Relation of Gut Lactobacillus Acidophilus and Atherosclerosis in type 2 diabetic patients with and without atherosclerosis

A Thesis

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Mark Nabil Bios

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List of Abbreviations

Abb.	Full term
2hr PP	Two hours post prandial plasma glucose
ABI	ankle-brachial index
ADA	American Diabetes Association
AGEs	Advanced glycation end products
AHA	American Heart Association
AHA/ACC	American Heart Association and American College of Cardiology
Angptl4	Angiopoietin-like 4
ApoB	Apolipoprotein B
CAD	Coronary artery disease
CDC	Center for Disease Control and Prevention
CVS	Cerebrovascular stroke
DM	Diabetes melitis
ESC/EAS	European Society of Cardiology and European Atherosclerosis Society.
FFAs	Free fatty acids.
FIAF	Fasting-induced adipose factor
FMO3	Flavin-containing monooxygenase form 3
FPG	Fasting plasma glucose
GDM	Gestational diabetes mellitus
GLP-1	Glucagon-like peptide-1
HBA1C	Glycosylated hemoglobin
HDL	High density lipoproteins

List of Abbreviations (Cont.)

Abb.	Full term
IDF	International diabetes federation
IL-6	Interleukin 6
IMT	Intima media thickness
Interferon-α	Interferon gamma
IR	Insulin resistance
LDL	Low density lipoproteins
LPL	Lipoprotein lipase
LPS	Lipopolysaccharides
NDR	Swedish National Diabetes registry
NF- κB	Nuclear factor kappa B
NO	Nitric oxide
PAD	Peripheral arterial disease
PCA	Protocatechuic acid
PCR CT	Polymerase chain reaction cut-off threshold
PCR	Polymerase chain reaction
ROS	Reactive oxygen species
SCFA	Short chain fatty acids
TLR5	Toll-like receptor 5
TMA	Trimethylamine
TMAO	Trimethylamine-N-oxide
TNF-α	Tumor necrosis factor alpha
VCAM-1	Vascular cell adhesion molecule-1
VLDL	Very low-density lipoproteins

INTRODUCTION

Atherosclerosis is a major burden of modern society and according to the 2018 report by the World Health Organization (WHO), ischemic heart disease, a major complication of atherosclerosis; is the leading cause of death worldwide ***(WHO, 2018).***

Diabetes is considered an important risk factor for the development and severity of all forms of atherosclerosis, including peripheral arterial disease (PAD), coronary artery disease (CAD), and cerebrovascular disease (CVD) ***(Centers for Disease Control and Prevention, 2014).***

In the past, there have been several studies suggesting that microbes may play a role in the development of atherosclerosis. And recently, colonic bacteria were considered as agents activating chronic inflammatory mechanisms. This is supported by multiple data showing the link between the gut microbiota, inflammation, and autoimmunity ***(Stefanie et al., 2018).***

Also, some animal models suggest that obesity, insulin resistance & the metabolic syndrome are associated with alterations of the composition and the functional properties of the gut microbiota ***(Chistiakov et al., 2015).***

More recent, a direct connection between microbiota and atherosclerosis has been established through directly atherogenic

compounds like trimethylamine-oxide (TMAO) which is produced by the action of gut microbiota ***(Brugere et al., 2014).***

On the other hand, many members of the gut microbiota are now considered to be probiotics; providing many health benefits ***(Ewaschuk et al., 2007).***

Bifidobacterium and *Lactobacillus* are two well-known probiotics that are widely used for improving human health. In vitro experiments showed that some of members of those families can assimilate cholesterol and deconjugate bile salts. Resulting in reduction of cholesterol levels and thus being protective against metabolic diseases ***(Lebeer et al., 2010).***

Moreover, ***Moroti et al.,*** reported that the administration of a synbiotic beverage contained *Lactobacillus acidophilus*, *Bifidobacterium bifidum* and *oligofructose*, markedly increased the plasma High Density Lipoproteins (HDL-C) and decreased fasting glycemia in elderly type 2 diabetic patients ***(Moroti et al., 2012).***

Other mechanisms also have been postulated for their athero-protective role including: increasing colonic short chain fatty acids decreasing low Density Lipoproteins (LDL) and cholesterol synthesis, lowering inflammatory cytokines ie: interleukin six (IL-6), interleukin eight (IL-8), Tumor Necrosis Factor Alpha (TNF-α) ***(Chistiakov et al., 2015).***

Aim of the Work

To study the association of gut Lactobacillus Acidophilus and the presence of atherosclerosis in type 2 diabetic patients.

Chapter 1

DIABETES AND ATHEROSCLEROSIS

Diabetes is a disorder characterized by chronic hyperglycemia resulting either from a lack of insulin production (type one), from insulin resistance (type two) or both. In the last several decades, an alarming increase in the global prevalence of diabetes has been reported. The financial impact of diabetes to the health care system is enormous causing a two to three times higher cost expenditure than the rest of the population. In ten years, the diabetic population will exceed 700 million, this has been attributed to rising obesity epidemic all over the world ***(CDC, 2014).***

According to International diabetes federation (IDF), 425 million people have diabetes in the world and more than 39 million people in the middle east and north Africa region; by 2045 this will rise to 67 million. In 2017, the number of diabetics in Egypt was 8,222.6 ***(IDF, 2018).***

Types of diabetes:

Diabetes can be classified into the next general types:

1. *Type 1 diabetes* (absolute insulin deficiency, mostly due to autoimmune β-cell destruction).

2. *Type 2 diabetes* (due to insulin resistance and progressive loss of β-cells of pancreas).

3. *Gestational diabetes mellitus (GDM)* (diagnosed in the second or third trimester of pregnancy that was absent prior to gestation).

4. *Specific types* of diabetes secondary to other etiology, e.g., monogenic diabetes syndromes (such as neonatal diabetes and maturity-onset diabetes of the young [MODY]), diseases of the exocrine pancreas (such as cystic fibrosis and pancreatitis), and drug- or chemical-induced diabetes (such as with glucocorticoid use).

(ADA, 2018)

Type two diabetes phenotypes:

Type 2 diabetes is the most prevalent diabetes form in adults worldwide, and is characterized by adulthood onset, a strong association with obesity, state of insulin resistance and gradual loss of islet β-cells functions. However, phenotypically, type 2 diabetes is more to be found a heterogeneous condition. In 2018, *Ahlqvist et al.,* identified five replicable clusters of patients with significantly distinguishing characteristics and risk of complications. This was done using a set of available data: age, BMI, HbA1c, estimates of β-cell function and insulin resistance, and presence or absence of autoantibodies. The clusters included

severely insulin-deficient diabetes (SIDD), severe autoimmune diabetes (SAID), severe insulin-resistant diabetes (SIRD), mild age-related diabetes (MARD) and mild obesity-related diabetes (MOD) ***(Ahlqvist et al., 2018).***

Table (1): Showing Diagnostic Criteria for Diabetes Mellitus. ***(ADA, 2018).***

American Diabetes Association Diagnostic Criteria for Diabetes Mellitus
1. Fasting plasma glucose (FPG) ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 hours. *or*
2. Two-hour plasma glucose ≥200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test (OGTT). The test should be performed as described by the World Health Organization, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water. *or*
3. Glycated hemoglobin (A1c) ≥6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is standardized to the Diabetes Control and Complications Trial (DCCT) assay. *or*
4. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).
*Criteria 1 to 3 require confirmatory testing; criterion 4 does not.

Table (2): Summarizing different diabetic complications ***(Rich et al., 2009).***

Acute complications	**Chronic complications**	
	Macro-angiopathy	**Micro-angiopathy**
Diabetic ketoacidosis	Coronary a. disease	Cardiomyopathy
hyperosmolar Hyperglycemia	Myonecrosis	Nephropathy
Hypoglycemic coma	Peripheral vas. dis.	Retinopathy
Diabetic coma	Stroke	Polyneuropathy
Respiratory infections		
Periodontal disease		

The chronic health complications of diabetes are mainly vascular. Diabetes is believed to shorten the lifespan of a 50-year-old person by approximately 6 years, more than half of which is caused by vascular disease. Vascular complications are usually divided into: microvascular and macrovascular complications. Where elevated blood sugar is found to be a driving force in both large and small vessel disease ***(Rao et al., 2011).***

Microvascular complications include: retinopathy and nephropathy. Diabetics have a 20-fold increased relative risk of blindness and a 25-fold higher relative risk of end-stage renal disease (ESRD).

While macrovascular disease is due to atherosclerosis. Diabetes is an important risk factor for the development and severity of all forms of atherosclerosis, including peripheral artery disease (PAD), coronary artery disease (CAD), and cerebrovascular disease (CVD) ***(Orasanu et al., 2009).***

Epidemiological studies show that atherosclerosis causes most of the morbidity and mortality in patients with diabetes ***(Rao et al., 2011).***

Cardiovascular disease accounts for about 44% of all-cause mortality in the diabetic patients' population, diabetes accounts for 60% of nontraumatic lower-limb amputations and diabetes