Research article

Effect of Ayurveda in management of dysbiosis with special reference to Bifidobacterium in children with autism spectrum disorders

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ABSTRACT

Introduction and Aim: The prevalence of autism spectrum disorder has risen in the last two decades. Disruption of homeostasis of several species and genus of bacteria in the gut termed as dysbiosis is having a pivotal role in ASD pathophysiology. Invariable correlations between the behavioral severities of ASD and dysbiosis management have been affirmed through various previous studies. Bifidobacterium make a significant species appraised well in microbial studies linked with ASD. A randomized controlled trial was conducted to assess the efficacy of Ayurveda therapies in improving Bifidobacterial abundance in Autism children.

Methodology: The study took place at the VPSV Ayurveda College Kottakkal's outpatient and in-patient units where 60 children with Autism were randomized to two groups. The intervention prolonged 30 days in total, with three rounds of assessment: baseline (0th day), interim (30th day), and final (60th day). Both the test and control groups received conventional multidisciplinary therapies for 60 days and additionally, the test group was given Ayurveda poly-herbal formulations and food and lifestyle instructions for first 30 days. The result was made through GI severity index measurement and 16SrRNA sequencing.

Results: After one month of intervention with poly herbal compounds of Ayurveda along with lifestyle guidelines and conventional therapies a statistically significant improvement observed in the relative abundance of Bifidobacterium in intervention group compared to control group.

Conclusion: Poly herbal compounds of Vilwadi Gulika and Rajanyadi choornam along with the dietary lifestyle guidelines and multidisciplinary interventions has played a big role in improving Bifidobacteria in autistic children.

Keywords: Ayurveda; poly herbal drugs; lifestyle guidelines; microbiome; ASD.

INTRODUCTION

Autism spectrum disorders (ASD) are a heterogeneous group of neurodevelopment impairments marked by deficits in communication, social interaction, and cognitive difficulties (1). A marked increase in the prevalence of ASD cases has been reported for the past decades assisting the claim of an 'autism epidemic' ahead (2). Associations between the Gastrointestinal (GI) problems in autistic children have been ascertained from the increasing shreds of evidence (3) and include symptoms like bloating, stomach discomfort, and diarrhea, burping/belching, gastro esophageal reflux symptoms, constipation, and flatulence (4). According to several pieces of research, the prevalence of gastrointestinal (GI) problems in people with ASD ranges from 9% to 84 percent, depending on the type of evaluation, compared to 9% to 37 percent in children without ASD. (5, 6) The causes of gastrointestinal problems in autistic children are obscure, however, dysbiosis is considered as one (7).

Disruption of homeostasis of several species and genera of bacteria in the gut termed dysbiosis is having a pivotal role in ASD pathophysiology (8). Invariable correlations between the behavioral severities of ASD and dysbiosis management have been affirmed through various previous studies (9). The promising role of the novel approach of prebiotics and probiotics in tackling GI morbidities by upgrading the entire gut microbiome is well established from the prior studies (10). Rather than colonizing and shifting the bacterial populations to the so-called good bacteria, a more comprehensive approach that is cost-effective with more durable benefits including near to the activities of daily living for managing dysbiosis is being considered. The repository of Ayurveda literature has bounteous references for such blanket perspectives enclosing polyherbal compounds along with lifestyle guidelines well applicable for managing...
the multi-pronged factor of dysbiosis. Bifidobacterium makes a significant species appraised well in microbial studies linked with ASD (11). Literature assists on the same have been escalated to such a disposition that alters the gut microbiota predominately Bifidobacterium for the management of autism symptoms (10). Novel approaches as management remedies like enhancing the Bifidobacterium composition in gut microbiota through enhanced functional foods like prebiotics and probiotics are springing up nowadays (12). Many of the medical findings laid intertwined with the emergence of associated industries are foreseen by the scientific community with the apprehension of underlying financial ties (13). Hence for a traditional population, culturally competent and ethnic background emanated alternatives are more enduring even with the disparities in health care access (14). In India, Ayurveda plays such an essential role right from its inception. The primary motive of this study is to assess the impact of drugs and dietary rules and activities of daily living (ADL) in Ayurveda on Bifidobacterium.

MATERIALS AND METHODS

Study setting

The current study is an open-label randomized controlled clinical trial conducted at VPSV Ayurveda College, Kottakkal out-patient and in-patient units. In this study, 60 children with ASD were randomly assigned to one of the two groups with 1:1 allocation. The intervention was prolonged 30 days in total, with three rounds of assessment: baseline (0th day), interim (30th day), and final (60th day; Table 1) lists the specifics of the trial schedule. The research protocol for the entire study has already been published in a reputed journal (15).

Eligibility criteria

Inclusion criteria

Children of all gender, between the ages of three and twelve who have been diagnosed with ASD, according to DSM-V criteria (16) were chosen. Children’s ethnicity was also checked to ensure that they had similar food patterns and lived in a similar environment.

Exclusion criteria

Children on antibiotics (within three months), antiepileptic medicines, probiotics, or ASD medications from any system of medicines including Ayurveda were excluded. Children having co-morbid problems such as cerebral palsy, mental retardation, or any other chronic neurological or metabolic diseases, as well as those in coma or paralysis, were excluded from the study.

Table 1: Specifics of trial schedule

<table>
<thead>
<tr>
<th>STUDY PERIOD</th>
<th>Time Period</th>
<th>Enrolment</th>
<th>Baseline phase 0</th>
<th>Interim phase 1</th>
<th>Final phase 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening</td>
<td>Preliminary data</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Physical examination</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eligibility screening</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Informed consent</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Allocation</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td>Poly-herbal formulations</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Parental guidelines</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lifestyle guidelines</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Interdisciplinary intervention</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Assessment</td>
<td>16SrRNA sequencing</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>
### Table 2: Multi modular interventions

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Particulars</th>
<th>Intervention Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sample Size</td>
<td>30</td>
<td>30</td>
</tr>
</tbody>
</table>
| 2     | Internal Administration and duration | 1. Rajanyadi churnam  
2. Vilwadigutika  
Duration – 30 days | NIL           |
| 3     | Dose                              | As per Ayurveda Posology drug is administered with Lukewarm water thrice daily, 30 minutes before meals | NA            |
| 4     | Other Interventions and duration   | Speech Therapy, Occupational Therapy and Behavioral Therapy  
Duration – 2 months | Speech Therapy, Occupational Therapy  
Behavioral Therapy  
Duration – 2 months |
| 5     | Diet modifications                 | As per the food and lifestyle guidelines mentioned in Ayurveda                       | NA            |

### Intervention

According to the group allocation, the study participants received a multi-modal intervention that included poly-herbal Ayurveda formulations, lifestyle guidelines, parental guidelines, and an interdisciplinary intervention (17). Pill counting, diary entry, and intermediate phone calls were used to assure patient involvement in the trial setting, and the interventions were carried out in two groups of 30 participants each. For two months, both the test and control groups received multidisciplinary therapies (Speech Therapy, Occupational Therapy, and Behavioral Therapy) and additionally, the intervention group was given Ayurveda poly-herbal formulations (Rajanyadi Churna, Vilwadi Guilka) following Ayurveda posology principles and food and lifestyle instructions for 30 days (Table 2).

### Primary and secondary outcome measures

During each visit, data were collected from the participants’ parents at the baseline (0th day), interim (30th day), and follow-up (60th day). The primary outcome assessed was the relative abundance of microbiomes present in the gut done by using 16S rRNA sequencing as well as the Alpha and Beta diversity of the gut microbiome on an Illumina HiSeq2500/Miseq to create 0.5 million 2x250 bp reads for each participant's fecal sample. DNA isolation and purification with the QiAmp mini stool kit, followed by quality check and sequence processing by QIIME (Quantitative Insight into Microbiological Ecology), was used for 16S rRNA illumina sequencing and from fungal, viral, bacterial, and archaeal populations, nucleic acid sequence data was analysed and interpreted. It covers a wide range of subjects, including sequence processing, alpha-diversity, beta-diversity, and taxonomic composition (18). The secondary outcome assessed was the changes in the organism of Bifidobacterium in the test group compared to the control group after treatment and thus to execute a comprehensive and least expensive autism management strategy in Ayurveda that can be implemented autonomously. Patient recruitment began in May 2016. In October 2020, the primary and secondary outcome measure was completed and in December 2020 the study was completed.

### Sample size

The study was done with a completely randomized design with 2 treatments. For a completely randomized design, the error degrees of freedom are \( t(r-1) \), where \( t \) is the number of treatments and \( r \) is the number of replications. The number of replications was 7 with two treatments, with a minimum required error degrees of freedom of twelve. However, because of the trial's novelty and the lack of prior information on the error variance, the number of replications for each treatment is put as 30. The precision of treatment comparison is expected to improve much more with repeated evaluations. After getting consent from the parents, they were randomly assigned to one of the two groups - an intervention group and a control group, each with 30 participants using computer-generated randomization (1:1; Fig.1)
Recruitment

During their visit to the hospital, the parents of children with ASD were approached to join in the trial with a participant information file that detailed the aim of the study, the procedures to be followed, and the risks and advantages of participation. Following that, screening was performed to ensure that the inclusion and exclusion criteria were met. After a brief conversation about the trial, the informed assent was taken. Participants were free to leave the study at any time and the reason for doing so was also documented.

Data collection methods

After recruitment, screening was done, and the data collected was entered on a paper-based Case Report Form. Data collection and assessment were repeated during the Baseline period, the Interim period (30th day), and the final phase (60th day). Stool samples were collected in sterile premises and were stored at a particular temperature of -40°C with care to avoid disruption in the cold chain. Later, the specimens were sent to Med Genome Labs, Bangalore, for the isolation and sequencing techniques maintaining the cold chain.

Data management

The preliminary data and assessments collected on a paper-based case report form were evaluated and approved by the Institutional Ethical Committee (IEC).

Statistical analysis

ANOVA was used in the statistical analysis of the primary result comparing the species of Bifidobacterium before and after treatment. Operational Taxonomic Unit (OTU) table, Principal Component Analysis Plot (PCA plot), Alpha and Beta Diversity through 16S rRNA sequencing was used to classify and sequence the microbiome.

Data monitoring

The Institute's National Pharmacovigilance Unit – Vidyaratnam P.S.Varier Ayurveda College monitored the study for any adverse impacts and to avoid any confounding factors, participants of the same ethnicity and food behaviors were only included.

Ethics and dissemination

On May 4, 2017, the IEC of Vaidya Ratnam P S Varier Ayurveda College, India (Proceedings No: IEC/CI/24/17) accepted the protocol for this clinical trial after an initial peer review. This is a short communication extracted from an entire study that was registered with the Clinical Trial Registry of India (CTRI) on May 21, 2018 (registration number CTRI/2018/05/014017).

Informed consent
After describing every requirement of the trial procedure, the parents of concerned participants gave their consent. Throughout the study, the individuals' privacy was maintained, and the study followed the Declaration of Helsinki's ethical principles, the International Conference on Harmonization's Good Clinical Practice Guidelines (ICH-GCP), and all applicable laws and regulations, such as Data Safety Management and Clinical Trial Disclosure Laws and Regulations.

RESULTS

The gender-wise distribution of children is given in (Table 3). As shown 71% of children were male and 29% female in intervention group, whereas in control group, 6% were male and 34% female.

Table 3: Gender wise distribution

<table>
<thead>
<tr>
<th>Details</th>
<th>Intervention</th>
<th>Control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>08</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td>Male</td>
<td>22</td>
<td>20</td>
<td>42</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>30</td>
<td>60</td>
</tr>
</tbody>
</table>

In socio-economic status wise distribution, most children belonged to Kuppuswamy score 4 (Table 4). In intervention group, 73% of deliveries were vaginal delivery and 27% by caesarean section whereas in control group, 70% were vaginal delivery and 30% were by caesarean section (Table 5).

Table 4: Socio-economic status

<table>
<thead>
<tr>
<th>Details</th>
<th>Intervention</th>
<th>Control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monthly Income</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>less than 10,002 (KSS-1)</td>
<td>02</td>
<td>00</td>
<td>02</td>
</tr>
<tr>
<td>&quot;10,002-29,972 (KSS-2)&quot;</td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>29,973-49,962 (KSS-3)</td>
<td>10</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>49,962-74,755 (KSS-4)</td>
<td>9</td>
<td>17</td>
<td>26</td>
</tr>
<tr>
<td>99,931-199,862 (KSS-10)</td>
<td>03</td>
<td>03</td>
<td>06</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>30</td>
<td>60</td>
</tr>
</tbody>
</table>

Table 5: Mode of delivery

<table>
<thead>
<tr>
<th>Details</th>
<th>Intervention</th>
<th>Control</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mode of Delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>22</td>
<td>21</td>
<td>43</td>
</tr>
<tr>
<td>LSCS</td>
<td>8</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>30</td>
<td>60</td>
</tr>
</tbody>
</table>

Top ten genus present in the samples were Faecalibacterium, Bifidobacterium, Prevotella, Roseburia, Bacteroides, Escherichia, Shigella, Blautia, Collinsella, Ruminococcaceae, and Christensenellaceae (Fig. 2).

Among them, the abundance of significant taxa bifidobacterium after treatment in control and intervention groups are shown in Fig.3a. It was relatively more abundant in intervention group when compared with controlled group.

Fig.3b represents the bifidobacterial presence during follow up period. In control group, no improvement was noticed, where as in test group, the increased abundance was maintained. In Fig.3c, the comparison was done between the after-treatment and follow up period in control group. An increase in bifidobacteria was noted in control group after one month, but it was reduced during follow up period.
DISCUSSION

Striking consistency of sexually dimorphic disease prevalence in ASD with male bias is well recognized.
from the already existing data (19). Herein this research study also with the presence of 71 and 66 percent of males in the intervention and control group, goes under the general inference on gender with male predominance. From major studies on ASD children, it was found that most autistic children are born to parents with high economic high socio-demographic profiles (20). However, in contrary has happened in this trial with most of the prevalence belonging to the middle class. Possible grounds for the same may be that most of the population in Kerala belongs to the middle class and the economic and social polarization is also the least here in the state. With increased accessibility to the health care facilities in the state, this studied population yet cannot be taken as a representative sample rather is of the district of Malappuram where the study setting was done. With 27 and 30 percent of study samples having Caesarean section as the mode of delivery, this goes per the average LSCS rate of the state. Association of higher rates of autism with Caesarean delivery has already been reported in many of the previous studies (21).

The Plotted results are representations of statistically significant change among the "In between group" and "single group" analyses. A P-value less than 0.05 is used to filter the significant species across the groups. Most studies support the relative abundance of Bifidobacterium is phenotypically good for autistic features (22). The relative abundance of Bifidobacterium is observed to increase after one month of intervention in the studied population. As the phenotypical character of ASD is not defined by the reduction or improvement of a particular organism, the phenotypical explanation is given only by observing the changes that occurred in Bifidobacteria is not a good trend. The administered medicine could help to improve the gut health of children. There might be an improvement in Phenotypical features as well. Increased abundance of Bifidobacteria is observed not only in the children treated with Ayurveda medicines but in the children given other therapies as well. This means the relative abundance of microorganisms can be altered by other means too.

A healthy gut is determined by multiple factors. Supplying medicine that can improve gut health is just one of them. The relative abundance of any bacterial colony cannot be maintained only by providing the same organisms or the probiotics. The presence of one organism also might have a role in determining the increased or decreased presence of another organism. Here the abundance is increased in the control group after one month of assessment. This indicates that the changes can occur even without administering medicines. So, the determination of gut health should not be done by evaluating a single organism. They must have a harmonious cohabitation in a well-maintained ecosystem. Certain studies have explained how the introduction of wolves determined the abundance of herbivore and vegetation abundance in the yellow stone national park (23) Maintaining an ecosystem is very important in a micro-level community as well. Diet has a significant impact on the bacterial flora composition by defining its richness and diversity and the changes are detectable in humans within 24 to 48 hours following dietary intake, regardless of body weight or adiposity (24). Improper dietary habits can lead to dysbiosis. Ayurveda brought forth an immense consciousness on food habits which leads to health (25). Bifidobacterium being a microbiome present in the healthy gut, the growth observed in the studied sample could be inferred that diet as well played a role in its abundance.

In the current study, a significant improvement was observed in bifidobacterial abundance in the test group after one month of assessment compared to the control group. The increased amount is persisting after one month of follow-up in the test group, but a reduction is observed in the control group. The drastic change that occurred in a single bacterial colony is not negligible. So, it can be inferred that polyherbal administration has played a big role in improving Bifidobacteria.

**CONCLUSION**

After one month of intervention with polyherbal compounds of Ayurveda along with lifestyle guidelines and conventional therapies such as occupational therapy, speech therapy, and behavioral therapy, a statistically significant improvement was observed in the relative abundance of Bifidobacterium in the intervention group compared to the control group where only behavioral therapy, occupational therapy, and speech therapy is administered. This increased relative abundance is maintained even after one month of follow-up in the intervention group but that was found to be reduced in the control group.

**ACKNOWLEDGEMENT**

The Ministry of Ayurveda, Yoga & Naturopathy, Unani, Siddha and Homeopathy, Government of India (grant no. Z28015/22/2018-HPC (EMR)-AYUSH-A) has provided the financial support as grant-in-aid for the project. This study is a short communication extracted from this.

**CONFLICT OF INTEREST**

There is no conflict of interest.

**REFERENCES**

Dinesh et al: Effect of Ayurveda in management of dysbiosis …… with autism spectrum disorders


