CHAPTER 1

INTRODUCTION

1.1 Introduction

The rise of metabolic diseases worldwide is one of the most serious public health issues. In 2019, a staggering number of 463 million people were diagnosed with diabetes. This number is expected to reach 578 million by 2030, and 700 million by 2045, according to the International Diabetes Federation (IDF) (2019). Type 2 diabetes mellitus (T2DM) which accounts for 90% of total diabetic cases, is also a leading cause of death among adults (Saeedi et al., 2019). According to the National Health and Morbidity Survey (2019), the prevalence of diabetes in Malaysia has increased from 11.2% in 2011 to 18.3% in 2019. Diabetes is also one of the risk factors for cardiovascular diseases, the leading cause of death in Malaysia (Institute for Public Health, 2019).

T2DM is a silent disease that is often associated with chronic complications like kidney failure, retinopathy, neuropathy, cardiovascular disease (CVD) and limb amputations (WHO, 2016). T2DM is characterized by raised blood glucose levels or hyperglycaemia due to the body cells' inability to respond to the insulin hormone leading to a condition termed 'insulin resistance' or can also be due to the failure of beta cells of the pancreas to produce insulin (WHO, 2022). Besides genetics, other causative factors such as age and obesity, diet, along with sedentary lifestyle have been implicated to have an equivocal role in the pathogenesis of T2DM (Brunkwall et al., 2017; Han et al., 2014). In addition, studies investigating the human gut microbiota have indicated that dysbiosis in the composition of these human co-habitants plays an

essential role in the pathogenesis of T2DM (Larsen et al., 2010; Qin et al., 2012).

Until recently, the understanding of human microbiology was entirely based on conventional culture methods (Lynch et al., 2016). The characterization of microorganisms (microbiota) and their encoded genes (microbiome) based on next generation-sequencing have given an insight into the complex ecosystem of microorganisms in different parts of the body (Lynch & Pedersen, 2016). The genes encoding 16S ribosomal RNA (rRNA) or the RNA component of small ribosomal subunits have been utilized for the identification and classification of prokaryotic microbes in different ecosystems (Fraher et al., 2012). It is now evident that the human gut is one of the largest microbial ecosystems consisting of trillions of microorganisms with more than 1000 species, primarily consisting of bacteria from phyla *Firmicutes* and *Bacteroidetes* along with fungi, archaea, and viruses residing in the gastrointestinal tract (Lynch & Pedersen, 2016; Neish, 2009; Peterson et al., 2009; Pflughoeft et al., 2012). Many factors such as ethnicity (Karlsson et al., 2013; Qin et al., 2012), age, gender (Takagi et al., 2019), and geographical location (Adachi et al., 2019) influence the composition of gut microbiota. Over the years, this human microbiota is proven to engage in several vital functions to maintain health and homeostasis in the host (Musso et al., 2010).

Numerous studies have indicated that gut microbiota dysbiosis i.e. imbalances in the composition and function of these intestinal microbes is associated with a wide range of diseases (Barlow et al., 2015). Accumulating evidence shows that gut microbiota alterations and systemic inflammation could result in insulin resistance, obesity and T2DM (Kau et al., 2011; Tremaroli et al., 2012; van Olden et al., 2015). It is also apparent that in comparison to normal individuals, subjects with T2DM had

reduced gut bacteria diversity and undergoes microbial dysbiosis with a shift in the proportions of *Firmicutes* and *Bacteroidetes* (Karlsson et al., 2013; Larsen et al., 2010). These alterations in gut microbiota with T2DM development have been explained by metabolic endotoxemia and decreased butyrate production mechanisms (Allin et al., 2015; Amar et al., 2011; Moreno-Indias et al., 2014). However, since the pathogenesis of TDM development is still not fully understood, it is still not clear if the gut microbiota alterations cause T2DM occurrence or if it's merely an effect of physiological changes that progress along with T2DM development.

Meanwhile, studies among healthy Malaysian adults found that gut microbiota changes are affected by ethnicity (Dwiyanto et al., 2021; Neoh et al., 2018). In comparing healthy, obese and T2DM Malaysian adults, gut microbiota changes were also seen to be affected by metabolic disorders such as obesity and T2DM (Kaliyappan et al., 2016). Studies among Malaysian children, further report the existence of a connection between gut microbiota and diet (Khine et al., 2019) as well as ethnicity and socioeconomic status (Chong et al., 2015). Considering the important role of gut microbiota in obesity, one Malaysian study also attempted to manipulate the gut microbiota of overweight children using a fermented probiotic drink (Joseph et al., 2019). However, to the best of our knowledge, no studies have collectively investigated the ethnic-specific gut microbiota changes among adult T2DM subjects from Malaysia. Hence, in a multi-ethnic community such as Malaysia, the profiling of gut microbiota in specific ethnic groups as well as identifying the confounding factors affecting the gut microbiota may enable us to understand the changes in gut microbiota specific to T2DM patients. This will provide a detailed insight into distinct alterations in microbial patterns specific to T2DM development among different ethnic groups in Malaysia.

On the other hand, the gut microbiota has been reported to change with the progression of glucose intolerance before T2DM development (Gurung et al., 2020). Indeed, a distinct difference in gut microbiota composition was found when comparing healthy nonDM individuals with prediabetics (preDM), treatment-naïve, newly diagnosed diabetic patients (newDM) (Zhang et al., 2013) and T2DM patients under medication (Almugadam et al., 2020a). Studies have also linked the intake of metformin, an oral glucose-lowering agent to further altering the gut microbiota composition among T2DM patients (Forslund et al., 2015). Hence, observation of dysbiosis or compositional changes in the gut microbiota of prediabetic and/or newly diagnosed diabetic individuals who have yet to begin pharmacotherapy may serve as a predictive tool for identifying individuals at high risk for developing T2DM. Therefore, a systematic review was conducted to summarize the existing evidence related to microbiota composition and diversity in preDM and newDM (Appendix A). Findings on the association between gut microbiota composition and clinical or dietary factors were also summarized.

Thus, in an attempt to help lead to a better understanding of the composition of the gut microbial profile in T2DM in the Malaysian population, the present study aims to determine the gut microbiota composition in T2DM subjects (T2DM) when compared to nondiabetic subjects (nonDM) among Malaysian adults across the three major ethnic groups. This, to the best of our knowledge, is the first study of its kind. Moreover, the systematic review summarizes the existing evidence on microbial alteration specific to the early stages of glucose intolerance. These findings may be comparable to the findings among study participants in this study to further understand the changes in gut microbiota with T2DM progression in the Malaysian community.

This may assist in the development of early gut microbiota-targeted interventions aimed at reducing the incidence of T2DM and preventing further life-threatening complications.

1.2 Research Questions

1. What are the differences in gut microbiota composition of healthy, non-diabetic (nonDM) Malaysian participants of the three main ethnic groups attending a health clinic in Ampang (Selangor)?
2. What are the differences in gut microbiota composition in type-2 diabetics (T2DM) when compared to (nonDM) among Malaysians of the three main ethnic groups?
3. What are the clinical characteristics that correlate with the gut microbiota profiles found in all study participants?
4. What are the differences in gut microbiota composition among pre-diabetics (preDM) and newly diagnosed diabetics (newDM) in comparison to nonDM participants when systematically reviewing the literature from related studies?

1.3 Objectives of the Study

1.3.1 General Objective

This study aims to investigate the gut microbiota composition in T2DM participants when compared to nonDM participants across the three major Malaysian ethnic groups in Ampang, Selangor.

1.3.2 Specific Objectives

1. To determine and compare the gut microbiota composition of nonDM Malaysian participants of Malay, Chinese and Indian ethnicity attending a health clinic in Ampang (Selangor), using 16S ribosomal DNA sequencing.
2. To assess the differences in diversity and abundance of gut microbiota between T2DM and nonDM participants among the three ethnic groups in Malaysia.
3. To investigate associations between various clinical characteristics (anthropometry, demographic, diabetic profile and biochemical parameters) and gut microbiota profiles in all participants
4. To systematically review the literature on gut microbiota composition of preDM and newDM as compared to nonDM.

1.4 Research Hypotheses

1. There are significant differences in the gut microbial composition of nonDM Malaysian participants of Malay, Chinese, and Indian ethnicity.
2. When compared to the Malaysian nonDM participants, the gut microbiota of T2DM participants were less diverse, less abundant, and appear to have specific compositional differences.
3. Impaired clinical characteristics (anthropometry, diabetic profile, biochemical parameters) in all participants are significantly associated with specific bacterial taxa.
4. The available literature to date shows that the gut microbiota in preDM and newDM is aberrant and shows significant changes in specific bacterial taxa when compared to nonDM.

1.5 The Significance of the Study

The increasing incidence of metabolic diseases like T2DM is a growing global concern. Prevention and further improvement in the current management of T2DM are needed to curb the surge in T2DM cases worldwide.

The gut microbiota and its role in T2DM pathogenesis have created new possibilities for disease prevention and intervention. The gut bacteria and its repertoire may significantly contribute to the prevention and treatment of T2DM (Brunkwall & Orho-Melander, 2017). While improved sequencing technology has enabled us to map out most of the gut bacterial genome, confounding factors like genetic diversity, environmental factors, dietary habits, ethnicity, age, and geography (Adachi et al., 2019; Gupta et al., 2017) have influenced the gut microbiota to vary greatly. More studies need to be done to characterize the gut microbial communities of residents of different regions of the world as we practice different lifestyles, dietary habits, and traditions that shape our genetic impositions accordingly. Although it is a global effort to control T2DM, the population-based intervention will be the best choice for gut microbial-based treatment (Gupta et al., 2017).

Hence, this study aims to determine the gut microbiota of a multi-racial Malaysian community in Ampang, Selangor to help establish specific microbial markers about ethnicity in health and disease i.e., T2DM. The T2DM-specific gut microbial profile could provide a target for nutritional interventions in the future to promote beneficial microbes as protection against T2DM disease progression.

1.6 Scope of the Study

This study was conducted at Klinik Kesihatan Ampang, Selangor and the research laboratory at the Faculty of Medicine and Health Science at Universiti Sains Islam Malaysia (FPSK USIM, Nilai). The population was chosen from patients and their families who visited the outpatient department in Klinik Kesihatan Ampang from October 2019 to October 2020. All the volunteers were approached for their consent to take part in our study. The eligibility criteria of participants were assessed and a set of questions from a questionnaire were asked. After recording their answers, an appointment was fixed to collect their samples (fasting blood and stool). Blood samples were analysed in the clinic for diabetic profile and biochemical parameters. The stool samples were analysed with 16S rRNA gene amplicon sequencing to obtain the gut microbiota composition. The microbiota composition was analysed in terms of its abundance and diversity. The gut microbiota analysis was also correlated with the clinical characteristics of all study participants. A systematic review was conducted to summarise the existing literature characterizing the gut microbial composition of preDM and newDM when compared to nonDM.