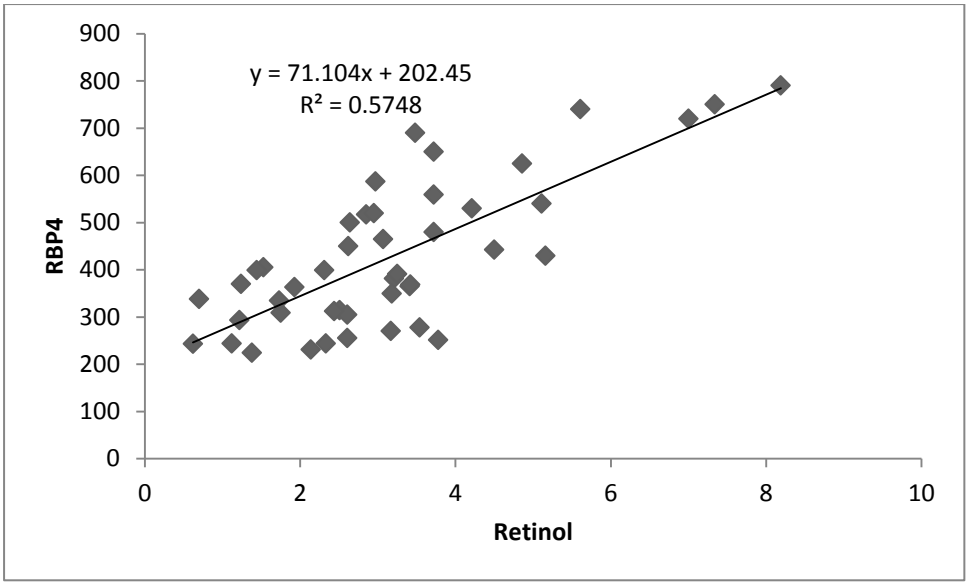


Figure (3-1): Correlation between serum RBP 4 and retinol in T2DM.

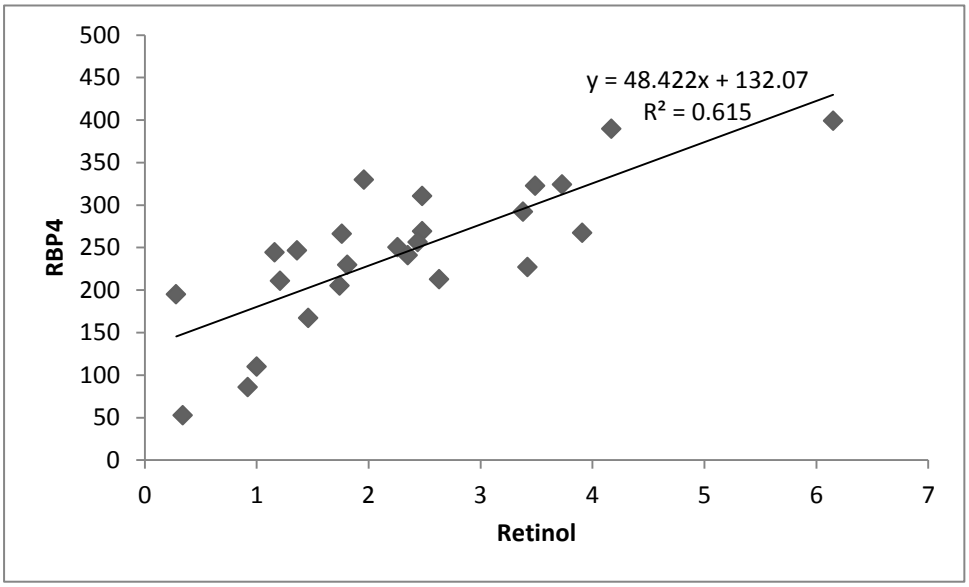


Figure (3-2): Correlation between serum RBP 4 and retinol in obese control

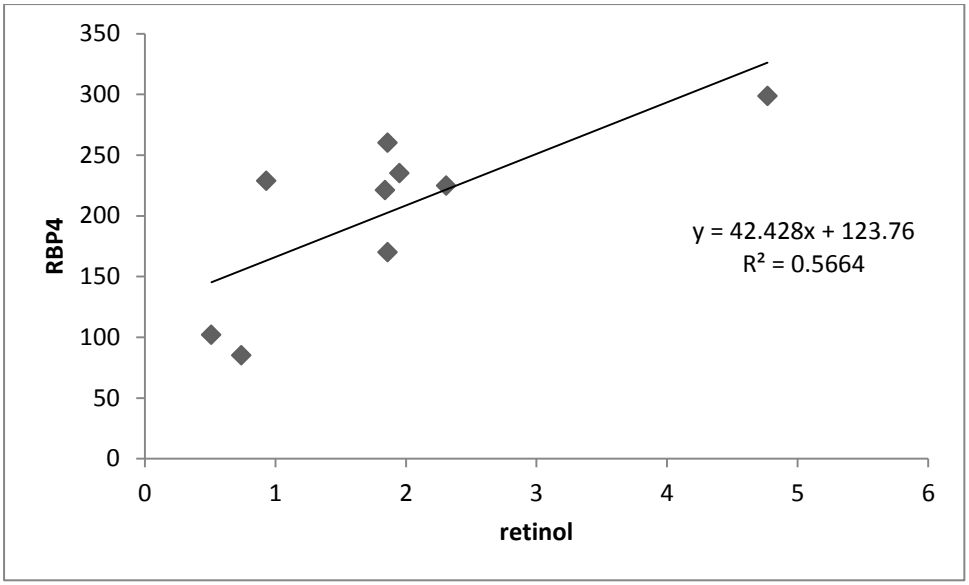


Figure (3-3): correlation between serum RBP 4 and retinol in normal control

3.10. correlation between serum retinol binding protein 4 and insulin resistance(HOMA-IR):

There was no significant correlation between serum retinol binding protein 4 and insulin resistance (HOMA-IR) in type 2 diabetic patients figure (3-4) (r=-0.008, P=0.958), normal control figure (3-5)(r=-0.278, p=0.546) and obese control figure (3-6) (r=0.116, P=0.580).

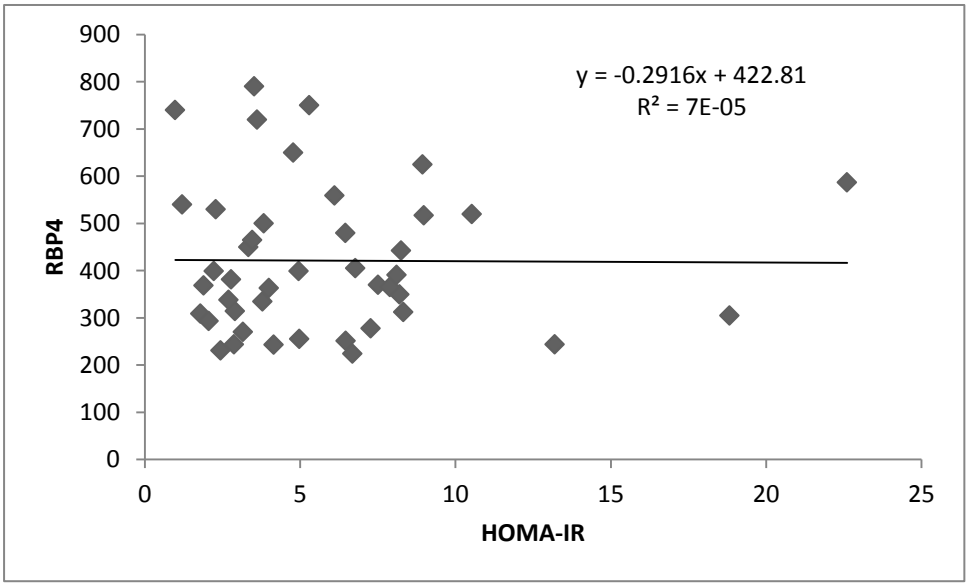


Figure (3-4): correlation between RBP 4 and IR in T2DM group

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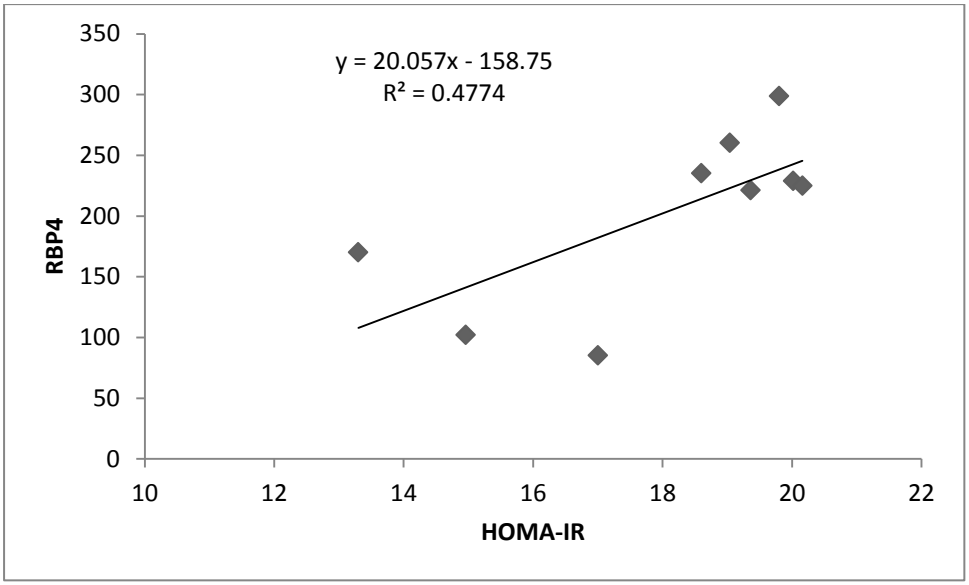

Figure (3-5): correlation between RBP 4 and IR in normal control group.

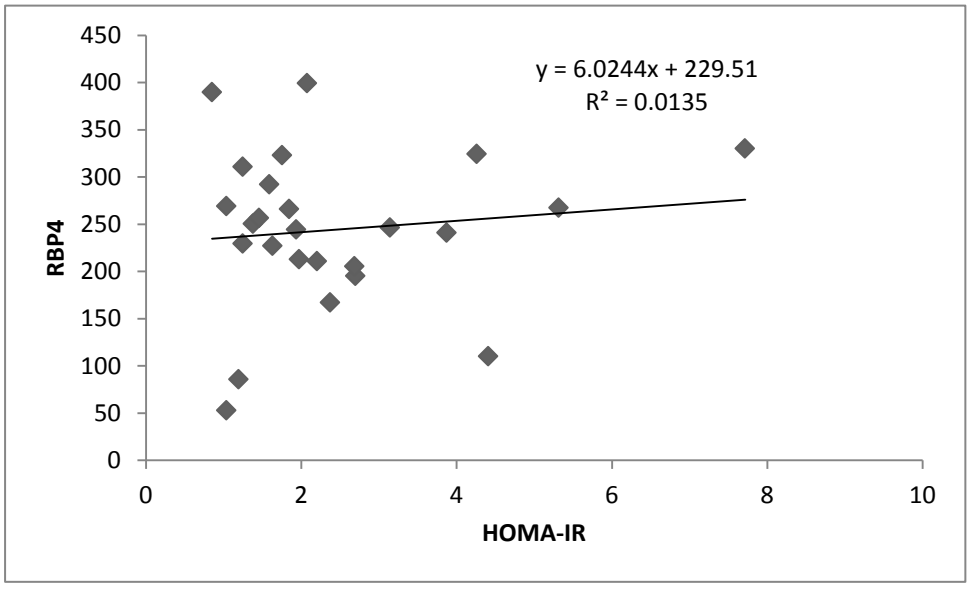

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Figure (3-6): correlation between RBP 4 and IR in obese control group.

3.11. ***Correlation between serum retinol binding protein 4 and lipid profile:***

Retinol binding protein 4 was not correlated with lipid profile parameter as seen in table (3-5).

Table (3-5): Correlation of RBP 4 with lipid profile parameters.

Groups	**Pearson's correlation**	**S. chol**	**S. tri**	**S.HDL**	**S.LDL**
normal control	r	0.530	0.237	0.523	0.350
	P	0.142	0.539	0.149	0.356
obese control	r	-0.174	0.042	0.068	-0.218
	P	0.405	0.842	0.748	0.294
T2DM	r	0.227	0.127	-0.035	0.217
	P	0.134	0.407	0.819	0.152

Chapter Four
Discussion

The mean age of patients participated in the present study was (52.14 ± 1.01 years), their age ranged between 35-65 years, and this was comparable with other studies by Takebayashi et al. (2007) in Japan, Raj and Rajan, (2013) in India and Heidari (2013) in Iran in which the mean age was (58.8 ± 12.1 years), (54.3 ±9.2 years), and (49.26 ± 6.39) respectively. The high prevalence of diabetes in this age group meant that majority of diabetes subjects were suffering from diabetes in their most productive years of life. The risk for developing diabetes also increases with age (Hartz et al., 1983), therefore type 2 diabetes mellitus is known as mainly a disease of older age group (Kahn 1994). High proportion of type 2 diabetic patients were above the age of 50 years (64.4%), and this is comparable with results reported by (WHO 1993) in Iraq, that the majority of type 2 diabetic patients were in the age group (50-69) years, & is also comparable with another study in USA where (54.15%) of diabetics were above 50 years (Janette et al., 2009).

In this study mean BMI of T2DM group was (36.62 ± 0.8 kg/m^2) and this value was comparable with other studies by Lewis et al., (2008) in New Zealand, in which mean BMI was 32.64 ± 4.52 kg/m^2 (Lewis et al., 2008).

Al-auqbi and Mustafa in Iraq (2005) found 71.2% of type 2 diabetic patients had BMI>25 kg/m^2 who categorized their patients as follows:

- Overweight, BMI 25-29.9 kg/m^2 included 832 patients.
- Obesity, BMI 30-39.9 kg/m^2 included 661 patients.

- Morbid obesity, BMI >40 kg/m2 included 64 patients.

And just 28.8% of the patients were non-obese, BMI≤25 kg/m2 (Al-auqbi and Mustafa, 2005).

In this present study the results showed that (84.4%) of diabetic patients were females, this percentage was in agreement with that found in Basrah were the percentage was (80.5%) (Abbas AM et al., 2007), but higher than that in other studies by Ozoh et al., (2010) & Liu Zhaolan et al., (2010) in China, in which the rates were (53.5%) & (58.2%) respectively.

This study showed that HbA1c was significantly elevated in T2DM patients compared with those obese controls ($p<0.001$) and normal controls ($p<0.001$) this confirms the previous report in India 2010 by Murugan et al.

Murugan et al. reported increased HbA1c level in obese diabetic patients compared to obese control and normal control groups (p value < 0.05) and the statistical analysis by one way ANOVA revealed that there was significant difference among group $p<0.05$ (Murugan et al., 2010).

These data showed poor glycemic control and this differs from the results obtained by Chavez et al. 2009 who reported that in 39 obese T2DM hemoglobin A1C was 6.5 ± 0.2 % indicating a reasonably good glycemic control.

Fasting blood glucose was significantly higher in patient group in comparable with obese control ($p<0.001$) and normal control groups ($p<0.001$), also F TEST shows significant difference between all groups.

These results were in agreement with those reported previously by Šindelka et al. (2002) in Prague and Kocak et al (2007) in Turkey.

Šindelka et al. found increased serum blood glucose levels in 18 obese diabetic patients (mean blood glucose 10.6 ± 3.9 mmol/l) compared to 14 normal controls (mean blood glucose 4.5 ± 0.7 mmol/l), 10 overweight controls (mean blood glucose 4.5 ± 0.5 mmol/l) and 17 obese controls (mean blood glucose 4.9 ± 0.8 mmol/l) p value < 0.01 (Šindelka et al., 2002).

Kocak et al. described that serum glucose levels were significantly higher ($p<0.001$) in 28 diabetic obese patients (mean BMI = 34.3 ± 2.6 kg/m^2, mean blood glucose = 10.41 ± 4.38 mmol/l) than the 14 healthy controls (mean BMI = 24.0 ± 1.2, mean blood glucose = 4.72 ± 0.39 mmol/l) (Kocak et al., 2007).

The high level of glucose in diabetic patients may be explained by that the glucose transporter GLUT4 facilitates the transport of glucose across plasma membranes into skeletal muscle cells and into adipocytes. This process is the rate-limiting step in glucose uptake into those tissue and is subject to stimulation by insulin. Insulin resistance occurs in the pathogenic conditions of obesity, metabolic syndrome, and type 2 (non-insulin-dependent) diabetes. The expression of GLUT4 is then down-regulated in adipose tissue, resulting in impaired glucose uptake (Wolf, 2007).

In a normoglycemic individual, in response to a rise in blood glucose, the β-cells of the islets of Langerhans, found within the pancreas, will synthesize and secrete insulin into the blood in a biphasic pattern (Moreira and Hamadeh, 2010).

Total cholesterol (TC) showed an elevation in diabetic patients group as compared to the control groups table (3-1). The present findings are consistent with other study that showed no different in cholesterol levels between 39 obese diabetes group (mean cholesterol ± SE was 180 ± 6 mg/dl) and 17 obese control (mean cholesterol 179 ± 7 mg/dl) and16 normal control (mean cholesterol 183 ± 7 mg/dl) in Texas (Chavez et al., 2009).

In the present work diabetic group had a significantly higher serum triglyceride when compared to normal control group and obese control group ($p<0.001$). Furthermore, there was not significantly elevation in the normal control group compared with obese controls group ($p=0.303$) whereas $p<0.001$ among all groups,(table 3-1). This results along with another report by Riaz et al. in Pakistan (2009) that triglyceride levels was highly significant elevated in diabetic obese (mean BMI = 33.56 ± 0.44 kg/m^2, mean TG = 250.26 ± 19.4 mg/dl) compared to normal control (mean BMI = 24.6 ± 0.61 kg/m^2, mean TG = 108.64 ± 13.91 mg/dl) $p<0.05$.

Yerlikaya et al in Turkey(2013) study that serum triglyceride of 41 patients was (2.79 ± 1.4 mmol/l) significant elevated ($p<0.05$) in comparison with a 50 normal control group triglyceride level (0.8 ± 0.4 mmol/l) and with 45 obese control group triglyceride level (1.41 ± 0.6 mmol/l)

Normal control group showed higher insignificant elevation of the Serum HDLc in comparison to the patient group. Obese control group showed no significant elevation of Serum HDLc level when compre with T2DM group and there was no significant difference between the two control groups as seen in table (3-1).

Similar results described by Wiegand et al. and Muscelli et al. who compared on HDL levels between normal glucose tolerance of obese control (mean BMI > 30) with obese type 2 diabetes (mean BMI > 30 ± 1.9).

Wiegand et al. and Muscelli et al. also found that there was no significant change ($p>0.05$) in control serum LDL in compared with diabetic group.

In this study a significant elevation in diabetic serum insulin in comparison to normal control group was observed but no significant elevation when compared to obese control group

These results were similar to those obtained previously (Pontiroli et al, 2004; EL-Said et al, 2011).

EL-Said et al. found a significant elevation (p value < 0.001) in diabetic serum insulin (mean BMI = 34.63 ± 4.22, mean serum insulin = 18.04 ± 2.69 mU/l) in comparable to control subjects (mean BMI = 22.13 ± 1.85 kg/m2 , mean serum insulin = 10.09 ±1.07 mU/l). While Pontiroli et al stated that there was no significant different in serum insulin of T2DM (mean BMI = 42.9 ± 1.21 kg/m2, mean serum insulin = 109.1 ± 8.13 pmol/l) when compared to obese control group (mean BMI = 44.5 ± 1.09 kg/m2, mean serum insulin = 127.4 ± 15.75 pmol/l) but a significant elevation was observed (p value < 0.001) when compared to normal control group (mean BMI = 22.5 ± 0.21kg/m^2, mean serum insulin = 63.9 ± 2.63 pmol/l). Also they found no significant difference in HOMA-IR between T2DM (mean HOMA-IR = 5.7 ± 0.49) and obese control group (mean HOMA-IR = 4.6 ± 0.68) this finding is in agreement with the present study.

In T2DM group insulin sensitivity index was significantly reduced ($p<0.001$) in comparison with each control group, this covenant with Wiegand et al. who found that ISI decreased significantly (p value <0.001) in T2DM (mean ISI = 1.2 ± 0.1) than normal glucose tolerance (mean ISI = 3.5 ± 0.3).

In current study a significant elevation in diabetic serum RBP 4 in comparison to normal and obese control groups was observed but no significant difference between the two control groups.

This finding was consistent with another studies (Bajzova et al, 2008; EL-Said et al, 2011; Shaker et al, 2011; Hassan et al, 2012).

Shaker et al. showed that plasma RBP 4 levels were significantly increased (p value < 0.001) in obese T2DM patients compared with obese control group. Hassan et al. reached to the same results when compared between RBP 4 in T2DM (mean BMI = 30.1 ± 3.8 kg/m2, mean RBP 4 = 136.5 ± 34.6 ng/ml) vs normal control group (mean BMI = 23.4 ± 1.3 kg/m2, mean RBP 4 = 78.8 ± 38 ng/ml) with high significant different in RBP 4.

Bajzova et al. found no significant difference ($p>0.05$) between obese subjects (mean BMI = 37.14 kg/m^2, mean RBP 4 = 26.0 ± 1.6 mg/l) and non-obese subjects (mean BMI = 24.5 ± 0.73, mean RBP 4 = 27.8 ± 1.5 mg/l).

Wolf G. explained the increase of serum RBP 4 by the down regulation of the insulin-responsive glucose transporter GLUT 4 accompanied with insulin resistance that occurs under conditions of obesity, metabolic syndrome, and type2 diabetes decreased adipocyte GLUT 4caused secretion by adipocytes of the serum retinol-binding

protein RBP 4. In mice increased levels of serum RBP 4 lead to impaired glucose uptake into skeletal muscle and increased glucose production by liver, whereas lowered serum RBP 4 levels greatly enhanced insulin sensitivity (Wolf, 2007).

In contrast to this study Erikstrup et al. found that RBP was lower in individuals with T2DM compared with individuals with NGT ($p<0.05$). But Lewis et al. found that RBP levels were not significantly different between subjects with diabetes (mean BMI = 31.7 ± 6.5, mean RBP = 39.9 ± 13.8) and subject without diabetes (mean BMI = 30.4 ± 8.7, mean RBP = 37.2 ± 12.5) p value=0.125

Vitamin A activity is very important for maintaining health and thus, humans and other animals have developed the capacity to store it. Storage lessens the need for regular intake of the vitamin, since it can be mobilized from these stores as retinal bound to retinol binding protein (Wongsiriroj and Blaner, 2004). Liver is the major storage organ for vitamin A, though other tissues, such as lungs, eyes adipose tissue and skin can also store it (Wongsiriroj and Blaner, 2004).

Vitamin A (retinol) and its provitamin beta-carotene play an essential role in the pancreatic secretions of insulin and glucagon that regulate glucose metabolism and gluconeogenesis in the body. The actual status of these vitamins in diabetes is still controversial because of inconsistent and conflicting results. Some studies reported lower serum retinol concentration in type 1 and type 2 diabetes (Harviv et al., 1991; Martinoli et al., 1993;Olmedilla et al., 1997), some reported higher serum vitamin A levels in patients with impaired glucose tolerance and type 2 diabetes (Sasaki et al., 1995; Tavriduo, 1997), others observed no significant differences in their levels between diabetics and non-diabetics (Basuldo

et al., 1997) whereas some established an inverse association between serum carotenoids and type 2 diabetes (Coyne et al., 2005). These controversies have been attributed to nutrition, environmental factors and the presence of other ailments.

In the current work retinol levels was found to be significantly higher in diabetics than in the control subjects . This may be attributed to the fact that the diabetics used in the study consumed a lot of vegetable rich in beta-carotene compared to the control group. Higher dietary vitamin A intake supplementation has been associated with higher serum levels (Comstock et al., 1988;Yelonen et al., 2003; Usoro et al., 2006). A similar finding was made by Hozumi et al. (1998). Biological activities of carotenoids include induction of cell-to-cell communication (Stahl and Sies, 1997). Junctional communication of beta cell was shown to contribute to the control of insulin secretion and glucose tolerance (Charollais et al, 2000). In non-insulin dependent diabetes mellitus (NIDDM), lipid or lipo-protein changes in blood are the most likely explanation for high serum retinol concentration also hyperinsulinemia might increase the level of vitamin A in the liver (Alam et al, 2006) however, lower retinol and beta carotene levels have been reported in diabetics independently by Baena et al. (2002) and Abahusain et al. (1999).

Onyesom et al. (2011) reported that serum levels of vitamin A levels for 50 NIDDM (mean BMI = 29.24 ± 2.34) and control subjects (mean BMI = 24.24 ± 1.43) were 14.38 ± 7.59 and 44.12 ± 11.79 µg/dl, respectively. Concentration of vitamin A in NIDDM subjects were significantly lower than the values obtained for the control subjects.

High levels are usually found in individuals receiving diets high in vitamin A (Cunningham, 1998). Low serum levels of vitamin A are also found in gastrointestinal, pancreatic and hepatic disease and in infections like fever and chronic nephritis (Sankale et al., 1992). However, women taking steroids or oral contraceptive have been reported to have slightly elevated serum concentrations of vitamin A.

In this study there was no significant difference in retinol binding protein 4 to retinol ratio between patient and controls groups in contrast to Erikstrup et al. who reported that RBP-to-retinol ratio was higher in T2DM ($P<0.001$) than non-glucose tolerance. In this study retinol to retinol binding protein 4 was found to be highly decreased ($p=0.025$) in patient group when compared to control group. Mills et al., 2008 reported that retinol:RBP significantly higher in obese subject compared to non-obese subjects.

This study showed there was no significant correlation between serum retinol binding protein 4 and insulin resistance in type 2 diabetic patients figure (3-4), normal control figure (3-5) and obese control figure (3-6).

This results support the idea that RBP 4 is not a useful marker of insulin resistance and in agreement with Lewis et al. results (Lewis et al., 2008).

In this present study retinol binding protein 4 was not correlated with the lipid profile parameters(table 3-5) in contrast to this finding Verges et al., reported that in T2DM, plasma RBP 4 level is associated with plasma triglyceride but there is no correlation between RBP 4 and HDL-c or LDL-c (Verges et al., 2012).

In this work retinol binding protein 4 was not correlated with the lipid profile parameters : total cholesterol (r=0.007, p=0.969); triglycerides (r=0.16, p=0.366); HDL-c (r=0.093, p=0.599); and LDL-c (r=-0.095, p=0.595) for the entire group of control subjects this results are in line with those from Broch et al. who reported that RBP 4 not correlated with the lipid profile parameters (Broch et al., 2009).

In the present work RBP 4 not correlated with HDL-c in normal control or obese control groups. Kowalska et al. reported that serum RBP 4 was negatively related to HDL-c in the obese group but such relationships were not found in the normal subjects and the entire group (Kowalska et al., 2008).

Conclusions and recommendations:

Conclusions

1. The present study results proved that there was a significant positive correlation between serum retinol binding protein 4 and serum retinol in type 2 diabetes mellitus and both controls groups.

2. The present result also support the concept that serum retinol binding protein 4 / serum retinol ratio is not related with insulin resistance.

3. The results also show that serum retinol / retinol binding protein 4 was significantly increased in type 2 diabetes mellitus and may be considered as association of T2DM than RBP 4 itself.

4. High significant ($p<0.05$) increase in serum retinol binding protein 4 and serum retinol in type 2 diabetes mellitus compared with control groups.

Recommendations

1. Other studies of other vitamins (mainly vitamin D) in sera of controls and patients with type 2 diabetes mellitus.

2. Measurement of serum retinol and retinol binding protein 4 in sera with other types of diabetes(type 1 diabetes mellitus, gestational diabetes mellitus).

References

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Serum retinol binding protein 4 and its ratio to serum retinol in obese type 2 diabetic patients

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Dhualhijjah / 1434 AH

October/ 2013 AD

Dedication

This effort is dedicated to

My father and mother

My husband ***ALI***

My sun Hussain

For their love & support

قُلْ لَوْ كَانَ الْبَحْرُ مِدَادًا
لِكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ
أَنْ تَنْفَدَ كَلِمَاتُ رَبِّي وَلَوْ
جِئْنَا بِمِثْلِهِ مَدَدًا

سورة الكهف

أية (١٠٩)

الخلاصة

الخلفية الأساسية ان داء السكري هو مجموعة من الأمراض الاستقلابية التي تتميز بارتفاع السكر في الدم الناتج عن عيوب في إفراز الأنسولين، عمل الانسولين أو كليهما، ويرتبط ارتفاع السكر في الدم المزمن لمرض السكري مع ضرر على المدى الطويل، وعجز وفشل في مختلف أجهزة الجسم، وخاصة العينين والكليتين، والأعصاب، والقلب، والأوعية الدموية التي تفرض عبئا هائلا على كاهل الفرد مع مرض السكري وعلى نظام الرعاية الصحية.

الهدف من الدراسة دراسة العلاقة بين نسبة البروتين الرابط للريتينول 4 الى الريتينول مع مقاومة الانسولين للمرضى المصابين بالنوع الثاني من داء السكري

الاشخاص والطرق تم اجراء دراسة مقارنة بين مجموعة المرضى ومجموعة السيطرة في فرع الكيمياء والكيمياء الحياتيه ،كلية الطب، جامعة النهرين، العراق-بغداد للفتره من اذار 2012 ولغايه حزيران 2012.

استندت الدراسه على متابعه 45 مريضا و34 شخصا سليما،تم الحصول على النماذج بطريقه عشوائيه.

تتضمن الدراسة الحالية قياس مستويات الهيموغلوبين السكري بطريقه التبادل الايوني لل(HPLC)، تم تحديد مستوى كل من السكر في الدم بعد الصيام، مستوى الدهون، اليوريا في الدم، والكرياتينين في مصل الدم بالطرق اللونية الأنزيمية. كما تم قياس مستوى الانسولين في مصل الدم بعد الصيام، مستوى البروتين الرابط للريتينول 4، مستوى الريتينول بطريقه ال(HPLC) لدى 45 مريضا مصابا بالسكري النوع الثاني(معدل العمر ± الخطأ القياسي، 52.14 ± 1.01 سنه).

تم مقارنه النتائج مع نتائج مجموعه من 35(اعمارهم متناظره مع اعمار المرضى) شخصا سليما ظاهرا (مجموعه السيطرة) كانت معدل اعمارهم (48.79 ± 1.17 سنه)، وقد تم تقسيمهم بالاعتماد على مؤشر كتله الجسم الى مجموعتين:

1. الاصحاء ذوي مؤشر كتله الجسم <25 كغم/م^2 : مجموعه السيطرة النحفاء (العدد 9 شخصا سليما، معدل العمر 49.22 ± 1.88 سنه).
2. الاصحاء ذوي مؤشر كتله الجسم ≤ 30 كغم/م2 : مجموعه السيطرة البدناء (العدد 25 شخصا سليما، معدل العمر 48.38 ± 1.45 سنه).

النتائج اوضحت النتائج ان المرضى المصابين بالسكري النوع 2 يعانون من ارتفاع هام في مستوى الكلوكوز في الدم، مستوى الهيموغلوبين السكري، الدهون الثلاثية في مصل الدم، البروتين الشحمي واطئ الكثافة جدا في مصل الدم ، مؤشر تصلب الشرايين من البلازما، مؤشر مقاومه الانسولين بعد الصيام، نسبه الكلوكوز بعد الصيام الى الانسولين بعد الصيام، قيم ال HOMA لخلايا بيتا (نشاط خلايا بيتا) ومستوى البروتين الرابط للريتينول 4 في مصل الدم مقارنه مع مجموعتي السيطرة.

مستوى البروتين الشحمي عالي الكثافة، مستوى الانسولين و قيم ال HOMA لمقاومه الانسولين اظهرت ارتفاعا هاما عند المرضى المصابين بالسكري النوع 2 عندما قورنت بالأشخاص الاصحاء النحفاء لكن لا يوجد فرق ذو اهميه عندما قورنت مع الاشخاص الاصحاء البدناء.

مستويات الانسولين ومقاومه الانسولين المحدده بHOMA اظهرت ارتفاعا هاما عند المرضى المصابين بالسكري النوع الثاني عندما قورنت بالاشخاص الاصحاء النحفاء لكن لا يوجد فرق ذو اهميه عندما قورنت مع الاشخاص الاصحاء البدناء.

مستوى الكوليسترول في مصل الدم والبروتين الشحمي واطئ الكثافة لم يظهر اختلاف هام في مجموعه المرضى عند مقارنتها مع كلتا مجموعتي السيطرة.

مستوى مؤشر حساسيه الانسولين كان منخفضا بشكل هام لدى مرضى السكري من النوع الثاني عند المقارنه مع كلتا مجموعتي السيطرة.

الاستنتاج نسبه الريتينول في مصل الدم الى البروتين الرابط للرتينول 4 ممكن اعتباره كدليل لمرض السكري من النوع الثاني بدلا من البروتين الرابط للرتينول 4 لوحده.

البروتين الرابط للرتينول 4 و نسبته للرتينول الحر في مرضى السكري من النوع الثاني البدناء

رسالة مقدمه الى مجلس كليه الطب / جامعه النهرين كجزء من متطلبات نيل درجه الماجستير في الكيمياء السريرية

من قبل

انس هاشم صادق الحسيني

بكالوريوس في علوم الكيمياء/جامعه بغداد 2009

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