

**COMPARISON OF GUT MICROBIOTA BETWEEN TYPE-2
DIABETES MELLITUS PATIENTS AND HEALTHY
POPULATION ACROSS THE THREE MAJOR MALAYSIAN
ETHNIC GROUPS IN AMPANG, SELANGOR**

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ABSTRAK

Diabetes Mellitus Jenis-2 (T2DM) merupakan faktor utama yang meningkatkan risiko penyakit kardiovaskular dan kadar kematian di Malaysia. Baru-baru ini, hasil pelbagai kajian telah mencadangkan disbiosis dalam mikrobiota usus memainkan peranan yang penting dalam penularan penyakit metabolik, termasuk T2DM. Walaubagaimanapun, di Malaysia, mikrobiota usus dalam kalangan etnik berbilang kaum yang mempunyai T2DM masih belum diterokai. Oleh itu, kajian ini bermatlamat untuk mencirikan komposisi mikrobiota usus di kalangan peserta kajian daripada 3 etnik utama di Malaysia (Melayu, Cina dan India) yang terdiri daripada peserta kajian T2DM dan peserta kajian tanpa diabetes (nonDM). Kajian kawalan kes mikrobiota usus di kalangan warga Malaysia ini terdiri daripada 45 T2DM dan 45 nonDM yang telah dipadankan dari segi kumpulan etnik (N=90). Mikrobiota usus ini telah dianalisa melalui proses '16S rDNA sequencing' yang menyasarkan bahagian V3-V4. Sebagai hasilnya, analisa 'sequencing' ini menunjukkan bakteria dominan di antara semua kumpulan kajian ialah filum *Firmicutes*, *Bacteroidetes*, *Proteobacteria* dan *Actinobacteria*. Dalam diversiti alpha, kumpulan T2DM menunjukkan pengurangan diversiti dalam kumpulan nonDM. Selain itu, terdapat perbezaan yang signifikan dalam diversiti beta di antara kumpulan nonDM dan T2DM. Secara keseluruhannya, berbanding dengan kumpulan nonDM, peserta kajian T2DM mempunyai peningkatan dalam komposisi filum *Proteobacteria*, *Synergistetes* dan genus *Escherichia-Shigella* berserta dengan penurunan komposisi genus *Anaerostipes*, *Fusicatenibacter* dan *Clostridium*. Tambahan pula, parameter klinikal yang berbeza dalam kalangan T2DM seperti umur, paras glukosa, alkaline phosphatase (ALP), urea, creatinine dan triglyceride (TG) telah berkorelasi secara positif dengan filum *Proteobacteria* berserta dengan genus *Escherichia-Shigella* dengan umur, BMI, urea dan TG. Selain itu, korelasi secara negatif antara BMI, FPG dan TG dengan genus *Anaerostipes*, BMI dan TG dengan genus *Fusicatenibacter* berserta dengan FPG dan TG dengan genus *Clostridium* turut direkodkan. Kajian ini juga telah menjalankan 'systematic review' untuk mengumpul bukti berkaitan dengan perubahan komposisi dan diversiti mikrobiota usus dalam peserta kajian pra-diabetes (preDM) dan pesakit yang baru di diagnosa dengan T2DM (newDM), yang telah dibandingkan dengan peserta kajian tanpa diabetes (nonDM). Secara keseluruhannya, hasil penyelidikan daripada 18 kertas kajian (5, 489 orang peserta kajian), kumpulan preDM dan newDM telah menunjukkan pengurangan diversiti mikrobiota usus dan pengurangan genus bakteria seperti *Faecalibacterium prausnitzii*, *Roseburia*, *Dialister*, *Flavonifractor*, *Alistipes*, *Haemophilus*, *Akkermansia muciniphila* dan peningkatan genus bakteria seperti *Lactobacillus*, *Streptococcus*, *Escherichia*, *Veillonella* dan *Collinsella*. Genus *Lactobacillus* juga berkorelasi secara positif dengan paras glukosa, HbA1c dan HOMA-IR. Secara kesimpulannya, hasil kajian ini boleh mengukuhkan pemahaman kita tentang perubahan diversiti dan komposisi mikrobiota usus dalam penyakit T2DM di kalangan tiga etnik utama dari Malaysia. Selain itu, 'systematic review' yang dijalankan telah meningkatkan pemahaman tentang disbiosis mikrobiota usus yang berlaku semasa perubahan toleransi glukosa pada peringkat awal penyakit T2DM.

ABSTRACT

Type 2 diabetes (T2DM) is a major risk factor for cardiovascular diseases and the leading cause of death in Malaysia. Accumulating evidence suggests that dysbiosis in the gut microbiota composition plays an important role in the pathogenesis of several metabolic disorders including T2DM. However, in Malaysia, the association between the gut microbiota composition of the multi-ethnic community with T2DM remains unexplored. Hence, this study aimed to investigate the gut microbiota composition in T2DM subjects when compared to non-diabetic subjects (nonDM) among the three major ethnic groups in Malaysia i.e., Malays, Chinese and Indians. This case-control study consisted of 45 T2DM and 45 nonDM participants, matched by ethnicity (N=90). The composition of gut microbiota was investigated using 16S rDNA sequencing targeting the V3 – V4 hypervariable regions. Phyla *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, and *Actinobacteria* were found to be dominant across all study groups. Alpha diversity measures found significantly reduced diversity in the overall T2DM group. Also, a significant difference in beta diversity between the T2DM and nonDM groups was reflected in Malay and Indian ethnicity. In comparison to the respective nonDM groups, T2DMs had increased prevalence and/or abundance of phyla *Proteobacteria*, *Synergistetes* and genus *Escherichia-Shigella* as well as the reduction of genera *Anaerostipes*, *Fusicatenibacter* and *Clostridium*. In addition, the altered clinical characteristics found among T2DMs, i.e., age, fasting plasma glucose (FPG), alkaline phosphatase (ALP), urea, creatinine, and triglyceride (TG) associated positively with phylum *Proteobacteria* as well as genus *Escherichia-Shigella* specifically with age, BMI, urea and TG. On the other hand, negative correlations were observed between BMI, FPG and TG with genus *Anaerostipes*, BMI and TG with genus *Fusicatenibacter* along with FPG and TG with genus *Clostridium*. This study also conducted a systematic review to summarise the existing evidence related to microbiota composition and diversity in prediabetic (preDM) and newly diagnosed T2DM (newDM) individuals in comparison to nonDM. In a total of 18 studies (5,489 participants), the preDM and newDM individuals exhibited low gut microbial diversity along with a decreased abundance of *Faecalibacterium prausnitzii*, *Roseburia*, *Dialister*, *Flavonifractor*, *Alistipes*, *Haemophilus*, *Akkermansia muciniphila* and increased abundance of *Lactobacillus*, *Streptococcus*, *Escherichia*, *Veillonella* and *Collinsella*. *Lactobacillus* was also found to positively correlate with FPG, HbA1c and HOMA-IR. In conclusion, findings from this study have provided us with a better insight into the diversity and composition of the diabetic gut microbiota in a multi-ethnic Malaysian population, while the systematic review has improved our understanding of gut microbial dysbiosis during the early stages of glucose intolerance.

الخلاصة

في ماليزيا، مرض السكري من النوع الثاني (T2DM) هو السبب الرئيسي للوفيات، حيث أنه من أخطر عوامل الأمراض القلبية والوعائية. وهناك الكثير من الأدلة التي تُشير إلى أن عدم التوازن في تركيبة البكتيريا الموجودة في الأمعاء، والمعروفة باسم "dysbiosis"، تُسهم بشكل كبير في تطوُّر العديد من اضطرابات التمثيل الغذائي، مُتضمِّناً مرض السكري من النوع الثاني. ومع ذلك، لم يُجرى أي تحقيق في دراسة العلاقة بين تركيبة بكتيريا الأمعاء لدى سكان ماليزيا المُتعدِّدة الأعراق ومرض السكري من النوع الثاني. يهدف هذا البحث إلى تحليل تركيب البكتيريا الموجودة في الأمعاء لدى مرضى السكري من النوع الثاني (T2DM) مُقارنَةً بذلك لدى الأفراد غير المصابين بالسكري (nonDM) بين ثلاثة عرقيات رئيسية في ماليزيا، وهم الملايو والصينيون والهنود. لقد شملت هذه الدراسة 90 مشارك إجمالاً، 45 منهم مصابون بالسكري من النوع الثاني (T2DM)، و45 آخرون غير مُصابين بمرض السكري (nonDM)، وقد أُختيروا حسب عرقهم ومُراعاة التوافق بين المجموعتين. استخدم الباحثون تقنية تسلسل 16S rDNA لتحليل تركيب البكتيريا الموجودة في الأمعاء والتي استهدفت المناطق القابلة للتباين العالي V3 - V4. وقد عُيِّرَ على الفصائل *Firmicutes*، و *Bacteroidetes*، و *Proteobacteria*، و *Actinobacteria* كأبرز الفصائل عبر جميع مجموعات الدراسة. وقد أشارت نتائج قياسات التنوع ألفا إلى انخفاض ملحوظ في التنوع داخل مجموعة الأشخاص المصابين بالسكري من النوع الثاني بشكل عام، وأظهرت الجماعات العرقية الماليزية والهندية اختلافاً ملحوظاً في تنوع القياس بيتا بين مجموعات الأشخاص المُصابين بالسكري من النوع الثاني والأشخاص الغير مُصابين بالسكري. بالمقارنة مع الأشخاص غير المصابين بالسكري، لُوِحِظَ أن مرضى السكري من النوع الثاني يزداد لديهم انتشار البكتيريا *Proteobacteria*، و *Synergistetes*، و *Escherichia-Shigella*، بالإضافة إلى قِلة الأنواع *Anaerostipes*، و *Fusicatenibacter*، و *Clostridium*. كما أن الخصائص السريرية المُعدَّلة المُكتشَفة لدى مرضى السكري من النوع الثاني - مثل العمر، وسكري الدم الصائم (FPG)، والفوسفاتيز القلوية (ALP)، واليوريا، والكرياتينين، وثلاثي الغليسريد (TG) - أظهرت علاقة ايجابية مع بكتيريا *Proteobacteria* وبكتيريا *Escherichia-Shigella*. وعلى وجه التحديد، كان العمر ومؤشر كتلة الجسم واليوريا وثلاثي الغليسريد (TG) مرتبطين بشكل ملحوظ بالنوع *Escherichia-Shigella*. وعلى الجانب الآخر، لُوِحِظت علاقات عكسية بين؛ مؤشر كتلة الجسم وسكر الدم الصائم وثلاثي الغليسريد (TG) مع بكتيريا *Anaerostipes*، مؤشر كتلة الجسم وثلاثي الغليسريد (TG) مع البكتيريا *Fusicatenibacter*، سكر الدم الصائم وثلاثي الغليسريد (TG) مع البكتيريا *Clostridium*. لقد أُجرت هذه الدراسة أيضاً مراجعة نظامية لتقديم نظرة عامة على الأدلة الحالية المتعلقة بتركيبية وتنوع البكتيريا الموجودة في الأمعاء لدى الأفراد المصابين بمرحلة ما قبل السكري (preDM) أو الذين شُخِّصوا حديثاً بالسكري من النوع الثاني (newDM) بالمقارنة مع الأفراد غير المصابين بالسكري. وشملت المراجعة 18 دراسة بمشاركة إجمالية بلغت 5489 مشارك، والتي أظهرت أن الأفراد المصابين بمرحلة ما قبل السكري والذين شُخِّصوا حديثاً بالسكري من النوع الثاني لديهم تنوع حيوي منخفض في أمعائهم، مع انخفاض وفرة البكتيريا *Faecalibacterium prausnitzii*، و *Roseburia*، و *Dialister*، و *Flavonifractor*، و *Alistipes*، و *Haemophilus*، و *Akkermansia muciniphila*، وزيادة وفرة البكتيريا *Lactobacillus*، و *Streptococcus*، و *Escherichia*، و *Veillonella*، و *Collinsella*. تبيَّن أيضاً أن البكتيريا *Lactobacillus* ترتبط بشكل إيجابي مع سكر الدم الصائم (FPG)، والهيموجلوبين المُعدَّل (HbA1c)، ومؤشر التحمل للأنسولين (HOMA-IR). لتلخيص النتائج، لقد أسهمت نتائج هذه الدراسة في إلقاء الضوء على تركيبة وتنوع البكتيريا الموجودة في الأمعاء لدى الأفراد من مُختلِّف الأعراق، والذين يعانون من السكري من النوع الثاني في ماليزيا. كما ساهمت المُراجعة النظامية في تحسين فهمنا لعدم التوازن في الميكروبات الموجودة في الأمعاء في المراحل الأولى من عدم تحمُّل الجلوكوز.

TABLE OF CONTENTS

CONTENT	PAGE
AUTHOR DECLARATION	ii
ACKNOWLEDGEMENTS	iii
ABSTRAK	iv
ABSTRACT	v
AL-MULAKHKHAS	vi
TABLE OF CONTENTS	vii-viii
LIST OF TABLES	ix – x
LIST OF FIGURES	xi - xii
LIST OF APPENDICES	xiii
LIST OF ABBREVIATIONS	xiv
CHAPTER 1: INTRODUCTION	
1.1. Introduction	1 – 5
1.2. Research Questions	5
1.3. Objectives of the Study	
1.3.1 General Objective	5
1.3.2 Specific Objectives	6
1.4. Research Hypotheses	6
1.5. The Significance of the Study	7
1.6. Scope of the Study	8
CHAPTER 2: LITERATURE REVIEW	
2.1. Introduction	9
2.2. Type 2 Diabetes	9 – 11
2.2.1. Pathogenesis of T2DM	11
2.2.2. Risk Factors for T2DM Development	12 – 13
2.2.3. Diagnosis and Management of T2DM	13 – 15
2.3 Human Gut Microbiota	15 – 17
2.3.1. Development of Gut Microbiota	17 – 18
2.3.2. Gut Microbiota and Ethnicity	19 – 22
2.3.3. Gut Microbiota and T2DM	22 – 29
2.3.4. Clinical Characteristics and Gut Microbiota in T2DM	30 – 31
2.3.5. Next-Generation Sequencing, 16S rDNA Sequencing	32 – 33
2.4 Conclusion	33
CHAPTER 3: RESEARCH METHODOLOGY	
3.1. Introduction	34
3.2. Research Approach and Design	34
3.3. Study Location/Setting	35
3.4. Target Population	35
3.5. Study Population	35
3.6. Sampling Method and Sample Size	35 – 36
3.7. Inclusion and Exclusion Criteria	
3.7.1. Inclusion and Exclusion Criteria for T2DM Participants	36
3.7.2. Inclusion and Exclusion Criteria for NonDM Participants	37
3.8. Methodology Flowchart	38
3.9. Research Instruments	39 – 40
3.10. Operational Definition	41

3.11. Definition and Normal Range of Variables Measured in this Study	42
3.12. Data and Samples Collection Method	43
3.13 Genomic DNA Extraction	44 – 45
3.14 DNA Quality Control and Sequencing	45 – 46
3.15. Data Analysis	
3.15.1. Sequence QC	46 – 47
3.15.2. Taxonomy Assignment	48
3.15.3. Diversity Analysis	49
3.15.4. Statistical Analysis	50
3.16. Research Ethics	51
3.17. Methodology for Systematic Review	52
3.18. Conclusion	53
CHAPTER 4: RESULTS	
4.1. Introduction	54
4.2. Characteristics of Study Participants	54 – 61
4.3. Genomic DNA Quantification	62 – 63
4.4. Gut Microbiota Analysis	64
4.4.1. Diversity Analysis	64 – 71
4.4.2. Gut Microbiota Composition	72 – 74
4.4.2.1. Gut Microbiota Composition in NonDM	75 – 77
4.4.2.2. Gut Microbiota Composition in T2DM when compared to NonDM by Ethnicity	78 – 84
4.5. Correlation Analysis	
4.5.1. Correlation of Top Nine Gut Phyla with Clinical Characteristics	85 – 86
4.5.2. Correlation of Top 20 Genera Clinical Characteristics	87 – 88
4.6. Systematic Review	
4.6.1. Study Characteristics	89 – 92
4.6.2. Systematic Review of the Gut Microbiota Composition	93 – 96
4.7. Conclusion	97
CHAPTER 5: DISCUSSION	
5.1. Introduction	98
5.2. Discussion of Study Results	98
5.2.1. Gut Microbiota Composition in NonDM	99 – 103
5.2.2. Gut Microbiota Composition in T2DM when compared to NonDM	103 – 113
5.2.3. Correlation Analysis	113 – 116
5.3. Systematic Review	117 – 119
CHAPTER 6: CONCLUSION	
6.1. Recommendations of the Study	120 – 121
6.2. Strengths and Limitations of the Study	122 – 123
6.3 Conclusions	123 – 125
REFERENCES	126 – 142
APPENDICES	143 - 172

LIST OF TABLES

TABLES		PAGES
Table 2.1:	Studies on Gut Microbiota Composition between Healthy and T2DM Adults using 16S rDNA Sequencing.	23 – 26
Table 2.2:	The Correlation of Clinical Characteristics with Gut Microbiota Composition in T2DM Participants.	31
Table 4.1:	The Characteristics of Study Participants in NonDM and T2DM Groups, n=90.	56
Table 4.2:	The Characteristics of All Study Participants by Ethnicity, n=90.	58
Table 4.3:	The Characteristics of NonDM Group by Ethnicity, n=45.	60
Table 4.4:	The Characteristics of T2DM Group by Ethnicity, n=45.	61
Table 4.5:	Assessment of gDNA Concentration and Purity Extracted for Optimisation of Bead Beating Time and Speed.	62
Table 4.6:	The gDNA Analysis of All Faecal Samples, n=90.	63
Table 4.7:	Bacterial Alpha Diversity in NonDM n=44.	66
Table 4.8:	Bacterial Alpha Diversity in NonDM and T2DM, n=89.	66
Table 4.9:	Pairwise Comparison of Bacterial Alpha Diversity in NonDM and T2DM by Ethnicity, n=89.	67
Table 4.10:	Beta Diversity of NonDM and T2DM Groups, n=89.	67

Table 4.11:	Bacterial Phyla and Genera in NonDM Group Arranged by Abundance, n=45.	76
Table 4.12:	Bacterial Phyla and Genera in NonDM and T2DM Arranged by Abundance in All Samples, n=90.	79
Table 4.13:	Pairwise Comparison of Gut Microbiota Abundances in NonDM and T2DM by Ethnicity, n=90.	83
Table 4.14:	Study Participant's Characteristics for Systematic Review.	91 - 92

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ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

LIST OF FIGURES

FIGURES	PAGES
Figure 3.1: Methodology Flowchart.	38
Figure 3.2: Flowchart for Gut Microbiome Analysis.	44
Figure 3.3: Sequence Quality Control using DADA2 Pipeline.	47
Figure 4.1: Agarose gel Showing gDNA Extracted for Optimisation of Bead Beating Time and Speed.	63
Figure 4.2: Rarefaction Curve in NonDM and T2DM Groups, n=90.	65
Figure 4.3: Principal Coordinate Analysis (PCoA) based on Bray-Curtis Dissimilarity Matrix between Microbial Communities of NonDM Participants by Ethnicity, n=44.	69
Figure 4.4: Principal Coordinate Analysis (PCoA) based on Bray-Curtis Dissimilarity Matrix between Microbial Communities from NonDM and T2DM Groups, n=89.	70
Figure 4.5: Principal Coordinate Analysis (PCoA) based on Bray-Curtis Dissimilarity Matrix between Microbial Communities from NonDM and T2DM Group of Similar Ethnicity, n=89.	71
Figure 4.6: The Gut Microbiota Composition at Phylum Level showing the top nine phyla that were present in at least 10% abundance in all 90 samples.	73
Figure 4.7: The Gut Microbiota Composition at Genus Level showing Top 20 Genera with more than 1% abundance in all 90 samples.	74
Figure 4.8: Gut Microbiota Abundances in (A) Phylum and (B) Genus Levels in NonDM Group by Ethnicity, n=45.	77

Figure 4.9:	Gut Microbiota Composition in (A) Phylum and (B) Genus Levels in NonDM and T2DM Groups, n=90.	80
Figure 4.10:	Gut Microbiota Composition in (A) Phylum and (B) Genus Levels in NonDM and T2DM by Ethnicity, n=90.	84
Figure 4.11:	Heatmap Showing Correlation of Bacterial Phyla with Clinical Characteristics in All Participants, n=90.	86
Figure 4.12:	Heatmap Showing Correlation of Bacterial Genera with Clinical Characteristics in All Participants, n=90.	88
Figure 4.13:	The Genera/Species found in PreDM and NewDM Groups.	96
Figure 5.1:	The Changes in Gut Microbiota Composition in this Study with the Postulated Effects in T2DM.	119

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 جامعة العلوم الإسلامية
 ISLAMIC SCIENCE UNIVERSITY OF MALAYSIA

LIST OF APPENDICES

APPENDICES	PAGES
Appendix A: Systematic Review on Gut Microbiota Composition in Prediabetes and Newly Diagnosed Type 2 Diabetes: A Systematic Review of Observational Studies	143 – 157
Appendix B: Blood Request Form from Klinik Kesihatan Ampang	158
Appendix C: Participant Information Sheet and Informed Consent Form	159 – 160
Appendix D: Questionnaire form	161 – 162
Appendix E: gDNA Quantification Report from Apical Scientific	163 – 166
Appendix F: PCR to QC gDNA Report from Apical Scientific	167 – 170
Appendix G: Abstract submitted for oral presentation at 4 th USIM International Health E-Conference 2020 (IHEC 2020) In Conjunction with the 3rd International Conference on Medicine and Health Sciences (ICMHS) on 16th - 17th December 2020	171
Appendix H: Poster submitted for Microbiome Interactions in Health and Disease 2022 conference on 24-26 th October 2022	172

LIST OF ABBREVIATIONS

2HPP	2-hour postprandial
Alb	Albumin
ALT	Alanine transaminase
ASV	Amplicon sequence variant
ALP	Alkaline phosphatase
BMI	Body Mass Index
CGI	Combined glucose intolerance
Cl	Chloride
Cr	Creatinine
CRP	C-reactive protein
CVD	Cardiovascular diseases
ECG	Electrocardiogram
FDR	False Discovery Rate
FPG	Fasting plasma glucose
FSL	Fasting serum lipids
gDNA	Genomic DNA
GLP-1	Glucagon-like Peptide-1 (GLP-1) receptor agonists
HbA1c	Hemoglobin A1c
HDL-C	High-density lipoprotein cholesterol
HDL/TC	Ratio of HDL-C/total cholesterol
HGM	Human gut microbiota
HOMA-IR	Homeostasis model assessment-estimated insulin resistance
IFG	Impaired fasting glucose
IL-6	Interleukin-6
K	Potassium
LBP	Lipopolysaccharide-binding protein
LDL-C	Low-density lipoprotein cholesterol
LFT	Liver function tests
LPS	Lipopolysaccharide
Na	Sodium
NGS	Next-generation sequencing
newDM	Newly diagnosed T2DM participants
nonDM	Non-diabetic/healthy participants
OAD	Oral anti-diabetic agents/medication
OGTT	Oral glucose tolerance test
PCoA	Principal coordinate analysis
PCR QC	Polymerase chain reaction quality control
preDM	Pre-diabetics
RP	Renal profile
SCFA	Short-chain fatty acids
Sp.	Species
T2DM	Type 2 diabetes Mellitus disease/participants
TB	Total bilirubin
TC	Total cholesterol
TG	Triglyceride
TP	Total protein
TNF	Tumor necrosis factor
Ur	Urea
WHR	Waist-to-hip ratio