DIFFERENTIATION OF *BLASTOCYSTIS* SUBTYPES AND THEIR ASSOCIATION WITH GUT MICROBIOTA IN TYPE 2 DIABETES MELLITUS PATIENTS AND NON-DIABETIC INDIVIDUALS

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ABSTRAK

Pengaruh anaerob protozoa usus *Blastocystis* terhadap kesihatan usus manusia masih kurang difahami, terutamanya dalam Diabetes Mellitus Jenis II (T2DM). Prevalens yang tinggi dalam jangkitan *Blastocystis* telah dilaporkan dalam pesakit T2DM, yang mana mencadangkan satu potensi terhadap hubungan dengan microbiota usus. Walaubagaimanapun, hubungan ini tidak didokumentasikan dengan baik dalam setting klinikal. Oleh itu, satu kajian keratan rentas telah dijalankan yang melibatkan 203 peserta, termasuk pesakit T2DM dan individu yang tidak menghidap diabetes (bukan-DM), untuk menilai prevalens subjenis *Blastocystis* yang berkaitan dengan mikrobiota usus. Subjenis *Blastocystis* dikenal pasti dengan menggunakan tindak balas berantai polimeras (PCR) dan mikrobiota najis dianalisis dengan menyasarkan pada kawasan V4 gen ribosomal 16S bakteria. Hasil kajian mendapati bahawa prevalens *Blastocystis* adalah lebih tinggi dalam kalangan T2DM (25.49%) berbanding bukan-DM (17.82%). Subjenis yang paling biasa dalam populasi keseluruhan adalah ST3, diikuti oleh ST1 dan ST2. Perubahan adalah signifikan dalam komposisi mikrobiota usus ditemui antara T2DM dan bukan-DM dengan menunjukkan kumpulan bukan-DM mempunyai kepelbagaian alfa yang lebih tinggi berdasarkan kekayaan spesies dan indeks kepelbagaian. Selain itu, kehadiran *Blastocystis* menunjukkan signifikan berkaitan dengan peningkatan kepelbagaian alfa dalam kedua-dua kumpulan T2DM dan bukan-DM. Pada peringkat filum, kumpulan T2DM menunjukkan peningkatan yang ketara dalam Bacteroidetes dan penurunan yang ketara dalam Actinobacteria dengan kehadiran *Blastocystis* berbanding dengan kumpulan *Blastocystis*-negatif. Selain itu, analisis profil genus juga mendedahkan bahawa subjenis *Blastocystis* yang berbeza menunjukkan kepelbagaian jumlah genus seperti *Prevotella 9, Bifidobacterium, Blautia* dan *Megamonas*. Secara khususnya, kekayan filum yang bukan dominan seperti Lentisphaerae dan Tenericutes dapat dilihat secara significans dalam *Blastocystis* ST1 dan ST3 berbanding subjenis yang lain. Menariknya. kajian ini juga mengaitkan hubungan antara sampel yang sangat terhad iaitu jangkitan daripada *Blastocystis* ST7 dengan peningkatan bakteria patogen seperti *Escherichia-Shigella* dalam individu dengan T2DM. Penemuan ini mencadangkan bahawa subjenis *Blastocystis* yang berbeza mungkin telah menyumbang kepada mikrobiota usus yang lebih sihat atau berpotensi menyebabkan dysbiosis. Walau bagaimanapun, analisis lanjutan pada tahap subjenis (ST) adalah perlu untuk menjelaskan peranan khusus subjenis *Blastocystis* dalam kesihatan usus, kerana mereka mungkin menunjukkan ciri-ciri komensal dan patogenik.

ABSTRACT

The influence of the anaerobic intestinal protozoan *Blastocystis* on human gut health remains poorly understood, particularly in type 2 diabetes mellitus (T2DM). A high prevalence of *Blastocystis* infection has been reported in T2DM patients, suggesting a potential link with gut microbiota. However, this association is not well documented in clinical settings. Hence, a comparative cross-sectional study was conducted involving 203 participants, including both T2DM patients and non-diabetic (NonDM) individuals, to evaluate the prevalence of *Blastocystis* subtypes associated with gut microbiota. *Blastocystis* subtypes were identified using polymerase chain reaction (PCR) and the faecal microbiome was analysed by targeting the V4 region of the bacterial 16S ribosomal gene. Our findings revealed that the prevalence of *Blastocystis* was higher in T2DM (25.49%) compared to NonDM (17.82%). The most common subtypes (STs) in the overall population were ST3, followed by ST1 and ST2. Notably, a significant change in the composition of gut microbiota was discovered between T2DM and NonDM with the NonDM group exhibiting higher alpha diversity based on species richness and diversity index. Furthermore, it was discovered that in both T2DM and NonDM, the presence of *Blastocystis* was significantly linked to increased alpha diversity. At the phylum level, the T2DM group showed a notable increase in Bacteroidetes and a marked decrease in Actinobacteria in the presence of *Blastocystis* compared with the negative *Blastocystis* group. Additionally, our genus profile analysis revealed that different *Blastocystis* subtypes exhibited a significant abundance of genera such as *Bacteroides*, *Prevotella 9*, *Bifidobacterium*, *Blautia* and *Megamonas.* Specifically, the abundance of Lentisphaerae and Tenericutes in less dominant phyla was significantly increased in *Blastocystis* ST1 and ST3 compared to other STs. Interestingly, a limited sample infected by *Blastocystis* ST7 was associated with an increase in pathogenic bacteria like *Escherichia-Shigella* in individuals with T2DM. These findings suggested that different *Blastocystis* subtypes may have contributed to a healthier gut microbiota or potentially led to dysbiosis. However, advanced analysis at the subtype (ST) level is necessary to elucidate the specific role of *Blastocystis* subtypes in gut health, as they may exhibit both commensal and pathogenic characteristics.

المُلَخَّص

على صحة الأمعاء البشرية لا يزال غير مفهوم تمامًا، خاصة *Blastocystis* تأثير البروتوزوا المعوية اللاهوائية في مرضى *Blastocystis* تم الإبلاغ عن انتشار مرتفع لعدوى .(T2DM) في مرض السكري من النوع 2 مما يشير إلى رابط محتمل مع ميكروبيوتا الأمعاء .ومع ذلك، فإن هذه العلاقة غير موثقة بشكل جيد في ،T2DM T2DM البيئات السريرية .ومن ثم، تم إجراء دراسة مقطعية مقارنة شملت 203 مشاركًا، بما في ذلك مرضى المرتبطة بميكروبيوتا *Blastocystis* لتقييم انتشار الأنماط الفرعية لـ ،(NonDM) وأفراد غير مصابين بالسكري وتم تحليل (PCR) الفرعية باستخدام تفاعل البوليميراز المتسلسل *Blastocystis* الأمعاء .تم تحديد أنماط للبكتيريا .كشفت نتائجنا أن انتشار Sمن الجين الريبي 16 V4 الميكروبيوم البرازي من خلال استهداف منطقة كانت الأنماط الفرعية .(17.82%) NonDM مقارنة بـ (25.49%) T2DM كان أعلى في *Blastocystis* ومن الملاحظ أن هناك تغييرًا كبيرًا في .ST2و ST1 تليها ،ST3 في السكان الإجمالي هي (STs) الأكثر شيوعًا تنوعًا أعلى بناءً على NonDM حيث أظهر مجموعة ،NonDM و T2DM تركيب ميكروبيوتا الأمعاء بين كانت وجود ،NonDMو T2DM ثراء الأنواع ومؤشر التنوع .علاوة على ذلك، تم اكتشاف أنه في كل من زيادة T2DM مرتبطًا بشكل كبير بزيادة التنوع الألفا .على مستوى الفصيلة، أظهرت مجموعة *Blastocystis* مقارنة *Blastocystis* في وجود Actinobacteria وانخفاضًا ملحوظًا في Bacteroidetes ملحوظة في السلبية .بالإضافة إلى ذلك، كشفت تحليل ملف الأنواع لدينا أن الأنماط الفرعية المختلفة *Blastocystis* بمجموعة أظهرت وفرة ملحوظة من الأجناس مثل *Blastocystis* لـ *Prevotella* 9، *Bacteroides*، Lentisphaerae على وجه الخصوص، كانت وفرة *Megamonas*. و *Blautia*، *Bifidobacterium* و *Blastocystis* ST1 في الفصائل الأقل هيمنة زادت بشكل ملحوظ في الأنماط الفرعية *Tenericutes*و كانت *Blastocystis* ST7 الأخرى .ومن المثير للاهتمام، أن عينة محدودة مصابة بـ ST مقارنة بأنماط ST3 .T2DM في الأفراد المصابين بـ *Escherichia-Shigella* مرتبطة بزيادة في البكتيريا المسببة للأمراض مثل قد ساهمت في ميكروبيوتا أمعاء أكثر صحة *Blastocystis* تشير هذه النتائج إلى أن الأنماط الفرعية المختلفة لـ ضروري (ST) أو قد أدت إلى اختلال التوازن الميكروبي .ومع ذلك، فإن التحليل المتقدم عند مستوى النمط الفرعي في صحة الأمعاء، حيث قد تظهر خصائص متعايشة *Blastocystis* لتوضيح الدور المحدد للأنماط الفرعية لـ ومسببة للأمراض."

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ASVs	Amplicon sequence variants
BCAAs	Branched-Chain Amino Acid
BMI	Body Mass Index
BP	Base-pair
COVID-19	Corona Virus Disease 2019
DESeq	Differential expression analysis for sequence count data
DSS	Dextran sulphate sodium
ELISA	Enzyme-Linked Immunosorbent Assay
FBS	Fasting Blood Sugar
FDR	False Discovery Rate
gDNA	Genomic Deoxyribonucleic Acid
GLP-1	Glucagon-like peptide 1
HbA1c	Haemoglobin A1c
IBD	Inflammatory bowel disease
IBS	Irritable bowel syndrome
IL1-β	Interleukin 1β
IL6	Interleukin 6
IgA	Immunoglobulin A
IR	Insulin resistance
LPS	Lipopolysaccharide
MAPKs	Mitogen- activated protein kinases
PBMCs	Peripheral Blood Mononuclear Cells
NonDM	non-Diabetic Mellitus
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGS	Next Generation sequencing
PCoA	Principal Coordinates Analysis
PCOS	Polycystic Ovary Syndrome
PCR	Polymerase Chain Reaction
PCR-QC	Polymerase Chain Reaction Quality Control
PGN	Peptidoglycans
rDNA	Ribosomal DNA
SCFA	Short-chain fatty acid
Sp.	Species
SPSS	Statistical Package for the Social Sciences
ST	Subtype
T2DM	Type- 2 Diabetes Mellitus
TNF-α	Tumour Necrosis Factor alpha