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Acceptability and feasibility of fecal microBIOME and serum metabolite sample collection in people with end-stage kidney disease and pain being treated with HemoDialysis: A pilot study (BIOME-HDp)

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ABSTRACT

Pain is known to reduce hemodialysis treatment adherence, reduce quality of life, and increase mortality. The absence of effective strategies to treat pain without medications has contributed to poor health outcomes for people with end-stage kidney disease (ESKD) on hemodialysis. It is now recognized that symbiotic microbiota in the gut play a critical role in health and disease, and new evidence sheds light on the role of the microbiome in chronic pain. The pilot study protocol presented here (BIOME-HDp) employs a longitudinal repeated measures design to interrogate the effects of a nonpharmacological pain intervention on the composition and function of the gut microbiome and circulating metabolites. This pilot study is an ancillary study of the HOPE Consortium Trial to reduce pain and opioid use in hemodialysis, which is part of the NIH's Helping to End Addiction Long-term (HEAL) initiative. The BIOME-HDp pilot study will establish clinical microbiome research methods and determine the acceptability and feasibility of fecal microbiome and serum metabolite sample collection.

1. Introduction

1.1. Background

Because most people with end-stage kidney disease (ESKD) prioritize how they feel and function over how long they live, there has been increasing effort to address the tremendous symptom burden that accompanies ESKD [1]. Pain is among the most common symptoms, with approximately 60% of ESKD patients reporting pain of moderate or severe intensity [2]. Pain has been found to reduce hemodialysis treatment adherence, reduce quality of life, and increase mortality [2].

Some pain among people with ESKD who are being treated with hemodialysis may be temporary, due to the hemodialysis procedure itself (e.g., needle insertions, fluid shifts, cramps, headaches). However, ESKD pain is often chronic and related to etiology (e.g., polycystic disease), complications (e.g., bone disease, neuropathy), or comorbidities (e.g., osteoarthritis, vascular disease, diabetes) [3]. Depending on its etiology, the pain may be categorized as nociceptive, neuropathic, or both [4]. Musculoskeletal pain is most common in ESKD, accounting for up to 59% of ESKD-related chronic pain [4].

Reduced kidney function alters the pharmacokinetic and pharmacodynamic properties of various analgesic agents, and this complicates medical management of pain conditions in ESKD [5–7]. Due to the combination of high pain prevalence and limited options for pain

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management, nearly 30% of US adults receiving maintenance hemodialysis are prescribed opioids for 90 days or more [3]. However, long-term opioid therapy is of questionable benefit for chronic pain [8], and long-term opioid use among maintenance hemodialysis patients is associated with increased rates of falls, hip fractures, hospitalizations, dialysis withdrawal, and death [3,9]. Nonpharmacologic approaches to pain such as cognitive behavioral therapy have demonstrated efficacy for chronic pain in the general population and are now being studied in adults with ESKD on hemodialysis. The HOPE consortium study is registered at ClinicalTrials.gov (NCT04571619).

1.1.1. *Microbiomics: The brain-gut-microbiome axis and chronic pain*

Research is emerging that symbiotic microbes in the gut (microbiota), and the metabolites they produce (microbiota-derived signaling molecules), may underlie a wide range of debilitating symptoms such as fatigue, anxiety, depression, and chronic pain experienced across multiple chronic diseases [10–13]. For example, Yang et al. (2019), observed a higher abundance of the phylum *Firmicutes* and lower *Verrucomicrobiota* and *Bacteroides* was associated with an anhedonia-like phenotype in rats with neuropathic pain [14]. Moreover, Guida, et al., 2019, used a chronic pain model of vitamin D deficiency, and found higher abundance of *Paracubacteria* and lower *Verrucomicrobiota* was closely correlated with altered nociception and the endocannabinoid system among vitamin D deficient mice with neuropathic pain [15]. While less is known about these relationships in the specific context of ESKD, evidence is emerging that microbiota in the gut interact with the host via immune, endocrine, and inflammatory pathways in the central and peripheral nervous systems involved in the pain experience [16]. Communication along this pathway, known as the brain-gut-microbiome axis (BGMA), occurs through activation of the central and enteric nervous systems, where microbiota in the gut synthesize neuroactive molecules that mediate central nervous system homeostasis via the vagal pathway or by crossing the blood-brain barrier directly into the brain [17]. The BGMA may thus provide an underlying mechanism to explain part of the relationship between chronic kidney disease and chronic pain [18,19].

1.1.2. *Metabolomics*

Microbiota in the gut are involved in regulating multiple metabolic pathways involved in chronic pain [20,21]. Tryptophan metabolites and short chain fatty acids are increasingly recognized as important signaling molecules involved in BGMA communication, and have been implicated in nociceptive and neuropathic pain [22]. Nociceptive and neuropathic pain are highly prevalent in people receiving hemodialysis, thus these relationships are explored in the BIOME-HDp study.

1.1.2.1. The role of tryptophan metabolism in pain. Tryptophan (Trp), an essential amino acid acquired through diet, is engaged in multiple vital functions in human physiology, including structural and functional processes of the cell, protein biosynthesis, and immunoregulation [23], and is posited to play a critical role in nociceptive and neuropathic pain [22]. Historically, accelerated metabolism of Trp was associated with clinical factors such as infection, inflammation, and certain malignancies; however, researchers are now focusing on the role of nutrition and the gut microbiome in tryptophan catabolism [24]. Gut microbiota are able to change the tryptophan availability in their host directly [25]. Several gut bacteria including *Clostridia*, *Bacteroides*, and *Escherichia* produce neuroactive metabolites involved in pain through tryptophan metabolism [26]. Tryptophan catabolism may play an important role in pain associated with kidney diseases, as derangement of Trp metabolic pathways has previously been observed in chronic kidney disease [23,27,28].

Approximately 99% of dietary tryptophan not used for protein synthesis is catabolized along the kynurenine pathway [29]. Tryptophan 2, 3-dioxygenase (TDO) and Indoleamine 2,3-dioxygenase (IDO) are the enzymes involved in the first rate-limiting step in tryptophan metabolism [16,30]. TDO is mainly expressed in the liver, while IDO is produced in tissues through the body [22]. Under physiological conditions, approximately 90% of tryptophan is degraded hepatically [22]. IDO is produced extrahepatically by the cells and tissues in response physiological or psychological stress, and has immunosuppressive properties through its ability to limit T-cell function [16]. Tryptophan (Trp) is a biochemical precursor to several critical neuroactive compounds involved in pain perception, including kynurenine (Kyn), kynurenic acid (KYNA), 3-hydroxykynurenine (3-HK), quinolinic acid (QUIN), 5-hydroxytryptamine (5-HT, serotonin), and melatonin [16,20]. The effect of Trp metabolism via the kynurenine pathway on chronic pain occurs as a result of two processes: (1) Under conditions of physiologic or psychological stress, IDO expression increases promoting accelerated Trp degradation, causing a shift away from the serotonin pathway to kynurenine pathway. This shift results in deprivation of Trp hydroxylase (a precursor of 5-HT, serotonin) available for 5-HT biosynthesis via the serotonin pathway. Overexpression of IDO is systemic and leads to reduced production of serotonin, a key mediator of pain and depression [23,27,31,32]; and, (2) Synthesis of neurotoxic metabolites (e.g., 3-HK, QUIN), which have been shown to be present in multiple neurodegenerative diseases, and can cross the blood-brain barrier [22]. Trp metabolites may directly regulate neuronal excitability of primary sensory neurons through activation of pain related receptors or ion channels, and their pro-and antioxidative properties make them a potential target for intervention [29].

1.1.2.2. The role of short chain fatty acids. Short-chain fatty acids (SCFAs) are products of gut microbial fermentation of dietary non-digestible carbohydrates and exhibit important anti-inflammatory effects in the intestines that may protect against nociceptive and neuropathic pain [22]. SCFAs contribute to maintaining gut wall epithelium integrity by providing nutrients to colonocytes; they also demonstrate neuroactivity through action in the central and peripheral nervous systems [33,34]. SCFAs exhibit anti-inflammatory effects in the gut and enhance the production of IL-8, thereby improving epithelial barrier integrity and reducing translocation of proinflammatory molecules into the general circulation [20]. Recent evidence suggests that microbiota-derived SCFAs influence the development and function of the microglia, which are specialized immune cells of the central nervous system, and play an essential role in initiating and maintaining chronic pain [35,36]. A model of chemotherapy-induced peripheral neuropathy recently showed the gut microbiome to be the primary determinant of pain sensitivity, where pain sensitivity was significantly correlated with the degree of microglial proliferation in the spinal cord [37]. Moreover, SCFAs can active G protein-coupled receptors (e.g. fatty acid free receptor 2 & 3 (FFAR2 & FFAR3), which are involved in regulation of leucocyte functions including the production of proinflammatory cytokines, eicosanoids, and chemokines involved in pain perception [36]. Studies have inferred SCFA exert analgesic effects by inhibition of histone deacetylases (HDACs) [38]. Epigenetic factors including chromatin remodeling via histone methylation and acetylation are known to play an important role in chronic pain [22].

Currently, there is a dearth of research exploring links between gut microbial community structure, SCFA production, and chronic pain in adults with chronic kidney disease. Microbiota involved in SCFA production may serve as targets for future randomized controlled trials. Notably, the microbiome is known to be amenable to patient-centered interventions, including plant-based nutrition, prebiotic and probiotic supplementation, physical activity, and stress reduction [39–41].

1.2. *Objectives of the BIOME-HDp pilot study*

The BIOME-HDp pilot study's immediate objective is to determine the acceptability and feasibility of collecting fecal microbiome and

microbiota–derived serum metabolite samples from people with ESKD-HD who experience chronic pain. A secondary aim is to interrogate the relationship between changes in fecal microbiome features, metabolites of the gut microbiome, and pain interference before and after pain coping skills training (PCST). Thus the four specific aims for this study are as follows:

Specific aim 1: Establish the feasibility and acceptability of collecting fecal samples for microbiome analysis in people with ESKD on hemodialysis.

Specific aim 2: Identify longitudinal changes in microbial community structure, diversity, and functional gene content among adults with ESKD and chronic pain receiving maintenance hemodialysis before and after pain interventions.

Specific aim 3: Interrogate changes in metabolic activity of the gut microbiome by directly measuring circulating SCFAs (acetic acid, propionic acid, and butyric acid) and tryptophan metabolites.

Specific aim 4: Determine if changes in gut microbiota are associated with patient-reported outcomes.

2. Design of the BIOME-HDp

2.1. The BIOME-HDp pilot study

This pilot study is an ancillary study of the HOPE Consortium Trial to reduce pain and opioid use in hemodialysis, which is part of the NIH's Helping to End Addiction Long-term (HEAL) initiative. The BIOME-HDp pilot study will establish clinical microbiome research methods and determine the acceptability and feasibility of fecal microbiome and serum metabolite sample collection.

This report on the BIOME-HDp pilot study was guided by the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) extension for pilot studies [42]. The pilot study is being conducted in accordance with the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use's Good Clinical Practice international ethical and scientific quality standard for designing, conducting, recording, and reporting trials that involve the participation of human subjects. The study protocol has been approved by the Office for the Protection of Research Subjects (OPRS) at the University of Illinois Chicago (IRB 2020 0005).

2.2. Design of the BIOME-HDp pilot study

The BIOME-HDp study uses a prospective sequential multiple-assignment randomized trial design. Participants are ESKD patients undergoing treatment with maintenance hemodialysis who have chronic pain, a subset of whom use prescribed opioid medications, and they are randomized in equal proportions to the intervention (PCST or usual care). The primary outcome for the BIOME-HDp study, pain interference, will be ascertained at Week 12, coinciding with the end of the PCST weekly coaching sessions. Pain interference, a broad measure of pain's influence on daily living, was selected as the outcome variable of interest. A timeline of events for the Biome-HDp pilot study is presented in Fig. 1.

3. Methods: participants, interventions, and outcomes

3.1. Study setting

The BIOME-HDp pilot study will be conducted at the dialysis unit at UI Health. UI Health serves a diverse population in the Chicagoland area of the United States. The Division of Nephrology at UI Health has over 50 years of experience treating people with kidney conditions and diseases, including ESKD, kidney transplantation, acute kidney failure, kidney stones, and immunological kidney diseases. The UI Health ESKD

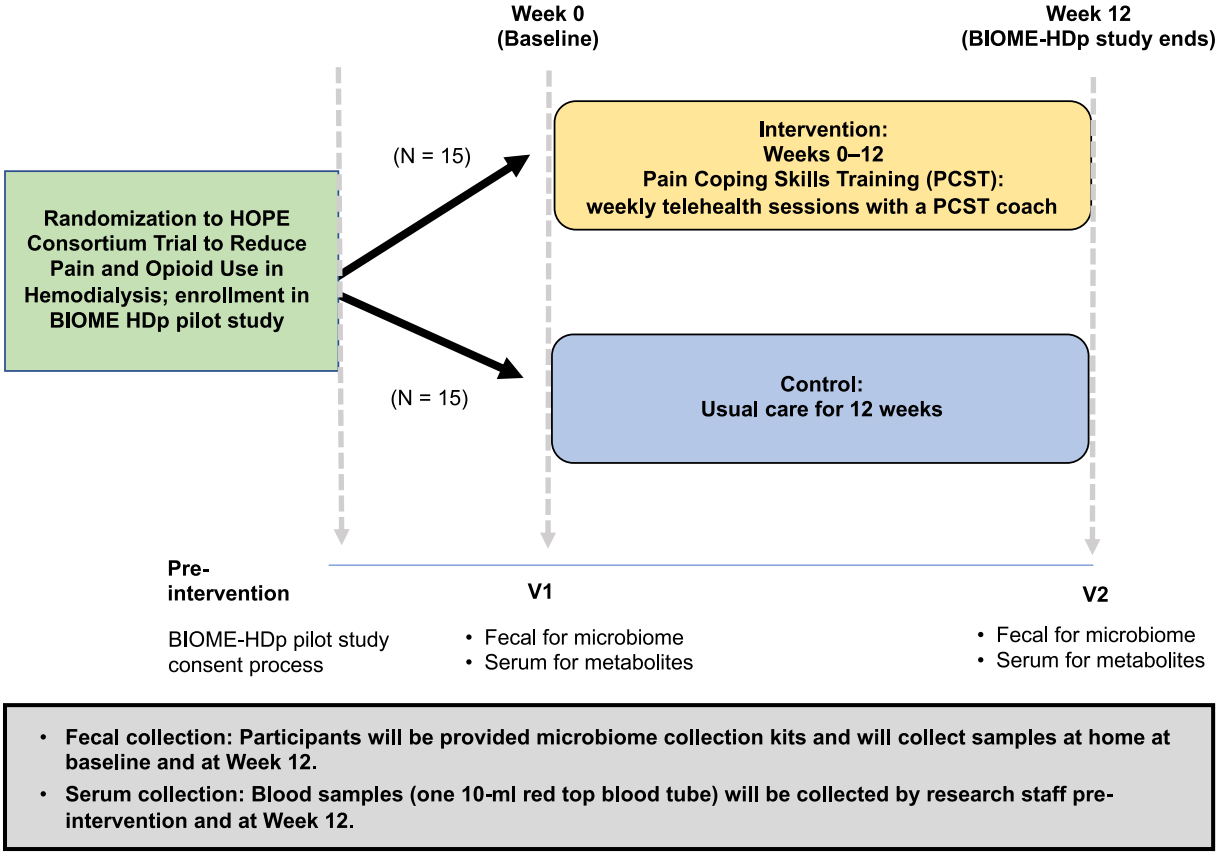

Fig. 1. Timeline of events for the Biome-HDp pilot study.

program's 23-chair outpatient dialysis unit is where study participants will be recruited.

3.2. Eligibility criteria

Patients treated at the UI Health dialysis unit are invited to complete a brief screening survey for the HOPE consortium study; the survey includes one item about the chronicity of pain plus the three-item Pain, Enjoyment of Life, and General Activity (PEG) scale. All people meeting the pain chronicity criterion (PEG ≥4) are approached for their willingness to participate in the HOPE consortium clinical trial. All HOPE trial participants enrolled at the UI Health dialysis unit are eligible for the BIOME-HDp pilot study, and are approached for the pilot study after they are randomized to the HOPE consortium trial. A list of inclusion and exclusion criteria for the BIOME-HDp study can be found in Table 1.

3.3. Interventions, outcomes, and participant timeline

Participants will collect fecal specimens for microbiome feature analysis and blood for targeted metabolomic analysis at two time points: prior to starting he PCST intervention (V1) and 3 months after initiation of the PCST intervention (V2). Once an individual signs the informed consent for the BIOME-HDp pilot study, they are given the fecal microbiome specimen collection kit and asked to collect a fecal sample at home before starting the PCST study intervention (V1). Generally, there is a 7-day window between the time of consent for the BIOME-HDp study and the start of the PCST intervention. Participants are encouraged to collect the fecal sample several days prior to the start of the PCST intervention to allow adequate time for fecal specimen collection. The procedure is followed for the second fecal specimen collection at Week 12, after the PCST intervention is completed (V2). Every pilot study participant receives training on the proper technique for sample collection and storage prior to each sample collection. Participants are provided with one fecal microbiome collection kit (Norgen Biotek Corp., ON, Canada) one week prior to each study visit; a list of the contents of this kit can be found in Table 2. Participants are contacted before their designated hemodialysis appointment and reminded to collect the fecal microbiome specimen and return the microbiome kit at that appointment, via a drop box in the reception area of the UI Health dialysis unit. In addition, one tube of blood (10-ml red top serum tube) is collected at their regularly scheduled hemodialysis appointment, prior to initiation of the dialysis treatment, for analysis of serum SCFAs and tryptophan metabolites. A timeline of study procedures and collection of variables for analysis can be found in Table 3.

Table 1
Participant inclusion and exclusion criteria for the BIOME HDp study.

Inclusion criteria	
1.	Age >18 years
2.	Undergoing in-center maintenance hemodialysis for >90 days
3.	Able to speak and understand English
4.	Chronic pain defined as a response of "Most days" or "Every day" to the following question: "In the past 3 months, how often have you had pain?" (Answer options: Never, Some days, Most days, Every day)
5.	Current PEG score ≥4
6.	Willing to provide informed consent
7.	Willing to allow the research team to obtain opioid pharmacy refill data
8.	Willing to allow the research team to contact and work with their opioid prescriber
Exclusion criteria	
1.	Current opioid use disorder
2.	Current use of heroin
3.	Current non-opioid substance use disorder (except for tobacco use disorder)
4.	Current use of methadone, buprenorphine, or naltrexone for opioid use disorder
5.	Current receipt of hospice care
6.	Cognitive impairment that, in the judgment of the research team, precludes trial participation
7.	Active suicidal intent based on an initial screening with PHQ-9 question #9, followed by further assessment when indicated
8.	Unstable bipolar disorder, schizophrenia, post-traumatic stress disorder, or other psychiatric disorder
9.	Life expectancy <6 months
10.	Expected to receive a kidney transplant, transfer to another dialysis facility, or transition to home dialysis within 6 months
11.	Current incarceration
12.	Any other condition that the investigator considers precludes participation in the clinical trial

Table 2
Contents of the fecal microbiome collection kit.

1	Fecal swab collection and preservation tube (tube only)[a]
2	Sterile fecal specimen collection swabs[a]
1	Shipping accessories[a,b]
1	Feces catcher[c]
1	Sample requisition form[d]
1	Pair of latex-free gloves
1	Written instructions with images for feces catcher[c]
1	Written instructions with images for sample collection[a]

[a] Norgen Biotek Corp. ON, Canada: https://norgenbiotek.com/product/fecal-swab-collection-and-preservation-system.
[b] Shipping accessories include: 1 biohazard specimen bag with absorbent pad; 1 bubble envelope labeled "Exempt Human Specimen"; and 2 blank labels.
[c] Zymo Research, Tusin, CA, USA: https://www.zymoresearch.com/products/feces-catcher.
[d] Sample requisition form includes: patient ID; investigator name and contact information; university IRB number; date and time of sample collection; quality of stool (hard, not too hard, not too soft, soft, liquid).

Table 3
Biome-HDp study enrollment and data collection timeline.

	HOPE Phase 1 (PCST or usual care)		
Study procedure	**Pre-screening**	**Baseline (Week 0)**	**Week 12**
HOPE study screening	X		
Confirmation of HOPE enrollment	X		
Confirmation of HOPE randomization arm	X		
Informed consent for Biome-HDp pilot study	X	X	X
Demographics	X		
Medical history	X		
Dialysis history	X		
Opioid history	X		
Patient-reported outcomes			
Brief Pain Index (BPI) Interference [55]		X	X
Brief Pain Index (BPI) Severity [55]		X	X
Pain Catastrophizing Scale (PCS) Short Form 6 [56]		X	X
McGill Quality of Life (MQOL) [57]		X	X
Patient Health Questionnaire (PHQ-9) [58]		X	X
Generalized Anxiety Disorder (GAD-7) [59]		X	X
Coping Strategies Questionnaire 24 (CSQ-24) [60]		X	X
PROMIS Fatigue Short Form 6a [61,62]		X	X
Dialysis Symptom Index (DSI) [63]		X	X
Multidimensional Scale of Perceived Social Support (MSPSS) [64]		X	X
Everyday Discrimination Scale [65]		X	X
Acceptability/feasibility			X
Biospecimen collection			
Fecal microbiome swab		X	X
Serum samples[a]		X	X

Abbreviations: HOPE, Hemodialysis Opioid Prescription Effort. PCST, pain coping skills training.
[a] Serum metabolite analysis to include tryptophan (TRP); kynurenine (KYN); KYN/TRP ratio; kynurenic acid (KYNA)' 3-hydroxykynurenine (3 HK); quinolinic acid (QA); serotonin (5-HT); neopterin; short-chain fatty acids (acetate, propionate, butyrate).

4. Methods: data collection, management, and analysis

4.1. Fecal microbiome sample preservation

Participants will collect fecal microbiome samples using the Norgen Fecal Swab Collection and Preservation System (Norgen Biotek Corp., ON, Canada) [43]. Fecal specimens will be collected at the participants home within 4 days of their scheduled study visit, and stored at room temperature. Upon delivery to the dialysis unit, fecal specimens will then be transferred to a −80° Celsius freezer until DNA extraction and metagenomic sequencing is performed. Research has shown the Norgen Fecal Swab and Collection and Preservation System preserves fecal microorganisms profile, up to 4 weeks at room temperature, with no significant changes in microbiome features (e.g., Simpson diversity index, differentially abundant features, and Bray-Curtis similarity index) when compared to immediate and rapid freezing to −80 °C [44].

4.2. Survey data collection

Participants' patient-reported outcomes will be captured using computer-assisted telephone interviewing (CATI), administered by a centralized team who will be masked to participants' treatment assignments. CATI is a highly reproducible approach for patient-reported outcomes, with successful implementation in several multi-center clinical trials in hemodialysis including the Frequent Hemodialysis Network studies and ASCEND (A Study of Cardiovascular Events in Diabetes) [45]. CATI is currently being used for the SLEEP-HD trial (NCT03534284) and the Hemodialysis Novel Therapies ACTION trial (NCT03141983). This approach allows for study participation by people with wide ranges of health literacy and limitations in vision and manual dexterity; it also reduces bias in assessing patient-reported outcomes.

An interviewer with no knowledge of participant treatment assignment will administer the English version of the study's patient-reported outcome measures (made available to the interviewer through the web-based study portal in a fixed sequence of screens), starting with the Brief Pain Inventory interference scale. A study coordinator will schedule the dates and times for the participants to receive their phone calls for outcome assessment. Each participant will choose whether to receive the phone call at home on a non-dialysis day (preferred) or while at the dialysis unit. (The research team at the dialysis unit will be equipped with mobile phones that can be made available to participants for these calls as needed.) Efforts will be made to ensure that all subsequent calls to participants occur at the same site as their baseline assessment. Each call is expected to last approximately 45 min when collecting the full set of patient-reported outcomes, or 25 min for the partial list of patient-reported outcomes. Pain interference is the primary outcome of interest for the BIOME-HDp study.

4.3. Data management

Data to be extracted from the electronic medical record include patient name, medical record number, and phone number; medical history; height, weight, and body mass index; hospitalizations; and concomitant medications. All fecal microbiome and serum for metabolite specimens will be stored in a −80-degree Celsius freezer. Once microbial DNA is extracted from the samples, any additional material will continue to be held in a −80-degree freezer for future research upon consent from the study participant.

4.4. Microbiome analysis

4.4.1. DNA extraction and library preparation

Microbial DNA will be extracted using the Qiagen MagAttract PowerSoil DNA KF Kit (formerly, MOBio PowerSoil DNA Kit) using a KingFisher robot. DNA quality will be evaluated visually via gel electrophoresis and will be quantified using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Libraries will be prepared using a Nextera library preparation kit (Illumina, San Diego, CA, USA) with an in-house protocol.

4.4.2. Sequencing, data curation, and sequence processing

Paired-end sequencing (150 base pairs x 2) will be performed on an Illumina NextSeq500 DNA sequences. Shotgun metagenomic sequence reads will be processed with the Sunbeam pipeline. Initial quality evaluation will be performed using FastQC version 0.11.5. Processing will take place in four steps: adapter removal, read trimming, low-complexity-read removal, and host-sequence removal. Adapter removal will be performed using cutadapt version 2.6 [46], and trimming with Trimmomatic version 0.36 [47] using custom parameters (LEADING:3 TRAILING:3 SLIDINGWINDOW:4:15 MINLEN:36). Low-complexity sequences will be detected with Complexity version 0.3.6 [48]. High-quality reads will be mapped to the human genome (Genome Reference Consortium Human Reference 37), and mapped reads will be removed from the analysis. The remaining reads will be taxonomically classified using Kraken2 with the MiniKraken2_v1 database [49]. For functional profiling, high-quality (filtered) reads will be aligned against the SEED database via translated homology search and annotated to Subsystems, or functional levels, 1 through 3 using Super-Focus [50].

4.4.3. Quantification of serum SCFA and tryptophan metabolites

Tryptophan metabolites will be extracted by protein precipitation protocol, followed by dryness under nitrogen and reconstitution in HPLC-grade water acetonitrile and formic acid before being subjected to LC/MS analysis. For SCFAs, the extracts will go through derivatization by 3-nitrophenylhydrazine before LC/MS analysis. The sample analysis will be carried out in the LC-MS/MS (Agilent 1290 UPLC coupled to AB Sciex QTRAP 6500). We will record the eluents' positive or negative ion mass spectra by reversed-phase C18 column, using the multiple reaction-monitoring mode. This mode uses the mass spectrometers MS1 and MS2 operated in static mode for single ions, which allows a higher sensitivity compared with the scan mode. The molecular and daughter ions for each target are selected for MS1 and MS2. Quantification will be done using Sciex Analyst software.

4.5. Statistical analysis

4.5.1. Descriptive analyses

Descriptive statistics on participant recruitment, retention, and adherence to specimen collection protocols will be used to report the feasibility and acceptability of the fecal microbiome and serum metabolite sample collection protocol used in the BIOME-HDp pilot study. To determine the overall longitudinal effects of the microbiome features and microbiota-derived metabolites on pain, we will employ longitudinal mixed-effects pain models on microbiome features and individual metabolites, with time variables. The time-specific longitudinal effects on pain of the microbiome features and microbiota-derived metabolites will be determined based on the interaction terms of time variables in the longitudinal mixed-effects models of pain. All longitudinal mixed-effects models will include potential covariates that may contribute to explaining the outcomes. Longitudinal structural equation modeling will be applied with different degrees of cross-lag structures to determine the dynamic temporal effect of microbiome features on pain over time.

4.5.2. Differential analysis of microbial taxa

Differential analyses of taxa as compared with experimental covariates are performed using the software package edgeR v3.28.1 on raw sequence counts [51]. Prior to analysis, the data are filtered to remove any sequence counts that were annotated as chloroplast or mitochondria in origin, as well as removing taxa that had less than 1000 total sequence counts, summed across all specimens, or were present in less than 30% of the specimens. Data are normalized as counts per million. TMM

normalized data are then fit using a negative binomial generalized linear model (GLM) using experimental covariates, and statistical tests are performed using a likelihood ratio test (i.e., glmFit and glmLRT functions in edgeR). Post-hoc pairwise tests are performed using the exactTest function in edgeR. Adjusted p values (q values) are calculated using the Benjamini-Hochberg false discovery rate (FDR) correction [52]. Significant taxa are determined based on an FDR threshold of 5% (0.05).

4.5.3. Alpha diversity analyses

Shannon indices are calculated with default parameters in R using the vegan library v2.5-6 [53]. Prior to analysis, the data are rarefied to a depth of 100,000 counts per sample. The resulting Shannon indices are then modelled with the sample covariates using a GLM assuming a Gaussian distribution. Significance of the model (ANOVA) was tested using the F test. Post-hoc, pairwise tests are performed using Mann-Whitney test. Plots are generated in R using the ggplot2 library [54].

4.5.4. Beta diversity/dissimilarity analyses

Bray-Curtis indices are calculated with default parameters in R using the vegan library v2.5-6 [53]. Prior to analysis, the normalized data are square root transformed. The resulting dissimilarity indices are modelled and tested for significance with the sample covariates using the PERMANOVA test (a.k.a. ADONIS). Additional comparisons of the individual covariates (e.g. age, race/ethnicity, body mass index, time on dialysis) are also performed using ANOSIM. Plots are generated in R using the ggplot2 library [54]. Additionally, since we utilized a repeated measures design, we will be explicitly controlling for individual differences in all microbiome feature analyses.

5. Conclusion

People with ESKD receiving maintenance dialysis often prioritize symptom relief above all else due to the devastating effects of high symptom burden on every aspect of quality of life. Chronic, debilitating pain is among the most common symptoms experienced by people with ESKD. This chronic pain is associated with comorbidities and systemic inflammation resulting from the accumulation of uremic toxins and significantly impacts the ability of people with ESKD to participate in and enjoy usual physical and social activities. Moreover, pain has been shown to reduce hemodialysis treatment adherence, reduce quality of life, and increase risk of mortality. At the same time, the opioid epidemic in the United States has resulted in high social and economic costs and made it clear that novel solutions are needed for people suffering from chronic pain. The lack of effective strategies to treat pain without medications has contributed to poor health outcomes for people with ESKD on hemodialysis.

Research on the connection between ESKD and its effects on microbiome features and associated metabolites is now emerging. It is now recognized that the symbiotic microbiota that comprises the human microbiome play a critical role in health and disease. New evidence is shedding light on the role of the microbiome in mediating chronic pain. With the acknowledgment that causes of chronic pain are complex and multifactorial, we posit that (a) significant changes in microbiome structure and composition result from impaired kidney function, (b) the change in microbiome structure and composition results in a change in the metabolic function of the microbiome via microbiota derived metabolites, and (c) changes in microbiome composition and function exacerbate the perception of pain in people with ESKD on hemodialysis.

The BIOME-HDp pilot study is an essential first step in understanding the relationships between renal function, microbiome features, associated metabolites, and pain in people with ESKD on hemodialysis. The longitudinal, repeated measures design is robust enough to show specific dynamic relationships between the structure and function of the microbiome, serum metabolites, and pain. Once we establish the acceptability and feasibility of sample collection protocols and identify fecal microbiome features and serum metabolites involved in chronic pain, we may be able to develop more extensive clinical trials that target nonpharmacological interventions—such as diet or lifestyle modifications or use of pre- and/or probiotics—to treat the epidemic of chronic pain.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] P.L. Kimmel, S.D. Cohen, S.D. Weisbord, Quality of life in patients with end-stage renal disease treated with hemodialysis: survival is not enough, J. Nephrol. 21 (Suppl 13) (2008) S54–S58.
[2] S.N. Davison, Pain in hemodialysis patients: prevalence, cause, severity, and management, Am. J. Kidney Dis. 42 (6) (2003) 1239–1247.
[3] P.L. Kimmel, C.-W. Fwu, K.C. Abbott, A.W. Eggers, P.P. Kline, P.W. Eggers, Opioid prescription, morbidity, and mortality in United States dialysis patients, J. Am. Soc. Nephrol. 28 (12) (2017) 3658–3670.
[4] A. Wyne, R. Rai, M. Cuerden, W.F. Clark, R.S. Suri, Opioid and benzodiazepine use in end-stage renal disease: a systematic review, Clin. J. Am. Soc. Nephrol. : CJASN 6 (2) (2011) 326–333.
[5] S. Dolati, F. Tarighat, F. Pashazadeh, K. Shahsavarinia, S. Gholipouri, H. Soleimanpour, The role of opioids in pain management in elderly patients with chronic kidney disease: a review article, Anesthesiol. Pain Med. 10 (5) (2020), e105754.
[6] M. Raouf, J. Bettinger, E.W. Wegrzyn, R.O. Mathew, J.J. Fudin, Pharmacotherapeutic management of neuropathic pain in end-stage renal disease, Kidney Dis. 6 (3) (2020) 157–167.
[7] H.S. Smith, Opioid metabolism, Mayo Clin. Proc. 84 (7) (2009) 613–624.
[8] J.W. Busse, L. Wang, M. Kamaleldin, et al., Opioids for chronic noncancer pain: a systematic review and meta-analysis, JAMA 320 (23) (2018) 2448–2460.
[9] C. Vangala, J. Niu, M.E. Montez-Rath, et al., Hip fracture risk among hemodialysis-dependent patients prescribed opioids and gabapentinoids, J. Am. Soc. Nephrol. : JASN (J. Am. Soc. Nephrol.) (2020).
[10] E.J. Corwin, G. Brewster, S.B. Dunbar, et al., The metabolomic underpinnings of symptom burden in patients with multiple chronic conditions, Biol. Res. Nurs. (2020), 1099800420958196.
[11] T.G. Dinan, J.F. Cryan, The impact of gut microbiota on brain and behaviour: implications for psychiatry, Curr. Opin. Clin. Nutr. Metab. Care 18 (6) (2015) 552–558.
[12] T.G. Dinan, J.F. Cryan, Microbes, immunity, and behavior: psychoneuroimmunology meets the microbiome, Neuropsychopharmacology 42 (1) (2017) 178–192.
[13] B.C. Song, J. Bai, Microbiome-gut-brain axis in cancer treatment-related psychoneurological toxicities and symptoms: a systematic review, Support. Care Cancer (2020).
[14] C. Yang, X. Fang, G. Zhan, et al., Key role of gut microbiota in anhedonia-like phenotype in rodents with neuropathic pain, Transl. Psychiatry 9 (1) (2019) 57.
[15] F. Guida, S. Boccella, C. Belardo, et al., Altered gut microbiota and endocannabinoid system tone in vitamin D deficiency-mediated chronic pain, Brain Behav. Immun. 85 (2020) 128–141.
[16] J. Gao, K. Xu, H. Liu, et al., Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism, Front. Cell. Infect. Microbiol. 8 (13) (2018).
[17] E. Sherwin, K. Rea, T.G. Dinan, J.F. Cryan, A gut (microbiome) feeling about the brain, Curr. Opin. Gastroenterol. 32 (2) (2016) 96–102.
[18] C.R. Martin, V. Osadchiy, A. Kalani, E.A. Mayer, The brain-gut-microbiome Axis, Cell. Mol. Gastroenterol. Hepatol. 6 (2) (2018) 133–148.

[19] S.M. O'Mahony, G. Clarke, Y.E. Borre, T.G. Dinan, J.F. Cryan, Serotonin, tryptophan metabolism and the brain-gut-microbiome axis, Behav. Brain Res. 277 (2015) 32–48.
[20] K. Ciapała, J. Mika, E. Rojewska, The kynurenine pathway as a potential target for neuropathic therapy design: from basic research to clinical perspectives, Int. J. Mol. Sci. 22 (20) (2021), 11055.
[21] P.J. Kennedy, J.F. Cryan, T.G. Dinan, G. Clarke, Kynurenine pathway metabolism and the microbiota-gut-brain axis, Neuropharmacology 112 (Pt B) (2017) 399–412.
[22] S. Li, D. Hua, Q. Wang, et al., The role of bacteria and its derived metabolites in chronic pain and depression: recent findings and research progress, Int. J. Neuropsychopharmacol. 23 (1) (2019) 26–41.
[23] N. Karu, C. McKercher, D.S. Nichols, et al., Tryptophan metabolism, its relation to inflammation and stress markers and association with psychological and cognitive functioning: tasmanian Chronic Kidney Disease pilot study, BMC Nephrol. 17 (1) (2016) 171.
[24] J.M. Gostner, S. Geisler, M. Stonig, L. Mair, B. Sperner-Unterweger, D. Fuchs, Tryptophan metabolism and related pathways in psychoneuroimmunology: the impact of nutrition and lifestyle, Neuropsychobiology 79 (1–2) (2020) 89–99.
[25] M. Dehhaghi, H. Kazemi Shariat Panahi, G.J. Guillemin, Microorganisms, tryptophan metabolism, and kynurenine pathway: a complex interconnected loop influencing human health status, Int. J. Tryptophan Res. 12 (2019), 1178646919852996.
[26] A.A. Badawy, Tryptophan metabolism: a versatile area providing multiple targets for pharmacological intervention, Egypt J. Basic Clin. Pharmacol. 9 (2019).
[27] S. Debnath, C. Velagapudi, L. Redus, et al., Tryptophan metabolism in patients with chronic kidney disease secondary to type 2 diabetes: relationship to inflammatory markers, Int. J. Tryptophan Res. 10 (2017), 1178646917694600-1178646917694600.
[28] A. Mor, B. Kalaska, D. Pawlak, Kynurenine pathway in chronic kidney disease: what's old, what's new, and what's next? Int. J. Tryptophan Res. 13 (2020), 1178646920954882.
[29] I. Davis, A. Liu, What is the tryptophan kynurenine pathway and why is it important to neurotherapeutics? Expert Rev. Neurother. 15 (7) (2015) 719–721.
[30] W. Roth, K. Zadeh, R. Vekariya, Y. Ge, M. Mohamadzadeh, Tryptophan metabolism and gut-brain homeostasis, Int. J. Mol. Sci. 22 (6) (2021).
[31] A. Agus, J. Planchais, H. Sokol, Gut microbiota regulation of tryptophan metabolism in health and disease, Cell Host Microbe 23 (6) (2018) 716–724.
[32] N.H. Jazani, J. Savoj, M. Lustgarten, W.L. Lau, N.D. Vaziri, Impact of gut dysbiosis on neurohormonal pathways in chronic kidney disease, Diseases 7 (1) (2019) 21.
[33] B. Dalile, L. Van Oudenhove, B. Vervliet, K. Verbeke, The role of short-chain fatty acids in microbiota–gut–brain communication, Nat. Rev. Gastroenterol. Hepatol. 16 (8) (2019) 461–478.
[34] A. Nallu, S. Sharma, A. Ramezani, J. Muralidharan, D. Raj, Gut microbiome in chronic kidney disease: challenges and opportunities, Transl. Res. 179 (2017) 24–37.
[35] Z. Dworsky-Fried, B.J. Kerr, A.M.W. Taylor, Microbes, microglia, and pain, Neurobiol Pain 7 (2020), 100045.
[36] R. Guo, L.H. Chen, C. Xing, T. Liu, Pain regulation by gut microbiota: molecular mechanisms and therapeutic potential, Br. J. Anaesth. 123 (5) (2019) 637–654.
[37] C. Ramakrishna, J. Corleto, P.M. Ruegger, et al., Dominant role of the gut microbiota in chemotherapy induced neuropathic pain, Sci. Rep. 9 (1) (2019), 20324.
[38] R. Negrete, M.S. García Gutiérrez, J. Manzanares, R. Maldonado, Involvement of the dynorphin/KOR system on the nociceptive, emotional and cognitive manifestations of joint pain in mice, Neuropharmacology 116 (2017) 315–327.
[39] J.J. Carrero, A. González-Ortiz, C.M. Avesani, et al., Plant-based diets to manage the risks and complications of chronic kidney disease, Nat. Rev. Nephrol. 16 (9) (2020) 525–542.
[40] L. Ortiz-Alvarez, H. Xu, B. Martinez-Tellez, Influence of exercise on the human gut microbiota of healthy adults: a systematic review, Clin. Transl. Gastroenterol. 11 (2) (2020) e00126-e00126.
[41] S.L. Schnorr, H.A. Bachner, Integrative Therapies in anxiety treatment with special emphasis on the gut microbiome, Yale J. Biol. Med. 89 (3) (2016) 397–422.
[42] L. Thabane, G. Lancaster, A guide to the reporting of protocols of pilot and feasibility trials, Pilot and Feasibility Studies 5 (1) (2019) 37.
[43] Norgen Biotek Corp, Fecal Swab collection and preservation system (Cat. 4570-B), Published, https://norgenbiotek.com/product/fecal-swab-collection-and-preservation-system, 2022. (Accessed 10 August 2022).
[44] W.S. Kim, M. Elmogy, M. Earle, Z. Haj-Ahmad, R. Bak, Y. Haj-Ahmad, Study of the Comparative Microbiome Profile from Different Fecal Preservation Methods, Norgen Biotek Corp, 2022. Published, https://norgenbiotek.com/sites/default/files/resources/App%20Note%2091%20-%20Comparative%20Microbiome%20Profiles%20from%20Fecal%20Preservation%20Methods%20-%20Rev%2002_2.pdf. (Accessed 10 August 2022).
[45] R. Mehrotra, D. Cukor, M. Unruh, et al., Comparative efficacy of Therapies for treatment of depression for patients undergoing maintenance hemodialysis, Ann. Intern. Med. 170 (6) (2019) 369–379.
[46] M. Martin, 2011, in: Cutadapt Removes Adapter Sequences from High-Throughput Sequencing Reads, 17, 2011, p. 3, 1.
[47] A.M. Bolger, M. Lohse, B. Usadel, Trimmomatic: a flexible trimmer for Illumina sequence data, Bioinformatics 30 (15) (2014) 2114–2120.
[48] E.L. Clarke, L.J. Taylor, C. Zhao, et al., Sunbeam: an extensible pipeline for analyzing metagenomic sequencing experiments, Microbiome 7 (1) (2019) 46.
[49] D.E. Wood, J. Lu, B. Langmead, Improved metagenomic analysis with Kraken 2, Genome Biol. 20 (1) (2019) 257.
[50] G.G. Silva, K.T. Green, B.E. Dutilh, R.A. Edwards, SUPER-FOCUS: a tool for agile functional analysis of shotgun metagenomic data, Bioinformatics 32 (3) (2016) 354–361.
[51] D.J. McCarthy, Y. Chen, G.K. Smyth, Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation, Nucleic Acids Res. 40 (10) (2012) 4288–4297, https://doi.org/10.1093/nar/gks042. Epub 2012/01/31.
[52] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing, J. Roy. Stat. Soc. B (Methodological) 57 (1) (1995) 289–300.
[53] J. Oksanen, F.G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R.B. O'Hara, G.L. Simpson, P. Solymos, M.H.H. Stevens, E. Szoecs, H. Wagner, Vegan: Community Ecology Package, 2.4.0, Computer Program), 2018.
[54] H. Ggplot2 Wickham, Elegant Graphics for Data Analysis, Springer, New York, NY, 2009.
[55] C.S. Cleeland, K.M. Ryan, Pain assessment: global use of the brief pain inventory, Ann. Acad. Med. Singapore 23 (2) (1994) 129–138.
[56] M.J.L. Sullivan, S.R. Bishop, J. Pivik, The pain catastrophizing scale: development and validation, Psychol. Assess. 7 (4) (1995) 524–532.
[57] S.R. Cohen, R. Sawatzky, L.B. Russell, J. Shahidi, D.K. Heyland, A.M. Gadermann, Measuring the quality of life of people at the end of life: the McGill Quality of Life Questionnaire-Revised, Palliat. Med. 31 (2) (2017) 120–129.
[58] R.L. Spitzer, K. Kroenke, J.B. Williams, Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. Primary care evaluation of mental disorders. Patient health questionnaire, JAMA 282 (18) (1999) 1737–1744.
[59] R.L. Spitzer, K. Kroenke, J.B. Williams, B. Löwe, A brief measure for assessing generalized anxiety disorder: the GAD-7, Arch. Intern. Med. 166 (10) (2006) 1092–1097.
[60] N. Harland, K. Georgieff, Development of the coping strategies questionnaire 24, a clinically utilitarian version of the coping strategies questionnaire, Rehabil. Psychol. 48 (2003) 296–300.
[61] D. Cella, W. Riley, A. Stone, et al., The Patient-Reported Outcomes Measurement Information System (PROMIS) developed and tested its first wave of adult self-reported health outcome item banks: 2005-2008, J. Clin. Epidemiol. 63 (11) (2010) 1179–1194.
[62] S. Schneider, S.W. Choi, D.U. Junghaenel, J.E. Schwartz, A.A. Stone, Psychometric characteristics of daily diaries for the patient-reported outcomes measurement information system (PROMIS®): a preliminary investigation, Qual. Life Res. : an international journal of quality of life aspects of treatment, care and rehabilitation 22 (7) (2013) 1859–1869.
[63] S.D. Weisbord, L.F. Fried, R.M. Arnold, et al., Development of a symptom assessment instrument for chronic hemodialysis patients: the dialysis symptom index, J. Pain Symptom Manag. 27 (3) (2004) 226–240.
[64] G.D. Zimet, S.S. Powell, G.K. Farley, S. Werkman, K.A. Berkoff, Psychometric characteristics of the multidimensional scale of perceived social support, J. Pers. Assess. 55 (3–4) (1990) 610–617.
[65] D.R. Williams, Y. Yan, J.S. Jackson, N.B. Anderson, Racial differences in physical and mental health: socio-economic status, stress and discrimination, J. Health Psychol. 2 (3) (1997) 335–351.