INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with longterm damage, dysfunction and failure of various organs such as; nephropathy, retinopathy, angiopathy and neuropathy **(American Diabetes Association, 2004).**

In many patients with diabetes mellitus, typical symptoms associated with the disease manifest only after sufficient cumulative effect of diabetes mellitus. As a result, diabetes related complications and associated clinical conditions may be present by the time it is clinically diagnosed, one such diabetic complication which remains subclinical is autonomic neuropathy **(Subbalakshmi et al., 2008).**

Diabetic autonomic neuropathy (DAN) is among the least recognized and understood complications of diabetes despite its significant negative impact on survival and quality of life in people with diabetes. A subtype of the peripheral polyneuropathies that accompany diabetes, DAN can involve the entire autonomic nervous system (ANS). ANS vasomotor, visceromotor and sensory fibers innervate every organ. DAN may be either clinically evident or subclinical. It is manifested by dysfunction of one or more organ systems (e.g., cardiovascular, gastrointestinal [GI], genitourinary, sudomotor or ocular) **(Vinki et al., 2003).**

Because of its association with adverse outcomes including cardiovascular deaths, cardiovascular autonomic neuropathy (CAN) is the most clinically important and well studied form of DAN **(Verrotti et al., 2009).**

Cardiovascular autonomic neuropathy (CAN) occurs when peripheral autonomic fibers (sympathetic and parasympathetic) of the cardiovascular system (CVS) are affected, thus resulting in neurohormonal regulation disturbances the sympathetic vagal balance (both tonic and phasic) modulates the function of the main CVS structures: the sinus node (heart rate), the ventricles (end systolic and end diastolic volumes) and the blood vessels including microcirculation (total peripheral resistance) **(Rolim et al., 2008).**

Cardiovascular autonomic neuropathy was detected by standard cardiovascular reflex tests: heart rate responses to deep breathing and standing (30/15 ratio) and the valsalva maneuver (valsalva ratio) assessing mainly parasympathetic function and blood pressure responses to standing and sustained handgrip assessing mainly sympathetic function **(Putz et al., 2009).**

Early detection of subclinical autonomic dysfunction in diabetic individuals is important for risk stratification and subsequent manegement, possibly including pharmacologic and lifestyle interventions **(Ahamed Seyd et al., 2008).**

AIM OF THE WORK

The aim of this work was to detect diabetic patients with subclinical autonomic neuropathy using reflex cardiovascular testing.

Chapter 1

DIABETES MELLITUS

Definition of diabetes mellitus

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes, this range from autoimmune destruction of the ß-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes, is deficient action of insulin on target tissues and this deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin, at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia **(American Diabetes Association, 2013).**

*Etiologic Classification of Diabetes Mellitus: *By (American Diabetes Association, 2010).*

I. Type 1 diabetes (β-cell destruction, usually leading to absolute insulin deficiency):

A. Immune-mediated.

B. Idiopathic.

Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers.

II. Type 2 diabetes (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly insulin secretary defect with insulin resistance).

The degree of hyperglycemia sufficient to cause pathologic and functional changes in various target tissues, but without clinical symptoms, may be present for a long period of time before diabetes is detected. During this asymptomatic period, it is possible to demonstrate an abnormality in carbohydrate metabolism by measurement of plasma glucose in the fasting state or after a challenge with an oral glucose load.

III. Other specific types of diabetes:

A. Genetic defects of β-cell function.

B. Genetic defects in insulin action:

1. Type A insulin resistance.

2. Leprechanism.

3. Rabson-Mendenhall syndrome.

4. Lipodystrophy syndromes.

C. Diseases of the exocrine pancreas-pancreatitis, pancreatectomy, neoplasia, cysticfibrosis, hemochromatosis, fibrocalculous pancreatopathy.

D. Endocrinopathies-acromegaly, Cushing's syndrome, glucagonoma, pheochromocytoma, hyperthyroidism, somatostatinoma, aldosteronoma.

E. Drug – or chemical-induced – Vacor, pentamidine, nicotinic acid, glucocorticods, thyroid hormone, diazoxide, β-adrenergic agonists, thiazides, Phenytoin, α-interferon, protease inhibitors, clozapine, beta blockers.

F. Infections-congenital rubella, cytomegalovirus, coxsackie.

G. Uncommon forms of immune-mediated diabetes- "Stiff- man" syndrome, anti-insulin receptor antibodies.

H. Other genetic syndromes sometimes associated with diabetes- Down's Syndrome, Klinefelter's syndrome, Turner's syndrome.

Wolfram's syndrome, Friedrikh's ataxia, Huntington's chorea, Laurence-Moon-Biedl syndrome, myotonic dystrophy, porphyria, Prader-Willi syndrome.

IV. Gestational diabetes mellitus (GDM**).**

Risk factors for diabetes

I. Genetics:

Genetic suscetibility is important for both types of diabetes. Family history of type 1 diabetes or other autoimmune diseases such as autoimmune thyroid disease is associated with a higher risk of developing type 1 diabetes in the family. Inhheritance in type 2 diabetes is far more complex as there are many underlying causes. Furthermore, the risk varies according to the particular sub-type of type 2 diabetes.A family history of type 2 diabetes in a first degree relative is a strong risk factor for diabetes in that individual **(Holt and Kumar, 2010).**

II. Obesity:

Obese persons have higher levels of blood pressure, blood glucose and atherogenic serum lipids, and on that account alone could be expected to increase stroke incidence. The pattern of obesity is also important. Central obesity and abdominal deposition of fat are more strongly associated with atherosclerotic disease ***(Folsom et al., 2003).***

Adipose tissue is now recognized to be a significant endocrine organ secreting a variety of hormones and cytokines. Data suggest that some of these cytokines arising from adipose tissue may be partly responsible for the metabolic, hemodynamic and haemostatic abnormalities associated with insulin resistance. Studies show a close relationship between obesity and circulating

C- reactive protein (CRP), tumor necrosis factor α (TNF–α) and interleukin-6 (IL-6), and some of these cytokines are predictors of cardiovascular disease ***(Fonseca et al., 2004)***.

III. Age:

Beta cell function declines with age, indeed if we live long enough all of us have the potential to develop diabetes at some stage. With an aging population an increase in prevalence of diabetes can be expected ***(Holt and Kumar, 2010).***

IV. Ethinicity:

People of South Asian or Afro-Caribbean origin are at higher risk of Developing diabetes. They are also more likely to have type 2 diabetes presenting at a young age and usually have poorer risk factor control ***(Holt and Kumar, 2010).***

V. Metabolic syndrome

Dysglycemia and type 2 diabetes are often manifestations of a much broader underlying disorder, including the metabolic syndrome-a highly prevalent, multifaceted condition characterized by a distinctive constellation of abnormalities that include abdominal obesity, hypertension, dyslipidemia, insulin resistance and dysglycemia. Individuals with the metabolic syndrome are at significant risk of developing diabetes and cardiovascular diseases (CVD). Evidence now exists to support an aggressive approach to identifying people with the metabolic syndrome and treating not

only the hyperglycemia but also the associated cardiovascular (CV) risk factors, such as hypertension, dyslipidemia and abdominal obesity, in the hope of significantly reducing CV morbidity and mortality **(Canadian diabetes association, 2008).**

Epidemiology of diabetes mellitus:

Diabetes mellitus is a serious condition with potentially devastating complications that affects all age groups worldwide. In 1985, an estimated 30 million people around the world were diagnosed with diabetes; in 2000, that figure rose to over 150 million; and, in 2012, the International Diabetes Federation (IDF) estimated that 371 million people had diabetes. That number is projected to rise to 552 million (or 1 in 10 adults) by 2030, which equates to 3 new cases per second. Although the largest increase is expected to be in countries with developing economies **(Cheng, 2013).**

Categories of increased risk for diabetes:

In 1997 and 2003, the Expert Committee on Diagnosis and Classification of Diabetes Mellitus recognized an intermediate group of individuals whose glucose levels do not meet criteria for diabetes, yet are higher than those considered normal. These people were defined as having impaired fasting glucose (IFG) [fasting plasma glucose (FPG) levels 100 mg/dl (5.6 mmol/l) to 125 mg/dl (6.9mmol/l)], or impaired glucose tolerance (IGT) [2-h values in

the oral glucose tolerance test (OGTT) of 140 mg/dl (7.8 mmol/l) to 199 mg/dl (11.0 mmol/l)]. Individuals with IFG and/or IGT have been referred to as having prediabetes, indicating the relatively high risk for the future development of diabetes **(Ameriacn diabetes association 2013).**

Table (1): Criteria for the diagnosis of diabetes (American Diabetes Association, 2013).

A1C ≥ 6.5%. The test should be performed in a laboratory using a method that is the national glycohemoglobin standardization program (NGSP) certified and standardized to the diabetes Control and Complication Trial (DCCT) assay.*
OR
FPG ≥ 126 mg/dl (7.0 mmol/l). Fasting is defined as no caloric intake for at least 8 h.*
OR
2-h plasma glucose ≥ 200 mg/dl (11.1mmol/l) during anOGTT. The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma Glucose ≥ 200 mg/dl (11.1 mmol/l).

*In the absence of unequivocal hyperglycemia, criteria 1–3 should be confirmed by repeat testing.

Type 1 Diabetes Mellitus

Type 1diabetes is the form of the disease due primarily to β-cell destruction in which insulin is required for survival. Type 1 diabetes usually is characterized by the presence of anti- Glutamic acid decarboxylase (GAD), anti-islet cell, or anti-insulin antibodies, which reflect the autoimmune processes that, have led to β-cell destruction. Individuals who have one of more of these antibodies can be sub-classified as having type 1A, immune-mediated type 1 diabetes mellitus **(WHO Consultation Group, 1999).**

Particularly in nonwhites, type 1 diabetes can occur in the absence of autoimmune antibodies and without evidence of any autoimmune disorder. Such individuals are classified as having type 1B, or idiopathic, diabetes. Type 1A diabetes shows strong associations with specific haplotypes or alleles at the DQ-A and DQ-B loci of the human leukocyte antigen (HLA) complex. The rate of β-cell destruction is quite variable, being rapid in some individuals, especially in infants and children and slower in adults. Some have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia or ketoacidosis, while others (particularly adults) may retain some residual β-cell function for many years and have sometimes been termed as having "latent autoimmune diabetes". Such individuals may become dependent on insulin for survival only many years after the detection of diabetes. Individuals with type 1 diabetes have low or undetectable levels of insulin and plasma C-peptide. Patients with type 1A diabetes are also more

likely to have other concomitant autoimmune disorders, such as Graves's disease, Hashimoto thyroiditis, Addison disease, vitiligo, or pernicious anemia **(Greenbaum et al., 2000).**

Type 2 diabetes Mellitus

Type 2 diabetes is the most common form of diabetes. It is characterized by disorders of insulin action and insulin secretion, either of which may be the predominant feature. Usually, both are present at the time diabetes becomes clinically manifest. Although the specific etiology of this form of diabetes is not known, autoimmune destruction of the β-cells does not occur. Patients with type 2 diabetes usually have insulin resistance and relative, rather than absolute, insulin deficiency. At the time of diagnosis of diabetes and often throughout their lifetimes, these patients do not need insulin treatment to survive, although ultimately many require it for glycemic control. This form of diabetes is associated with progressive β-cell failure with increasing duration of diabetes **(Turner et al., 1999)**

Most patients with type 2 diabetes are obese when they develop diabetes, and obesity aggravates the insulin resistance. Type 2diabetes frequently pass undiagnosed for many years, because the hyperglycemia develops gradually and in the earlier stages is not severe enough to produce the classic symptoms of diabetes. However, such patients are at increased risk of developing macro-vascular and micro-vascular complications. Their circulating insulin levels may be normal or elevated yet,

insufficient to control blood glucose levels within the normal range because of their insulin resistance. Thus, they have relative, rather than absolute, insulinopenia. Insulin resistance may improve with weight reduction or pharmacologic treatment and results in normalization of their blood glucose. The risk of developing type 2 diabetes increases with age, obesity, and physical inactivity. Type 2diabetes shows strong familial aggregation, so that persons with a parent or sibling with the disease are at increased risk, as are individuals with obesity, hypertension, or dyslipidemia and women with a history of gestational diabetes. Although the disease is most commonly seen in adults, the disease can occur at any age and is now seen in children and adolescents **(Fagot-Campagna et al., 2000).**

Pathogenesis of type II Diabetes Mellitus

The pathogenesis of type II diabetes remains unclear. Environmental factors, such as a sedentary life style and dietary habits, clearly play a role, as will become evident when obesity is considered. Nevertheless, genetic factors are even more important than in type I diabetes. Among identical, twins, the concordance rate is 50% to 90% while among first-degree relatives with type II diabetes (and in fraternal twins), the risk of developing the disease is 20% to 40% compared to 5% to 7% in the population at large. Unlike type II diabetes, however, the disease is not linked to genes involved in immune tolerance and regulation, and there is no evidence to suggest an autoimmune basis for type II diabetes **(Saltiel and Kahn, 2001).**

The two metabolic defects that characterize type II diabetes are: (1) a decreased ability of peripheral tissues to respond to insulin (insulin resistance) and (2) β-cell dysfunction that is manifested as inadequate insulin secretion in the face of insulin resistance and hyperglycemia. In most cases, insulin resistance is the primary event and is followed by increasing degrees of B-cell dysfunction **(Saltiel and Kahn, 2001).**

I. Insulin resistance

Insulin resistance is defined as resistance to the effects of insulin on glucose uptake; metabolism, or storage. Insulin resistance is a characteristic feature of most patients with type II diabetes and is an almost universal finding in diabetic individuals who are obese. The role of insulin resistance in the pathogenesis of type II diabetes can be explained from the followings**:**

(1) Insulin resistance is often detected 10 to 20 years before the onset of diabetes in predisposed individuals (e.g., offspring of type II diabetics) and

(2) In prospective studies, insulin resistance is the best predictor for subsequent progression to diabetes **(Shulman, 2000).**

Insulin resistance leads to decreased uptake of glucose in muscle and adipose tissues and an inability of the hormone to suppress hepatic gluconeogenesis. Functional studies in individuals with insulin resistance have demonstrated numerous quantitative and qualitative abnormalities of the insulin signaling pathway,

including down regulation of the insulin receptor; decreased insulin receptor phosphorylation and tyrosine kinase activity; reduced levels of active intermediates in the insulin signaling pathway and impairment of translocation, docking, and fusion of glucose transporter type 4 (GLUT4)-containing vesicles with the plasma membrane **(Saltiel and Kahn, 2001).**

A. Genetic Defects of the Insulin Receptor and Insulin Signaling Pathway:

Loss-of-function abnormalities of either the insulin receptor or its downstream intermediates are obvious candidates for mediating insulin resistance in type II diabetes. Analysis of candidate genes involved in insulin secretion or insulin action, as well as whole genome linkage studies of affected families have yielded many polymorphisms that associate with the type II diabetic phenotype, but in most cases, the associations have been weak, or the studies were not reproducible. From these analyses, it appears that while the population risk associated with any particular genetic variant (polymorphism) may be significant, the increase risk for developing diabetes for a given individual harboring that variant is small at best. The genetic basis of insulin resistance and by extension type II diabetes, therefore remains unclear **(Saltiel and Kahn, 2001).**

B. Obesity and insulin resistance. The association of obesity with type II diabetes has been recognized for decades, visceral