INTRODUCTION

Symbiotic interactions of microorganisms are widespread in nature, and support fundamentally important processes linking health and disease to the bacterial ecology. Intestinal microbiota is the largest source of microbial stimulation that exerts both harmful and beneficial effects on human health. It participates in the development of the postnatal immune system as well as oral tolerance and immunity ***(Delcenserie et al., 2008).***

The recently explored impact of the microbiota on energy metabolism, gut hormone regulation and the gut–brain axis was judged to be a fascinating topic and of great value in the future, and can have a clinical role in the management of obesity, diabetes,…*etc*. ***(Rowland et al., 2010).***

Many of the published studies described the differences between gut microbiota, in multiple sclerosis, rheumatoid arthritis, inflammatory bowel diseases, obesity and diabetes ***(Larsen et al., 2010).***

No one can judge the true link between diabetes mellitus and gut microbiota, however, it was known that high fat feeding changed the gut microbiota toward decreasing the number of beneficial bacteria together with an increase in the number of hazardous bacteria resulting in

a state of metabolic endotoxaemia which might trigger an inflammatory response and play a role in the development of diabetes ***(Khan et al., 2014).***

Lactobacillus Acidophilus has been shown in one of the studies to be increased in patients with type 2 Diabetes Mellitus ***(Tilg and Moschen, 2014).***

However, another analysis demonstrated a low yield of Lactobacillus Acidophilus in uncontrolled versus controlled type 2 Diabetes Mellitus cases ***(Abo Ali et al., 2013).***

The present study evaluates the impact of Lactobacillus Acidophilus in type 2 Diabetes Mellitus.

Aim of the Work

The aim of this work is to assess the impact of the gut microbe, Lactobacillus Acidophilus, in patients with type 2 Diabetes Mellitus (controlled and uncontrolled) compared to healthy individuals, as a preliminary approach to future treatment with probiotics, prebiotics or diet modulation.

Chapter (1): Diabetes Mellitus

Definition:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of various organs especially the eyes, kidneys, nerves, heart, and blood vessels ***(American Diabetes Association (ADA), 2013).***

Symptoms of marked hyperglycemia include polyuria, polydipsia, and weight loss, associated sometimes with polyphagia and blurring of vision. Impairment of growth and susceptibility to certain infection may also accompany chronic hyperglycemia. Acute life threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non ketotic hyperosmolar syndrome ***(ADA, 2008).***

Epidemiology of Diabetes:

Diabetes Mellitus is a major public health problem worldwide. Its prevalence has been Longley used as one of the parameters in the assessment of the quality of health care by the world health organizations (WHO) ***(Metelko et al., 2008).***

In 2015, 415 million people in the world had diabetes and more than 35.4 million people in the Middle East and North Africa (MENA) Region; by 2040 this will rise to 72.1 million. In Egypt There were 7.8 million cases of diabetes in 2015 ***(International Diabetes Federation, 2017).***

Factors Influencing the Prevalence of Diabetes:

1- Age:

Populations worldwide continue to show a consistent increase in the prevalence of diabetes with increasing age, with values reaching a plateau or even declining slightly in the very old ***(Colagiuri & Davies, 2009).***

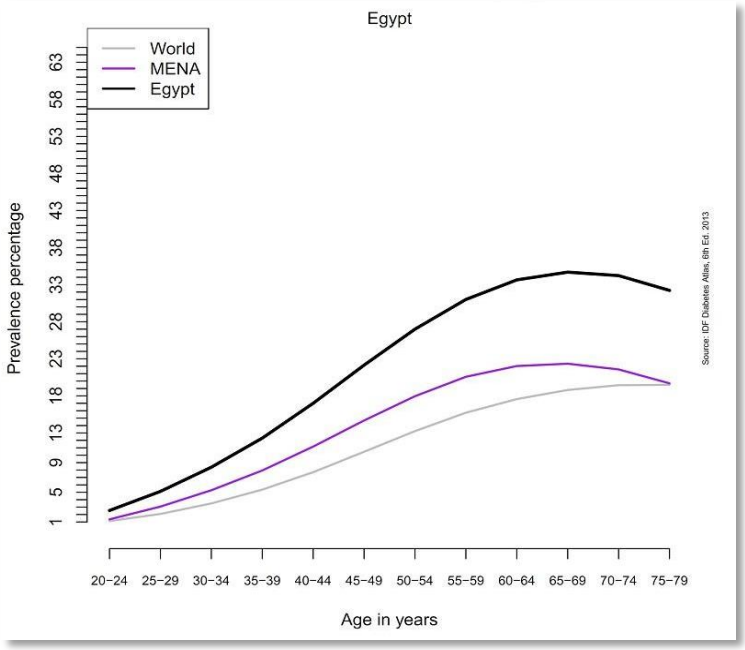

Figure (1): Prevalence of diabetes in adults by age
(International Diabetes federation, 2014)

2- Body Weight:

In 2009, the worldwide prevalence of obesity has grown to alarming levels of at least 300 million people. A body mass index (BMI) of > 30% increases the absolute risk of Type 2 Diabetes Mellitus by approximately two fold ***(Mokdad et al., 2009).***

3- Family History:

Individuals with a family history of T2DM are at an increased risk for the disease. The life time risk of developing T2DM has been estimated at 40% if one parent has T2DM ***(Colagiuri & Davies, 2009).***

4- Pre-Existing Impaired Glucose Homeostasis :

Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) were said to increase the risk between 10 and 20 fold compared to subjects with normal glucose tolerance ***(Magliano et al., 2008)***.

5- Ethnicity:

It is well documented that the prevalence of diabetes varies among different ethnic groups. It was found to be significantly higher among Hispanics (33.4%) than among blacks (29.6%), Asians (24.3%) and whites (18.4%)**.** Certain ethnic groups were particularly susceptible to

develop diabetes when they move from traditional to urbanized sedentary life style, with higher prevalence rate in urban rather than rural environments within the same country ***(Colagiuri & Davies, 2009).***

6- Other Factors Predisposing To DM:

- Dyslipidemia: high (triglyceride, cholesterol, low density lipoprotein LDL) or low (high density lipoprotein HDL
- Hypertension >140/90 mmHg in adults was noted with an approximate two fold increase in undiagnosed T2DM.
- Diabetes is common in people with cardiovascular and cerebrovascular diseases ***(Colagiuri & Davies, 2009).***

Assigning a type of diabetes to an individual often depends on the circumstances present at the time of diagnosis, and many diabetic individuals do not easily fit into a single class. For example, a person diagnosed with gestational diabetes mellitus (GDM) may continue to be hyperglycemic after delivery and may be determined to have, in fact, type 2 diabetes. Alternatively, a person who acquires diabetes because of large dose of exogenous steroids may become normoglycemic once the

glucocorticoids are discontinued, but then may develop diabetes many years later after recurrent episodes of pancreatitis. Another example would be a person treated with thiazides, who develops diabetes years later, because thiazides themselves seldom cause sever hyperglycemia. Such individuals probably have type 2 diabetes that is exacerbated by the drug. Thus, for the clinician and the patient, it is less important to label the particular type of diabetes than it is to understand the pathogenesis of the hyperglycemia and to treat it effectively. Figure 2 shows an overview for etiological classification of Diabetes Mellitus ***(ADA, 2014).***

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

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

III. Other specific types
   A. Genetic defects of β-cell function
      1. MODY 3 (Chromosome 12, HNF-1α)
      2. MODY 1 (Chromosome 20, HNF-4α)
      3. MODY 2 (Chromosome 7, glucokinase)
      4. Other very rare forms of MODY (e.g., MODY 4: Chromosome 13, insulin promoter factor-1; MODY 6: Chromosome 2, *NeuroD1*; MODY 7: Chromosome 9, carboxyl ester lipase)
      5. Transient neonatal diabetes (most commonly ZAC/HYAMI imprinting defect on 6q24)
      6. Permanent neonatal diabetes (most commonly KCNJ11 gene encoding Kir6.2 subunit of β-cell K_{ATP} channel)
      7. Mitochondrial DNA
      8. Others
   B. Genetic defects in insulin action
      1. Type A insulin resistance
      2. Leprechaunism
      3. Rabson-Mendenhall syndrome
      4. Lipoatrophic diabetes
      5. Others
   C. Diseases of the exocrine pancreas
      1. Pancreatitis
      2. Trauma/pancreatectomy
      3. Neoplasia
      4. Cystic fibrosis
      5. Hemochromatosis
      6. Fibrocalculous pancreatopathy
      7. Others
   D. Endocrinopathies
      1. Acromegaly
      2. Cushing's syndrome
      3. Glucagonoma
      4. Pheochromocytoma
      5. Hyperthyroidism
      6. Somatostatinoma
      7. Aldosteronoma
      8. Others
   E. Drug or chemical induced
      1. Vacor
      2. Pentamidine
      3. Nicotinic acid
      4. Glucocorticoids
      5. Thyroid hormone
      6. Diazoxide
      7. β-Adrenergic agonists
      8. Thiazides
      9. Dilantin
      10. γ-Interferon
      11. Others
   F. Infections
      1. Congenital rubella
      2. Cytomegalovirus
      3. Others
   G. Uncommon forms of immune-mediated diabetes
      1. Stiff-man syndrome
      2. Anti-insulin receptor antibodies
      3. Others
   H. Other genetic syndromes sometimes associated with diabetes
      1. Down syndrome
      2. Klinefelter syndrome
      3. Turner syndrome
      4. Wolfram syndrome
      5. Friedreich ataxia
      6. Huntington chorea
      7. Laurence-Moon-Biedl syndrome
      8. Myotonic dystrophy
      9. Porphyria
      10. Prader-Willi syndrome
      11. Others

IV. Gestational diabetes mellitus

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

Figure (2): Classification of Diabetes Mellitus and Other Categories of Glucose Regulation ***(ADA, 2014)***

Screening For Diabetes Mellitus:

BMI >25 kg/m^2 and have additional risk factors;

- Physical inactivity
- First degree relative with diabetes.
- A member of high risk ethnic population (Such as African American, Hispanic, Native American, Asian or Pacific Islander).
- A History of vascular disease.
- Previous testing revealing IGT or IFG.
- Blood pressure >140/90 mmHg.
- Giving birth to a baby weighing > 4 Kg.
- HDL-Cholesterol levels < 35 mg/dl or TG levels > 250 mg/dl.
- Previous diagnosis with GDM.
- Other clinical conditions associated with insulin resistance, such as PCOS or Acanthosis nigricans.

In the absence of the above criteria, testing for diabetes should begin at age 45 years.

If results are normal, testing should be repeated at least at 3-year intervals, with consideration of more frequent testing depending on initial results (e.g., those with prediabetes should be tested yearly) and risk status ***(Craig and Kamer, 2016).***

Diagnosis of Diabetes Mellitus:

Symptoms include:

The classic symptoms of diabetes are loss of weight, polyuria, polydipsia and polyphagia. Symptoms may develop rapidly (weeks or months) in type 1 diabetes, while they usually develop much more slowly and may be subtle or absent in T2DM ***(Cooke & Plotnick, 2008)***.

Diagnostic criteria: WHO criteria *(Longmore et al., 2010):*

Diabetes is diagnosed if:

- Symptoms of hyperglycemia (e.g. polyuria, polydipsia, unexplained weight loss, visual blurring, genital thrush, lethargy)
- **AND American Diabetes Association criteria *(ADA, 2016)One or more of following criteria;***
  - HbA1C≥.6.5%.
  - Fasting plasma glucose (FPG). ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.

- Two-hour plasma glucose ≥.200 mg/dL(11.1 mmol/L) during an OGTT. The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 gram anhydrous glucose dissolved in water. *

- In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥ 200 mg/dL (11.1 mmol/L).

➢ IFG and IGT refer to individuals whose glucose homeostasis is abnormal, but who not quite qualify as having diabetes**.**

- HBA1c ≥ 5.7% to 6.4%.

- Fasting glucose levels ≥100 mg/dl but <126 mg/dl.

- 2 hours values in the OGTT.140 mg/dl but <200 mg/dl.

Complications of Diabetes Mellitus

Chronic complications of diabetes
Microvascular
Retinopathy
Impaired vision, blindness
Nephropathy
Proteinuria, chronic kidney disease, dialysis
Neuropathy
Peripheral: sensory (pain, numbness, paresthesias) and motor neuropathy
Autonomic: gastroparesis, postural hypotension, impotence
Macrovascular
Coronary artery disease
Myocardial infarction
Peripheral vascular disease
Claudication, ulcers, amputation
Cerebrovascular disease
Stroke
Perioral diseases
Gingivitis
Periodontitis
Xerostomia
Candidiasis
Oral lichen planus
Leucoplakia (premalignancy)
Oral cancer
Acute complications of diabetes
Hyperosmolar hyperglycemia
Diabetic ketoacidosis
Acute infections

Figure (3): Chronic and acute complications of diabetes ***(Skamagas et al., 2008)***

Metabolic Syndrome (Syndrome X)

Metabolic syndrome is a multiplex risk factor that arises from insulin resistance accompanying abnormal adipose deposition and function. It is a risk factor for coronary heart disease, as well as for diabetes, fatty liver, and several cancers ***(Wang, 2015).***

Signs and symptoms

Clinical manifestations of metabolic syndrome include the following:

- Hypertension
- Hyperglycemia
- Hypertriglyceridemia
- Reduced high-density lipoprotein cholesterol (HDL-C)
- Abdominal obesity
- Chest pains or shortness of breath: Suggesting the rise of cardiovascular and other complications
- Acanthosis nigricans, hirsutism, peripheral neuropathy, and retinopathy: In patients with insulin resistance and hyperglycemia.
- Xanthomas or xanthelasmas: In patients with severe dyslipidemia ***(Wang, 2015).***

Diagnosis

According to guidelines from the National Heart, Lung, and Blood Institute (NHLBI) and the American Heart Association (AHA), metabolic syndrome is diagnosed when a patient has at least 3 of the following 5 conditions:

- Fasting glucose ≥100 mg/dL (or receiving drug therapy for hyperglycemia)
- Blood pressure ≥130/85 mm Hg (or receiving therapy for hypertension)
- Triglycerides ≥150 mg/dL (or receiving therapy for hypertriglyceridemia)
- HDL-C < 40 mg/dL in men or < 50 mg/dL in women (or receiving drug therapy for reduced HDL-C)
- Waist circumference ≥102 cm (40 in) in men or ≥88 cm (35 in) in women; if Asian American, ≥90 cm (35 in) in men or ≥80 cm (32 in) in women ***(Wang, 2015).***