CHAPTER 6

CONCLUSION AND RECOMMENDATION

6.1 Recommendation of the study

Blastocystis always has been controversial for the past few years because of its interactions seen between specific intestinal microbiota that influence whether *Blastocystis* will function in a commensal or parasitic role. This study has provided preliminary evidence on *Blastocystis* subtypes in gut microbiota composition among Type-2 Diabetes Mellitus (T2DM) and non-diabetic (NonDM) participants. This study has shown that *Blastocystis* could promote a healthy gut and also possibly alter gut health depending on their subtypes. With this in mind, several recommendations have to be considered for future research to improve the quality of significant studies related to a complex interplay between *Blastocystis* and gut microbiota.

Future work should employ multi-omics approaches including metagenomics, meta transcriptomics, metabolomics or animal models to comprehensively characterize the interactions between *Blastocystis* and the gut microbiota composition with longitudinal studies. Tracking individuals at multiple time points would probably provide insight into the stability and resilience of the gut composition in response to *Blastocystis*. From there, *Blastocystis* subtypes ranging from ST1-ST7 should include involving more representatives of gut bacteria. The conflicting results may appear due

to the differential effects of various subtypes on the host and gut microbiota. Therefore, the need for a larger sample size regarding each of the *Blastocystis* subtypes of infection helps to produce more conclusive evidence as it controls or stratifies for *Blastocystis* subtypes to avoid self-limiting results. This could provide a clear picture of where the *Blastocystis* subtypes stand on gut health and disease.

Furthermore, mechanistic studies are required to unravel the host-microbe interactions underlying these associations, including the role of immune responses and microbial metabolites in shaping the gut environment. In particular, some metabolic disorders of the host have been associated with an inflammation-related environment caused by the imbalance of the specific gut bacterial strains. This mechanistic study could facilitate in understanding parasite bacteria interaction and the pathogenicity of *Blastocystis* subtypes. The differences in the gut microbiota in individuals are also related to various factors. So, it is important to note that future studies to consider external stimuli such as dietary patterns, antibiotics as well as probiotics and prebiotics, medication, life stresses, travel and migrations. These factors will contribute to the host's health and disease state.

Overall, further studies should combine advanced bioinformatics analyses with detailed sociodemographic and clinical data to comprehensively investigate the role of *Blastocystis* in gut health and disease.

6.2 Strengths and Limitations of the Study

The present study gives a preliminary finding, particularly focusing on the association of *Blastocystis* subtypes with gut microbiota composition in type 2 diabetes mellitus (T2DM) and non-diabetic (NonDM) individuals in Malaysia. As a consequence, a cross-sectional study comparing gut composition between T2DM and NonDM participants may provide valuable insights into the potential differences in gut microbiota associated with T2DM and NonDM. By employing advanced molecular techniques, including high throughput sequencing and bioinformatic analysis, composition and gut microbial diversity had been characterised in individuals with and without *Blastocystis* colonisation. This finding allowed us to elucidate specific microbial taxa associated with *Blastocystis* infection.

However, several limitations need to be considered in this study. Firstly, the study was only limited to diabetic patients who were coming to the two selected centres with a small number of patients because of Corona Virus disease 2019 (COVID-19) pandemic. Due to that, a relatively small sample size of our study population may have limited the statistical power to detect subtle variations in gut microbiota composition associated with *Blastocystis* subtypes colonisation. Moreover, the T2DM participants in this study were older than the NonDM as most of the volunteers from NonDM were from among staff and students. Thus, a comparative analysis of gut composition between groups may not accurately reflect the influence of diabetes on gut microbiota due to the potential confounding factor of age group.

Generally, this study was conducted to determine the association of *Blastocystis* subtypes with gut microbiota, but it would not merely confirm the pathogenesis of

Blastocystis subtypes particularly for the pathogenic *Blastocystis* ST7 in the participants due to an unknown mechanism interfering with the issue. In addition, another potential limitation lies in the methodologies employed for *Blastocystis* detection and gut microbiota profiling. By using qualitative Polymerase Chain Reaction (PCR), only the absence and presence of subtypes can be identified. Throughout the experimentation by using molecular techniques such as PCR and high-throughput sequencing, it could introduce some technical artifacts mainly in the faecal sample that may impact sample accuracy and reproducibility of the results.

Finally, it is important to acknowledge the potential risk factors that influenced the complexity of the gut microbiota with *Blastocystis* infection as the data in this study might be biased such as formation of health status, diet, activity involving the risk of parasitic infection, medication or supplements used and underlying health conditions. In this study, the participants stated not to recall several of the concerns asked throughout the questionnaire, mostly related to the presence of gastrointestinal symptoms and health status, which could imply bias in the result. Plus, the difference in glycaemic control between the groups may have affected the risk of infection in these individuals. Although the NonDM group consisted of people considered healthy participants, the presence of other underlying diseases may have affected the study results.

6.3 Conclusion

This is to our knowledge, the first study on *Blastocystis* subtype infection among the type 2 diabetes mellitus (T2DM) population in Malaysia associated with gut microbiota composition. This was a preliminary study highlighting the complex interaction between *Blastocystis* and gut composition depending on *Blastocystis* subtypes. In conclusion, through a comprehensive faecal analysis from participants, several important pieces of knowledge on the prevalence of *Blastocystis* among T2DM and NonDM contribute to our understanding of *Blastocystis* colonisation in the gut microbiota.

Firstly, the results of the present study demonstrated a high prevalence of *Blastocystis* infections in individuals with T2DM compared to NonDM with the most common subtypes detected being ST3 followed by ST2 and ST1. Some gastrointestinal symptoms especially bloating symptom was associated with the presence of *Blastocystis* detection. Looking at its high prevalence in T2DM symptomatically, *Blastocystis* may serve as an opportunistic pathogen and should be considered in patients with diabetes. However, it should be noted that the pathogenic role of *Blastocystis* might depend on the subtype level as different subtypes could exhibit different potentials. Overall, this study did not find any significant relationship exists between the presence and subtypes of *Blastocystis* and symptoms.

Furthermore, this study aimed to determine and compare the gut microbiota among T2DM and NonDM with the presence of *Blastocystis*. Generally, the alteration to the gut microbiota diversity was observed in T2DM participants compared to NonDM with a lower microbiota richness and diversity. The dominant phyla among the

study groups include Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria. In T2DM participants, the increased abundance of pro-inflammatory bacteria such as *Escherichia-Shigella* belonging to phylum Proteobacteria was found along with a decrease in alpha diversity.

Interestingly, the presence of *Blastocystis* could potentially contribute to good gut health as the study observed *Blastocystis* being associated significantly with higher alpha microbiota richness and diversity in T2DM participants. All groups were found to have a similar gut microbiota structure by beta diversity analysis. Commonly, microbiota richness is considered an indicator of ecosystem stability and human health. Regarding the gut microbiota composition, the phyla Bacteroidetes, Proteobacteria and Tenericutes were higher in *Blastocystis*-positive participants with a slightly lower in Firmicutes phylum whereas Actinobacteria, Lentisphaerae and Euyarchaeota were higher in *Blastocystis* negative participants among T2DM and NonDM. At the genus level, SCFA bacteria producers like *Bacteroides*, *Prevotella 9* and *Faecalibacterium* were more abundant in *Blastocystis* positive in T2DM patients. The abundance of those genera was promoted by a healthy gut microbiota. Overall, *Blastocystis* colonisation was associated with eubiosis, a condition characterised by a higher proportion of beneficial bacteria (Firmicutes and Bacteroides).

In addition to that, the higher probable pathogenic bacteria (Proteobacteria) in the presence of *Blastocystis* has also been observed in T2DM and NonDM. The pathogenic bacteria like *Escherichia-Shigella* belonging to the Proteobacteria phylum was slightly higher in T2DM, especially in the absence of *Blastocystis*. However, the finding suggested that the current ambiguity regarding the role of *Blastocystis* could be related to subtypes where only ST7 was significantly associated with *Escherichia-Shigella*

belonging to the Proteobacteria phylum. This might potentially indicate that *Blastocystis* ST7 may have pathogenic properties, which can lead to disruption of the microbiota balance in the gut composition. In line with this proposition, it has been reported that *Blastocystis* colonisation is more frequent in T2DM patients compared to NonDM. Also, *Blastocystis* ST1 and ST2 were found to be significantly different in IL-6 and IL-1β which suggested *Blastocystis* subtypes infection has the potential to modulate the various immune responses including both pro-inflammatory markers but the mechanism is not fully understood.

In short, *Blastocystis* could be considered a commensal member of the gut microbiome and also possibly can lead to dysbiosis depending on the subtypes, particularly *Blastocystis* ST7. A reduction of beneficial bacteria in patients like T2DM together with inflammation may highlight dysbiosis in T2DM which could play the progression of diabetes disease in the Malaysian population. So, it is worth noting that a certain member of *Blastocystis* subtypes could promote a healthy gut environment. Future study on *Blastocystis* subtypes and the gut microbiota holds great promise for the complex host-protist interaction, understanding the role of *Blastocystis* subtypes in their clinical significance.