Summary

Introduction.

Recently, there have been a large number of reports on the intestinal microbiota in the pathogenesis of obesity and diabetes and, among others, the impact on the development of colorectal cancer or behavioral disorders. Today it is known fact that the human phenotype is not a simple sum of the genetic expression recorded in the human nuclear genome, because the overall picture is outlined at least to a significant by the metabolism of microorganisms inhabiting it. The human phenotype is therefore the resultant of the human genotype and the environment affecting it from all sides. According to various analyzes, there are over 3,000,000 different genes in the human intestine, in addition, the number of bacteria is about 10 times more cells than the entire body of a healthy adult human. In recent years, much attention has been paid to the analysis of the diversity of microbiomes present in the human digestive tract, emphasizing that the intestinal genome is in third place after the nuclear and maternally inherited (mitochondrial) genomes. Unfortunately, the exact role of the bacterial flora is still unknown. Implementation of a project that would describe the impact of microflora on the development of multiple myeloma would be a key role in filling the gap to determine gut role, and the presented study is the first such study conducted on the Polish population.

Aim of the work.

The main aim of my work was to study the microbiome in the population of Polish patients with newly diagnosed multiple myeloma (NDMM) in relation to the composition of the microbiome of healthy Poles.

The following questions were posed in the work:

1. Are there differences in the composition of the microbiome between the Polish population of NDMM patients and healthy people?

2. Can differences in the composition of the microbiome between NDMM patients and healthy people contribute to the development of the disease?

3. Are there differences in relative abundance between the study groups among bacteria involved in short-chain fatty acid metabolism?

An additional aim of the work was to bank information on the microbiome of people from the Polish population, with the possibility of using it for further analysis.

Material and Methods:

Samples from 39 people were collected for the study. The control group consisted of 20 healthy people who gave their stool samples for examination. The study group consisted of 19 people diagnosed with multiple myeloma before hematological treatment.

In the present study, the 16S ribosomal RNA (rRNA) gen was sequenced to profile the microbial community. Properly isolated DNA was the basis for the preparation of the NGS sequencing library (Next Generation Sequencing). The preparation of the libraries for sequencing involved two main steps. In the first stage, sets of amplicons of the hypervariable regions of the 16S rRNA gene were prepared, in the presented study they were V3-V4 and V7-V9 regions, these regions allow the identification of bacteria and archaea. The amplified fragments were then purified on magnetic beads.

Then, after proper preparation of the material, the samples were sequenced.

Paired-end sequencing (2x276) of the 10 pM DNA library was performed on a MiSeq sequencer (Illumina) using the MiSeq Reagent Kit v3 (600 cycles-600 cycles) (Illumina), yielding data in FASTQ format (FASTQ format). Bioinformatics analysis of microbiomes was carried out using the QIIME2 2021.8 program

The analysis performed in the study was carried out by combining the V3-V4 regions (V3-V4 regions) with V7-V9 (V7-V9 regions) using the SMURF method in the q2-side implementation (q2-side implementation). In the bioinformatics analysis, it was decided to measure alpha-diversity and beta-diversity and use the ANCOM method

Results

In the context of alpha-diversity, no statistical significance was obtained ($p>0.05$). However, in the aspect of beta-diversity in the analysis, statistically significant differences were obtained in all 4 metrics. In the performed analysis, statistical significance was obtained in the Jaccard ($p=0.001$), Bray-Curtis ($p=0.001$), Unweighted-unifrac ($p=0.011$), Weighted-unifrac ($p=0.029$) metrics. In addition, the ANCOM method showed differences in relative abundance among bacteria belonging to the following families:

-Peptostreptococcaceae ($p < 0.016$)

-Lachnospiraceae ($p<0.016$)

-Staphylococcaceae ($p<0.012$)

The highest in the taxonomy, the ANCOM analysis obtained information on the differences in the taxa included in the genus: Lachnospiraceae UCG-008 ($p < 0.001$), Erysipelotrichaceae UCG-003 ($p < 0.0015$) and Anaerostipes ($p < 0.001$). The presented analysis showed differences in relative abundance among bacteria involved in the metabolism of short-chain fatty acids.

Conclusions

1. The microbiome of patients with newly diagnosed multiple myeloma and the microbiome of healthy people differ in terms of beta diversity

2. Differences between the microbiomes of NDMM patients and healthy people occur in each of the 4 basic metrics: Jaccard, Bray-Curtis, Unweighted-unifrac, Weighted-unifrac

3. Specific taxa occur in varying abundance in the intestinal microbiome of healthy people and patients diagnosed with multiple myeloma. Differences in relative abundance occur at different taxonomic levels.

4. Bacterial families that show differences in relative abundance between sick and healthy

people are as follows: Peptostreptococcaceae, Lachnospiraceae, Staphylococcaceae. Bacterial genus showing differences in relative abundance were demonstrated among bacteria belonging to: Lachnospiraceae UCG-008, Erysipelotrichaceae UCG-003, Anaerostipes.

5. In the microbiome of sick and healthy people, there are differences in the relative abundance of bacteria involved in the metabolism of short-chain fatty acids.

6. The microbiome of patients with newly diagnosed multiple myeloma and the microbiome of healthy people do not differ in terms of alpha diversity, in any of the four basic measures: Shannon Index, Pielou's Evenness, Observed Features (OF), Faith's Phylogenetic Diversity (PD).