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(RESEARCH ARTICLE)

GUT MICROBIOTA DYSBIOSIS FOLLOWING AUTOLOGOUS BONE MARROW TRANSPLANTATION IN MULTIPLE MYELOMA PATIENTS: A PILOT METAGENOMIC STUDY

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ABSTRACT

Background: Gut microbiota plays a crucial role in immune regulation, nutrient metabolism, and maintenance of intestinal homeostasis. Bone marrow transplantation (BMT) is associated with significant alterations in gut microbial communities due to conditioning chemotherapy, antimicrobial exposure, and immune suppression. However, data on microbiota changes following autologous transplantation in multiple myeloma patients remain limited.

Methods: This pilot longitudinal study evaluated gut microbiota alterations in two male patients with multiple myeloma undergoing autologous BMT. Both patients received four cycles of lenalidomide, bortezomib, and dexamethasone (RVD) followed by melphalan conditioning. Fecal samples were collected before transplantation and on Day +29/+30 post-transplantation. Microbial DNA was extracted and subjected to 16S rRNA gene sequencing using the Ion 16S Metagenomics Kit on the Ion Torrent platform. Taxonomic profiling and diversity analyses were performed using Ion Reporter software.

Results: Significant microbiota restructuring was observed after transplantation. Patient 1 exhibited marked depletion of Bacteroidetes with increased Firmicutes and Proteobacteria, accompanied by expansion of Enterococcaceae and Enterobacteriaceae and reduction of beneficial butyrate-producing taxa. In contrast, Patient 2 demonstrated increased abundance of Actinobacteria and Bacteroidetes, with dominance of Bifidobacteriaceae and enrichment of beneficial species including *Bifidobacterium longum*. Phylum-, family-, and species-level analyses revealed substantial inter-individual variability in microbial responses following transplantation.

Conclusion: Autologous BMT in multiple myeloma patients is associated with pronounced alterations in gut microbial composition. The observed dysbiosis and loss of beneficial commensal bacteria highlight the impact of transplantation-related interventions on intestinal microbial ecology. Larger longitudinal studies are required to clarify the clinical significance of these microbiome changes and their potential role in transplant outcomes.

Keywords: Multiple Myeloma; Autologous Bone Marrow Transplantation; Gut Microbiota; Dysbiosis; 16S rRNA Sequencing; Metagenomics

1 INTRODUCTION

The human gastrointestinal tract harbors a complex microbial ecosystem comprising bacteria, archaea, viruses, and fungi collectively known as the gut microbiota. These microorganisms play essential roles in nutrient metabolism, maintenance of intestinal barrier integrity, production of short-chain fatty acids (SCFAs), and regulation of host immune responses. In healthy individuals, the gut microbiota is predominantly composed of members of the phyla Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria, which exist in a balanced symbiotic relationship with the host [1,2].

Disruption of this microbial balance, commonly referred to as dysbiosis, has been associated with numerous pathological conditions, including inflammatory bowel disease, obesity, diabetes, cardiovascular disorders, autoimmune diseases, and cancer [3]. Dysbiosis is typically characterized by reduced microbial diversity, depletion of beneficial commensal bacteria, and expansion of opportunistic pathogens. Such alterations may contribute to disease progression through immune dysregulation, chronic inflammation, and impaired intestinal barrier function [3,4].

Multiple myeloma (MM) is a plasma cell malignancy characterized by uncontrolled proliferation of clonal plasma cells within the bone marrow. Despite substantial advances in treatment, MM remains a significant cause of morbidity and mortality worldwide. Current therapeutic strategies include immunomodulatory drugs, proteasome inhibitors, monoclonal antibodies, and autologous hematopoietic stem cell transplantation (auto-HSCT), which remains a standard treatment option for eligible patients [5].

Autologous bone marrow transplantation (BMT) involves administration of high-dose chemotherapy followed by reinfusion of the patient's own hematopoietic stem cells to restore bone marrow function. High-dose melphalan is widely used as the conditioning regimen prior to transplantation. Although autologous transplantation is generally associated with lower treatment-related complications than allogeneic transplantation, patients frequently experience gastrointestinal toxicity, mucosal injury, immune suppression, and infectious complications during the peri-transplant period [6].

Several transplantation-associated factors can profoundly influence gut microbial composition. Conditioning chemotherapy damages the intestinal epithelium and alters the gut environment, while broad-spectrum antibiotics further reduce microbial diversity by eliminating susceptible bacterial populations. Additional factors such as hospitalization, dietary changes, and immune disturbances contribute to microbiota disruption during transplantation [7].

Recent studies have demonstrated that hematopoietic stem cell transplantation is associated with significant reductions in gut microbial diversity and expansion of opportunistic bacterial taxa, particularly members of the Enterococcaceae and Enterobacteriaceae families. Conversely, preservation of beneficial anaerobic bacteria and butyrate-producing microorganisms has been associated with improved intestinal health and favorable clinical outcomes [8,9]. However, most microbiome investigations have focused on allogeneic transplantation, whereas information regarding microbiota alterations following autologous transplantation in multiple myeloma patients remains limited [10].

Advances in next-generation sequencing technologies have enabled comprehensive characterization of microbial communities. Among these approaches, 16S ribosomal RNA (rRNA) gene sequencing is widely utilized for taxonomic profiling of bacterial populations and evaluation of microbial diversity. This technique provides valuable insights into microbiota dynamics and their potential clinical implications in transplantation settings [11].

Therefore, the present pilot study aimed to investigate gut microbiota alterations before and after autologous bone marrow transplantation in two multiple myeloma patients using 16S rRNA gene sequencing. By comparing pre-transplant and post-transplant fecal microbiota profiles, we sought to characterize transplantation-associated dysbiosis and identify major microbial shifts occurring during the early post-transplant recovery period.

2 MATERIALS AND METHODS

2.1 Study Design and Patient Recruitment

This pilot longitudinal research study was conducted to investigate alterations in gut microbiota composition before and after autologous bone marrow transplantation (BMT) in patients diagnosed with Multiple Myeloma (MM). Two male patients undergoing autologous hematopoietic stem cell transplantation at a tertiary care center were enrolled in the study. Both patients received four cycles of induction chemotherapy consisting of lenalidomide, bortezomib, and dexamethasone (RVD), followed by high-dose melphalan conditioning and autologous stem cell transplantation.

Patient 1 was a 56-year-old male and Patient 2 was a 55-year-old male. Both patients received antimicrobial prophylaxis comprising amikacin, meropenem, Zostom, and fluconazole during the transplantation period. Stool samples were collected before transplantation (pre-BMT) and approximately one month after transplantation (Day +29 to Day +30 post-BMT) for microbiome analysis.

The study protocol was approved by the Institutional Ethics Committee of GeneXplore Diagnostics and Research Centre Pvt. Ltd., Ahmedabad, India (Approval No. 004/24). All procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki and Indian Council of Medical Research (ICMR) guidelines. Written informed consent was obtained from all participants before enrollment.

2.2 Fecal Sample Collection and Storage

Fresh fecal samples were collected from each patient at two different time points:

- Pre-transplantation (baseline sample before conditioning therapy)
- Post-transplantation (Day +29 to Day +30 following autologous BMT)

Samples were collected in sterile fecal collection containers under aseptic conditions. Immediately after collection, samples were transported to the laboratory and processed within 24 hours. Aliquots of approximately 200 mg of stool were prepared for DNA extraction. Samples were stored at −80°C until further molecular analysis to preserve microbial DNA integrity and minimize degradation.

2.3 Microbial DNA Extraction

Microbial genomic DNA was extracted from fecal samples using the **QIAamp DNA Stool Mini Kit** (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

Briefly, approximately 200 mg of stool sample was suspended in lysis buffer and subjected to mechanical and chemical disruption to release microbial DNA. Cellular debris and inhibitory substances commonly present in fecal material were removed through a series of washing and purification steps. Purified DNA was subsequently eluted in nuclease-free elution buffer.

The concentration and purity of extracted DNA were initially evaluated using a **Q5000 Micro-volume UV–Visible Spectrophotometer** (Quawell Technology Inc., San Jose, CA, USA). DNA integrity and concentration were further confirmed before library preparation. Extracted DNA samples were stored at −80°C until amplification and sequencing.

2.4 16S rRNA Gene Amplification

Bacterial community profiling was performed using amplification of the 16S ribosomal RNA (16S rRNA) gene. Amplification targeted multiple hypervariable regions using the **Ion 16S™ Metagenomics Kit** (Thermo Fisher Scientific, USA).

For amplification of the V2–V3 region, the following primer pair was utilized:

- U341F-MiSeq: 5′-CCTACGGGRSGCAGCAG-3′
- 805R-MiSeq: 5′-GACTACHVGGGTATCTAATCC-3′

PCR reactions were prepared according to the manufacturer's protocol. Amplification was performed in a thermal cycler under the following conditions:

- Initial denaturation: 95°C for 3 minutes
- 35 amplification cycles:

- Denaturation: 95°C for 30 seconds
- Annealing: 53°C for 30 seconds
- Extension: 72°C for 60 seconds
- Final extension: 72°C for 5 minutes

Successful amplification was confirmed by agarose gel electrophoresis.

2.5 Library Preparation and Purification

Amplicon libraries were prepared using the **Ion 16S Metagenomic Sequencing Library Preparation Kit** following the manufacturer's recommendations.

Following PCR amplification, products were purified using the **QIAquick PCR Purification Kit** (Qiagen, Germany) to remove residual primers, nucleotides, enzymes, and amplification artifacts.

Additional cleanup and size selection were performed using the **NGS Clean-up and Size Selection Kit** (Macherey-Nagel, Germany). Purified amplicons underwent indexing PCR to incorporate sample-specific barcodes required for multiplex sequencing.

Library concentration was quantified using a **Qubit 2.0 Fluorometer** (Thermo Fisher Scientific, USA). Library quality assessment was performed prior to pooling. Individual libraries were normalized and pooled at an equimolar concentration of 8 nM to ensure balanced sequencing coverage across all samples [12,13].

2.6 High-Throughput Sequencing

Sequencing was performed using the **Ion Torrent sequencing platform** (Thermo Fisher Scientific, USA) according to the manufacturer's standard workflow.

Template preparation, enrichment, and chip loading were carried out following Ion Torrent protocols. Sequencing generated high-quality reads corresponding to bacterial 16S rRNA gene fragments from the fecal microbiome.

Sequencing quality metrics including total reads, valid reads, mapped reads, and mean sequence length were obtained for each sample and used to assess sequencing performance.

2.7 Bioinformatics Analysis

Raw sequencing reads were analyzed using **Ion Reporter™ Software v5.20** (Thermo Fisher Scientific, USA). Quality filtering, adapter trimming, and removal of low-quality sequences were performed prior to taxonomic classification. Microbial taxa were assigned using the Ion Reporter Metagenomics 16S workflow, and relative abundance profiles were generated at phylum, class, order, family, genus, and species levels. Comparative analysis of pre-transplant and post-transplant samples was conducted to evaluate transplantation-associated changes in gut microbiota composition [13, 14].

2.8 Statistical Analysis

Descriptive analyses were performed to summarize clinical characteristics, sequencing statistics, and taxonomic abundance profiles.

Relative abundance percentages were calculated for individual taxa and compared between pre-transplant and post-transplant samples. Diversity indices including Shannon, Simpson, and Chao1 metrics were used to assess changes in microbial diversity associated with transplantation [15,16].

Due to the pilot nature of the study and the limited sample size (n = 2 patients), formal inferential statistical testing was not performed. Data were interpreted descriptively, focusing on biologically meaningful changes in microbial composition and diversity following transplantation.

2.9 Ethical Approval and Consent to Participate

The study was approved by the Institutional Ethics Committee of GeneXplore Diagnostics and Research Centre Pvt. Ltd., Ahmedabad, India (IEC Approval No. 004/24). Written informed consent was obtained from all participants prior to sample collection and enrollment in the study. All procedures were conducted in accordance with the Declaration of Helsinki and applicable national ethical guidelines for human research.

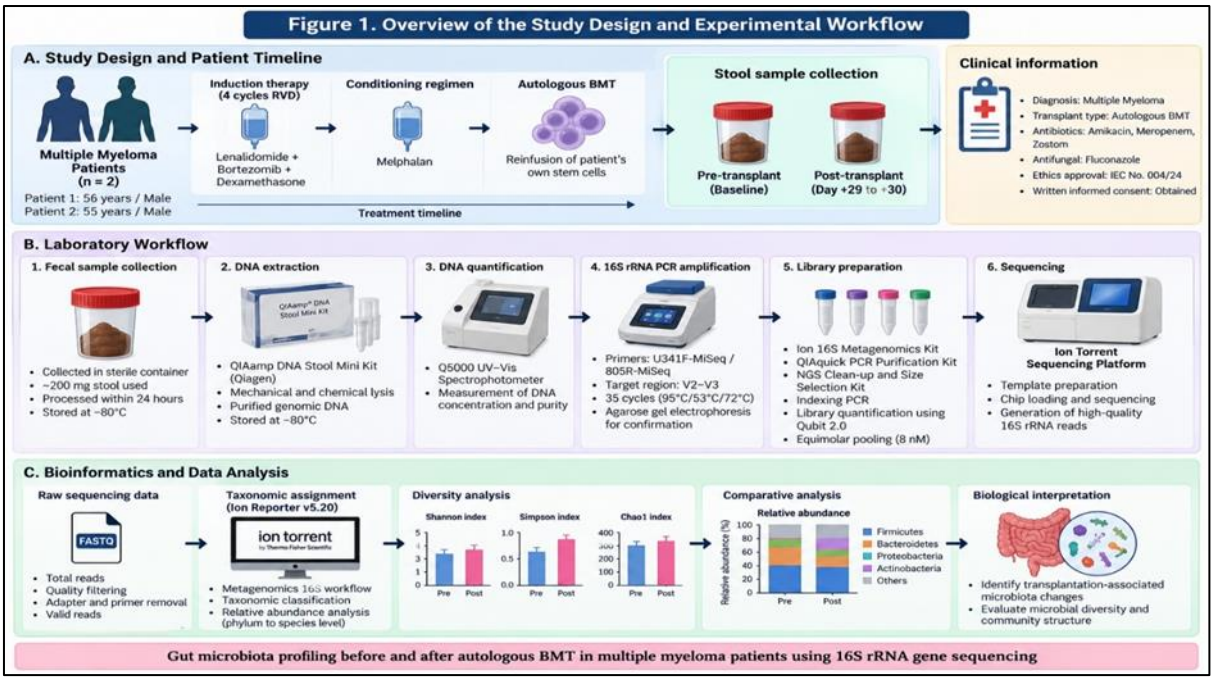


Figure 1: Study Workflow

3 RESULTS

3.1 Clinical Characteristics of the Study Participants

Two male patients diagnosed with Multiple Myeloma underwent autologous bone marrow transplantation following four cycles of induction therapy comprising lenalidomide, bortezomib, and dexamethasone (RVD), followed by high-dose melphalan conditioning. Stool samples were collected before transplantation and approximately one month after transplantation (Day +29 and Day +30). Both patients received broad-spectrum antimicrobial therapy including amikacin, meropenem, Zostom, and fluconazole during the transplantation period.

Table 1: Clinical Characteristics of the Study Participants

Parameter	Patient 1	Patient 2
Age/Sex	56/M	55/M
Diagnosis	Multiple Myeloma	Multiple Myeloma
Transplant Type	Autologous BMT	Autologous BMT
Induction Therapy	4 cycles RVD	4 cycles RVD
Conditioning Regimen	Melphalan	Melphalan
Antibiotics	Amikacin, Meropenem, Zostom	Amikacin, Meropenem, Zostom
Antifungal	Fluconazole	Fluconazole
Post-BMT Sampling	Day +30	Day +29

3.2 Sequencing Performance

High-throughput sequencing generated sufficient reads for comprehensive taxonomic characterization of the fecal microbiota in all samples. Quality filtering and mapping procedures retained a substantial proportion of reads for downstream taxonomic analysis, enabling evaluation of microbial composition from phylum to species level.

3.3 Gut Microbiota Alterations Following Autologous BMT

3.3.1 Patient 1

3.3.1.1 Phylum-Level Changes

Before transplantation, the intestinal microbiota was predominantly composed of **Firmicutes (46.95%)** and **Bacteroidetes (44.72%)**, with smaller proportions of **Proteobacteria (6.05%)** and **Actinobacteria (2.28%)**. This profile is generally characteristic of a relatively balanced gut microbial ecosystem.

Following autologous transplantation, a substantial shift in microbial composition was observed. Firmicutes increased to **77.82%**, while Bacteroidetes dramatically declined to **0.09%**. Proteobacteria expanded to **18.44%**, indicating a marked alteration in microbial community structure. This finding suggests severe post-transplant dysbiosis characterized by loss of microbial balance and expansion of opportunistic bacterial groups.

3.3.1.2 Family-Level Changes

Pre-transplant microbiota was dominated by **Bacteroidaceae (43.56%)**, **Lachnospiraceae (28.01%)**, and **Ruminococcaceae (9.14%)**, families commonly associated with gut health and short-chain fatty acid production.

Post-transplant analysis demonstrated pronounced enrichment of **Enterococcaceae (60.33%)** and **Enterobacteriaceae (16.16%)**, accompanied by substantial reductions in Lachnospiraceae and Ruminococcaceae. Such alterations are frequently associated with antibiotic exposure and transplantation-induced intestinal injury.

3.3.1.3 Species-Level Changes

The pre-transplant microbiome contained beneficial commensal organisms including:

- *Bacteroides vulgatus*
- *Pseudobutyrivibrio ruminis*
- *Ruminococcus coprocola*
- *Faecalibacterium bromii*
- *Roseburia prausnitzii*

Following transplantation, these taxa were markedly reduced and replaced by dominance of:

- *Enterococcus faecium*
- *Streptococcus thermophilus*
- *Weissella confusa*
- *Lactobacillus delbrueckii*

The emergence of *Enterococcus faecium* as the dominant species indicates a substantial ecological disruption of the gut microbiota after transplantation.

- Krona chart

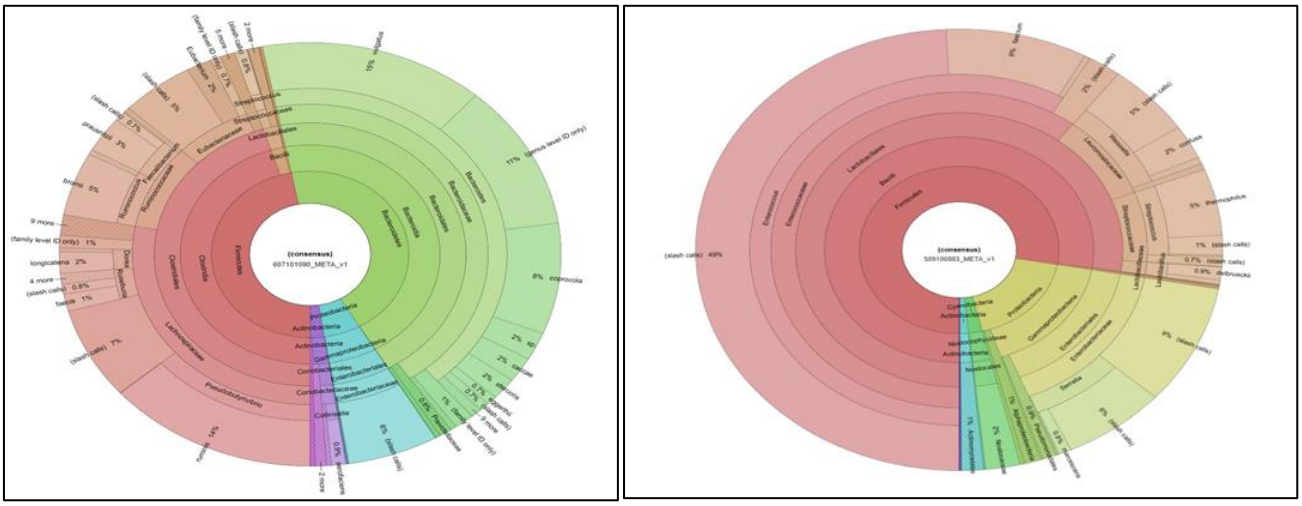


Figure 2: Krona charts showing gut microbiota composition in Patient 1 before (A) and after (B) autologous bone marrow transplantation.

3.3.2 Patient 2

3.3.2.1 Phylum-Level Changes

The pre-transplant microbiota of Patient 2 was characterized by predominance of **Firmicutes (49.25%)** and **Proteobacteria (35.82%)**, with lower abundances of **Bacteroidetes (8.21%)** and **Actinobacteria (5.97%)**.

Post-transplant analysis revealed a dramatic restructuring of the microbial community. **Actinobacteria increased to 45.92%** and **Bacteroidetes increased to 35.82%**, while **Firmicutes decreased to 11.99%** and **Proteobacteria decreased to 6.12%**. This shift suggests a distinct microbial response compared with Patient 1 and may indicate partial restoration of beneficial microbial populations.

3.3.2.2 Family-Level Changes

Before transplantation, the predominant families included:

- Enterobacteriaceae (24.63%)
- Veillonellaceae (13.43%)
- Streptococcaceae (8.96%)
- Lachnospiraceae (4.48%)

Following transplantation, the microbial community became dominated by:

- Bifidobacteriaceae (39.10%)
- Bacteroidaceae (35.02%)
- Coriobacteriaceae (6.53%)
- Enterobacteriaceae (6.02%)

Notably, the abundance of Enterobacteriaceae decreased substantially, while beneficial Bifidobacteriaceae became the dominant family.

3.3.2.3 Species-Level Changes

The pre-transplant microbiota was dominated by:

- Morganella morganii (1.82%)
- Prevotella copri (1.79%)
- Streptococcus salivarius (1.61%)
- Mannheimia varigena (1.27%)
- Clostridium ramosum (1.21%)

Post-transplant samples demonstrated enrichment of:

- Bacteroides plebeius (28.87%)
- Bifidobacterium longum (9.48%)
- Collinsella aerofaciens (6.51%)
- Bacteroides coprocola (4.58%)
- Bifidobacterium catenulatum (4.25%)

The increased abundance of *Bifidobacterium* species suggests recovery of beneficial commensal bacteria and potential restoration of microbial homeostasis.

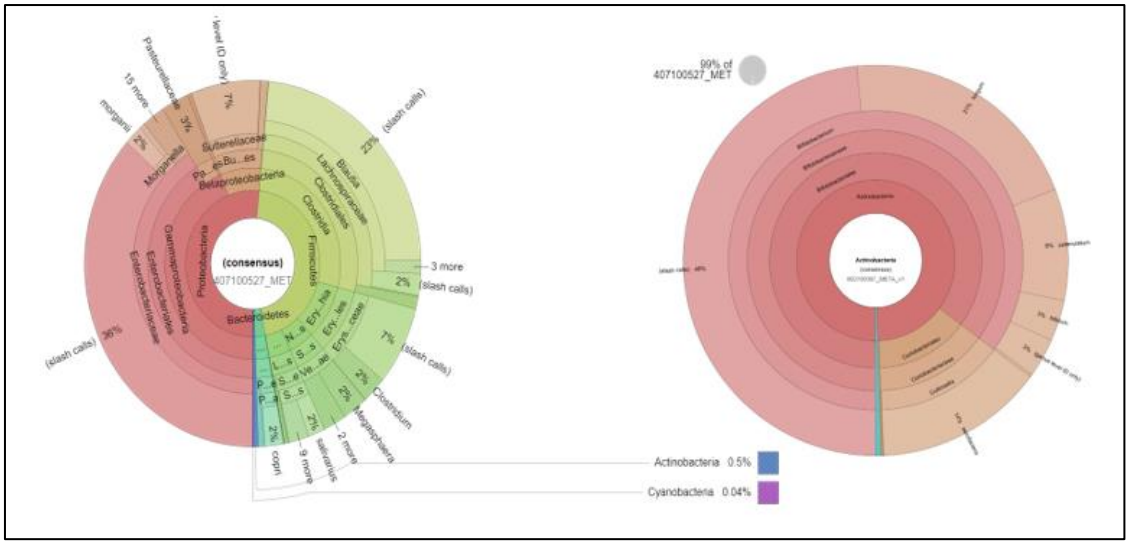


Figure 3: Krona charts showing gut microbiota composition in Patient 2 before (A) and after (B) autologous bone marrow transplantation.

3.4 Comparative Analysis of Both Patients

Despite receiving similar treatment regimens, both patients exhibited distinct microbiota responses following autologous transplantation.

Patient 1 demonstrated severe dysbiosis characterized by:

- Loss of Bacteroidetes
- Expansion of Firmicutes and Proteobacteria
- Dominance of Enterococcaceae
- Reduction of butyrate-producing bacteria

In contrast, Patient 2 exhibited:

- Increased abundance of Actinobacteria and Bacteroidetes
- Expansion of Bifidobacteriaceae
- Reduction of Enterobacteriaceae
- Increased representation of beneficial commensal microorganisms

These observations indicate substantial inter-individual variability in microbiota responses following autologous BMT.

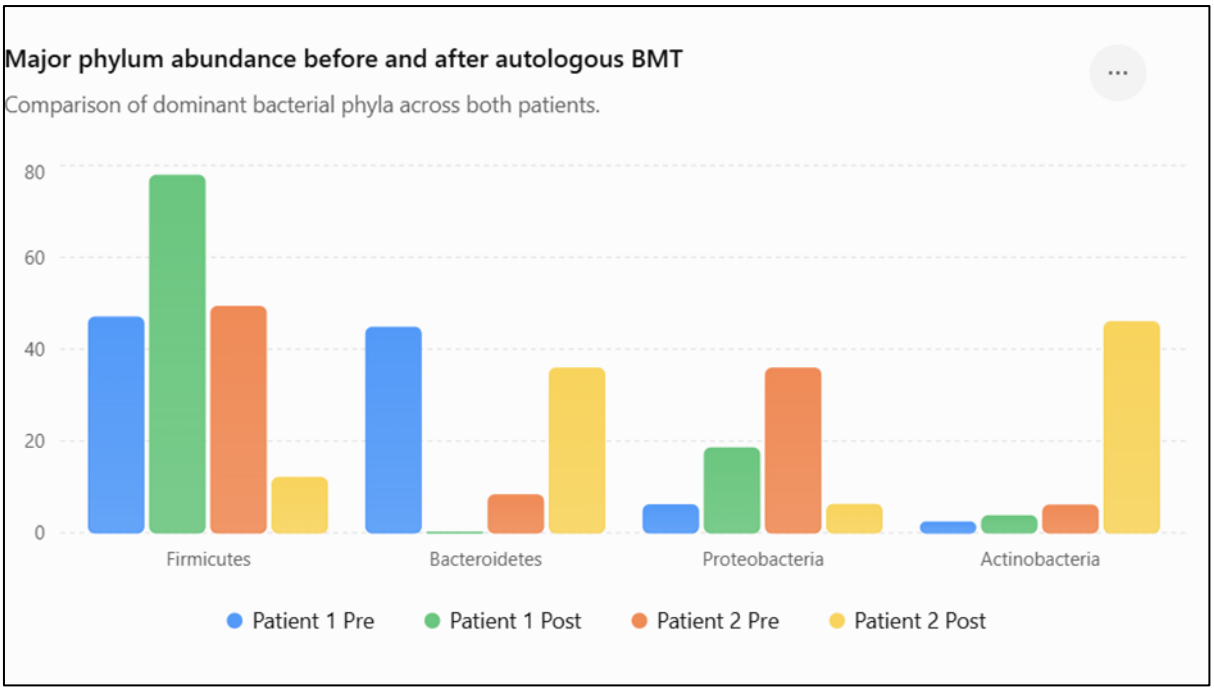


Figure 4: Relative abundance of major bacterial phyla in pre- and post-transplant fecal samples from two Multiple Myeloma patients undergoing autologous bone marrow transplantation.

3.5 Overall Interpretation of Findings

The present study demonstrated that autologous bone marrow transplantation is associated with significant alterations in gut microbial composition. Both patients experienced considerable microbiota restructuring during the first month following transplantation. However, the direction and magnitude of microbial changes differed between patients.

Patient 1 exhibited a dysbiotic microbial profile characterized by depletion of beneficial anaerobic bacteria and enrichment of opportunistic taxa such as *Enterococcus faecium*. Such patterns have been associated with antibiotic exposure, mucosal injury, and immune perturbation during transplantation.

Conversely, Patient 2 demonstrated enrichment of beneficial taxa including *Bifidobacterium longum* and *Bifidobacterium catenulatum*, suggesting partial microbiota recovery despite intensive treatment.

Collectively, these findings indicate that autologous BMT profoundly influences intestinal microbial ecology and highlight the potential importance of microbiome monitoring in multiple myeloma patients undergoing transplantation.

4 DISCUSSION

The present pilot study investigated gut microbiota alterations before and after autologous bone marrow transplantation (BMT) in two patients with Multiple Myeloma using 16S rRNA gene sequencing. Significant changes in microbial composition were observed following transplantation, indicating substantial microbiota restructuring during the early post-transplant period.

Patient 1 demonstrated marked dysbiosis characterized by depletion of Bacteroidetes and enrichment of Firmicutes and Proteobacteria. Increased abundance of Enterococcaceae and Enterobacteriaceae, together with reduction of beneficial butyrate-producing bacteria such as *Faecalibacterium*, *Roseburia*, and *Ruminococcus*, suggests disruption of intestinal microbial homeostasis. Similar findings have been reported in transplantation studies where antibiotic exposure and chemotherapy promote expansion of opportunistic microorganisms and loss of protective commensal bacteria [8,9].

In contrast, Patient 2 exhibited increased abundance of Actinobacteria and Bacteroidetes following transplantation, with enrichment of *Bifidobacterium longum* and other beneficial commensal bacteria. The increase in Bifidobacteriaceae and reduction in Enterobacteriaceae may indicate partial restoration of microbial balance and a more favorable post-transplant microbial profile.

Despite receiving similar treatment regimens, both patients displayed distinct microbiota responses, highlighting considerable inter-individual variability. Differences in baseline microbial composition, host immune status, antimicrobial exposure, and environmental factors may contribute to these variations. These findings support previous reports suggesting that gut microbiota recovery following transplantation is highly individualized.

This study has several limitations, including the small sample size and assessment at a single post-transplant time point. Additionally, 16S rRNA sequencing provides taxonomic information but does not evaluate microbial function or metabolite production. Therefore, larger longitudinal studies are needed to validate these findings and determine their clinical significance.

Overall, the results demonstrate that autologous BMT can substantially alter gut microbial composition in Multiple Myeloma patients. Monitoring microbiota dynamics may improve understanding of post-transplant recovery and support the development of microbiome-based therapeutic strategies in transplantation settings.

5 CONCLUSION

This pilot study demonstrates that autologous bone marrow transplantation (BMT) is associated with significant alterations in gut microbiota composition in patients with Multiple Myeloma. Comparative analysis of pre-transplant and post-transplant fecal samples revealed substantial microbial restructuring, including changes in bacterial diversity and taxonomic abundance. Patient 1 exhibited pronounced dysbiosis characterized by expansion of opportunistic taxa and depletion of beneficial commensal microorganisms, whereas Patient 2 showed enrichment of beneficial bacteria, particularly members of the family *Bifidobacteriaceae*. These findings highlight the considerable inter-individual variability in microbiota responses following transplantation.

The observed reduction of beneficial butyrate-producing bacteria and shifts in dominant microbial taxa suggest that conditioning chemotherapy, antimicrobial exposure, and transplantation-related factors can profoundly influence intestinal microbial ecology. Although limited by the small sample size, this study provides preliminary evidence that gut microbiota dynamics may play an important role in post-transplant recovery in Multiple Myeloma patients undergoing autologous BMT.

Further large-scale longitudinal studies incorporating functional metagenomic analyses and extended follow-up are required to better understand the clinical significance of these microbial alterations and to explore the potential of microbiome-based therapeutic interventions for improving transplantation outcomes.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

Statement of ethical approval

The study protocol was approved by the Institutional Ethics Committee of GeneXplore Diagnostics and Research Centre Pvt. Ltd., Ahmedabad, India (IEC Approval No. 004/24). All procedures were performed in accordance with the Declaration of Helsinki and applicable ethical guidelines.

Statement of Informed consent

Informed consent was obtained from all individual participants included in the study.

Data Availability Statement

The datasets generated and analyzed during the current study are part of an ongoing doctoral research project and are available from the corresponding author upon reasonable request.

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