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Investigation of the Association of the PGC-1α (Gly482Ser) Gene's Polymorphism with Type 2 Diabetes in Palestinian Patients.

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Dedication

This work is dedicated to....

My father and mother,

My dear husband,

My brothers and sister,

My lovely son,

For their patience and encouragement, with love and respect.

Anmar Mustafa Tawfiq Abu-Zahrah

Declaration

I certify that this thesis submitted for the degree of Master, is the result of my own research, except where otherwise acknowledged, and that this thesis (or any part of the same) has not been submitted for a higher degree to any university or institution.

Signature: ----------

Anmar Mustafa Tawfiq Abu-Zahrah

June,2010

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Abstract

Diabetes mellitus is a group of metabolic disorders 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.

Type 2 diabetes accounts for ~90-95% of those with diabetes. The cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response.

Type 2 diabetes is a common disease due to both genetic and environmental factors together with lifestyle and behavioral factors. Genome-wide association studies (GWAS) have identified several type 2 diabetes susceptibility loci in many genes including peroxisome proliferator-activated receptor γ gene.

PGC-1α, originally identified as a co-activator of PPARγ, has since been shown to increase the transcriptional activity of PPARγ and many additional nuclear receptor families. PGC-1α regulates biological programs linked to energy homeostasis and adaptive thermogenesis. In humans PGC-1α is expressed in high quantities in liver, heart, kidney, and skeletal muscle and to a lesser extent in white adipose tissue, pancreas, and brain. An important association with Type 2 diabetes has been reported for a miss-sense variation (Gly482Ser) in the peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1α) gene. The Gly482Ser variation in PGC-1α gene was reported to be associated with type 2 diabetes in Danish and Japanese subjects. In contrast, no association was found in French, Caucasians or in Pima Indians.

In this study, the allelic variation Gly482Ser polymorphism of the PGC-1α gene were investigated, in association with type 2 diabetes and its related clinical parameters among Palestinian patients in the West Bank by using RFLP-PCR technique. The samples comprised of 341 subjects in which 160 were clinically diagnosed as diabetic and 181 as control.

SPSS was used to analyze the correlation between the PGC-1α gene allelic polymorphism and the various clinical parameters characteristic of the disease. T-test was used to test the difference between the means of allelic variation of the PGC-1α gene. Also, Chi-square analysis was used to analyze the data of the gene's haplotypes when they were compared with respect to the various clinical and biochemical parameters. Data analysis revealed that there was no statistical significant difference between the G (GG) and S (GS and SS) haplotypes of PGC-1α gene among diabetic cases vs. control subjects (P=0.160). Also the results indicated that there was no statistically significant difference between the PGC-1α gene haplotypes and gender (P=0.115). In addition, no statistically significant difference could be detected between these haplotypes and the various biochemical parameters (i.e. total cholesterol (P=0.124), High Density Lipoprotein (HDL) (P=0.992), Low Density Lipoprotein (LDL) calculated (P=0.232), LDL direct (P=0.553), Triglyceride (TG) (P=0.345), Hemoglobin A1c (HbA1c) (P=0.051)) or other parameters (i.e. Blood Pressure (BP) (Systolic Blood Pressure (SBP) (P=0.401), Diastolic Blood Pressure (DBP) (P=0.514)) and Body Mass Index (BMI) (P=0.139)) among all subjects. Therefore, our collective results indicate that the Gly482Ser allelic polymorphism in the PGC-1α gene is not correlated with diabetes among the Palestinian population studied. So that, more investigations and studies should be done to have more information about magnitude genetic risk factors that are correlated to type 2 diabetes among our population.

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List of abbreviations

aP2: adipocyte Protein 2.

BAT: Brown adipose tissue.

BMI: Body Mass Index.

bp: base pair.

BP: Blood pressure.

cAMP: cyclin Adenosine Monophosphate.

CBP: CREB binding protein.

CDKAL1: Cyclin-dependent kinase 5 (CDK5) regulatory subunit associated protein-1-like1.

CDKN2B: Cyclin-dependent kinase inhibitor 2 B.

CK1: Casein kinase 1.

CREB : cAMP Response element binding protein.

CVD: Cardiovascular disease.

DBP: Diastolic blood pressure.

DCCT: Diabetes control and complications trial.

DNA: Deoxy ribo Nucleic Acid.

ER: Estrogen receptor.

ERR: Estrogen-related Receptor.

FPG: Fasting plasma glucose.

GAD: Glutamic acid decarboxylase.

Glut-4: Glucose Transporter – 4.

GR: Glucocorticoid Receptor.

GSK: Glycogen synthase kinase.

GWAS: Genome-wide association studies.

HAT: Histone Acetyl Transferase.

HbA1c: Hemoglobin A1c.

HDL: High density lipoprotein.

HHEX: Hematopoietically expressed homeobox.

HNF: Hepatocyte Nuclear Factor.

IFG: Impaired fasting glucose.

IGF2BP2: Insulin like growth factor 2 mRNA binding protein 2.

IGT: Impaired glucose tolerance.

KCNJ11: Potassium inwardly-rectifying channel, subfamily J, member 11.

LDL: Low density lipoprotein.

LXR: Liver X Receptor.

MEF2: Myocyte enhancer factor 2.

mRNA: messenger ribo nucleic acid.

mtDNA: mitochondrial deoxy ribo nucleic acid.

mtTFA: mitochondrial Transcription factor A.

NRFs: Nuclear respiratory factors.

OGTT: Oral glucose tolerance test.

p38MAPK: p38 Mitogen-Activated Protein Kinases.

PEPCK: Phosphoenolpyruvate carboxy kinases.

PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha.

PKA: Protein Kinase A.

PPARγ: Peroxisome proliferator-activated receptor gamma.

RNA: Ribo Nucleic Acid.

RRM: RNA Recognition motif.

RS domain: Arginine-serine- rich domain.

RXR: Retinoid X Receptor.

SBP: Systolic blood pressure.

SLC30A8: Solute carrier family 30 (zinc transporter) member 8.

SNP: Single nucleotide polymorphism.

SRC-1: Steroid receptor coactivator -1.

STOP-NIDDM: Study to prevent Non-Insulin-Dependent Diabetes Mellitus.

TCF7L2: Transcription factor 7-like 2 (T-cell specific).

TG: Triglyceride.

TR: Thyroid hormone receptor.

UCP-1: Uncoupling protein-1.

UGDP: University group diabetes program.

UKPDS: United Kingdom prospective diabetes study.

WAT: White adipose tissue.

WHO: World Health Organization.

Chapter One

Introduction

1.1 Historical Background

Diabetes mellitus has been observed and reported throughout written history since at least 1500 B.C. (Poretsky, 2002). The Egyptologist George Ebers (1874) described the first written reference to diabetes by ancient Egyptian physicians which described a condition of "too great emptying of the urine" (Poretsky, 2002). Around the same time, Indian physicians observed that the urine from people with diabetes attracted ants and flies. They named the condition "madhumeha" or "honey urine". They also noted that patients with "madhumeha" suffered from extreme thirst and ketosis. Around 230 B.C. Apollonius of Memphis used for the first time the term "diabetes", which in Greek means "to pass through" (dia-through, betes-to go) (Poretsky, 2002).

Diabetes mellitus has been characterized by researchers throughout history. In the 5^{th} century Surata and Charaka, two Indian physicians, were the first to differentiate between the two types of diabetes (Poretsky, 2002). Between 16^{th} and 18^{th} centuries, the understanding of some aspects of diabetes was traced to discoveries made in Europe. In 1776, British physiologist Matthew Dobson, determined that the sweet tasting substance in the urine of diabetics was sugar (Poretsky, 2002). Thomas Cawley, in 1788, was the first to suggest link between the pancreas and diabetes after he observed that people with pancreatic injury developed diabetes (Poretsky, 2002). In 1869, Paul Langerhans, German

pathologist, described a small cell clusters in the pancreas, that were not drained by the pancreatic ducts. These cell clusters later named "Islets of Langerhans". In 1889, Oscar Minkowiski, Joseph ven Mehring from Germany observed that removal of the pancreas in the dogs causing immediate development of diabetes. In 1921-1922, Frederick Banting, Charles Best, James Collip and John Macleod from Canada found that dog's pancreatic extracts shown to decrease glucosuria. This extract was named insulin. In 1969, Dorothy Hodgkin, Great Britain, described the three-dimensional structure of porcine insulin using x-ray crystallography.

In 1998, United Kingdom Prospective Diabetes Study (UK PDS, a clinical research trial) found the relation of the metabolic control of type 2 diabetes to the development of diabetic complications. Diabetes is a metabolic disorder primarily characterized by elevated blood glucose levels and by microvascular and cardiovascular complications that substantially increase the morbidity and mortality associated with the disease and reduce the quality of life. An association between the complications of diabetes and elevated blood glucose levels was postulated. Animal experimental studies and human observational studies and clinical trials directly linked hyperglycemia with the development of diabetic complications (Genuth, 1995). Many of the observational studies support a correlation between glycemic control and diabetic complications in patients with type 2 diabetes. These studies attempting to test the benefit of lowering blood glucose on the incidence of complications. The first of these studies was the University Group Diabetes Program (UGDP), which showed no benefit of glycemic control in new-onset type 2 diabetic patients. However, in the UGDP, there were only 200 subjects in each treatment group, HbA_{1c} was not available as a reliable method for measuring chronic glycemia, and the difference in glucose control between the most intensively treated group and the other treatment groups was only a fasting plasma glucose of ~30 mg/dl (1.7 mmol/l). The second controlled trial in type 2 diabetes was only recently reported (Ohkubo et al., 1995). This small study conducted in 110 lean Japanese subjects showed that multiple insulin injections resulting in better glycemic control (HbA_{1c} = 7.1%) compared with conventional treatment (HbA_{1c} = 9.4%) significantly reduced the microvascular complications of diabetes. The third trial in type 2 diabetes was a pilot study that randomized 153 men to intensive or conventional therapy (Albraira et al., 1997). Despite a 2% absolute HbA_{1c} difference in glycemic control between the two groups, the

trial reported no significant difference in cardiovascular events (when adjusted for baseline characteristics) in a follow-up period of only 27 months. With this background, we now have the results of the largest and longest study on type 2 diabetic patients that has ever been performed (UK Prospective Diabetes Study Group, 1998-A; UK Prospective Diabetes Study Group, 1998-B; UK Prospective Diabetes Study Group, 1998-C; UK Prospective Diabetes Study Group, 1998-D). The UKPDS results establish that retinopathy, nephropathy, and possibly neuropathy are benefited by lowering blood glucose levels in type 2 diabetes with intensive therapy, which achieved a median HbA_{1c} of 7.0% compared with conventional therapy with a median HbA_{1c} of 7.9%. The overall microvascular complication rate was decreased by 25% (American Diabetes Association, 2002).

1n 2001, Diabetes Prevention Program, in the USA, found the relation of diet and exercise to the rate of development of type 2 diabetes in high risk population (Poretsky, 2002).

1.2 Definition and Description of Diabetes Mellitus

1.2.1. Definition:

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (WHO, 1999). 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 (American Diabetes Association, 2006).

Several pathogenic processes are involved in the development of diabetes. They range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency (type 1 diabetes) to abnormalities that result in resistance to insulin action (type 2 diabetes) (American Diabetes Association, 2006). The abnormalities of carbohydrate, fat and protein metabolism in diabetes are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin (WHO, 1999)

1.2.2. Signs and Symptoms:

The general characteristics symptoms of marked hyperglycemia are polyuria (excessive urine production), polydipsia (thirst and increased fluid intake), weight loss, sometimes with polyphagia (increased appetite), and blurred vision (Cooky and Plotnick, 2008).

Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or a non-ketotic hyperosmolar state that may develop and lead to stupor, coma and, in absence of effective treatment, death (WHO, 1999). Diabetic ketoacidosis usually occurs among type 1 diabetes and has very sever symptoms like: nausea, vomiting, sweet-smelling fruity acetone breath, breathing difficulty, rapid pulse and abdominal pain (Professional Guide to Disease, 2005).

Long-term complications of diabetes include microvascular complications like (retinopathy with potential loss of vision, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcers, amputations), and Charcot joints (American Diabetes Association, 2006), and features of autonomic dysfunction, including sexual dysfunction (WHO, 1999). Patients with diabetes are at increased risk of macrovascular diseases like (cardiovascular, peripheral vascular, and cerebrovascular disease) (WHO, 1999).

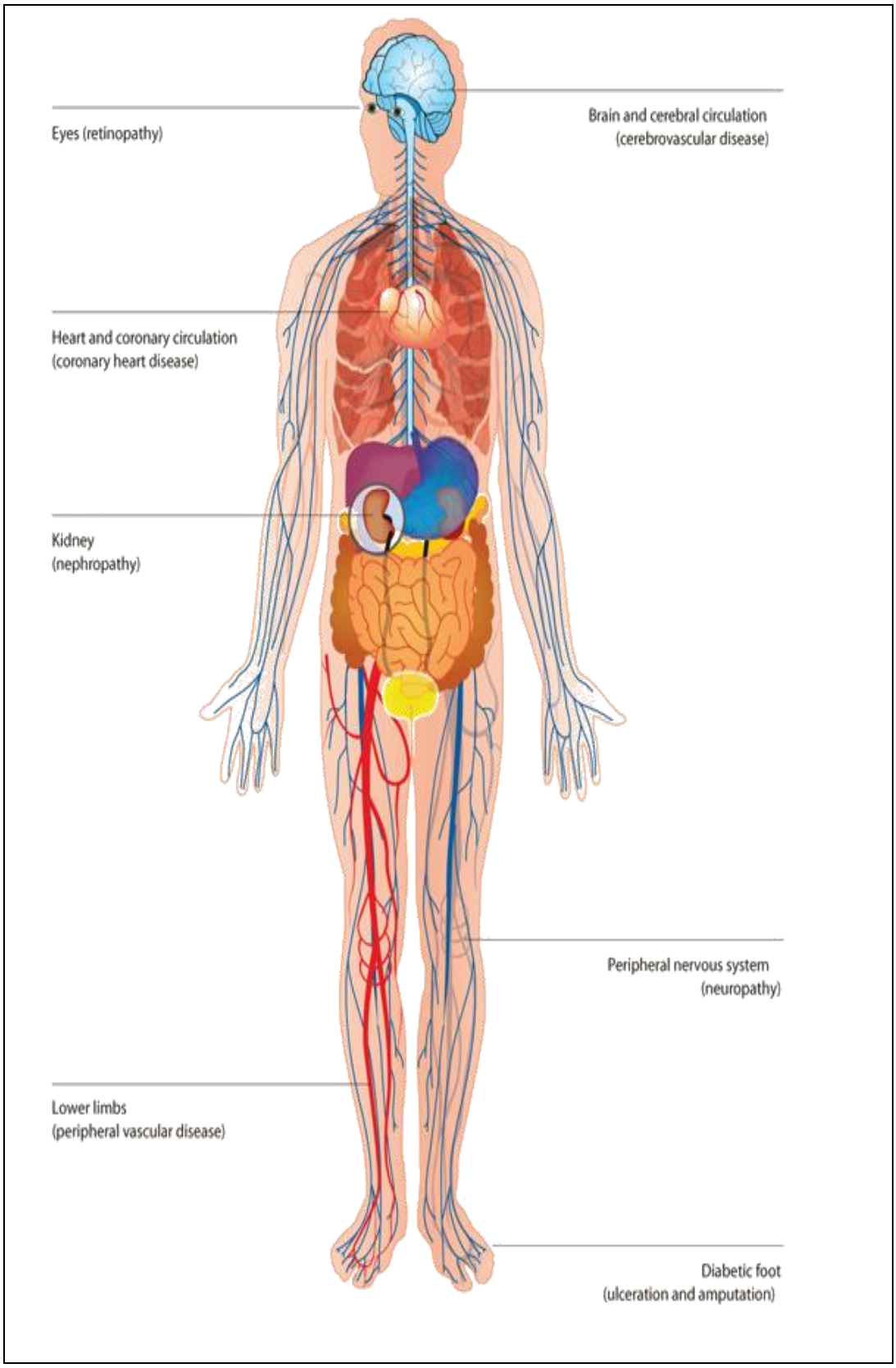

Fig. 1.1: The Major Diabetes Complications. This figure describes the major chronic complications associated with diabetes which are cardiovascular disease (CVD); nephropathy; neuropathy; amputation; and retinopathy (International Diabetes Federation, 2009).

1.3 Classification of Diabetes Mellitus and other Categories of Glucose Regulation:

The vast majority of cases of diabetes fall into two broad etiopathogenic categories:

1.3.1. Type 1 Diabetes (β-Cell Destruction, Usually Leading to Absolute Insulin Deficiency):

This form of diabetes, which accounts for only 5-10% of those with diabetes (American Diabetes Association, 2006), previously encompassed by the terms insulin-dependent diabetes mellitus (IDDM), type 1 diabetes, or juvenile-onset diabetes. The cause of this form of diabetes is an absolute deficiency of insulin secretion results from a cellular-mediated autoimmune destruction of the β-cells of the pancreas (American Diabetes Association, 2006). The rate of destruction is quite variable, being rapid in children and slowly in adults (Zimmet et al., 1994; Humphrey et al., 1998).

Type 1 is usually characterized by the presence of autoantibodies to glutamic acid decarboxylase (GAD) (Verge et al., 1996), islet cell autoantibodies and autoantibodies to insulin that lead to β-cell destruction that may ultimately lead to diabetes mellitus in which " insulin is required for survival " (Willis et al., 1996) to prevent the development of ketoacidosis, coma and death (WHO, 1999).

Autoimmune destruction of β-cells has multiple genetic predispositions and is also related to environmental factors including viral infections, rat poison and cow milk formula. Although patients are rarely obese when they present with this type of diabetes, the presence of obesity is not incompatible with the diagnosis (American Diabetes Association, 2006).

1.3.2. Type 2 Diabetes (Ranging from Predominantly Insulin Resistance with Relative Insulin Deficiency to Predominantly an Insulin Secretary Defect with Insulin Resistance):

Diabetes mellitus of this type previously encompassed non insulin-dependent diabetes mellitus (NIDDM), or adult-onset diabetes (WHO, 1999). It accounts for 90-95% of those with diabetes. The cause is a combination of resistance to insulin action and an inadequate

compensatory insulin secretory response (American Diabetes Association, 2006). So type 2 diabetes is a term used for individuals who have insulin resistance (DeFronzo et al., 1997; Lillioja et al., 1993) and usually have relative (rather than absolute) insulin deficiency. At least initially, and often throughout their lifetime, these individuals do not need insulin treatment to survive (WHO, 1999; American Diabetes Association, 2006).

The majority of patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance (Campbell et al., 1993; Bogardua et al., 1985). Many of those who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region (Kissebah et al., 1982), Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another illness such as infection (Banerji et al., 1994; Umpierrez et al., 1995). This form of diabetes is frequently undiagnosed for many years because the hyperglycemia is often not severe enough for the patients to notice any of the classic symptoms of diabetes (Mooy et al., 1995; Harris et al., 1993). Nevertheless, such patients are at increased risk of developing macrovascular (coronary artery disease, peripheral arterial disease and stroke (Michael and Fowler, 2008)) and microvascular complications (diabetic nephropathy, neuropathy, and retinopathy (Michael and Fowler, 2008)) (Mooy et al., 1995; Harris et al., 1993).

Oxidative stress is produced under diabetic conditions and possibly causes various forms of tissue damage especially pancreatic β cells in patients with type 2 diabetes. Under diabetic conditions, reactive oxygen species (free radicals) are produced mainly through the glycation reaction, which occurs in various tissues and cells, especially β cells (Kaneto et al., 1999). Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose level in these diabetic patients would be expected to result in even higher insulin values had there β-cell function been normal (Polonsky et al., 1996). Thus, insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. Insulin resistance may improve with weight reduction, increased physical activity, and/or pharmacological treatment of hyperglycemia but is not restored to normal (Simonson et al., 1984; Wing et al., 1994).

The risk of developing type 2 diabetes increases with age, obesity, and lack of physical activity (Zimmet et al., 1992; Harris et al., 1995). It occurs more frequently in individuals

with hypertension or dyslipidemia, and its frequency varies in different racial/ethnic subgroups (Zimmet et al., 1992; Harris et al., 1995; Valle et al., 1997; de Courten et al., 1993). It is often associated with a strong genetic predisposition (Valle et al., 1997; de Courten et al., 1993; Knowler et al., 1993) However, the genetics of this form of diabetes are complex, it is now fully defined, and Genome-wide association studies (GWAS) have identified several type 2 diabetes susceptibility loci in several genes including CDKN2B, IGF2BP2, CDKAL1, HHEX, SLC30A8, TCF7L2, KCNJ11 and PPARG (Tan et al., 2009).

1.3.3. Impaired Glucose Regulation (Impaired Glucose Tolerance (IGT) and Impaired Fasting Glucose (IFG)):

Impaired glucose regulation (IGT and IFG) refers to a metabolic state intermediate between normal glucose homeostasis and diabetes (WHO, 1985). IFG and IGT represent different abnormalities of glucose regulation, one in the fasting state and one post-prandial.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus recognized an intermediate group of subjects whose glucose levels, although not meeting criteria for diabetes, are nevertheless too high to be considered normal. This group is defined as having fasting plasma glucose (FPG) levels ≥100 mg/dl (5.6 mmol/l) but <126 mg/dl (7.0 mmol/l) or 2-h values in the oral glucose tolerance test (OGTT) of ≥140 mg/dl (7.8 mmol/l) but <200 mg/dl (11.1 mmol/l). Thus, the categories of FPG values are as follows:

- FPG <100 mg/dl (5.6 mmol/l) = normal fasting glucose;
- FPG 100–125 mg/dl (5.6–6.9 mmol/l) = IFG (impaired fasting glucose or prediabetes);
- FPG ≥126 mg/dl (7.0 mmol/l) = provisional diagnosis of diabetes.

The corresponding categories when the OGTT is used are the following:

- 2-h postload glucose <140 mg/dl (7.8 mmol/l) = normal glucose tolerance;
- 2-h postload glucose 140–199 mg/dl (7.8 –11.1 mmol/l) = IGT (impaired glucose tolerance);
- 2-h postload glucose ≥ 200 mg/dl (11.1 mmol/l) =provisional diagnosis of diabetes.

IGT and IFG are not clinical entities in their own right, but rather risk categories for future diabetes as well as cardiovascular disease (Fuller et al., 1980; Alberti, 1996).

IFG and IGT are often associated with the metabolic syndrome (Reaven, 1998),which includes obesity (especially abdominal or visceral obesity), dyslipidemia of the high-triglyceride and/or low-HDL type, and hypertension (American Diabetes Association, 2006).

Individuals with IGT often manifest hyperglycemia only when challenged with an oral glucose load (American Diabetes Association, 2006).

1.4 Diagnosis and Diagnostic Criteria for Diabetes Mellitus

The criteria for the diagnosis of diabetes are shown in (Table 1.1). Three ways to diagnose diabetes are possible, and each, in the absence of unequivocal hyperglycemia, must be confirmed, on a subsequent day, by any one of the three methods given in table 2 (American Diabetes Association, 2006).

Table 1.1: Criteria for the Diagnosis of Diabetes Mellitus (American Diabetes Association, 2006)

1. Symptoms of diabetes plus casual plasma glucose concentration ≥200 mg/dl (11.1 mmol/l). Casual is defined as any time of day without regard to time since last meal. The classic symptoms of diabetes include polyuria, polydipsia, and unexplained weight loss.
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 postload glucose ≥200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.
In the absence of unequivocal hyperglycemia, these criteria should be confirmed by repeat testing on a different day. The third measure (OGTT) is not recommended for routine clinical use.

An elevated level of glucose irreversibly bound to hemoglobin (termed glycosylated hemoglobin or HbA1c) of 6.0% or higher (the 2003 revised US standard) is considered abnormal by most labs; HbA1c is primarily used as a treatment-tracking test reflecting average blood glucose levels over the preceding 90 days (approximately). However some physicians may order this test at the time of diagnosis to track changes over time. The

higher the glucose concentration in blood, the higher the level of HbA1c. Levels of HbA1c are not influenced by daily fluctuations in blood glucose concentration but reflect the average glucose levels over a prior six to eight weeks. Therefore, HbA1c is a useful indicator of how well the blood glucose level has been controlled in the past and may be used to monitor the effects of diet, exercise, and drug therapy on blood glucose in diabetic patients (American Diabetes Association, 2010). Glycated haemoglobin (HbA1c) has been recommended by some international organisations as a diagnostic tool for detecting type 2 diabetes and impaired glucose regulation. The HbA1c cut-point of ≥6.5% (48 mmol/mol) has been selected as diagnostic for type 2 diabetes, while the cut-points for IGR are debated by the different international organisations: an International Expert Committee has suggested using HbA1c 6.0-6.4% (42-46 mmol/mol); however, the American Diabetes Association has recommended using HbA1c 5.7-6.4% (39-46 mmol/mol) (Mostafa et al., 2010). Some countries will adopt a new method of reporting HbA1c values in millimoles per mole (mmol/mol). Use of HbA1c has some logistical advantages over using an oral glucose tolerance test (OGTT). As patients do not need to fast, appointments do not need to be limited to the morning. The HbA1c result reflects longer term glycaemia and is less affected by recent physical/emotional stress. However, there is some debate as to whether HbA1c should replace fasting plasma glucose or the OGTT. As the two tests detect different people, some individuals with diabetes detected on OGTT will no longer be classified as having type 2 diabetes using HbA1c ≥6.5% criteria.

The importance of HbA1c comes from the facts that:

- It is a simple way to evaluate average glucose levels over the past two to four weeks.
- It is the best single test for evaluating the risk for glycemic damage to tissues (e.g., nerves, and small blood vessels in the eyes and kidneys) and, thus, risk of complications of diabetes.
- Clinical trials, such as the Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) have shown that improving HbA1c measures will decrease the development and progression of eye, kidney and nerve complications in both type 1 and type 2 diabetes (Michigan Diabetes Research and Training Center, 2010).

Furthermore, some medical conditions can result in HbA1c assay measurements not reflecting glycaemic control over the last 2-3 months; these include haematological disorders, renal failure, and chronic excess alcohol consumption (Mostafa et al., 2010).

1.5 Epidemiology of Type 2 Diabetes

Diabetes mellitus is one of the most common endocrine disorders affecting almost 6% of the world's population. The number of diabetic patients will reach 300 million in 2025. More than 97% of these patients will have type 2 diabetes. The incidence of type 1 diabetes ranged from 1.9 to 7.0/100,000/yr in Africa, 0.13 to 10/100,000/yr in Asia, 4.4/100,000/yr in Australasia, 3.4 to 36/100,000/yr in Europe, 2.62 to 20.18/100,000/yr in the Middle East, 7.61 to 25.7/100,000/yr in North America, and 1.27 to 18/100,000/yr in South America. The epidemiology of type 2 diabetes is equally bleak. The prevalence of type 2 diabetes ranged from 0.3 to 17.9% in Africa, 1.2 to 14.6% in Asia, 0.7 to 11.6% in Europe, 4.6 to 40% in the Middle East, 6.69 to 28.2% in North America, and 2.01 to 17.4% in South America (Adeghate et al., 2006).

The incidence of diabetes in Palestine is relatively high, ranging between 7 and 10% of the population (World Diabetes Foundation, 2007).

The incidence is highest in Arab countries. It is 18.7% in United Arab Emirates, 16.8% in Saudi Arabia and 15.4% in Qatar and Bahrain (International Diabetes Federation, 2009).

1.6 Treatment and Management of Type 2 Diabetes

Diabetes mellitus is currently a chronic disease, without a cure, and medical emphasis must necessarily be on managing/avoiding possible short-term as well as long-term diabetes-related problems. There is an exceptionally important role for patient education, dietetic support, sensible exercise, self glucose monitoring, with the goal of keeping both short-term blood glucose levels, and long-term levels as well, within acceptable bounds. Careful control is needed to reduce the risk of long term complications. This can be achieved with combinations of diet, exercise and weight loss (type 2), various oral diabetic drugs (type 2 only), and insulin use (type 1 and increasingly for type 2 not responding to oral

medication). In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications should be undertaken to control blood pressure (Rolka et al., 2001).

Cardiovascular complications, characterized by endothelial dysfunction and accelerated atherosclerosis, are the leading cause of morbidity and mortality associated with diabetes.

There is growing evidence that excess generation of highly reactive free radicals (such as superoxide ($^{\bullet}O_2^-$), hydroxyl ($^{\bullet}OH$), peroxyl ($^{\bullet}RO_2$), hydroperoxyl ($^{\bullet}HRO_2$), nitric oxide ($^{\bullet}NO$), and nitrogen dioxide ($^{\bullet}NO_2^-$)), largely due to hyperglycemia, causes oxidative stress, which further exacerbates the development and progression of diabetes and its complications. Overproduction and/or insufficient removal of these free radicals result in vascular dysfunction, damage to cellular proteins, membrane lipids and nucleic acids. Since numerous studies demonstrated that oxidative stress, contributes to the development and progression of diabetes and related contributions, it became clear that ameliorating oxidative stress through treatment with antioxidants might be an effective strategy for reducing diabetic complications. So, several clinical trials investigated the effect of the antioxidant vitamin E on the prevention of diabetic complications. However, these trials failed to demonstrate relevant clinical benefits of this antioxidant on cardiovascular disease (Guigliano et al., 1996; Ceriello, 2003; Ceriello and Motz, 2004). The negative results of the clinical trials with antioxidants prompted new studies focusing on the mechanisms of oxidative stress in diabetes in order to develop causal antioxidant therapy that should be part of the cardiovascular treatment plan in diabetes (Johansen et al., 2005).

1.7 Molecular Genetics of Type 2 Diabetes

Type 2 diabetes is caused by a combination of genetic susceptibility (hereditary factors), environmental factors together with lifestyle and behavioral factors (Scott et al., 2007). Genome-wide association studies (GWAS) have identified several type 2 diabetes susceptibility loci in the genes CDKN2B, IGF2BP2, CDKAL1, HHEX, SLC30A8, TCF7L2, KCNJ11, and PPARG (Tan et al., 2009). Association analysis of Single Nucleotide Polymorphisms (SNPs) in and around these genes is summarized in Table 1.2 to identify genetic variants that predispose to type 2 diabetes (Scott et al., 2007).

Table 1.2: Confirmed Susceptibility Variants Loci of Type 2 Diabetes Associated Genes (Scott et al., 2007; Genome-Wide Association Analysis Identifies Loci for Type 2 Diabetes, June 2007).

Chromosome	Position (bp)	Risk Allele	Genes
3	186,994,389	T	IGF2BP2
6	20,769,229	C	CDKAL1
9	22,124,094	T	CDKN2B
10	94,452,862	C	HHEX
8	118,253,964	C	SLC30A8
3	12,368,125	C	PPARG
11	17,366,148	T	KCNJ11
10	114,748,339	T	TCF7L2

Many studies show a difference in the degree of association of these variants with type 2 diabetes (Scott et al., 2007). A cluster of variants in the IGF2BP2 (insulin-like growth factor 2 mRNA binding protein 2) region was associated with type 2 diabetes (Scott et al., 2007). IGF2BP2 is a paralog of IGF2BP2, which binds to the 5' untranslated region of the insulin-like growth factor 2 (IGF2) mRNA and regulates IGF2 translation (Nielsen et al., 1999). IGF2 is a member of the insulin family of polypeptide growth factors involved in the development, growth, and stimulation of insulin action. The most strongly associated IGF2BP2 SNPs are located in a 50-kb region within intron 2 diabetes-predisposing variants may therefore affect regulation of IGF2BP2 expression. SNPs, located within intron 5 of CDKAL1, showed modest evidence for type 2 diabetes association (Scott et al., 2007). CDKAL1 [cyclin-dependent kinase 5 (CDK5) regulatory subunit associated protein-1-like 1] shares protein domain similarity with CDK5 regulatory subunit-associated protein 1 (CDK5RAP1), which specifically inhibits activation of CDK5 by CDK5 regulatory subunit 1 (CDK5R1) (Ching et al., 2002). The associated SNPs within intron 5, may regulate expression of CDKAL1 and so affect the expression of CDK. CDK5 and CDK5R1 activity is influenced by glucose and may influence beta-cell processes (Ubeda et al., 2004; Wei et al., 2005); overactivity of CDK5 in the pancreas may lead to beta-cell degeneration, specially under glucotoxic conditions (Ubeda et al., 2006). SNP is located upstream of CDKN2B. CDKN2B inhibits the activity of CDK4 and CDK6. In mice, CDK4 activity has been shown to influence beta-cell proliferation and mass, with loss of Cdk4 leading diabetes (Rane et al., 1999; Tsutsui et al., 1999). SNPs in a region that includes HHEX (hematopoietically expressed homeobox) showed modest evidence of type 2 diabetes association (Scott et al., 2007). HHEX is critical for development of the ventral pancreas (Bort et al., 2004). SNP in the pancreatic beta cell-specific zinc transporter SLC30A8

(Chimienti et al., 2004), showed evidence for type 2 diabetes association. SLC30A8 transports zinc from the cytoplasm into insulin secretory vesicles (Chimienti et al., 2004; Chimienti et al., 2006), where insulin is stored as a hexamer bound with two Zn^{2+} ions before secretion (Dunn, 2005). Variation in SLC30A8 may affect zinc accumulation in insulin granules, affecting insulin stability, storage or secretion.

Candidate gene has suggested many putative susceptibility variants, but unequivocal replications are so far limited to variants in just three genes: PPARG, KCNJ11, and TCF7L2 (Altshuler et al., 2000; Gloyn et al., 2003; Grant et al., 2006). The strongest association is observed for single-nucleotide polymorphisms (SNPs) in TCF7L2 (Zeggini et al., 2007). The ability to construct a list of robust type 2 diabetes-associated loci (Tale 1.2) represents a land mark in efforts to identify genetic variants that predispose to complex human diseases, although the specific predisposing variants and even the relevant genes remain to be defined (Scott et al., 2007).

1.8 PGC-1α: Transcriptional Coactivator and Metabolic Regulator

1.8.1. Introduction:

Gene transcription is now understood to involve several distinct protein complexes that are necessary to get proper regulation in space and time. The transcription machinery must function at the correct time and chromosomal location to unwind compacted chromatin, recruit RNA polymerase and related factors, and initiate RNA synthesis (Puigserver et al., 2003). The key components can be organized into several categories. First, transcription factors generally refer to proteins that bind to DNA in a sequence-specific manner, typically as hetero- or homodimers. Most commonly, this occurs in the region 5' to the transcription start site. Second, the region around the start of transcription binds the general transcription apparatus, a complex that functions to correctly orient RNA polymerase II at the specific site for initiation of transcription. This complex, involving many proteins, usually contains TATA binding protein. Finally, mediating the functional connection between transcription factors and the general transcription apparatus are the coactivators (Puigserver et al., 2003).

Coactivator refers to a protein or protein complex that increases the rate of transcription by interacting with transcription factors but does not itself bind to DNA in a sequence-specific manner. The binding of coactivators to transcription factors can also be regulated, for example, by the binding of ligands to nuclear hormone receptor (Puigserver et al., 2003).

Coactivators usually exist and function as multiprotein complexes. These complexes contain individual proteins that mediate docking on transcription factors and others that mediate functions necessary for transcription itself. These include modifications of histones by acetyltransferase activity, modification of histones by phosphorylation or methylation, and the unwinding and remodeling of chromatin in an ATP-dependent fashion (Puigserver et al., 2003).

1.8.2. PGC-1α: A Versatile Coactivator:

PGC-1α, originally identified as a coactivator of PPAR γ (Puigserver et al., 1998), has since been shown to increase the transcriptional activity of PPAR γ and many additional nuclear receptor families, including members of the estrogen (Tcherepanova et al., 2000; Huss et al., 2002), retinoid X (Delerive et al., 2002), glucocorticoid (GR) (Knutti et al., 2000), liver X (LXR) (Oberkofler et al., 2002), vitamin D and thyroid hormone receptor families (Dietz, 2004). PGC-1α can also bind unliganded nuclear receptors, as in the case of the orphan hepatocyte nuclear factor (HNF) 4α (Rhee et al., 2003), and estrogen-related receptor (ERR)α, suggesting that their conformation are conductive to ligand-independent mechanisms of gene regulation (Kallen et al., 2004).

PGC-1α target are not confined to the nuclear receptor superfamily, however, and this versatile coactivator associates with a diverse array of other transcription factors involved in the insulin and glucagon signaling pathway, including the forkhead/winged helix protein family member FOXO1 (Puigserver et al., 2003).

1.8.3. PGC-1α Regulates Biological Programs Linked to Energy Homeostasis:

1.8.3.1. PGC-1α and Adaptive Thermogenesis:

1.8.3.1.1. Concepts and Tissues Involved in Adaptive Thermogenesis:

Adaptive thermogenesis refers to changes in heat dissipation in response to environmental temperatures, nutritional status, or infection. In each of these challenges, physiological mechanisms are activated to increase heat production in different tissues, particularly brown fat and muscle (Cannon et al., 1998; Rothwell and Stock, 1979).

Most adaptive thermogenesis in small mammals takes place in brown adipose tissue (BAT). BAT is morphologically and metabolically different from white adipose tissue (WAT) and plays an opposite physiological function: dissipation vs. storage of energy. Brown adipocytes contain multiple small droplets of triglycerides and a high number of mitochondria. In addition, there mitochondria contain a specific uncoupling protein-1 (UCP-1), which is expressed only in brown adipocytes. UCP-1 is an essential component for cold-induced thermogenesis. Adaptive thermogenesis in BAT is achieved through two main inputs: adrenergic stimulation (Cannon et al., 1996; Ricquier, 1998) and thyroid hormone action (Silva, 1995; Reitman et al., 1999).

Another tissue involved in adaptive thermogenesis is skeletal muscle. Skeletal muscle represents about 40% of the total body mass and has high oxidative metabolism linked to a high mitochondrial number (Simonsen et al., 1992).

1.8.3.1.2. PGC-1α Regulates Components of Adaptive Thermogenesis and Mitochondrial Biogenesis:

1.8.3.1.2.1. Identification of PGC-1α:

UCP-1 gene expression is highly cold-inducible through the activation of the sympathetic nervous system and is mediated by β-adrenergic receptors and cAMP (Cassard-Doulcier et al., 1993; Kozak et al., 1994). In addition, several activated nuclear hormone receptors play an important role in differentiation of brown fat cells and in UCP-1 gene expression,

including thyroid hormone receptor (TR), and PPARs (Barbera et al., 2001; Sears et al., 1996). The potential involvement of PPARγ in this regulation is particularly interesting because of its key role in white fat-specific gene expression and differentiation. Activation of PPARγ by synthetic ligands, the antidiabetic thiazolidinediones, has been shown to promote the differentiation of brown fat precursor cells when given to rats. Furthermore, PPARγ knockout animals lack brown adipose tissue, indicating that it is required for the formation of this tissue, as well as white fat (Barak et al., 1999; Rosen et al., 1999). Although these data strongly support the importance of PPARγ in brown fat differentiation, it is clear that the same factor alone cannot determine whether adipocyte cells are white (energy storage) or brown (energy dissipation). These data suggested that the possibility that a specific cofactor might augment and alter PPARγ function in brown fat cells, resulting in activation of the thermogenic genes that characterize this cell type. Based on this hypothesis, a PPARγ-interacting protein that is expressed preferentially in brown fat compared with white fat was cloned, in which endogenous levels are very low. This factor was called PGC-1α (Puigserver et al., 1998).

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha is a protein that in humans is encoded by the PPARGC1A gene. The protein encoded by this gene is a transcriptional coactivator that regulates the genes involved in energy metabolism. This protein interacts with the nuclear receptor PPAR-γ, which permits the interaction of this protein with multiple transcription factors. This protein can interact with, and regulate the activities of, cAMP response element binding protein (CREB) and nuclear respiratory factors (NRFs) (Wu et al., 1999). It provides a direct link between external physiological stimuli and the regulation of mitochondrial biogenesis (Wu et al., 1999), and is a major factor that regulates muscle fiber type determination (Lin et al., 2002). In humans PGC-1α is expressed in high quantities in liver, heart, kidney, and skeletal muscle and to a lesser extent in white adipose tissue, pancreas, and brain (Larraouy et al., 1999; Esterbaur et al., 1999).

1.8.3.1.2.2. Adrenergic Regulation of PGC-1α in Brown Fat and Muscle:

The function of the β-adrenergic receptors in brown fat and muscle is critical for the response of these tissues to cold and alterations in diet. Physiologically, this stimulation is mediated through the sympathetic nervous system that innervates the brown adipocytes.

PGC-1α is strongly induced at the transcriptional level in brown fat and skeletal muscle when mice are exposed to the cold. Consistent with these data, PGC-1 mRNA is induced in a brown fat cell line treated with a β-adrenergic receptor agonist, isoproterenol (Puigserver et al., 1998). The effect is mediated through the β3-adrenergic pathway, in that knockout animals for this receptor lack the cold induction of PGC-1α in brown fat (Boss et al., 1999). In addition, injection of specific β3-agonists can specifically induced PGC-1α in brown fat (Gomez-Ambrosi et al., 2001).

PGC-1α strongly coactivates several nuclear receptors that bind to the UCP-1 enhancer. Thus, a likely model is that a major protein of the cAMP effect is mediated via the induction of PGC-1α and subsequent interaction of this factor with nuclear hormone receptor PPARγ, PPARα, RAR, and TR.

1.8.3.1.2.3. PGC-1α and the Adipocyte Cell Fate Decision:

Several pieces of data suggested that PGC-1α is likely to be involved in the decision to become either white or brown fat cells (Puigserver et al., 1998). First, PGC-1α is the only protein thus far describe that can powerfully activate the UCP-1 enhancer in non-BAT cell lines. Second, when introduced into white fat cells, PGC-1α induces endogenous UCP-1 gene expression and mitochondrial biogenesis, two decisive features of brown fat.

1.8.3.1.2.4. PGC-1α Regulates Respiration, Mitochondrial Biogenesis, and Gene Expression:

Enhanced mitochondrial biogenesis is an important component of adaptive thermogenesis, especially in brown fat and skeletal muscle, in which PGC-1α is highly expressed and inducible by cold or adrenergic stimuli. Exposure to cold temperatures induces mitochondrial proliferation and increased uncoupling of respiration.

Two novel transcription factors, nuclear respiratory factor (NRF)-1 and -2, that bind to the promoter region of a broad range of mitochondrial genes encoded in the cell nucleus, including β-ATP synthase, cytochrome-c, cytochrome-c-oxidase subunit IV, and mitochondrial transcription factor A (mtTFA). It is of particular interest that the NRFs turn

on mtTFA, a key transcriptional activator that translocates to the mitochondria and activates mitochondrial DNA replication and transcription.

PGC-1α has a major impact on the NRF system (Fig. 1.2). When introduced into muscle cells, PGC-1α dramatically induces gene expression for NRF-1, NRF-2, and mtTFA. Furthermore, PGC-1α physically interacts with NRF-1 and coactivates its transcriptional activity (Wu et al., 1999).

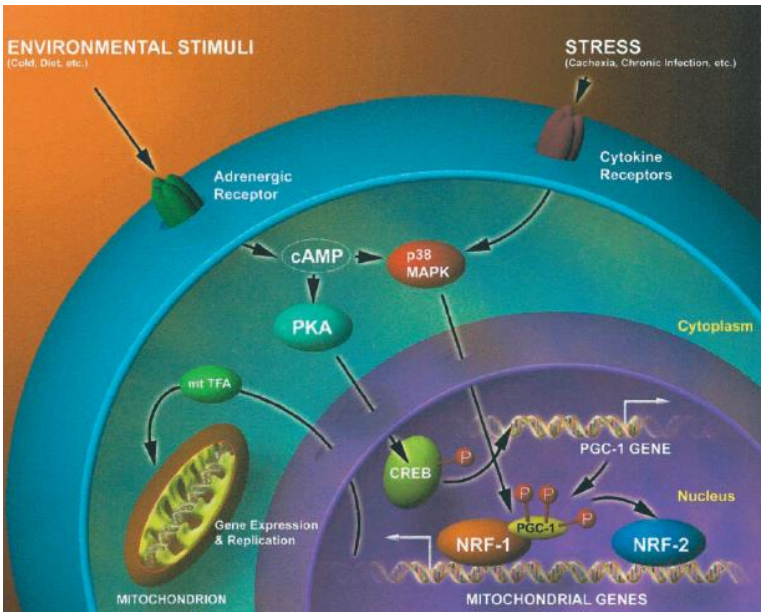

Fig. 1.2: Mitochondrial Biogenesis and Gene Expression through PGC-1α. Adrenergic and cytokine cell surface receptors trigger signaling cascades involving the PKA and p38 MAPK pathways. PKA phosphorylates CREB transcription factor, which is involved in the induction of PGC-1α gene expression. Stimulation of p38 MAPK directly phosphorylates the PGC-1α protein, resulting in its activation and stabilization. PGC-1α activates the expression of the subunits of respiratory chain and mtTFA through the induction of the expression of NRFs and the coactivation of NRF-1-mediated transcription. mtTFA subsequently translocates into the mitochondrion and directly increases the transcription and replication of mtDNA (Wu et al., 1999).

1.8.3.2. PGC-1α and Fuel Homeostasis: Glucose Uptake and Gluconeogenesis:

1.8.3.2.1. PGC-1α and Glucose Uptake:

The activation of increased energy expenditure ultimately requires an increased uptake and metabolism of fuels. PGC-1α has been shown to stimulate genes of fatty acid oxidation in cardiac cells, and induction is associated with an increase in fatty acid oxidation in adipocytes and in heart (Lehman et al., 2000; Vega wt al., 2000).

The effects of PGC-1α on glucose uptake and metabolism are of particular interest in diabetes because there are numerous studies indicating that the rates of mitochondrial oxidation can affect glucose uptake (Randle et al., 1994). Simultaneous to the effects of PGC-1α on mitochondrial respiration in skeletal muscle cells, this coactivator also induces gene expression for the insulin-sensitive glucose transporter (Glut-4) and increases glucose uptake (Michael et al., 2001). This effect on Glut-4 gene expression is partially mediated through PGC-1α binding to and coactivating the muscle transcriptional regulator MEF2 (myocyte enhancer factor-2).

1.8.3.2.2. PGC-1α and Hepatic Gluconeogenesis:

Mammals have highly regulated systems to maintain blood glucose levels within tight limits, despite intermittent access to food. Blood glucose levels are controlled by the hormonal modulation of glucose production and peripheral glucose uptake.

The liver is the major producer of glucose and does this through two different pathways. One is glycogenolysis, or the breakdown of glycogen that occurs in a relatively short-term fast (< 24 hour). A second pathway, gluconeogenisis is turned on with a medium-to long-term fast (after approximately 30 hours of fasting) and involves synthesis of glucose from precursors such as lactate, pyruvate, glycerol, and alanine. The rate of gluconeogenesis is controlled by the activities of three key enzymes: phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase, and glucose-6-phosphotase. Hormonal modulation of these enzymes is mainly controlled at the transcriptional level with insulin, glucagons, and glucocorticoids (Puigserver and Spiegelman, 2003). Gluconeogenesis occurs in fasting or diabetic states in which insulin is low or the liver is insulin resistant. In particular, insulin blocks the actions of the progluconeogenic hormones, glucagon and glucocorticoids (Puigserver and Spiegelman, 2003). Recent studies illustrate that PGC-1α expression in liver is dramatically increased by fasting (Yoon et al., 2001; Herzig et al., 2001). A key role of insulin as a suppressor of PGC-1α expression in liver was shown using mice having liver-specific mutations in the insulin receptor. The expression of PGC-1α correlated well with hepatic gluconeogenesis. Therefore the ability of gluconeogenic hormones to activate PGC-1α expression in primary cultures of hepatocytes was examined (Yoon et al., 2001). Glucagon, acting via cAMP, and glucocorticoids are the major positive factors activating the genes of gluconeogenesis in liver. Treatment of hepatocytes with 8-bromo-cAMP, a

cell-permeable analog of cAMP, induced mRNA expression of PEPCK and glucose-6-phosphatase, key enzymes of gluconeogenesis. Dexamethason, a synthetic glucocorticoid, increased PGC-1α gene expression only slightly but markedly synergized with 8-bromo-cAMP in the induction of PGC-1α mRNA (Yoon et al., 2001).

These correlative studies led to directly test a direct effect for PGC-1α in the activation of gluconeogenesis (Fig. 1.3). Primary hepatocytes cultures were infected with recombinant adenoviruses encoding the expression of PGC-1α gene. Remarkably, PGC-1α expression stimulated the expression of mRNA for all three key genes of gluconeogenesis: PEPCK, fructose 1,6-bisphosphatase, and glucose-6-phosphatase (Puigserver and Spiegelman, 2003). Experiments performed at different doses of the same virus indicated that maximal induction of these genes occurred at approximately the same levels of PGC-1α protein that are present in fasted liver (Yoon et al., 2001).

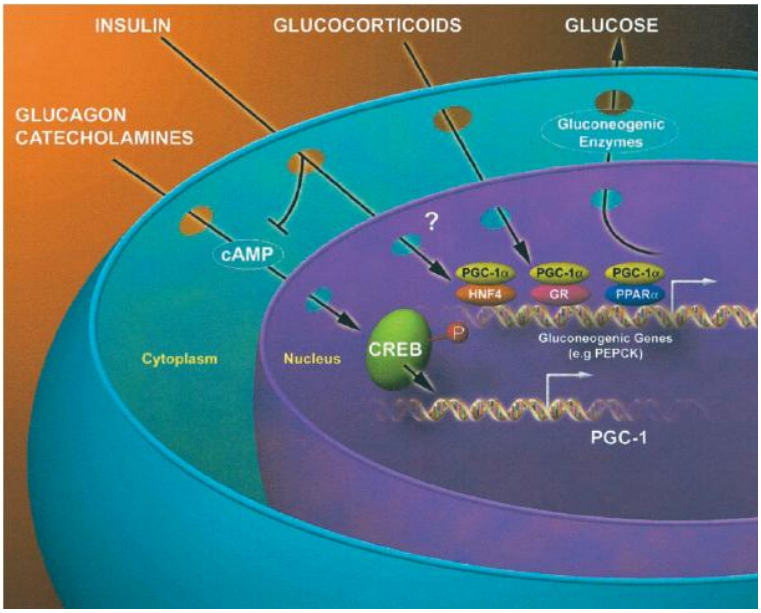

FIG. 1.3: Transcriptional Regulation of Hepatic Gluconeogenesis by PGC-1α. Hepatic glucose production in the liver is tightly controlled by hormones. Glucagon and catecholamines stimulate the cAMP pathway and CREB, which activates PGC-1α gene expression. PGC-1α is then recruited to different transcription factors that bind to the promoter of gluconeogenic genes such as PEPCK. Glucocorticoids induce a specific interaction between PGC-1α and GR-activating PEPCK. Insulin represses cAMP activation on gluconeogenic genes, but how it blocks PGC-1α gene expression is unknown (Puigserver and Spiegelman, 2003).

As might be predicted from gene expression data, PGC-1α stimulated a 3-fold increase in the ability of hepatocytes to secrete glucose when provided with gluconeogenic precursors. cAMP response element binding protein (CREB), a transcription factor activated by

glucagon and cAMP, directly activated the expression of PGC-1α through binding to its promoter (Puigserver and Spiegelman, 2003) .

1.8.4. Molecular Mechanisms of PGC-1α Function:

1.8.4.1. The PGC-1 Gene Family:

PGC-1α is now part of a small family of transcriptional coactivators that includes the close homolog PGC-1β (Lin et al., 2002; Kressler et al., 2002) and PGC-1-related coactivator (Andersson and Scarpulla, 2001) (Fig. 1.4). All these proteins share a high degree of homology in the N terminus and at the C terminus. The N terminus of all of these proteins contains a transcriptional activation domain and includes the major nuclear hormone receptor-interacting motif (LXXLL). The C-terminal region contains an RNA-binding motif (RMM) and a serine-arginine-rich (RS) domain. Therefore, an unusual feature of the PGC-1 family is the presence of transcriptional activation domains and RNA processing motifs in the same molecule.

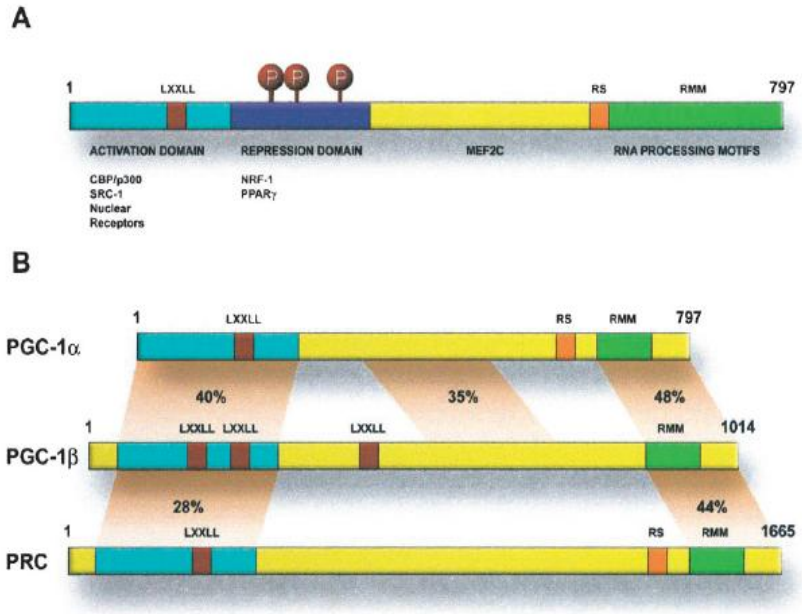

Fig. 1.4: Functional Domains of the PGC-1 Gene Family. A, Architecture of PGC-1α protein. PGC-1α contains a potent activation domain at its N terminus that interacts with other transcriptional coactivators. The LXXLL motif is responsible for ligand-dependent interaction with certain hormone nuclear receptors. A central suppression domain contains several p38 MAPK phosphorylation sites. The C terminus contains RNA processing motifs such as an RS domain and an RMM. B, Protein sequence alignment of the PGC-1 gene family. The percentage homology between the different domains of PGC-1 proteins is indicated. Note that the more conserved domains are at the N terminus and C terminus (Puigserver and Spiegelman, 2003) .

Computer-assisted searches reveal that domains at the N and C terminus of the PGC-1 family are conserved in proteins in many species, suggesting that these proteins may play important common roles in these species. It seems likely, that these proteins in cold-blooded animals may be linked to mitochondrial function and/or regulation of other cellular processes related to energy metabolism.

1.8.4.2. Analysis of PGC-1α/Transcription Factor Interactions:

1.8.4.2.1. Interaction with Different Transcription Factor Families:

PGC-1α was first identified as a protein of 798 amino acids that interacts with the nuclear receptor PPARγ, the master regulator of adipocyte differentiation (Puigserver et al., 1998). Structure-function analysis of PGC-1α revealed that the N-terminal 200 amino acids contain a potent transcriptional activation domain that is very rich in acidic amino acids (Fig. 1.4). Embedded in this region is an LXXLL sequence (amino acids 142-146), a motif known to be responsible for ligand-dependent interaction of other coactivators with nuclear hormone receptors (Heery et al., 1997). The LXXLL motif on PGC-1α is absolutely required for the ligand-dependent interaction with estrogen receptor (ER) (Tcherepanova et al., 2000), PPARα (Vega et al., 2000), and probably other nuclear hormone receptors. Interestingly, HNF4α also interacts with motif of PGC-1α without addition of any ligand, suggesting that this nuclear hormone receptor is in an active conformation even without the addition of exogenous ligand (Yoon et al., 2001). PGC-1α also uses different non-LXXLL domains to interact with certain other transcription factors: a domain between amino acids 200 and 400 interacts with PPARγ (Puigserver et al., 1998) and NRF-1 (Wu et al., 1999) and a region between amino acids 400 to 500 that interacts with MEF2-C (Michael et al., 2001).

1.8.4.2.2. PGC-1 Coactivates PPARγ in a Promoter-Specific Manner:

PGC-1α interacts with several nuclear hormone receptors present in the UCP-1 enhancer. PGC-1α coactivates both PPARγ and the thyroid receptor in the UCP-1enhancer. This activation was clearly synergistic after stimulation of cells with cAMP, a potent inducer of UCP-1 gene expression.

Because not all promoters of endogenous genes with functional PPARγ binding sites are activated by PGC-1α, there is apparent promoter specificity even when this coactivator is working through the same nuclear receptor. A good example of this is the selectively shown with the genes for UCP-1 and adipocyte protein 2 (aP2). Expression of PGC-1α in white fat cells turn on UCP-1 gene expression but failed to activate aP2 gene (Puigserver et al., 1998), the first gene actually identified as a target of PPARγ (Tontonoz et al., 1994).

This clearly illustrates that transcriptional coactivators are not only boosters of transcription but also play a key role in specification of the gene targeted by a transcription factor.

1.8.4.3. PGC-1α Recruits other Histone Acetyle Transferase (HAT) Containing Coactivator Proteins:

Most coactivaters potentiate transcriptional activity by having specific enzymatic functions that are required to remodel chromatin and initiate transcription (Struhl, 1998; Glass and Rosenfeld, 2000; Naar et al., 2001).

PGC-1α does not have significant amino acid sequence homology to other transcriptional coactivator families. In addition, PGC-1α does not contain any recognizable HAT domain and does not appear to have this intrinsic enzymatic activity. However, the N-terminal activation domain of PGC-1α recruits proteins that contain HAT activity, such as SRC-1 and CREB binding protein (CBP) (Puigserver et al., 1999). In addition, PGC-1α is also present in a complex containing RNA polymerase II.

The model of recruitment of transcriptional coactivators portrays the DNA binding transcription factor, functioning mainly to localize the coactivator complexes to genes that are marked for activation. Conversely, the coactivator complex has been thought to be constitutively active, requiring only proper positioning in the genome to initiate transcription. Interestingly, the binding of SRC-1 and CBP to the N terminus of PGC-1α is dependent upon docking of transcription factors such PPARγ and NRF-1 to the amino acids 200-400 region of PGC-1α (Puigserver et al., 1999). This suggests that PGC-1α is in a relatively quiescent state when not bound to a transcription factor; however, it becomes highly active when the transcription factor binds and induces a conformation change that

recruits SRC-1 and CBP into the complex. Thus, transcription factor docking switches on the activity of this coactivator protein.

1.9 Association of PGC-1α (Gly482Ser) Gene Polymorphism with Type 2 Diabetes

An important association with Type 2 diabetes has been reported for a missense variation (Gly 482 Ser) in the peroxisome proliferator-activated receptor γ coactivator-1 (PGC-1α) gene (Florz et al., 2003). The Gly 482 Ser variation in the PGC-1α gene has been reported to be associated with type 2 diabetes in Danish (Anderson et al., 2001) and Japanese (Hara et al., 2002) subjects. In contrast, no association was found in French, Caucasians (Lacquemnt et al., 2002) or in Pima Indians (Muller et al., 2003).

PGC-1α is a transcriptional coactivator that has been implicated in the regulation of gene involved in energy metabolism, adipogenesis, oxidative metabolism, thermogenesis. Given the critical role of PGC-1α in glucose uptake (it acts as a 'molecular switch' in pathways controlling glucose homeostasis), and the fact that human PGC-1α is mapped to a chromosomal region (4p15.1) that in Pima Indians has been shown to be linked to fasting serum insulin concentrations (Pratley et al., 1998), it was hypothesized that variability in the PGC-1α gene confers susceptibility to type 2 diabetes (Anderson et al., 2001) and may be a critical link in the pathogenesis of type 2 diabetes (Soyal et al., 2006).

1.10 Genetic Studies in Humans

Since transcriptional coregulators act at the amplification step of gene expression, minor imbalances in coregulator expression or activity levels may contribute to the pathogenesis of multifactorial diseases such as type 2 diabetes. Thus, functional sequence substitutions in PGC-1α may affect one or more of the three pathophysiological hallmarks of type 2 diabetes, insulin sensitivity, insulin secretion and hepatic gluconeogenesis. PGC-1α has been mapped to chromosome 4p15.1-2 (Esterbauer et al., 1999). This chromosomal region has been associated with basal insulin levels in Pima Indians (Pratley et al., 1998), abdominal subcutaneous fat in the Quebec Family Study (Perusse et al., 2001), high BMI in women of Utah pedigrees (Stone et al., 2002), obesity indices in Mexican Americans (Arya et al., 2004) and systolic blood pressure in families from the Netherlands (Allayee et al., 2001). Diabetes-related phenotypes have also been associated with single-nucleotide

polymorphisms or haplotypes at the PGC-1α locus (Ek et al., 2001; Andrulionyte et al., 2004; Andersen et al., 2005; Oberkofler et al., 2003; Esterbauer et al., 2002; Franks et al., 2003).

Associations of a Gly482Ser polymorphism with diabetes and related traits were studied in several populations. The Ser variant increased the relative risk of type 2 diabetes in a Danish population (Ek et al., 2001). Carriers of the Ser variant, in comparison with Gly482Gly subjects, showed a 1.6-fold higher risk of conversion from IGT to type 2 diabetes in the Study to Prevent Non-Insulin-Dependent Diabetes Mellitus (STOP-NIDDM) trial (Andrulionyte et al., 2004). No associations of Gly482Ser with type 2 diabetes were noted in French and Austrian populations or in Pima Indians (Lacquemant et al., 2002; Oberkofler et al., 2004; Muller et al., 2003). No associations between the Gly482Ser SNP and diabetes-related traits, such as insulin resistance was observed in non-diabetic German and Dutch populations (Stumvoll et al., 2004). Possible explanation for the lack of replication across studies are numerous and may include statistical fluctuations, insufficient power of some studies, genetic or phenotypical heterogeneity within and among study samples and differences in environmental factors.

The Gly482Ser polymorphism is located two amino acids downstream of the DEAD box motif, yet its functionality has not been proven (Fig. 1.5).

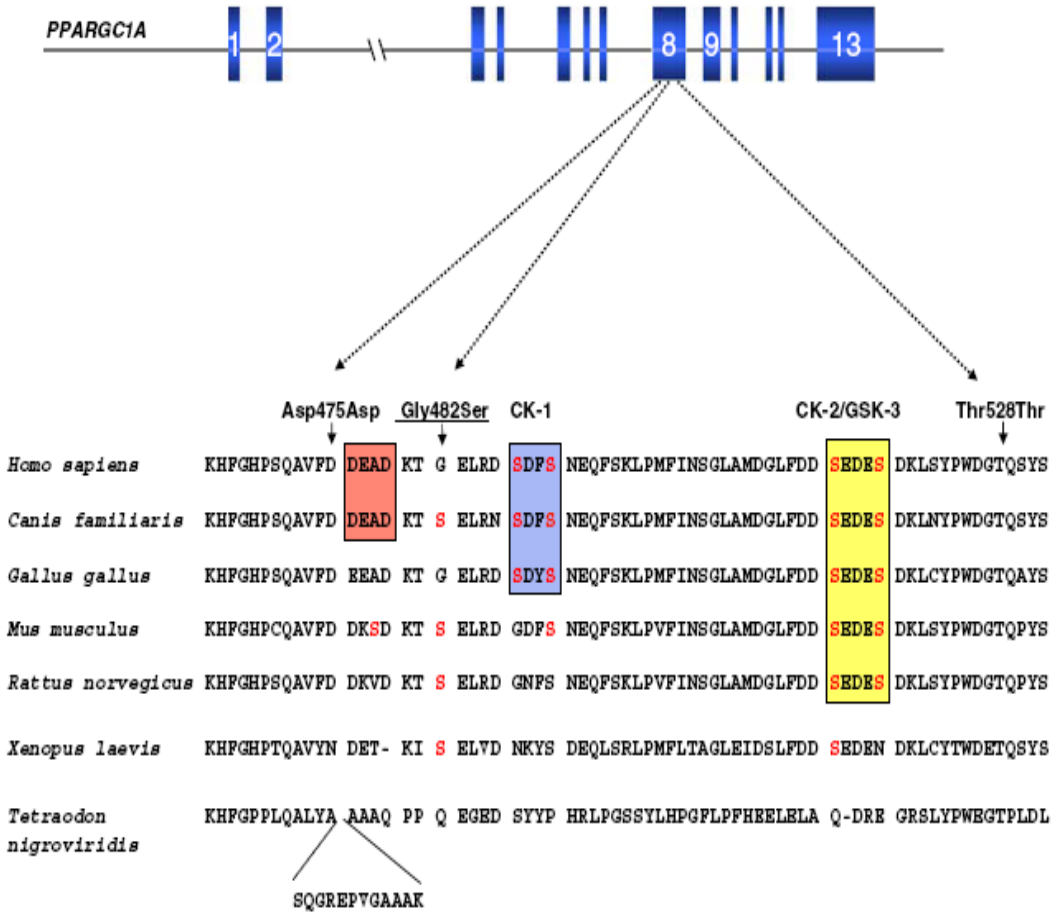

Fig. 1.5: Amino Acid Alignment of Sequences Spanning a Newly Identified DEAD Box Domain in the Human PGC-1α Protein. A Gly482Ser substitution and adjacent polymorphic sites are indicated as are a putative CK1 [phospho(P)Ser-X-X-Ser] and overlapping CK2 [Ser/Thr-X-XD/E] /glycogen synthase kinase (GSK) 3 sites (Soyal et al., 2006) .

The Gly482Ser polymorphism may not be the main or sole causative site, but may be part of a haplotype harbouring other functional sites. By characterizing and typing sequence substitutions across the entire PGC-1α locus, two distinct haplotype blocks were identified. Haplotype block 1 comprised the promoter and extended into intron 2, while haplotype block 2 extended from intron 2 beyond the poly A signals. Several promoter SNPs were located in transcription factor binding sites and affected transactivation of reporter constructs in an allele-specific manner. Score testing revealed moderate associations of specific block 1 haplotypes with beta cell indices. A common block 2 haplotype (containing Gly at codon 482) was associated with strongest insulin secretory response to glucose in glucose-tolerant subject. Thus, effects of PGC-1α on beta cell function added to the risk of type 2 diabetes (Soyal et al., 2006).

1.10 Objectives of the Study

1- To study the association of the polymorphism in the PPAR-α co activator 1α (PGC-1α) gene with type 2 diabetes among Palestinian patients in the West Bank.

2- To study the correlation between the polymorphism of this gene and different variables of collected clinical data (laboratory analysis) such as sex, age, blood pressure, BMI, fasting plasma glucose, HbA1c, cholesterol, HDL, LDL and TG.

Chapter Two

Materials and Methods

2.1 Study Subjects

The study sample comprised of 341 subjects (160 diabetic and 181 control) were included in our investigation. All subjects were recruited from three clinics in Ramallah district administered by UNRWA; Al Jalazon camp clinic, Al Amari camp clinic and Qalandia clinic. All subjects were interviewed to fill a special questioner to collect personal and demographic data (Appendix 1). All subjects were tested on site to obtain weight, hight, blood pressure and blood sample collection for DNA preparation and for testing several blood parameters related to diabetes.

2.2 Blood Sample Collection and DNA Preparation

2.2.1. Blood Sample Collection:

Fifteen ml whole blood samples were collected from each subject and distributed as follows:
Five ml whole blood were collected in potassium EDTA tubes and kept on ice then transported to Al-Quds University for processing i.e. DNA preparation, three ml in separate EDTA tubes for HbA1c measurement and the rest (seven ml) were processed for serum collection.

2.2.2. DNA preparation:

Genomic DNA was prepared from blood samples according to the following protocol:

1. Blood samples were centrifuged at 600xg for 12 minutes and 300µl of the buffy coat layer were collected and transferred to a sterile 0.5 ml eppendorf tube.
2. 600µl of lysis buffer 1 (MasterPure DNA Purification Kit) were added, and the tubes were mixed by inverting three times and flicking the bottom of the tubes to suspend any remaining material. Samples were incubated at room temperature for five minutes, mixed by inverting three times and then flicking the bottom of the tubes as before.
3. Incubation were continued at room temperature for an additional five minutes, the tubes were inverted three times for a final mix and flicked again to ensure full suspension.
4. White blood cells (WBCs) were pelleted by centrifugation at 10,000 RPM for 25 seconds in a micro centrifuge (Hettich) and most of the supernatant was poured off.
5. The pellets (WBCs) were suspended in 1ml distilled water to lyse the remaining RBCs, mixed and centrifuged immediately at 10,000 RPM for 25 seconds.
6. The pellets were suspended in 350µl water, 25µl of 10٪ SDS, and 100µg/ml of protinase k and incubated at 65˚C for 90 minutes. 100µl of 6 M NaCl were then added to the tubes, mixed vigorously by vortex for 30 seconds and centrifuged for 10 minutes at 12,000 RPM.
7. The supernatant was carefully transferred to a clean sterile eppendorf tube and 1ml of ice cold isopropanol was added , the tubes were capped , mixed gently for several times and were kept on -20˚C overnight.
8. DNA was collected by centrifugation for 10 minutes at 12000 RPM in a microcentrifuge. Supernatant was carefully poured off without dislodging the pellet. The pellet was allowed to air dry for at least 30 minutes and then suspended in 200 µl distilled water.
9. Some samples which showed some turbidity after suspension were centrifuged again at 12000 RPM for 10 minutes and the supernatant was transferred to new sterile eppendorf tube.

All samples were stored at -20˚C until further use.

2.3 Gel Electrophoresis for DNA Qualification

Genomic DNA qualification was tested by resolving on agarose gel. Agarose gel was prepared by dissolving 0.8g agarose (Cambrex Bio Science) in 100ml 1X TAE working buffer (Tris-accetate-EDTA buffer, PH 8). The mixture was boiled and allowed to cool. Ethidium bromide was then added to the agarose solution to a final concentration of 10 μg /100 ml and mixed. The warm agarose solution was poured into a prepared clean ultra violet (UV) transparent casting unit with a comb inserted vertically in the gel. The gel was left to set for 15 minutes at room temperature, and the casting unit was then placed into an electrophoresis tank filled with 1X TAE buffer.

1μl of extracted genomic DNA suspension was mixed with 1μl loading dye (Blue/orange 6X loading dye, Promega) and 6μl TE buffer, and then loaded onto the gel. 1μl high molecular weight DNA marker (Bioneer) was mixed with 1μl loading dye and 6μl TE buffer, and loaded in separate well. Electrophoresis was performed at 90 volts for 30 minutes. DNA was visualized under UV light (Transilluminator UV Sigma 10B201413) and photographed using gel documentation apparatus (Fujifilm/gel documentation system / Ramco company-80-6247-61 / 007-11-0-70).

2.4 Polymerase Chain Reaction (PCR) Analysis

The Gly482Ser polymorphism haplotypes of the PGC-1α gene were analyzed by polymerase chain reaction (PCR) amplification. The primers (Invitrogen) used in the PCR reaction as described by Andrulionyte et al., 2004 were as follows:
Forward primer 5'-TGC TAC CTG AGA GAG ACT TTG-3'.
Reverse primer 5'-CTT TCA TCT TCG CTG TCA TC-3'.

The PCR reaction mixture was prepared as follows:
1μl genomic DNA was mixed in 25μl reaction volume of ready mix PCR tubes (PCR-Ready™ High Yield Syntezza) with the addition of 1μl of each primer (150 ng/μl water) and 22μl of distilled water for final mixing of the components.
A 260 bp DNA fragment of the PGC-1α gene was amplified according to the following program:

Initial denaturation at 94˚C for 5 minutes, followed by 35 cycles of denaturation at 95˚C for 30 seconds, annealing at 56˚C for 30 seconds, extension at 72˚C for 30 seconds, and final extension at 72˚C for 6 minutes.

Following amplification, 10µl of the PCR mixture was analyzed on 2.5% agarose gel, after mixing with the loading mixture that included two stains for tracking of gels. 1µl of 100bp DNA marker (ladder marker (Bioneer)) was mixed with 2µl loading dye, 3µl TE buffer, and loaded onto the gel. Electrophoresis was run at 120 volts for 30 minutes. PCR product was visualized and photographed as described above.

2.5 Restriction Enzyme analysis

A polymorphism in the PGC-1α gene, leads to a Glycine to Serine substitution at nucleotide 482. The Gly482Ser polymorphism was analyzed after digestion of the 260bp DNA fragment with the HpaII digestion enzyme (Promega, USA). The recognition site for HpaII enzyme is:

5'… C▼CGG …3'

3'… GGC▲C …5'

Which includes a CCG codon for Glycine. Substitution of the first nucleotide (C) with (T) caused a change for the codon TCG which corresponds to Serine.

CCG

TCG

Therefore, when the nucleotide sequence which includes the CCG triplicate at this location, codes for Gly, the amplified DNA fragment will be digested with HpaII to produce two 157 and 103 bp fragments. However, when the first C in the triplicate is changed to T, the new triplicate (TCG) codes for Ser and the amplified DNA at this location will not be digested.

Restriction enzyme digestion:

10µl of the amplified PCR products were digested with 2 units of HpaII restriction enzyme (Promega) in a final reaction volume of 15µl containing 1X enzyme buffer (Promega). The reaction was incubated at 37˚C overnight.

After digestion, 10µl of the digested PCR products were mixed with 2µl loading dye and loaded onto 3% agarose gel. 1µl of the 100bp ladder marker was mixed with 2µl loading dye and 3µl TE buffer, and loaded onto the gel. Electrophoresis was carried out at 120 volts for 30 minutes. The gel was visualized and photographed as described above.

2.6 Blood Chemistry Analysis

Blood chemistry analysis was performed in the UNRWA labs using a multianalyser machine (Automatic chemistry analyzer (Cemwel, Awareness Technology, USA)).

2.6.1. HbA1c Assay:

The Glycated hemoglobin was measured by boronate affinity assay using NycoCard HbA1c Kit (Axis-shield, Oslo, Norway). HbA1c measures the total glycated hemoglobin but reports a standardized HbA1c value according to IFCC recommendations.

2.7 Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) version 17 for Windows. A p-value equal to or less than 0.05 was considered to be statistically significant. Analysis was done using T-test and Chi Square analysis.

Chapter Three

Results

3.1 Extracted Genomic DNA Qualification

Agarose gel (0.8%) was used for resolving genomic DNA that was extracted from all participants in this study in order to asses its integrity.

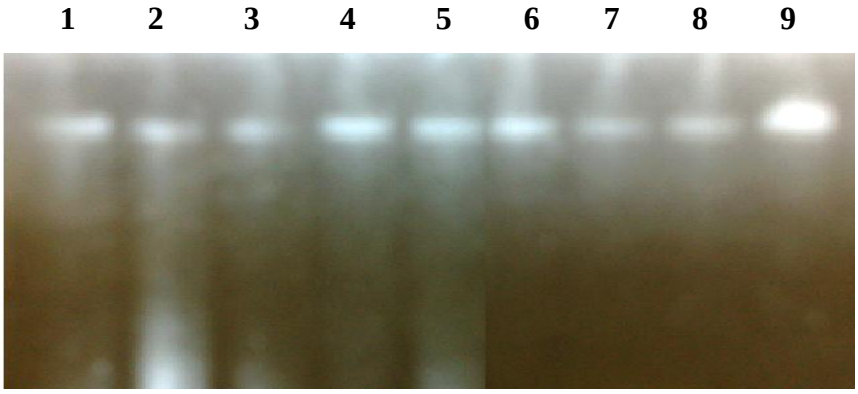

Fig. 3.1: Participants Genomic DNA Gel Electrophoresis (Samples 1-8). 0.8% agarose gel was performed to test genomic DNA qualification. Lane (1-8) showed DNA samples (1 µl of the extracted genomic DNA was loaded), lane 9 showed high molecular weight DNA marker (1 µl of DNA marker was loaded). The results showed intact genomic DNA samples with no significant degradation.

Fig. 3.1 shows a gel of representative samples of extracted genomic DNA (lanes 1-8). Each sample contains 1 µl of the extracted genomic DNA suspension. Lane 9 contains high

molecular weight DNA marker. The results show intact genomic DNA with no significant degradation. All genomic DNA preparations were evaluated in a similar manner.

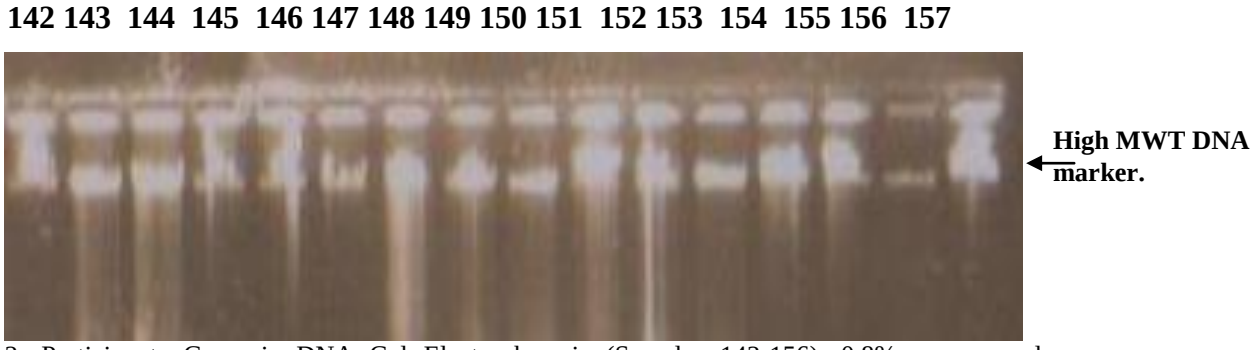

Fig. 3.2: Participants Genomic DNA Gel Electrophoresis (Samples 142-156). 0.8% agarose gel was performed to test genomic DNA qualification. Lane (142-156) showed another DNA samples (1 µl of the extracted genomic DNA was loaded), lane 157 showed high molecular weight DNA marker (1 µl of DNA marker was loaded). The results showed intact genomic DNA samples with no significant degradation.

Fig. 3.2 shows another gel of representative samples of extracted genomic DNA (lanes 142-156). Lane 157 contains high molecular weight DNA marker.

3.2 Amplified PGC-1α PCR Product

Fig. 3.3 describes the detection of representative amplified fragment of the PGC-1α gene (lanes 1-19).

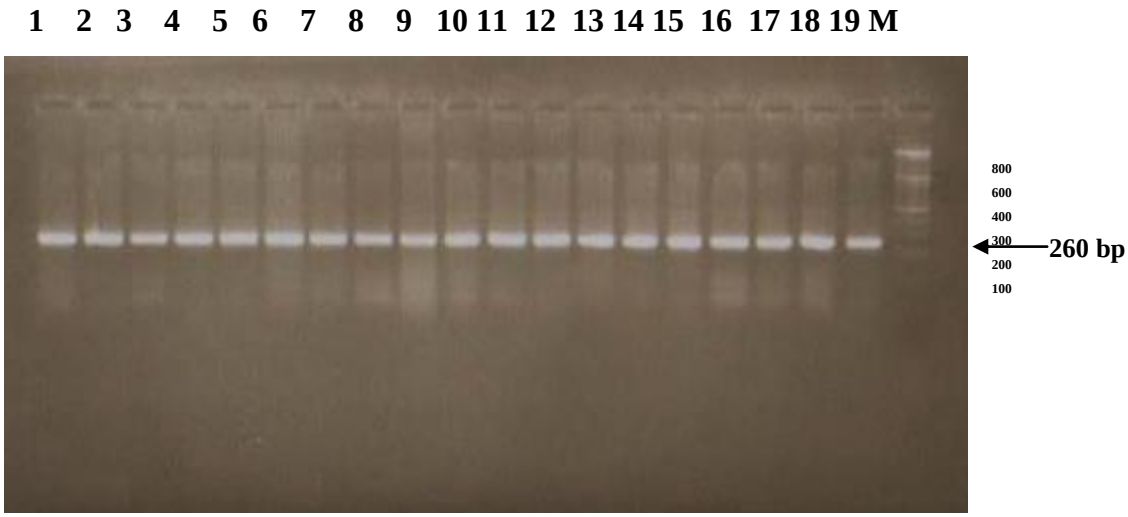

Fig. 3.3: PGC-1α Gene PCR Products Gel Electrophoresis (Samples 1-19). 2.5% agarose gel was performed for resolving the amplified PCR products of PGC-1α gene. PCR reaction was done using specific primers to have a specific 260 bp DNA fragment that contains the PGC-1α gene haplotypes. Lane (1-19) showed amplified DNA samples (10 µl of the amplified DNA samples was loaded), lane M showed 100 bp DNA ladder (1 µl of DNA ladder was loaded). The results showed a specific 260 bp of the amplified PGC-1α gene fragments.

The results show the size of the amplified PGC-1α gene fragments which was 260 base pairs as expected. No additional bands are visualized which indicated the specificity of the PCR amplification protocol. Lane M represent molecular weight marker (100 bp ladder).

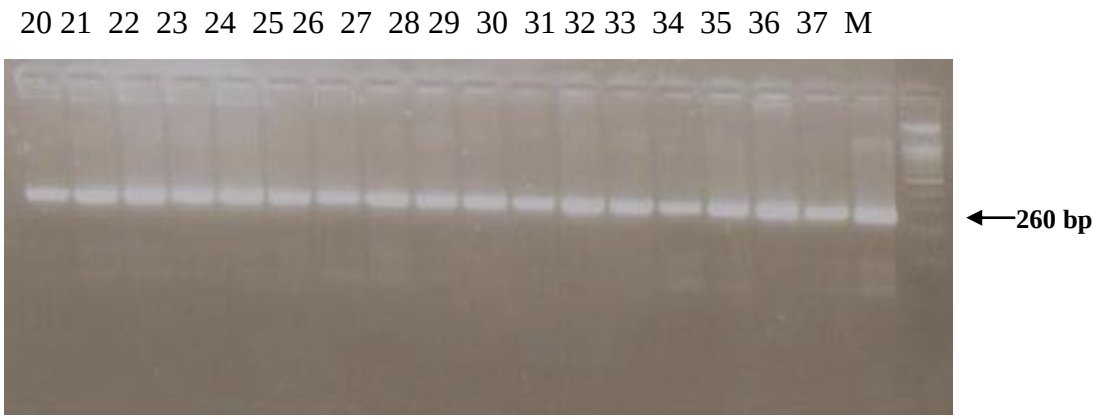

Fig. 3.4: PGC-1α Gene PCR Products Gel Electrophoresis (Samples 20-37). 2.5% agarose gel was performed for resolving another amplified PCR products of PGC-1α gene. PCR reaction was done using specific primers to have a specific 260 bp DNA fragment that contains the PGC-1α gene haplotypes. Lane (20-37) showed amplified DNA samples (10 µl of the amplified DNA samples was loaded), lane M showed 100 bp DNA ladder (1 µl of DNA ladder was loaded). The results showed a specific 260 bp of the amplified PGC-1α gene fragments.

Fig. 3.4 describes other amplified fragments of PGC-1α gene (lane 20-37). Lane M represents molecular weight marker (100 bp DNA ladder).

3.3 Restriction Enzyme Analysis

Following digestion with HpaII enzyme, the resulting fragments of the first above 19 samples were resolved on 3% agarose gel as shown in (Fig. 3.5). Lane M represent molecular weight DNA marker (100 bp DNA ladder).

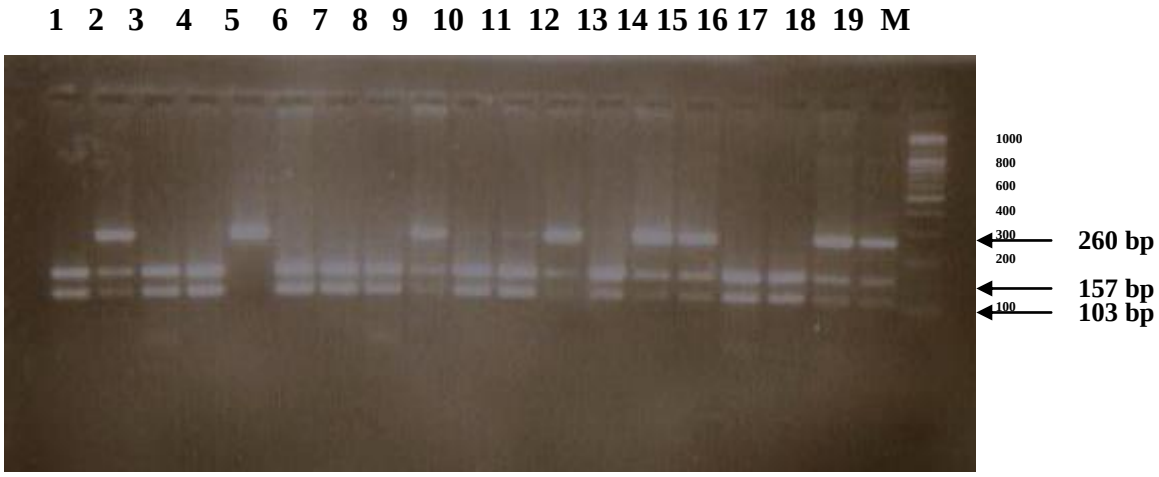

Fig. 3.5: PGC-1α Gene Digested PCR Products Gel Electrophoresis (Samples 1-19). 3% agarose gel was performed for resolving the digested PCR products of PGC-1α gene. Digestion was done using HpaII digestion enzyme. The results of the samples (lane 1-19) showed the following: samples 1,3,4,6,7,8,10,11,13,16,17 were fully digested (157 bp, 103 bp) and have the CC homozygous haplotype, samples 2,9,12,14,15,18,19 were partially digested which indicate that they have the CT haplotype (260 bp, 157 bp, 103 bp), sample #5 was completely undigested and therefore represents the TT haplotype (260 bp). Lane M showed 100 bp DNA ladder (1 µl of DNA ladder was loaded).

The results indicate the haplotype of the indicated samples which was as follows:
Samples 1,3,4,6,7,8,10,11,13,16,17 are fully digested (157 bp, 103 bp) and have the CC homozygous haplotype. Samples 2,9,12,14,15,18,19 were partially digested which indicate that they have the CT haplotype (260 bp, 157 bp, 103 bp). Sample #5 was completely undigested and therefore represents the TT haplotype (260 bp).

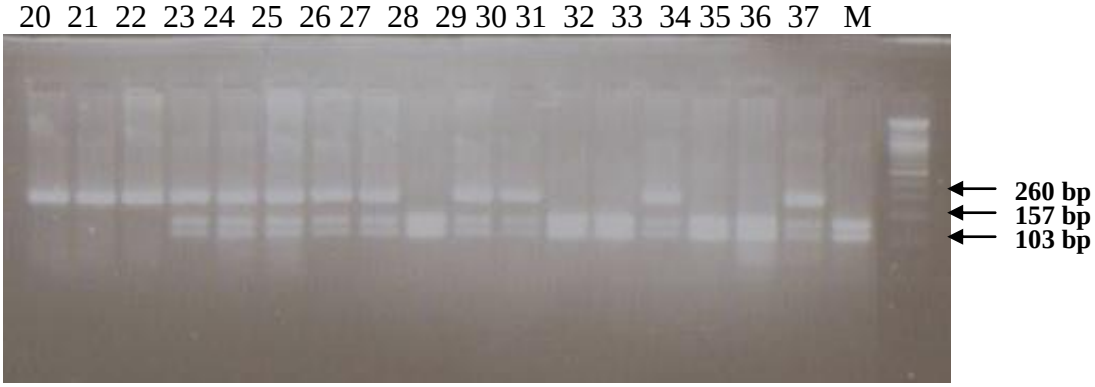

Fig. 3.6: Another PGC-1α Gene Digested PCR Products Gel Electrophoresis (Samples 20-37). 3% agarose gel was performed for resolving the digested PCR products of PGC-1α gene. Digestion was done using HpaII digestion enzyme. The results of the samples (lane 20-37) showed the following: samples 28,31,32,34,35,37 were fully digested and have the CC homozygous haplotype, samples 23,24,25,26,27,29,30,33,36 were

partially digested and have CT haplotype, samples 20,21,22 were completely undigested and therefore represent TT haplotype. Lane M showed 100 bp DNA ladder (1 µl of DNA ladder was loaded).

Fig. 3.6 showed the results of digested fragments of the second above 18 samples of PCR products.

Samples 28,31,32,34,35,37 are fully digested and have the CC homozygous haplotype. Samples 23,24,25,26,27,29,30,33,36 were partially digested and have CT haplotype. Samples 20,21,22 were completely undigested and therefore represent TT haplotype.

3.4 Study Subjects: Demographic and Clinical Data

Table 3.1: Comparison between Diabetic and Normal Subjects Concerning Clinical Parameters Related to Diabetes. Each different clinical parameter like age (year), systolic blood pressure (mm Hg), diastolic blood pressure (mm Hg), Total Cholesterol (mmol/L), Triglyceride TG (mmol/L), High density lipoprotein (HDL) (mmol/L), Low density lipoprotein (LDL) calculated (mmol/L), Fasting blood sugar (FBS) (mmol/L), LDL direct mmol/L, HbA1c (%), Body Mass Index, were compared between control subjects and diabetic. T-test was used to test the difference between the two means.

Parameter	Mean ± STD (N) Control	Mean ± STD (N) Diabetic	P-value
Gender Male 113 Female 228	41 87	72 141	
Age (years)	49.1 ± 9.11 (128)	55.0 ± 8.35 (209)	< 0.001
Systolic BP (mm Hg)	126 ± 17.8 (128)	134 ± 16.9 (212)	< 0.001
Diastolic BP (mm Hg)	77.5 ± 13.1 (128)	80.4 ± 12.2 (212)	0.035
Total Cholesterol mmol/L	5.10 ± 0.98 (128)	5.28 ± 1.07 (213)	0.124
TG mmol/L	1.64 ± 1.51 (128)	2.18 ± 1.34 (213)	0.003
HDL mmol/L	1.22 ± 0.27 (128)	1.09 ± 0.23 (213)	< 0.001
LDL calculated mmol/L	3.17 ± 0.77 (126)	3.23 ± 0.98 (202)	0.583
FBS mmol/L	4.77 ± 0.44 (128)	10.2 ± 4.27 (212)	< 0.001
LDL direct mmol/L	2.88 ± 0.98 (127)	3.24 ± 1.10 (212)	0.002
HbA1c (%)	5.70 ± 0.65 (126)	8.11 ± 1.75 (209)	< 0.001
Body Mass Index	30.5 ± 5.52 (128)	33.3 ± 6.16 (213)	0.004

Table (3.1) shows detailed demographic and clinical parameters of the study subjects. It includes information about gender, age (years), BMI (kg/m^2), blood pressure (mmHg)) and several biochemical clinical characteristics related to diabetes. The number of females (87 control and 141 diabetic) was higher than the number of males (41 control and 72 diabetic) who attended these clinics (0.293 ratio of ♂/♀ for controls, 0.758 ratio of ♂/♀ for diabetic subjects).

The mean of age in diabetic subjects (55.0 ± 8.35) was significantly ($P < 0.001$) higher than that among controls (49.1 ± 9.11).

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus defines diabetic subjects as having fasting blood sugar (FBS) levels ≥ 125 mg/dl (7.0 mmol/L), while FBS < 100 mg/dl (5.6 mmol/L) as normal subjects and FBS 100-125 mg/dl (5.6-6.9 mmol/L) as impaired fasting glucose subjects. The mean of FBS (mmol/L) among the

diabetic subjects (10.2 ± 4.27) was significantly higher (P <0.001) than that among controls (4.77 ± 0.44).

Another demographic data analyzed in this study was blood pressure. It is worth reporting that most of our patients did not suffer from diabetic related complications including cardiovascular complications, retinopathy or renal problems. This could be due to close monitoring of these patients and/or most patients are fairly young and very few patients are old (>70 years) to show these complications.

The mean (126 ± 17.8) of systolic BP was significantly lower (P <0.001) in the control group compared to diabetic subjects (134 ± 16.9). This result is in agreement with the increase in systolic blood pressure with diabetes. The mean (77.5 ± 13.1) of diastolic BP in the control group was also significantly lower (P=0.035) than that of diabetic subjects (80.4 ± 12.2).

The risk of developing dyslipidemia occurs more frequently in individuals with type 2 diabetes. Abnormal lipid profile consists of the following abnormalities either singly or in combination. These include elevated total cholesterol, hypertriglyceremia, elevated low-density lipoprotein (LDL) levels, and decreased high-density lipoprotein (HDL). Diabetes is associated with a marked increase in the risk of coronary heart disease. Total serum cholesterol level is a strong predictor of the risk of cardiovascular events in patients with diabetes (Steven et al., 1998). Our study revealed that the mean of total serum cholesterol in control subjects (5.10 ± 0.98) wasn't significantly different (P=0.124) from that in diabetic subjects (5.28 ± 1.07). This result was not expected since total cholesterol is a well known complication in type 2 diabetes. This result is due to close monitoring of serum cholesterol in clinics and taking lipid-lowering therapies. In addition, most of our subjects are fairly young as mentioned before and they do not suffer from cardiovascular complications.

The type of dyslipedemia that is most characteristic of type 2 diabetes is elevated triglyceride (TG) (Geltrude et al., 1999). Consistent with this fact, TG was significantly lower (P=0.003) in control subjects (1.64 ± 1.51) in comparison to diabetic subjects (2.18 ± 1.34). This result is consistent with the association of elevated triglyceremia and type 2 diabetes.

The risk of the development of cardiovascular complications in diabetes mellitus is often predicted by several factors which include lipid disorder including low high-density lipoprotein (HDL) levels and elevated low-density lipoprotein. Prevention studies have shown that lowering atherogenic low-density lipoprotein (LDL) whilst raising high-density lipoprotein (HDL) significantly decrease the risk for coronary disease (Kashyap, 1997) which is one of many complications of diabetes.

Our data showed that the mean of HDL in control subjects (1.22 ± 0.27) was significantly higher (P <0.001) than that among diabetic patients (1.09 ± 0.23). This result agrees with what is known about diabetic patient that their blood HDL level is usually lower compared to normal subjects. However, the mean of calculated LDL in control subjects (3.17 ± 0.77) wasn't significantly different (P=0.583) from that of diabetic subjects (3.23 ± 0.98). This result was not as expected, because diabetic patients often have an elevated level of LDL.

With respect to direct LDL, the mean of LDL in control subjects was (2.88 ± 0.98) and for diabetic subjects (3.24 ± 1.10). The increase in LDL measured directly is significantly higher (P=0.002) than that of controls. The direct LDL is more accurate because of the high TG.

In addition to LDL, HbA1c was also monitored in our study subjects. Evidently, the mean value of HbA1c was significantly higher (P <0.001) among diabetic subjects (8.11 ± 1.75) compared to control subjects (5.70 ± 0.65). This result is consistent with the levels that characterize diabetic patients from control and agrees with the data of FBS level in diabetic subjects (high FBS leads to high HbA1c).

The mean (30.5 ± 5.52) of BMI in control subjects was significantly lower (P=0.004) from that of diabetic subjects (33.3 ± 6.16). BMI was well known to be high in diabetic patient while normal or approximately normal in healthy control subjects but here, BMI was also high in both groups. According to Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, control subjects have a normal FBS (they are non diabetic), but their high BMI is due to many factors like diet and sedentary lifestyle. This may leads them to diabetes since obesity or high BMI plays a critical role in increasing the risk for diabetes, or any other diseases depending on their genetic susceptibility, family history and many other factors of the disease.

3.5 Association between the G (GG) and S (GS & SS) haplotypes in the PGC-1α gene and several clinical blood parameters linked to diabetes type 2 among all subjects

Table 3.2: Comparison between Mean Values of the Haplotype of PGC-1α Gene (the G (GG) Allele and the S Alleles (GS and SS)) and Several Clinical Blood Parameters Linked to Diabetes Type 2 Among All Subjects. Mean values of allelic variation (haplotypes) of the PGC-1α gene were compared among some different clinical blood parameters like Systolic BP (mm Hg), Diastolic BP (mm Hg), Body Mass Index, HbA1c (%), Total Cholesterol (mmol/L), TG (mmol/L), HDL (mmol/L), FBS (mmol/L), LDL Direct (mmol/L), LDL Calculated (mmol/L). T-test was used to test the difference between the two means of allelic variation of the PGC-1α gene.

Parameters	GG Mean ± STD (N)	GS+SS mean ± STD (N)	P-value
Systolic BP (mm Hg)	131 ± 17.5 (122)	132 ± 17.4 (211)	0.706
Diastolic BP (mm Hg)	80.1 ± 14.2 (122)	79.4 ± 13.2 (211)	0.620
Total Cholesterol mmol/L	5.18 ± 1.01 (121)	5.30 ± 1.10 (212)	0.396
TG mmol/L	1.84 ± 1.10 (121)	2.11 ± 1.64 (212)	0.077
HDL mmol/L	1.16 ± 0.37 (121)	1.14 ± 0.29 (212)	0.570
LDL calculated mmol/L	3.24 ± 0.87 (118)	3.23 ± 0.98 (201)	0.879
LDL direct mmol/L	3.14 ± 1.05 (122)	3.17 ± 1.13 (210)	0.750
FBS mmol/L	8.48 ± 4.25 (121)	8.10 ± 4.25 (212)	0.474
HbA1c (%)	7.37 ± 1.97 (122)	7.13 ± 1.78 (207)	0.303
Body Mass Index	33.8 ± 14.7 (122)	34.1 ± 22.2 (212)	0.927

Table (3.2) describes the association between Gly482Ser allelic variations (haplotypes) of PGC-1α gene and the different clinical parameter among all participants (male and female, diabetic and normal subjects). In order to study if the haplotypes of PGC-1α gene is a risk of diabetes and if it affects the complications of diabetes, all participants were separated into two groups according to their haplotypes: GG haplotype group, GS and SS haplotype group. The values in this table represent the mean for each parameter among all participants in the study and the Gly482Ser alleles. The results of mean values of allelic variation in PGC-1α gene such as G (GG) and S (GS and SS) did not differ significantly (as shown in Table 3.2) between the PGC-1α gene haplotypes. This revealed that there was no association between the alleles of PGC-1α gene (G allele and S alleles) and clinical parameters related to diabetes, and there was no association between the alleles of PGC-1α gene and each different parameter among all participants in the study (male and female). So there was no association between Gly482Ser allelic variations (haplotypes) of PGC-1α

gene and diabetes, also there was no association between the haplotypes of PGC-1α gene and complications of diabetes.

3.6 Correlation between the Gly482Ser Allelic Variation and Various Variables with respect to Gender, Diabetic Status

Table 3.3: Comparison between the Numbers (%) of Subjects with GG Allele and (GS and SS) Alleles with Respect to Different Parameters Observed. Participants with GG allele and (GS and SS) alleles were classified into males and females, diabetic and normal. Different clinical parameters were also showed with its normal and abnormal values like Systolic blood pressure (normal < 140 mm Hg, abnormal ≥ 140), Diastolic blood pressure (normal < 90 mm Hg, abnormal ≥ 90 mm Hg), Total cholesterol (normal < 5.5 mmol/L, abnormal ≥ 5.5 mmol/L), HDL (normal < 1.0 mmol/L, abnormal ≥ 1.0 mmol/L), LDL calculated (normal < 3.5 mmol/L, abnormal ≥ 3.5 mmol/L), LDL direct (normal < 3.5 mmol/L, abnormal ≥ 3.5 mmol/L), TG (normal < 2.0 mmol/L, abnormal ≥ 2.0 mmol/L), BMI (normal < 25 kg/m^2, abnormal overweight (≥ 25 to < 30) kg/m^2 , obese > 30 kg/m^2), HbA1c (normal ≤ 6.5 %, abnormal > 6.5 %). The numbers (%) of subjects with GG allele and GS, SS alleles were compared with respect to these parameters. Chi square analysis was used to analyze the data.

Parameter		GG (%)	GS+SS (%)	P-Value
Gender	Male	33 (30%)	77 (70.0%)	0.115
	Female	88 (39.5%)	135 (60.5%)	
Diabetic status	Diabetic	82 (39.2%)	127 (60.8%)	0.160
	Normal	39 (31.5%)	85 (68.5%)	
Systolic blood pressure (mm Hg)	< 140 mm Hg	76 (34.9%)	142 (65.1%)	0.401
	≥ 140 mm Hg	45 (39.8%)	68 (60.2%)	
Diastolic blood pressure (mm Hg)	< 90 mm Hg	87 (35.4%)	159 (64.6%)	0.514
	≥ 90 mm Hg	34 (40.0%)	51 (60.0%)	
Total cholesterol (mmol/L)	< 5.5 mmol/L	84 (39.4%)	129 (60.6%)	0.124
	≥ 5.5 mmol/L	37 (30.8%)	83 (69.2%)	
HDL (mmol/L)	< 1.0 mmol/L	36 (36.7%)	62 (63.8%)	0.992
	≥ 1.0 mmol/L	85 (36.2%)	150 (63.8%)	
LDL calculated (mmol/L)	< 3.5 mmol/L	85 (38.6%)	135 (61.4%)	0.232
	≥ 3.5 mmol/L	36 (31.9%)	77 (68.1%)	
LDL direct (mmol/L)	< 3.5 mmol/L	81 (37.7%)	134 (62.3%)	0.553
	≥ 3.5 mmol/L	40 (34.2%)	77 (65.8%)	
TG (mmol/L)	< 2.0 mmol/L	81 (38.4%)	130 (61.6%)	0.345
	≥ 2.0 mmol/L	40 (32.8%)	82 (67.2%)	
BMI (kg/m2)	< 25	6 (21.4%)	22 (78.6%)	0.139
	≥ 25 to < 30	33 (33.7%)	65 (66.3%)	
	> 30	82 (39.6%)	125 (60.4%)	
HbA1c (%)	≤ 6.5%	48 (31.0%)	107 (69.0%)	0.051
	> 6.5%	72 (41.6%)	101 (58.4%)	

This table describes the association between Gly482Ser haplotypes (allelic variation) of PGC-1α gene and various diabetic correlated variables. In this table, participants were divided into groups according to the gender (male or female), diabetic status found in the clinic (diabetic or normal subjects) and according to normal and abnormal values of each clinical parameter in the study. The association of PGC-1α gene haplotypes and these groups were studied. The values here represent the number (%) of alleles of PGC-1α gene (GG allele and (GS and SS) alleles) and their association with each different group among participants in this study. Chi Square analysis was used to analyze the data.

First, the association of PGC-1α gene haplotypes was compared and studied in participants with respect to gender among all subjects (diabetic and control) in the study. Participants were divided into two groups, male and female. The percent of males who had G allele was 30%, while 70% of males had S allele. In the female group, the percent of females that had G allele were 39.5% while 60.5% of them had S allele. The results here showed that there was no significant difference ($P>0.05$) when these values were compared between the same group and between different groups (male and female). So the result indicated that there was no statistically significant difference ($P=0.115$) between males and females. Therefore, there was no association between the Gly482Ser allelic variation (haplotype) of PGC-1α gene and gender.

Participants were classified into two groups according to their diabetic status. This classification was carried out to evaluate the association of allelic variations between diabetic and control subjects. Among the diabetic group, 39.2% had G allele, while 60.8% of them had S allele. The results of control subjects showed that 31.5% had G allele, while 68.5% of them had S allele. No statistical significant difference ($P=0.160$) of allelic variation (haplotypes) of PGC-1α gene between diabetic and control groups was observed on the effect of diabetes on the allelic variation of PGC-1α gene. So this revealed that there was no association between Gly482Ser allelic variation in this gene and diabetes.
Since no association between allelic variations (haplotypes) of PGC-1α gene was detected between the diabetic and control groups, the association between allelic variation (haplotype) of PGC-1α gene and the different parameters related to diabetes were studied. So, for each parameter, the participants were divided into two groups: subjects with normal level of the indicated clinical parameter and those with abnormal higher value.

Normal systolic BP group (<140 mmHg) showed 34.9% G allele and 65.1% of them had S allele. Abnormal systolic BP group (≥140 mmHg) had 39.8% G allele while 60.2% of them had S allele. No staistical significant difference (P=0.401) was observed between Gly482Ser allelic variation in this gene and systolic BP.

The percentage (35.4%) of participants with normal diastolic BP group (<90 mmHg) showed G allele while 64.6% had the S allele. Abnormal diastolic BP (≥140 mmHg) had 40.0% G allele while 60.0% of them had S allele. Also here, No statistical significant difference (P=0.514) was observed. So, there was no association between allelic variation (haplotype) of PGC-1α gene and diastolic blood pressure.

Another two groups of total cholesterol level were also compared. 39.4% of normal total cholesterol group (<5.5 mmol/L) showed G allele and 60.6% of them had S allele. In the abnormal total cholesterol group (≥5.5 mmol/L), 30.8% had G allele and 69.2% of them had S allele. There was no statistical significant difference (P=0.124) between the two types of alleles and the two groups (normal and abnormal) of this parameter. So there was no association between allelic variation of PGC-1α gene and total cholesterol.

HDL (mmol/L) parameter was also compared. 36.7% of normal level of HDL group (<1.0 mmol/L) had G allele while 63.3% of them had S allele. In the abnormal HDL level group (≥1.0 mmom/L), 36.2% of subjects had G allele and 63.8% of them had S allele. No statistical significant difference (P=0.992) between two types of alleles and normal, abnormal HDL level was observed. As a result, there was no association between allelic variation of PGC-1α gene and HDL level in the participants of this study.

The association between the haplotype (allelic variation) of PGC-1α gene and LDL level (either calculated or direct "measured by a kit") was also studied. Normal level of calculated LDL group (<3.5 mmol/L) had 38.6% G allele while 61.4% had S allele. The abnormal level of calculated LDL group (≥3.5 mmol/L) has 31.9% G allele while 68.1% of them had S alleles. Also there was no statistical significant difference (P=0.232) between two types of PGC-1α alleles and the (normal and abnormal) calculated LDL group. So there was no association between PGC-1α gene haplotype and calculated LDL in this study. In addition, normal level of LDL direct group (<3.5 mmol/L) had 37.7% G allele, while 62.3% of them had S allele. Abnormal direct LDL group (≥3.5 mmol/L) had 34.2%

G allele, while 65.8% of them had S allele. There was no statistically significant difference (P=0.553) between PGC-1α gene haplotype and the two normal and abnormal direct LDL groups, so there was no association between LDL levels (either direct or calculated) and allelic variation of PGC-1α gene in this study.

The association between triglyceride parameter and PGC-1α gene haplotype was also tested. Normal TG level group (<2.0 mmol/L) had 38.4% G allele while 61.6% of them had S allele. On the other hand, abnormal TG level group (≥2.0 mmol/L) had 32.8% G allele while 67.2% of them had S allele. There was no statistical significant difference (P=0.345) between the two types of the indicated alleles and (normal and abnormal) levels of TG. So there was no association between allelic variation of PGC-1α gene and TG levels in our participants.

BMI (kg/m^2) parameter was divided according to the World Health Organization guidelines into three groups: normal weight group (<25) which had here 21.4% of G allele while 78.6% of this group had S allele. 33.7% of abnormal overweight group (≥25 to <30) had G allele while 66.3% of them had S allele. The last group was abnormal obese group (>30), it had 39.6% G allele while 60.4% of this group had S allele. No statistical significant difference (P=0.139) was observed between PGC-1α gene allelic variation and the previous groups of BMI parameter. So there was no association between Gly482Ser allelic variation of PGC-1α gene and BMI.

The last parameter studied here is HbA1c (%). The two groups were: Normal HbA1c group (≤6.5%) which had 31.0% G allele, while 69.0% of this group had S allele. Abnormal HbA1c group (>6.5%) had 41.6% G allele while 58.4% of this group had S allele. There was no statistical significant difference (P=0.051) was observed when the allelic variation of PGC-1α gene was compared between normal HbA1c and abnormal HbA1c group. So this revealed that there was no association between Gly482Ser allelic variation of PGC-1α gene and HbA1c among participants of this study.

Finally, the overall results from this study showed that there was no association between allelic variation (Gly482Ser haplotype) of PGC-1α gene and gender among Palestinian participants in the West Bank (Central West Bank). In addition, no association between allelic variation (Gly482Ser haplotype) of PGC-1α gene and diabetes among Palestinian

participants in the West Bank (Central West Bank) was detected. Furthermore, no association between allelic variation (Gly482Ser haplotype) of PGC-1α gene and each different clinical parameter related to diabetes among Palestinian participants in the West Bank (Central West Bank) was observed.

Chapter Four

Discussion

Type 2 diabetes is a common disease due to both genetic and environmental factors (Kahn et al., 1996; Medici et al., 1999; Lehtovirta et al., 2000; Florez et al., 2003). Many genes have been reported to be associated with this disease (Florez et al., 2003) and there is extensive circumstantial evidence from studies on families from various populations in favour of genetic determinants for the onset of diabetes Type 2 with age (Ek et al., 2001). It is also evident that type 2 diabetes is polygenic and families from various populations display various polymorphisms in different genes and thereby contributing to the genetic heterogeneity of the disease. Among the type 2 diabetes susceptibility gene variants are the Pro12Ala polymorphism of the PPAR-γ gene and other polymorphisms in other genes including the CDKN2B, IGF2BP2, CDKAL1, KCNJ11 and TCF7L2 genes (Tan et al., 2009). In one study, the Pro-allele of PPAR-γ2 gene confered a 1.25-fold increases in the risk of Type 2 diabetes (Altshuler et al., 2000). These findings prompted us to examine the PGC-1α gene for variability, which might be associated with Type 2 diabetes because PGC-1α, besides being a co-activator of the nuclear receptor PPAR-γ, it has a critical role in glucose uptake by regulating the expression of GLUT-4 in skeletal muscle (Michael et al., 2001), adaptive thermogenesis (Puigserver et al., 1998), and has an effect on regulation of gluconeogenesis in the liver (Yoon et al., 2002). Additionally, a trait linkage analysis in Pima Indians suggested a link between basal insulin concentrations and chromosome 4p15.1 (Pratley et al., 1998) in cases where the PGC-1α gene has been mapped (Esterbauer

et al., 1999). Thus, PGC-1α can be viewed as a functional and positional candidate for the susceptibility gene for Type 2 diabetes mellitus.

Our study aimed to investigate the correlation between the allelic variations Gly482Ser polymorphism of the PGC-1α gene and Pro12Ala polymorphism of the PPAR-γ gene and Type 2 diabetes and the various clinical parameters that are affected by the disease among Palestinian Type 2 diabetes patients. This work will provide preliminary information about the magnitude of the genetic risk factors that are associated with the incidence of Type 2 diabetes among our population. The investigation of the PPAR-γ gene polymorphism was eventually terminated due to similar investigation performed by another group in the university that was published during the performance of this work.

Our data showed a comparison between various clinical parameters related to diabetes among the patients and control group. Many studies revealed that the risk of developing Type 2 diabetes increases with age and obesity (Zimmet et al., 1992; Harris et al., 1995; Valle et al., 1997). Among our study subjects, the mean age among the control subjects was lower than diabetic subjects (49.1±9.11 vs. 55.0±8.35 respectively). Although the analysis showed a significant difference between the two groups ($P<0.001$) however the observed variation in age is apparently small and does not seem to affect the overall variation in the PGC-1α gene polymorphisms among both groups. It is definitely ideal to compare the exact same ages between the two groups in the study; however, this observed age variation is related to the random recruitment of subjects in the various clinics. Subset of data with the same age also showed no significant difference in all parameters tested.

Many studies found a strong positive association between obesity as measured by body mass index (BMI), and the risk of type 2 diabetes (Chan et al., 1994). Measurement of BMI is potentially useful tool for clinics in counseling patients regarding type 2 diabetes risk and risk reduction (Carey et al., 1997). Based on the current World Health Organization guidelines, BMI was divided into three categories: normal weight, less than 25; overweight, 25 to less than 30; and obese, 30 or higher (Amy et al., 2004).

In our study, a significant difference ($P=0.004$) was observed in BMI value between control (30.5±5.52) and diabetic subjects (33.3±6.16). Although BMI value was high in both groups, control group has normal FBS and this indicated that most of our subjects

(diabetic and control) are obese and this may indicate that the control subjects are at risk to acquire diabetes in the future since obesity is considered a risk factor of diabetes.

The correlation between the Gly482Ser allelic variation in PGC-1α gene and different clinical parameters related to diabetes among all participants was investigated. The objective here was to investigate the possible correlation between the Gly482Ser allelic variation and each of the indicated clinical parameters related to diabetes to see if this allelic variation plays any role in the severity of these clinical complications independent of the disease itself. No significant difference between the indicated haplotypes of PGC-1α gene and FBS among diabetic and control subjects ($P=0.474$) which confer that the observed variation in FBS level between both groups is a consequence of the disease and not affected by the indicated haplotype variation in the gene. Similarly, the HbA1c showed no significant difference between the indicated haplotypes of PGC-1α gene among all participants ($P=0.303$), even though HbA1c level showed a significant difference ($P<0.001$) between diabetic and control subjects. This also indicates that the observed variation in HbA1c is a consequence of the disease and not affected by the haplotype variation.

No correlation could be detected between PGC-1α gene haplotypes and biochemical or other parameters related to diabetes. The observed variation in systolic BP, total cholesterol, TG, HDL, LDL is a consequence of the disease and not affected by the indicated haplotype variation.

Furthermore, the correlation between Gly482Ser allelic variation in PGC-1α gene and various variables including gender, diabetic status, normal and abnormal values of each of the clinical parameters related to diabetes was separately investigated in order to asses whether the indicated haplotypes play any role in determining the change in these parameters as a consequence of diabetes. Collectively, no significant correlation could be detected between the haplotypes and the various value of all the indicated parameters including age, incidence of diabetes, SBP, DBP, total cholesterol, HDL, LDL, TG, BMI and HbA1c

In conclusion, our data did not provide any evidence for a significant association between allelic variation of PGC-1α gene and the different biochemical and clinical parameters.

These findings are in agreement with studies in French Caucasians or Pima Indians (Lacquemant et al., 2002; Muller et al., 2003) which showed that the Gly482Ser allele doesn't predict diabetes among these populations. In other studies the correlation between Gly482Ser polymorphism in the PGC-1α gene and diabetes showed an increase in risk for the disease among Danish Caucasians (Ek et al., 2001) and Japanese subjects (Hara et al., 2002). These studies were carried out in single populations i.e. they are population dependent. Therefore even though the PGC-1α Gly482Ser variant is associated with Type 2 diabetes in the certain populations, this observed association could be in linkage with an as yet unidentified etiological variant (Ek et al., 2001).

Many other variants including Thr612Met were not associated with Type 2 diabetes in initial association study (Ek et al., 2001). In contrast, these variants were found to be associated with Type 2 diabetes both in replication study and in the combined study (Ek et al., 2001). It is not clear whether this observation is due to linkage with the Gly482Ser variant or whether it is a true functional variant providing a reduced risk of developing Type 2 diabetes or a chance finding due to multiple testing of various gene variants. The Gly482Ser varient is located in a part of the protein whose function is not well defined. However, a recent study showed that residues 403-570 of PGC-1α are critical for its interaction with MEF2C and thereby the ability of PGC-1 to restore insulin sensitive GLUT4 expression (Michael et al., 2001). The interaction between the Gly482Ser variant and Pro12Ala variant in the PPAR-γ gene gave no indication for additive effects on diabetes status (Ek et al., 2001). However, due to the polygenic nature of the common forms of Type 2 diabetes, future studies should examine the potential interactions of the PGC-1α gene, PPAR-γ gene and TCF7 gene and other potential genes haplotypes to see if they have additive impact on the susceptibility to common subsets of Type 2 diabetes mellitus.

Finally, our data analysis revealed that there is no statistical significant difference between the G (GG) and S (GS and SS) haplotypes of PGC-1α gene and diabetes. In addition, no correlation could be detected between these haplotypes and the various biochemical or other clinical parameters (i.e. BP and BMI) among all subjects. Therefore, our collective results indicate that the Gly482Ser allelic polymorphism in the PGC-1α gene is not correlated with diabetes in the Palestinian population tested.

Whether the control group being at high risk because of obesity has effected, the results need further investigations.

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Appendix 1: Questionnaire

No:
***Date*:**

Demographic Data	
Name	
Gender	a- Male b - Female
Age	
Level of education	a-Illiterate b- Less than Tawjihi c-Tawjihi c- Bachelor and above
Place of Residence	a- City b-Village c- Camp
Address, Tel	
Marital status	a- Single b- Married c-Widow/er No. of children: _____
Profession	

Diabetic and Nondiabetic Patients	
Do you have diabetes?	a - Yes b - No
When your diabetes was first diagnosed?	

Physical Activity	
Do you walk (or do other moderate activity) for at least 30 minutes on most days, or at least 150 min per week?	a - Yes. b - No.

Smoking Tobacco	
Do you smoke tobacco?	a- Current smoker. b- Ex-smoker. c- Never smoker.
What kind of tobacco do /did you use?	a- Cigarette. b- Waterpipe. c- Both.
Current smokers:	
For cigarette smokers	a- < 10 cigarettes /day. b- 10-19 cigarettes /day. C- 20-29 cigarettes /day. d- 30-39 cigarettes/day. e - > 40 cigarettes/day.
For waterpipe users: How many times do you use waterpipe per week?	_____
For ex-smoker: I have quitted smoking for	a-Less than 1 year. b-More than 1 year and less than ten years. c- More than ten years.

Family History	
Do other members of the family have cardiovascular diseases (mother, father, sister, brother)?	a - Yes. b - No.
Do other members of the family have diabetes (mother, father, sister, brother)?	a- Yes. b- No

Complications: Do you have any of the following diseases? If yes for how many years	
Hypertension	a - Yes b - No years:
Ischemic heart disease	a - Yes b - No years:
Stroke	a - Yes b - No years:
Peripheral arterial disease	a - Yes b - No years:
Retinopathy	a - Yes b - No years:
Nephropathy	a - Yes b - No years:
Neuropathy	a - Yes b - No years:
Amputation	a - Yes b - No years:
Diabetic Foot	a - Yes b - No years:
Hyperlipidemia	a - Yes b - No years:
Rheumatoid arthritis	a - Yes b - No years:
Other diseases	

Current medications:	

Blood pressure	
Blood pressure	mmHg

Blood sugar	
Fasting blood sugar level	mg/dl
HbA1c	

Lipid profile	
Total cholesterol	mg/dl
Triglycerides	mg/dl
HDL	mg/dl
LDL (calculated)	mg/dl
LDL (measured)	mg/dl

Anthropometric indices		
Body mass index (BMI)	Weight =	Kg
	Height =	m
	BMI =	kg/m2

Antioxidants	
Have you taken any vitamins during the past 24 hours.	a. Yes b. No

التحقق من العلاقة في التغيرات المحدثة في جينيّ PPARγ و PGC-1α ومرضى السكري من النوع الثاني للمرضى الفلسطينيين.

اعداد: أنمار مصطفى توفيق أبو زهرة

اشراف: بروفيسور هشام درويش

ملخص:

مرض السكري هو مجموعة من الاضطرابات الايضية التي تتسم بارتفاع السكر في الدم الناتجة عن خلل في افراز الانسولين، عمل الانسولين، او كليهما. ويرتبط ارتفاع السكر المزمن في الدم بالخلل الوظيفي والفشل في مختلف اجهزة الجسم وخاصة العينين والكليتين والاعصاب والقلب والاوعية الدموية.

اكثر من 90-95% من المصابين بداء السكري هم من المصابين بالنوع الثاني. السبب هو مزيج من المقاومة لعمل الانسولين و عدم الاستجابة لافراز الانسولين. السكري من النوع الثاني هو مرض شائع على حد سواء نظرا للعوامل الوراثية والبيئية معا مع اسلوب الحياة والعوامل السلوكية. وقد حددت الدراسات عدة مواقع في العديد من الجينات لها علاقة بذلك المرض بما في ذلك الجين PPARγ.

PGC-1α هو عبارة عن جين منشط لجين PPARγ حيث تبين انه يزيد من فعالية هذا الجين والعديد من الجينات الاخرى. PGC-1α ينظم برامج التوازن البيولوجي المرتبطة بطاقة الجسم والتكيف الحراري مع المحيط. وقد سجلت عدة دراسات وجود علاقة بين التغير في هذا الجين والنوع الثاني من مرض السكري. فقد ذكرت تقارير عدة وجود هذه العلاقة في الدنمارك واليابان في المقابل لم يتم العثور على وجود علاقة بين هذا المرض والتغير في الجين لدى الفرنسيين والقوقازيين والهنود الامريكان.

في هذه الدراسة تم البحث عن وجود تغيرات في هذا الجين وعلاقته بمرض السكري من النوع الثاني (وما يرتبط به من معايير اكلينيكية مثل ضغط الدم، قياس مؤشر كتلة الجسم، نسبة السكر، السن .....) في عدد من الفلسطينيين في الضفة الغربية. وقد تم تحليل هذه التغيرات باستخدام تقنية تفاعلات البلمرة المتسلسلة (RFLP-PCR) لدى 341 شخص منهم 160 شخص مصاب و181 سليم.

وقد تم تحليل النتائج باستخدام برنامج SPSS حيث كانت نتيجة هذه الدراسة هو عدم وجود علاقة بين التغيرات في هذا الجين (haplotypes) وارتباطها بالسكري من النوع الثاني لدى الفلسطينيين في الضفة الغربية.