CHAPTER 6

CONCLUSION

6.1 Recommendations of the Study

The present study gives a preliminary finding on ethnic-based differences in gut microbiota composition among healthy participants and participants with T2DM. This proves that it is important to consider ethnic-related lifestyle/environmental factors when determining gut microbiota composition, specifically diet and geography. Diet is one of the determinants of gut microbiota composition where dietary constituents could directly or indirectly promote or inhibit the selected gut bacteria, thus shaping the microbial community in the gut (Zmora et al., 2019). On the other hand, well-documented differences in gut microbiota composition had been seen among study populations from varied ethnic backgrounds residing in the same or different geographical regions (Gupta et al., 2017). Thus, a further study with a larger sample size among the Malaysian community should link the role of diet and geography specific to ethnic groups to understand the influence of these factors along with T2DM progression.

Besides that, the difference in gut microbial composition observed between T2DM and nonDM participants in the present study could have been contributed by other confounding factors such as the medication intake of T2DM participants. A past study observed varying changes in the gut microbiota composition with the intake of

antidiabetic medications even after adjusting for age, sex and dietary factors, i.e. metformin among T2DM participants across different ethnic groups residing in a similar geographical region (Balvers et al., 2021). Moreover, the usage of metformin associated with changes in the gut microbiota composition was found to contribute to both therapeutic effects and known intestinal adverse effects (Forslund et al., 2015). Thus, further studies in the Malaysian community should investigate the effects of specific types of medication on the gut microbiota composition.

Furthermore, in the systematic review conducted by this study, gut microbiota changes were observed in the earlier stage of glucose intolerance among preDM and newDM participants. Thus, gut microbiota changes in the earlier stages of glucose intolerance should be investigated along with ethnic factors in Malaysia. This will help to identify the specific gut bacterial signatures that may predict the occurrence of T2DM in different ethnic groups. Essentially, this may serve as an alternative screening method to identify individuals at risk of developing T2DM. This, of course, needs to be strengthened by interventional studies investigating various other confounding factors, including diet, lifestyle (Hu, 2011), exposure to antimicrobial agents (Forslund et al., 2015), host immunity (Levy et al., 2016), ethnicity, geography and environmental influences (Gupta et al., 2017) that are known to affect gut microbiota in health and disease, including T2DM. The effect of these factors on gut microbiota changes in T2DM could identify specific bacterial genera/species that either have beneficial or detrimental roles in T2DM. This allows further studies to analyse the cause and effect of gut microbiota changes with T2DM progression and improve our understanding of the complex pathogenesis of T2DM which is not fully understood to date.

6.2 Strengths and Limitations of the Study

The strength of this study is the homogeneity of the study population. The participants in this study were urban dwellers and adults from three major ethnic groups residing in the same geographical region in Malaysia. This contrasts with previous studies looking into gut microbiota composition among the Malaysian population. Studies led by Khine et al. (2019) and Chong et al. (2015) investigated the gut microbiota composition among pre-adolescent children living in different geographical regions, while Dwiyanto et al. (2021) investigated the gut microbiota composition among adults from four major ethnic groups living in different sub-districts in Segamat. Additionally, the chosen design of this study (case-control) simultaneously analysed the gut microbiota composition in the control group (nonDM) as a representation of the community in the study and conducted a comparative analysis of gut microbiota composition between controls (nonDM) and cases (T2DM).

On the other hand, one of the limitations of this study was the small sample size of each ethnic group which meant low statistical power to evaluate the taxonomical differences between the ethnic groups. Moreover, the cases (T2DM) chosen in this study were older than the controls in this study. This is because a higher number of T2DM participants attending the health clinic were from the older age group in comparison to non-diabetic individuals.

It is also important to emphasize that gut microbial sequencing and taxonomic analyses of microbiota have several biases. The sequencing output yields an ASV table that contains the relative information of each taxon instead of the absolute proportions of each taxon summed to 100% in total. Thus, if one bacterial group is reduced, this

would make the abundance of other bacteria to be naturally increased and vice versa. This may influence the analyses of differentially abundant taxon among study groups.

6.3 Conclusions

Gut microbiota has been associated with various health benefits and is known to be affected by various confounding factors, including ethnicity. Moreover, dysbiosis or alteration to the gut microbiota diversity as well as the abundance of gut bacteria is observed in T2DM. Thus, it is necessary to identify the ethnic-specific changes in gut microbiota composition among the nonDM and T2DM participants to determine the gut microbiota structure in the health and disease of a population. The present study was conducted in Malaysia which has a multi-ethnic community consisting of three major ethnic groups, namely Malay, Chinese and Indian. To the best of our knowledge, this is also the first study to have analysed the gut microbiota composition among the three major ethnic groups with T2DM in comparison to the healthy nonDM controls in Malaysia.

One of the goals of this study was to determine and compare the gut microbiota composition among the three major ethnic groups in Malaysia. The dominant phyla found among nonDM participants were *Firmicutes, Bacteroidetes, Actinobacteria* and *Proteobacteria*. These are the dominant groups of bacteria commonly observed in the healthy human gut. As reflected by the diversity analyses, this study shows that healthy adults from Malay, Chinese and Indian ethnicity living in a similar geographical region in Klang Valley shared a similar gut microbiota profile, thus exhibiting no notable difference in the abundance of gut bacteria.

On the other hand, the increased abundance of pro-inflammatory bacteria (phylum *Proteobacteria* and genus *Escherichia-Shigella*) and the reduction of SCFA-producing bacteria (genera *Anaerostipes, Fusicatenibacter* and *Clostridium*) found along with decreased alpha diversity among T2DM participants indicate a dysbiosis in T2DM. Also, as evidenced by beta diversity analyses, both Malay and Indian ethnicity were found to have a distinct gut microbiota structure between T2DM and nonDM groups. Meanwhile, one of the minor phyla, *Synergistetes* which was significantly increased among T2DM participants in this study, was also a consistent finding among T2DM participants in past studies. However, the relevance of this bacteria to T2DM is not clear. This proves that there is a need to characterize the minor constituents of the gut microbiota and determine their role in health and disease.

This study also found that the significantly increased abundance of phylum *Proteobacteria* in T2DM also positively correlated with the commonly altered parameters in T2DM, i.e., age, FPG, ALP, ALT, urea, creatinine and TG. Thus, the increased abundance of *Proteobacteria* which is also an important indicator of gut dysbiosis that precedes the appearance of low-grade inflammation could probably serve as a biomarker in analysing T2DM progression among Malaysians. Also, positive correlations were noted between genus *Escherichia-Shigella* with age, BMI, urea and TG along with genus *Fusicatenibacter* with height and genus *Clostridium* with HDL. Meanwhile, negative correlations were observed between genus *Fusicatenibacter* with BMI and TG, genus *Anaerostipes* with BMI, FPG and TG as well as genus *Clostridium* with FPG and TG. Together, the association of these clinical parameters with the respective gut bacteria which was found to be significantly altered among T2DM participants in this study could be important indicators of dysbiosis in T2DM in the Malaysian community. Future studies could investigate the quantification of

inflammatory markers in plasma in association with gut microbiota changes in T2DM. This will help to link the inflammatory responses and specific changes in gut microbiota composition along with T2DM progression.

Moreover, in comparing the gut microbial changes in preDM and newDM, it is understood that gut dysbiosis occurs in earlier stages of T2DM development. When comparing with the gut microbiota composition of T2DM participants in this study, the preDM and newDM exhibited increased F/B ratio, increased abundance of *Escherichia-Shigella* and *Lactobacillus* as well as a decreased abundance of *Faecalibacterium* and *Roseburia*. The small sample size in this study had caused insignificant findings when analysing the differences in gut microbiota composition between T2DM and nonDM groups. Still, the trend of gut bacteria abundance found to be increasing and decreasing among the ethnic groups in T2DM in the present study proves that a larger sample size of ethnic groups could exhibit more significant gut bacteria changes among the Malaysian community in T2DM.

Since ethnicity has been associated as one of the major determinants for health disparities, the predominant gut microbial changes in T2DM Malay, Chinese and Indian ethnic groups in this study emphasize the importance of studying gut microbiota composition in diseases by ethnicity. Therefore, larger studies with increased reproducibility are needed to identify and replicate specific ethnic-gut signatures among ethnic groups with T2DM residing in different regions of Malaysia. Also, by considering the ethnic differences in a population, specific gut microbial biomarkers for T2DM could be determined. This population-based medicine will lead to the development of effective preventive and management strategies for T2DM.