Brief Report

Association of plasma microbial composition with a leaky gut in obesity-related osteoarthritis: An exploratory study

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ABSTRACT

Objective: To examine the plasma microbiome for differences between obese individuals with and without osteoarthritis (OA) and its association with serum lipopolysaccharide (LPS).

Design: Blood samples from 70 participants with body mass index (BMI) ≥ 30 kg/m² and age ≥ 55 years, with (cases) or without (controls) hand plus knee OA, were analyzed for serum LPS and composition of the plasma microbiome. The Dirichlet-multinomial recursive partitioning model (DM-RPart) was applied to microbiome compositional data to test the hypothesis that LPS levels distinguish plasma microbiome, accounting for BMI and age.

Results: No significant differences in alpha diversity, or compositional differences between groups at the genus level, were seen between cases and controls (p = 0.11). β-Diversity was significantly associated with serum LPS levels (p = 0.01). DM-RPart resulted in an optimal tree with 3 divisions: 1) based on age (split at 69 years); 2) those older than 69 were split based on BMI; 3) those with BMI < 39 kg/m² were split based on LPS level (at 65 EU/ml). This resulted in 4 groups (nodes 2, and 5–7). Participants in node 2 were younger and the majority had no or mild OA. Those in nodes 5 and 6 were comparable in age and BMI but node 6 had higher LPS and more severe OA. Individuals in node 7 were older, had higher BMI, and the most severe OA.

Conclusions: Our results suggest a relationship between serum LPS and the plasma microbiome in a subgroup of obese individuals with hand plus knee OA that could reflect differences in intestinal permeability.

1. Introduction

Being overweight or obese is one of the most significant risk factors for developing osteoarthritis (OA) [1]. Obesity is an intriguing risk factor for OA in non-weight bearing joints, such as hands, indicating that the link between obesity and OA cannot be fully explained by increased joint load. Low-grade inflammation and altered metabolism have been suggested to play a role in obesity-related OA [1]. A link between the gut microbiota and human OA, particularly in obesity-related OA, is not fully understood. However, a causal link between the gut microbiome and systemic inflammation has been proposed through several biological mechanisms [2]. Studies have shown that an intestinal barrier disorder caused by gut microbial dysbiosis can lead to increased transport of microbes and their products, particularly lipopolysaccharide (LPS), into the systemic circulation [2]. Known by its capacity to induce inflammation, higher LPS levels have been found in serum and synovial fluid from individuals with knee OA, supporting a potential role of LPS in OA [3].

In a prior study, we tested the hypothesis that an altered gut microbiota plays a role in obesity associated with knee plus hand OA and found no differences between obese participants with and without OA [4]. However, we observed increased serum LPS levels in the obese individuals with OA relative to obese controls, suggesting that a leaky gut allowing for greater absorption of LPS, rather than a dysbiotic microbiota, may contribute to development of obesity-associated OA.

Although the gut microbiota is a major source of LPS, it is also produced by bacteria in the oral cavity, genitourinary tract, or potentially can be derived from food. Additionally, blood, which had been thought to be a sterile environment in healthy individuals, is populated by members of a microbial population that may be in a dormant state [5].

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Therefore, there is a possibility of translocation of living bacteria from gut to joints through blood. This could explain how DNA of the gut microbiome, found in human cartilage, was different between OA cases and controls [6]. In the present study, we analyzed and compared the plasma microbiome and serum LPS of obese individuals with and without hand plus knee OA to estimate the relationship among the plasma microbiota, LPS, and obesity-associated OA.

2. Methods

2.1. Participants

This cross-sectional study included participants from the Johnston County Osteoarthritis Project (JoCoOA) who were enrolled in a microbiome sub-study (IRB#15–1834) to study gut microbiota in the development of OA associated with obesity. Details of the study design and primary outcomes have been published [4]. Briefly, OA cases had clinical and/or radiographic hand OA, defined as involvement of at least 3 joints across both hands and radiographic knee OA defined as Kellgren-Lawrence (KL) grade 2–4 (or had undergone total knee replacement for OA). Controls had no hand OA and KL grade 0–1 of both knees. Both cases and controls were obese (body mass index (BMI) ≥ 30 kg/m²) and were older than 55 years. The present exploratory analysis included 70 participants (36 cases and 34 controls) who had a blood sample taken at the time of their study visit for the microbiome study.

2.2. LPS and microbiome analysis

Serum samples were analyzed for LPS using the EndoZyme® Recombinant Factor C (rFC) Endotoxin Detection Assay from Hyglos (#890030) as described [3]. The results of the LPS analysis have been previously reported in the main outcomes publication [4]. Isolation of total DNA from plasma samples and analysis by Illumina MiSeq 16S rRNA amplicon sequencing was performed in the University of North Carolina (UNC) Microbiome Core following standard protocols [7] with additional details provided in Supplementary Methods. Data sequencing output was converted to fastq format and demultiplexed using Illumina Bcl2Fastq 2.20.0. The resulting paired-end reads were processed with the QIIME ver.2.20.2006 wrapper for DADA2 including merging paired ends, quality filtering, error correction, and chimera detection. Amplicon sequencing units from DADA2 were assigned taxonomic identifiers using the Greengenes database. Alpha diversity with respect to Shannon index and entropy was estimated using QIIME2 at a rarefaction depth of 5000 sequences per subsample. Beta diversity estimates were calculated within QIIME2 using Bray Curtis distance between samples at a subsampling depth of 5000. Results were summarized and visualized through principal coordinate analysis as implemented in QIIME2.

2.3. Statistical analysis

Alpha diversity values were compared between OA cases and controls with t-tests, and the association between LPS and beta diversity measure was quantified with Permutational Multivariate Analysis of Variance (PERMANOVA). The Dirichlet-multinomial recursive partitioning model (DM-RPart) was applied to microbiome compositional data to test the hypothesis that LPS levels distinguish plasma microbiome composition [8]. DM-RPart uses recursive partitioning that separates samples into non-overlapping subgroups based on individual covariate values, similar to the classification and regression trees approach. It allows the regression of the individual’s microbial composition (the outcome) on the covariates (LPS, BMI, and age) without having to analyze one taxon at a time or requiring other data reduction. Overfitting was avoided through use of 10-fold cross-validation and cost-complexity pruning. The algorithm returned the best tree (i.e., the biggest pruned tree within one standard error of the lowest MSE). The analyses were performed using the R-package HMP. The bar charts were created to show the taxa composition and OA severity for each terminal node. Linear discriminant analysis effect size (LEfSe) of the 16S rRNA gene sequences was used to determine specific differences in the proportion of blood taxa for participants in 4 groups identified by DM-RPart [9].

3. Results

OA cases were slightly older (mean age 73 ± 7 versus 70 ± 6 years, p-value from unpaired t-test = 0.06) with a higher mean BMI (35.7 ± 3.9 versus 33.5 ± 3.1 kg/m², p = 0.01), and included more females (80.6% versus 55.9%) and fewer Black participants (27.8% versus 47.1%) compared to the controls.

At the phylum level, the blood microbiome was composed primarily of Firmicutes, followed by Proteobacteria, Bacteroidetes, and Actinobacteria, comprising approximately 90% of all phyla. No significant differences in alpha diversity or compositional differences at the genus level were observed between cases and controls (p = 0.11). Beta diversity was significantly associated with LPS levels (Pseudo-F = 2.84, p = 0.01).

DM-RPart results (in an optimal tree with 3 divisions (Fig. 1A): 1) based on age (split at 69 years); 2) those older than 69 were split based on BMI; 3) those with BMI <39 kg/m² were split based on LPS level (at 65 EU/ml). This resulted in 4 groups (nodes 2, 5, 6 and 7). Participants in node 2 were younger and the majority (68%) had no or mild OA (Fig. 1A). Individuals in nodes 5 and 6 were comparable in age and BMI, but those in node 6 had higher LPS and more severe OA. Half of participants in node 5 were OA free (KL grade 0–1 knees and without hand OA), and 25% had only mild knee OA in at least one knee. Individuals in node 7 were older with high BMI and the most severe OA. More than half of participants in node 7 had KL grade 4 knee OA; approximately one third of participants in this group had undergone total knee replacement. Fig. 1B shows the microbiome composition at the genus level in these groups. Analysis of the 16S rRNA gene sequences using the LEfSe showed specific differences in the proportion of blood taxa in the 4 groups found by DM-RPart (Fig. 2A and B). Eleven genera showed different relative abundance in participants from Node 5 compared to those from Node 6 (p < 0.1, Wilcoxon tests adjusted for multiple comparisons, Supplemental Table 1).

4. Discussion

Here we identified 4 groups of obese, older adult, individuals with and without hand plus knee OA having different compositions of blood microbiome. We used a novel statistical methodology to discover covariates driving differences in the microbial compositional data. We chose age, BMI, and LPS levels, which are known risk factors for OA to test the hypothesis that differences in these covariates, indicative of underlying physiological processes, are associated with differences in the blood microbial composition.

We observed a higher relative abundance of Firmicutes in the oldest group with the highest BMI (node 7). This phylum has been previously associated with visceral fat [10]. Lachnospiraceae and Ruminococcaceae were also increased in node 7 and different taxa of Lachnospiraceae were also previously related to visceral fat [10].

Interestingly, the participants in nodes 5 and 6, although comparable in age and BMI, were differentiated by LPS level, with those in node 6 having higher LPS and more severe OA compared with those in node 5. Within such a small cross-sectional study we can only speculate what contributed to the differences in microbial composition in these groups. However, our results can generate hypotheses about the nature of LPS in the blood microbiome and their interaction for future studies. Halomonas and Stenotrophomonas, both reported in blood previously [11], were more abundant in node 5 which was characterized by lower LPS levels and less severe OA. The role of Halomonas in OA is not clear, as this genus was recently reported at higher abundance in RA patients, but also has been identified as a possible contaminant [12].
Fig. 1. Optimal tree and plasma microbiome taxa distribution identified by DM-RPart. A, Optimal tree found by fitting the full tree and using cross-validation pruning. DM-RPart resulted in an optimal tree with 3 divisions 1) based on age (split at 69 years); 2) those older than 69 were split based on BMI; 3) those with BMI <39kg/m² were split based on LPS level (at 65). Each horizontal bar represents a tree node. The percentages in each horizontal node represent the percent of all 70 samples. The terminal nodes 2, 5, 6, and 7 represent the subgroups. The numbers within the segments of the vertical bars are proportions of participants from the corresponding subgroup having KLG of 0–4 or total knee replacement in their worse knee. B, The taxa frequency composition differences for the 4 terminal nodes. DM-RPart, the Dirichlet-multinominal recursive partitioning model; BMI, Body Mass Index; LPS, lipopolysaccharide; KLG, Kellgren-Lawrence Grade.

Fig. 2. Taxonomic differences of plasma microbiome between four subgroups identified by DM-RPart algorithm. A, LDA scores indicating differences in microbiome between the four subgroups. B, Taxonomic cladogram produced from LEfSe analysis. Red, green, blue and purple show taxa enriched in the nodes 2, 5, 6, and 7, respectively. DM-RPart, The Dirichlet-multinominal recursive partitioning model; LEfSE, linear discriminant analysis effect size; LDA, linear discriminant analysis.
Most bacteria found within blood in apparently healthy individuals may be in a dormant state; the ability to reactivate may represent a disease phenotype. Kell and colleagues discussed the role of iron dysregulation in the reactivation of dormant microbes acquired earlier via different sources, particularly in rheumatoid arthritis [13]. Iron overload can also be associated with OA development, as in patients with hemochromatosis [14]. Several mechanisms have been proposed to understand the relationship between iron metabolism and joint damage, but the causal mechanisms have not been defined. We cannot determine if iron overload is "causal" in an iron-overload-microbiome-OA relationship, which is one of the limitations of a cross-sectional study. Therefore, it is not clear if the differences observed in the blood microbiome are risk factors for disease, or if they are a result of elevated iron levels associated with OA. However, these questions could be investigated in future detailed studies with longitudinal samplings.

Additional limitations of the present work are the small sample size and lack of external validation due to unavailability of similar data. However, DM-Rpart allowed for cross-validation and cost-complexity pruning which can partially address the overfitting issues. In the present study, we did not correlate fecal and blood microbiomes. Future work could consider these two commensal populations to understand whether changes in blood microbiome composition are caused by changes in the gut microbiota and determine the potential role of the blood microbiome in the development and progression of OA.

There are several factors that pose a significant challenge to interpretation of the results from blood microbiome research. Although the existence of bacteria in the blood of healthy individuals is accepted [11], it is not easy to distinguish the presence of live microorganisms and their products from dead bacteria. Sequencing technologies (including 16S rRNA amplicon sequencing) can differentiate DNA from harmless taxa in the blood of healthy individuals; however, some of the taxa DNA identified by amplicon sequencing may be contaminants from reagents and lab equipment. For example, Lactobacillus, which is normally present in the gut microbiome and is considered a potential contaminant in the blood microbiome, has been identified as a causal agent of bacteremia in clinical reports [15]. Interestingly, this genus was more abundant in the oldest group with high BMI and severe OA. It is important to note that the blood samples from the cases and the controls were collected at the same clinic site, by the same study personnel, and processed together so that any differences between the case and control groups are unlikely to be due to differences in the collection protocol or sample processing.

In conclusion, our results suggest a relationship between LPS and the plasma microbiome in obese individuals with hand and knee OA. Although preliminary, these results open possible avenues in the field of understanding the nature of obesity-related OA. Future studies are needed to determine if increased intestinal permeability is responsible for the increased LPS and its contribution to the plasma microbiome.

Author contributions

The authors all made substantial contributions to this work as follows, including to the conception and design of the study (LA, RFL), acquisition of data (LA, M AA-P, YC), and analysis and interpretation of the data (all authors); in drafting the article (LA, RFL) or revising it critically for important intellectual content (M AA-P, YC, AEN); and in providing final approval of the version of the manuscript to be submitted (all authors).

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Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jocart.2022.100317.

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