Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterized by T-cell mediated destruction of the pancreatic b-cells, resulting in insulin deficiency and elevated blood glucose levels ***(Daneman, 2006).*** Poor glycemic control of T1DM negatively affects the development and progression of diabetic complications ***(Nathan et al., 2005)***.

Diabetic patients frequently develop severe chronic complications like cardiovascular disease, nephropathy, neuropathy, and retinopathy. Characteristics of these complications are macro- and microvascular damage, extracellular matrix (ECM) accumulation, and eventually chronic fibrosis ***(Gupta et al., 2003)***.

Vascular co-morbid diseases include retinopathy, which may cause reduced vision and blindness, and nephropathy, which may result in renal failure and require dialysis or kidney transplantation. This is in addition to hypertension, which is linked to peripheral, cardio- and cerebrovascular disease, the end points of which are limb amputations, cardiac failure, stroke and sudden death. As vascular complications curtail both life expectancy and quality of life **(*Marshall and Flyvbjerg, 2009)*** development at younger ages when people are typically establishing careers and families is particularly detrimental ***(James et al., 2014)***.

Diabetic nephropathy is a common complication in patients with diabetes. It is currently the leading cause of

end stage renal disease ESRD in Western societies and the main factor responsible for the increase in ESRD prevalence ***(Collins et al., 2007).*** Owing to the clinical and economic burden of diabetic nephropathy, considerable effort is made to identify ways to prevent it and delay its progression ***(Saraheimo et al., 2008).***

In diabetic nephropathy(DN), hyperglycemia and the formation of advanced glycated end products (AGEs) generate reactive oxygen species (ROS) and upregulate the transcription of many matrix genes (especially in mesangial cells), which leads to the expansion of the mesangial matrix and thickening of the glomerular basement membrane ***(Mason and Wahab, 2003).*** It has been shown that growth factors play an important role in the development of these diabetes complications ***(Chaturvedi et al., 2002).***

Connective tissue growth factor (CTGF) was first identified in conditioned media of endothelial cells as a 36- to 38-kDa polypeptide containing chemotactic activity toward fibroblasts ***(Bradham et al., 1991).*** CTGF has been acknowledged as a key factor in extracellular matrix production and other profibrotic activity mediated by transforming growth factor-β1 ***(Leask et al., 2004)***. Other biological functions of CTGF include angiogenesis, chondrogenesis, osteogenesis, and cell adhesion, migration, proliferation, and differentiation ***(Perbal, 2004)***.

CTGF has been shown to be up-regulated in various chronic diseases including liver fibrosis, systemic sclerosis, diabetic nephropathy and non-diabetic chronic kidney disease, idiopathic interstitial pneumonias, cardiomyopathy,

atherosclerotic plaques, nephrogenic systemic fibrosis, peritoneal fibrosis in peritoneal dialysis patients and urethral stricture ***(Paradis et al., 1999; Sato et al., 2000; Yoshisue et al., 2002; Cicha et al., 2005; Zhang et al., 2008; Kono et al., 2010; Schieren et al., 2010; Dendooven et al., 2011).***

Carotid intima media thickness (CIMT) is a simple, non-invasive, sensitive, screening tool for the assessment of atherosclerosis risk/prognosis in type 2 diabetic patients ***(Kota et al., 2013)*** but there are limited studies evaluating its role in type 1 diabetes in developing countries ***(Gupta et al., 2013; Adly et al., 2014).*** The relation between CTGF and CIMT as an index for atherosclerosis has not been widely explored.

Aim of the Work

The aim of this study is to determine connective tissue growth factor (CTGF) levels in children and adolescents with type 1 diabetes mellitus as a potential marker for diabetic vascular complications including diabetic nephropathy and assess its relation to the clinicopathological characteristics of patients, glycemic control and carotid intima media thickness.

Chapter (1): Diabetes Mellitus

Definition and Description

Diabetes Mellitus is a metabolic disorder characterized by the prescence of hyperglycemia due to defective insulin secretion, defective insulin action, or both ***(International Society of Pediatric and Adolescent Diabetes ISPAD, 2014).*** The chronic hyperglycemia of diabetes is associated with relatively specific long term microvascular complications affecting the eyes, kidneys and nerves, as well as an increased risk for cardiovascular disease (CVD) ***(American Diabetes Association, 2012).***

Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Often symptoms are not severe, or may be absent, and consequently hyperglycemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made ***(World Health Organization, 1999).***

Several pathogenic processes are involved in the development of diabetes. These include processes which destroy the beta cells of the pancreas with consequent insulin deficiency, and others that result in resistence to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin ***(World Health Organization, 1999)***.

The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease ***(McCance et al., 1994)***.

Global burden

Diabetes in all its forms imposes unacceptably high human, social and economic costs on countries at all income levels ***(IDF Diabetes Atlas, 2014)*** (Figure 1):

- **387 million** people have diabetes; by 2035 this will rise to **592 million** (table 1).
- The number of people with type 2 **diabetes is increasing** in every country.
- **80%** of people with diabetes live in **low- and middle-income countries**.
- The **greatest number** of people with diabetes are between **40 and 59** years of age.
- **175 million** people with diabetes are **undiagnosed**.
- Diabetes caused **4.9 million deaths** in 2014; Every seven seconds a person dies from diabetes.

- Diabetes caused at least **USD 612 billion dollars** in health expenditure in 2014 – **11% of total spending** on adults.
- More than **79,000 children** developed **type 1 diabetes** in 2013.
- More than **21 million live births** were affected by diabetes during pregnancy in 2013.

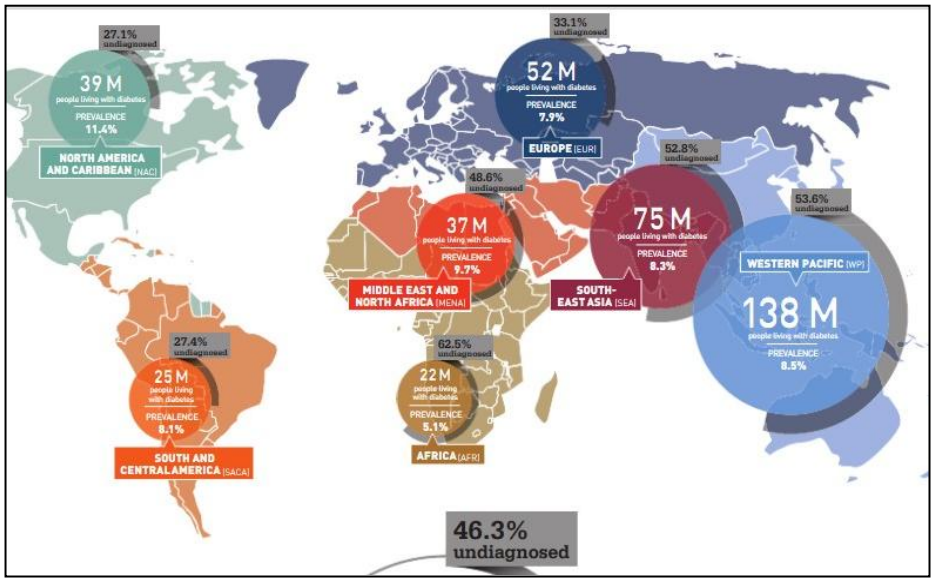

Figure 1: Global diabetes prevalence ***(IDF Diabetes Atlas, 2014)***

Table 1: Top 10 countries/ territories for number of people with diabetes (20-79 years), 2013 and 2035

Country / territory	2013 Millions	Country / territory	2035 Millions
China	98.4	China	142.7
India	65.1	India	109.0
United States of America	24.4	United States of America	29.7
Brazil	11.9	Brazil	19.2
Russian Federation	110.9	Mexico	15.7
Mexico	8.7	Indonesia	14.1
Indonesia	8.5	Egypt	13.1
Germany	7.6	Pakistan	12.8
Egypt	7.5	Turkey	11.8
Japan	7.2	Russian Federation	11.2

(IDF Diabetes Atlas, 2013)

Classification

The ADA classification of DM encompasses 4 groups: Type 1, Type 2, other specific types of diabetes, and gestational diabetes. Type 1 DM is further subclassified into Type 1A which is associated with the presence of islet cell autoantibodies, and Type 1B characterized by the absence of such antibodies ***(Sperling, 2002; Devendra et al., 2004)***. A classification of diabetes is presented in Table 2.

Table 2: Etiologic classification of diabetes mellitus

<table>
<tr><td>A. Type I-DM:
β-Cell destruction, usually leading to absolute insulin deficiency
a. Immune mediated
b. Idiopathic</td><td>B. Type II-DM:
May range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with or without insulin resistance</td></tr>
<tr><td>C. Genetic defects of β -cell function
1. Chromosome 12, HNF−1α (MODY3)
2. Chromosome 7, glucokinase (MODY2)
3. Chromosome 20, HNF−4α (MODY1)
4. Chromosome 13, insulin promoter factor- (IPF-1; MODY4)
5. Chromosome 17, HNF−1β (MODY5)
6. Chromosome 2, NeuroD1 (MODY6)
7. Mitochondrial DNA mutation
8. Chromosome 7, KCNJ11 (Kir6.2)
9. Others</td><td>D. Genetic defects in insulin action
1. Type A insulin resistance
2. Leprechaunism
3. Rabson-Mendenhall syndrome
4. Lipoatrophic diabetes
5. Others</td></tr>
<tr><td>E. Diseases of the exocrine pancreas
1. Pancreatitis 2. Trauma / pancreatectomy
3. Neoplasia 4. Cystic fibrosis
5. Haemochromatosis
6. Fibrocalculous pancreatopathy 7. Others</td><td>F. Endocrinopathies
1. Acromegaly 2. Cushing’s syndrome
3. Glucagonoma 4. Phaeochromocytoma
5. Hyperthyroidism 6. Somatostatinoma
7. Aldosteronoma 8. Others</td></tr>
<tr><td>G. Drug- or chemical-induced
1. Vacor 2. Pentamidine
3. Nicotinic acid 4. Glucocorticoids
5. Thyroid hormone 6. Diazoxide
7. β-adrenergic agonists 8. Thiazides
9. Dilantin 10. α –Interferon
11. Others</td><td>H. Infections
1. Congenital rubella
2. Cytomegalovirus
3. Enterovirus
4. Others</td></tr>
<tr><td>I. Uncommon forms of immune-mediated diabetes
‘‘Stiff-man’’ syndrome
Anti-insulin receptor antibodies
autoimmune Polyendocrine decencies’
(APS) I and II
Others</td><td>J. Other genetic syndromes sometimes associated with diabetes
Down syndrome
Klinefelter syndrome
Turner syndrome
Wolfram syndrome
Friedreich’s ataxia
Huntington’s chorea
Laurence-Moon-Biedl syndrome
Myotonic dystrophy
Porphyria
Prader-Willi syndrome
Others</td></tr>
<tr><td colspan="2">K. Gestational Diabetes.</td></tr>
</table>

MODY: Maturity onset diabetes of the young; HNF-4α: Hepatocyte Nuclear Factor 4; NeuroD1: Neurogenic differentiation 1; KCNJ11 (Kir6.2): inward-rectifier potassium ion channel gene.

(International Society of Pediatric and Adolescent Diabetes ISPAD, 2014)

Different types

Although the vast majority of children and young people with diabetes in the UK have type 1 diabetes, an increasing number are diagnosed with type 2 diabetes, recently reported at around 2% ***(Shepherd and Cropper, 2013).*** Other causes of childhood diabetes include neonatal diabetes, maturity-onset diabetes of the young (MODY), secondary diabetes and syndromic diabetes ***(Royal College of Pediatrics and Child Health, 2009).*** However, the correct classification of pediatric diabetes can be challenging (table 4); there may be confusion between the presenting features of different types of diabetes, which can lead to erroneous diagnosis ***(Ehtisham et al., 2004).***

Monogenic diabetes results from mutations in single genes that regulate beta-cell function, but is commonly misdiagnosed as type 1 or type 2 diabetes ***(Lambert et al, 2003; Shepherd, 2008)***, and only a minority of estimated cases have been confirmed by genetic testing in the UK ***(Shields et al., 2010).*** Individuals with monogenic diabetes do not need to be insulin resistent or obese to develop diabetes ***(Ehtisham et al., 2004).***

Guidelines have been developed to highlight when a diagnosis of monogenic diabetes should be considered in children (table 3) and when to suspect that a diagnosis of type 1 or type 2 diabetes is not correct ***(Hattersley et al., 2006).*** The approaches are separated into:

- Those diagnosed less than 6 months of age (indicating neonatal diabetes).
- Those who may otherwise have been labelled as having type 1 diabetes (where a child with diabetes has an affected parent and evidence of endogenous insulin production outside the honeymoon period).
- Those who may otherwise have been labelled as having type 2 diabetes who are not markedly obese, do not have acanthosis nigricans or other evidence of insulin resistance and are from a low-prevalence ethnic group ***(Hattersley et al., 2006).***

These guidelines should be used in the clinical setting to identify cases of atypical type 1 diabetes or type 2 diabetes, which warrant further investigation.

Table 3: When to consider monogenic diabetes in children.

When to consider monogenic diabetes in children
▪ Diabetes diagnosed below the age of 6 months (neonatal diabetes)
▪ Family history of diabetes with an affected parent
▪ Mild fasting hyperglycemia (5.2-8 mmol/L), especially if young or familial
▪ Diabetes associated with extra-pancreatic features

(Hattersley et al., 2006)

Table 4: Characteristic Phenotypes of the commonly encountered diabetes subtypes, illustrating the clinically useful differences between type 1 and type 2 diabetes, and monogenic forms of diabetes.

Features associated with diabetes	Type 1 diabetes	Young onset Type 2 diabetes	Monogenic diabetes					
			GCK*	HNF1A#	HNF4A#	HNF1B#	Neonatal diabetes	MIDD≠
DKA	Yes	No	No	No ∞	No ∞	No	Yes	Yes/No
Parent affected	2%-4%	Yes	Yes	Yes	Yes	Yes	Variable	Mother
Age of onset	6 months to adulthood	Adolescence and young adulthood	Birth	Teens to young adulthood	Teens to young adulthood	Teens to young adulthood	< 6 months	Young adulthood
Obesity	Population frequency	Increased Frequency	Population frequency	Population frequency	Population frequency	Population frequency	Population frequency	Rare
Glycaemic pattern	Acute General hyperglycemia	Progressive hyperglycemia	Stable, mild fasting glycemia	Post-prandial, 12nitially progressing to general hyperglycemia	Post-prandial, 12nitially progressing to general hyperglycemia	Post-prandial, 12nitially progressing to general hyperglycemia	Acute General hyperglycemia	Variable dysglycaemic pattern either acute or slowly progressive
B cell antibodies	Yes	**No**	**No**	**No**	No	No	No	No
C-peptide	**Very low/Absent (< 5 years)**	**Raised/ Normal**	**Normal**	**Low but detectable**	**Low but detectable**	**Low but detectable**	Absent but detectable once treated with SU	**Low but detectable**
hsCRP	Normal	**High/ High normal**	Normal	**Very low**	Normal	Normal	Normal	Normal
Additional clinical features	**Other autoimmune disease (Thyroid, celiac, etc.**	**Dyslipidaemia, PCOS, Hypertension, Acanthosis nigricans**	**Absence of microvascular and macrovascular complications**	**Low renal threshold for glucose in early stages of diabetes**	Macrosomia and transient neonatal hypoglycemia	High renal involvement e.g., cysts, etc,	Transient in 50% of cases although may relapse	Deafness, short stature, macular dystrophy

(Carroll & Murphy, 2013)

*= Glucokinase, #= Hepatocyte nuclear factor, ≠ = Mitochondrial diabetes and deafness, ∞= Excellent responses to sulfonylurea therapy are commonly noted

Type I Diabetes Mellitus

Type 1 diabetes is a disorder that arises following the autoimmune destruction of insulin-producing pancreatic b cells ***(Atkinson 2001; Bluestone et al. 2010)***. The disease is most often diagnosed in children and adolescents, usually presenting with a classic trio of symptoms (i.e., polydypsia, polyphagia, polyuria) alongside of overt hyperglycemia, positing the immediate need for exogenous insulin replacement-a medicinal introduction to the disorder whose therapeutic practice lasts a lifetime ***(Thunandera et al., 2008).***

Epidemiology of type I diabetes

Incidence and Prevalence

T1DM is without question one of the most common chronic diseases of childhood ***(Karvonen et al. 2000; Gale 2005).*** There are approximately 500,000 children aged under 15 with type 1 diabetes in the world ***(Patterson et al., 2014)***. The International Diabetes Federation estimates that 79,000 children developed type 1 diabetes in 2013 ***(IDF Diabetes Atlas 2013)***.

Of the 500,000 children with type 1 diabetes in the world, the most live in Europe (129,000) and North America (108,700). Countries with the highest estimated numbers of new cases annually (highest incidence) were the United States (13,000), India (10,900) and Brazil (5000) ***(Patterson et al., 2014).***

The incidence of type 1 diabetes is increasing in all population at a rate of approximately 3% per year and the onset of the condition is occurring at younger age. Its incidence varies dramatically between populations and even within the same population, much of this variation is due to genetic defect. Among Eastern Mediterranean and Middle Eastern countries, the largest contribution to the total number of estimated childhood T1DM cases comes from Egypt which accounts for about a quarter of the region's total. The incidence varies between 1/100 000 per year (Pakistan) and 8/100 000 per year (Egypt) in children under the age of 15 years ***(*Soltèsz *et al., 2006)*.**

An Egyptian study of incidence and prevalence of T1DM in children and adolescents in four Egyptian Governorates (Fayoum, Minofeya, North Sainai and Sues) was held by ***Salem et al. (2007)***, showing a prevalence rate of 0.7/1000 and an incidence rate of 4.01/100.000.

Classification of type I diabetes

Type 1 diabetes may be subdivided in three groups from the etiological point of view: autoimmune, idiopathic and double. The autoimmune group is represented by: type 1A, which is polygenic and it is the most frequent type of this disease, corresponding to approximately 80-90% of all T1DM cases ***(Daneman, 2006).***

The other subtype of this group is the latent autoimmune diabetes in adults (LADA) ***(Fourlanos et al., 2006)***, which appears after the age of 35 and is frequently associated with other autoimmune endocrine diseases.

The third subtype includes called "monogenic" T1DM. They correspond to T1DM of the autoimmune polyglandular syndrome type 1A ***(Su and Anderson, 2004)*** and of IPEX syndrome (Immune Dysfunction, Polyendocrinopathy, Enteropathy, X-linked) ***(Wildin and Freitas, 2005)***.

Type 1B, also called idiopathic, has all the clinical features of type 1A, but the autoimmune component is not detected ***(American Diabetes association, 2008).*** Another 1B subtype is the fulminant diabetes most described in Asian peoples, mainly Japan, China and Korea, characterized by a short clinical history, before to the first acute metabolic decompensation, presents the impairment of beta and alpha cells of the pancreatic islet and no autoimmune etiology ***(Imagawa et al., 2000)***.

Finally, the denomination of mixed, 1.5 or double (type 1 plus type 2) diabetes has been proposed when we have the type 1A (autoimmunity) plus type 2 (obesity, insulin resistance, dyslipidemia) diabetes characteristics in the same individual ***(Libman and Becker, 2003).***

Etiology of type I diabetes

The etiology of type 1 diabetes remains poorly understood, but it is likely that an environmental factor triggers an autoimmune process in a predisposed individual (figure 2). Although the genetic susceptibility to type 1 diabetes is inherited, only 12–15% of type 1 diabetes occurs in families. Twin studies have shown that the concordance rate for type 1 diabetes in monozygotic twins is around 20–30% ***(Holt, 2004).***