Effect of Probiotic supplementation on the fasting blood glucose and immune status in Type II diabetes mellitus.

M Shiva Prakash¹*, P Amruth Rao², K B Chathyushya,³ G Haricharan⁴, G Madhavi⁵, G Sumalata⁶, M Sravanti⁷, M Mounika⁸, P Sai Tejaswi⁹ & R Hemalatha¹⁰

1,2,3,5,6&10 Department of Microbiology & Immunology, National Institute of Nutrition (ICMR), Jamai-Osmania (P.O), Tarnaka, Hyderabad-07, Telangana, India.

4 Seasons Hospitals, Street no 7, Vaibhav Nagar Colony, Bagh Amberpet, Hyderabad-13, Telangana.

7-9 Project Trainees, KL University, Green Fields, Vaddeswaram, Guntur-02, A.P, India

Abstract: Diabetes mellitus is a worldwide, affecting almost all major sections of society and have an association with cell mediated responses (CMI). Adenosine deaminase (ADA), an enzyme which is a good marker of cell mediated immunity. Probiotics have recently emerged as the prospective bio therapeutics with proven efficacy. The present study is to assess the effect of probiotic curd on blood glucose levels and immune status in diabetes mellitus. A total of about 50 individuals in the age group between 30-50 years of either sex were selected and divided into two groups i.e. with and without diabetes. The subjects in the intervention group were supplemented with 100 gm/day of probiotic curd (Lactobacillus bulgaricus & Streptococcus thermophilus). The fasting blood samples were analyzed for glucose along with ADA before and after supplementation. The results indicated that there was a significant decrease in the blood glucose levels in the supplemented group in comparison with non-supplemented group of diabetes. It has been concluded that Probiotic curd has a significant role in the management of blood glucose and on immune status in Diabetes mellitus.

Key Words: Type-II Diabetes, Probiotics, Adenosine deaminase.

1. Introduction

Diabetes is a major metabolic syndrome affecting almost all socioeconomic groups worldwide. It is of multifactorial origin, including genetic and environmental factors. As of 2014, it was estimated that about 387 million people have diabetes worldwide, with type 2 diabetes of about 90% of the cases. Immunological disturbances in type 2 Diabetes Mellitus have an association with cell mediated responses and inappropriate T-lymphocyte function which is vital in this pathogenic condition and has a link with insulin defect [1].

Adenosine deaminase (ADA) is an enzyme distributed in all the human tissues and considered as an important immuno-enzyme marker of cell mediated immunity [2]. Probiotics are recently emerged as the prospective immuno-enzyme marker of cell mediated immunity [2]. Probiotics have recently emerged as the prospective bio therapeutics with proven efficacy demonstrated in various in vitro and in vivo animal models adequately supported with their established multifunctional roles and mechanism of action for the prevention and disease treatment [3]. Few studies were found the Lactic Acid Bacteria (LAB) as a treatment for diabetes especially in experimental animals, despite the evidence showing that they have the potential to reduce the incidence of diabetes [4,5,6].

This study highlighted the new developments in probiotic interventions and prospects for exploring probiotherapy in the prevention and/or control of lifestyle diseases like T2D.

2. Materials & Methods

2.1. Preparation of Probiotic Curd:

The Probiotic curd was prepared by fermenting the pasteurized milk with Lactobacillus bulgaricus (UBLB-38) (1x10⁶ cfu/gm) and Streptococcus thermophilus (UBST-50) (1x10⁷ cfu/gm) for 5-6 hours at 40-45°C.

Photograph showing 100g probiotic curd container box supplied for subjects under study
2.2. Selection of subjects

2.2.1. Inclusion Criteria:

Subjects with not less than 1 year onset of diabetes and no change in oral anti-diabetic medications during the last 6 months

*Age 30 to 60 years

*Provision of written informed consent

Exclusion criteria:

*Chronic gastrointestinal disease (except irritable bowel syndrome)

*Antibiotics and probiotics usage within 6 weeks before inclusion

*Regular intake of insulin or insulin analogues.

*Other secondary health complications such as hypertension, Cardio Vascular Diseases and Thyroid were excluded from the study.

2.2.2. Study Design:

This is a case-control study in which a total of about 50 individuals in the age group between 30-50 years of either sex was recruited in and around Hyderabad. These were divided into two groups i.e. with (Group A) and without (Group B) Type-2 Diabetes. The subjects with Diabetes are sub grouped as Group A1 (Supplemented) and Group A2 (Non-Supplemented). All the individuals were allowed to take their regular diet.

2.3. Supplementation:

The subjects in Group A1 were supplemented with about 100gm/day of freshly prepared probiotic curd (LB+ST) for 30days. They were advised to stop consuming alcohol. Group A2 (non-supplemented) individuals are asked to take milk and/or other curds along with their regular diet. Both the groups are advised to continue their regular medication.

2.4. Laboratory Investigations:

About 2 ml of fasting blood samples were drawn into vacutainer tubes at before and after supplementation and subjected to centrifugation at 3,000 rpm for 10 min for serum separation. The Blood Glucose test [7] and ADA estimation [8] were carried out using commercially available kits.

2.5. Subject withdrawal:

Subjects were given the liberty to withdraw at any point of time in case of any inconvenience during the study.

2.5. Statistical analysis:

The results obtained were tabulated for the values of Mean ± SD and statistically analyzed with paired t-test using computer software, SPSS version 12.0.

3. Result:

The demographic and physical characteristics of the recruited subjects have not shown any significant difference between the intervention group and control group (Table-1).

It was found that there were elevated levels in Fasting Blood Glucose in the diabetes subjects compared to Control Group at ‘0’day. When the Fasting Blood sugar levels in the supplemented Group were compared with Non-Supplemented and Control Group, there was a significant decrease after probiotic supplementation indicating the additive effect of Probiotics along with their regular treatment in the management of Blood Glucose Levels.

It was also observed that ADA levels are elevated levels in the above diabetic subjects when compared to healthy controls. In the supplemented group the ADA levels were significantly decreased but not to the extent of normal levels. In non-supplemented group the ADA levels did not show any significant changes between 0 & 30 days. However, there was no significant difference in healthy controls.

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group A1</th>
<th>Group A2</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Subjects</td>
<td>18</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>Males</td>
<td>11</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Females</td>
<td>7</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Age (Mean SD)</td>
<td>53.4±6.81</td>
<td>54.8±2.97</td>
<td>52±3.91</td>
</tr>
<tr>
<td>Weight (Kgs) (Mean SD)</td>
<td>73.04±8.6</td>
<td>76.4±5.25</td>
<td>70.2±5.7</td>
</tr>
<tr>
<td>Height (cm) (Mean SD)</td>
<td>163.6±6.9</td>
<td>164.3±5.2</td>
<td>162.2±2.9</td>
</tr>
<tr>
<td>BMI (Mean SD)</td>
<td>27.2 ±2.45</td>
<td>28.21±1.7</td>
<td>26.6±1.9</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of Subjects</th>
<th>Blood Glucose 0 Day</th>
<th>Blood Glucose 30 Day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A1 (Diabetic Supplemented)</td>
<td>18</td>
<td>157.55±83.99</td>
<td>134.5±40.7</td>
</tr>
<tr>
<td>Group A2 (Diabetic Non-Supplemented)</td>
<td>18</td>
<td>172.8±73.6</td>
<td>203.7±84.7</td>
</tr>
</tbody>
</table>

*a* no significant difference between Subjects and controls
Probiotics are live microorganisms which confer health benefits when taken in adequate amount (WHO) [9]. Modulation of intestinal microbiota by probiotics may facilitate the management of many clinical conditions [10]. Probiotics are also involved in the maintenance of a healthier gut microbiota, and have also been identified as effective adjuvants in insulin resistance therapies [11,12]. These are often proved for their different beneficial effects as anti-diarrheal [13], anti-cancerous [14], antimicrobial [15], hypocholesteremia [16] and anti-diabetic etc. In a study, Lactobacillus GG was shown the antidiabetic effect in diabetic rats [17]. In other study conducted in Saudi Arabia, a combination of probiotics has shown the potential benefits on circulating endotoxin levels and other markers for systemic low-grade inflammation in patients with T2DM [18]. Earlier our own study has shown the effect of probiotic curd on the management of lipid profile on obese subjects [19]. The mechanisms associated with gut flora–mediated pathology of obesity and diabetes are through (1) increased energy harvest, (2) increased blood LPS levels (endotoxemia), and (3) low-grade inflammation [20]. Therefore, modulation of gut flora has been considered as a potential target to treat against diabetes associated obesity.

In a recent study conducted by researchers of Cornell University discovered a probiotic, commonly found in the human gut, can 'rewire' the body to help combat diabetes. The probiotic treatment lowered diabetic rats' blood glucose levels by up to 30%. Scientists say higher doses could reverse both type 1 and 2 diabetes.

In the same study done by the scientists of Cornell University it was observed that the serum ADA activity was increased with an increase in insulin resistance in the diabetic population. ADA may be used as a marker of insulin resistance and can be employed as an effective tool in screening for insulin resistance in diabetes mellitus.

In addition to the above data the present study showed the additive effect of probiotic curd along with the regular medication in the maintenance of blood glucose levels and immunomodulation in the subjects with type-2 diabetes mellitus. It can be noted that since there are a number of probiotics available with large and narrow spectrum of activity, different combinations and prolonged period of supplementation may be tried for better beneficial effects.

5. Conclusion

Diabetes can usually be controlled with the right diet and medication. If we keep a close eye on the disease and our blood glucose levels we can greatly minimize the risk of further complications. Probiotics are GRAS (Generally Recognized As Safe) microorganisms which can be used for the management of diabetes. From this study it can be concluded that Probiotic Curd (Indian Dahi) has immense beneficial effect in the management of T2 DM, which has dual affect i.e. Blood Glucose and immune status. However, it is advisable to take food based probiotic supplements rather than drug based to obtain better beneficial effects in DM subjects.

It also be concluded that supplementation of probiotic curd eventually maintained the levels of ADA and thus imparting immuno-modulatory activity. The data also suggests that an increasing effort should be made to understand the cellular and molecular mechanisms behind the role played by probiotic organisms for identifying their other benefits.

The detailed studies in this area will bring newer dimensions to the projected area of safe and natural probiotic microflora. Our studies are providing a foundation that may lead to new approaches for diagnosing, preventing and treating diabetes mellitus in addition to development of new nutritional formulations. Our findings also give a scope to further carry out studies on large number of diabetic subjects and to evaluate the beneficial role of Probiotic microflora for better management of diabetes mellitus.
6. Acknowledgements

We wish to acknowledge Dr (Mrs) Ratna Sudha Managing Director of Unique Biotech Private Limited, Hyderabad, India for supplying the required probiotics at free of cost. We wish to thank Mr. T. Longvah, the Director-in-charge, NIN, for encouraging the present study.

7. References

19. Shiva Prakash M, Madhavi.G and Hemalatha.R. Effect of supplementation of...
probiotic curd on lipid profile in obese subjects. IJFANS Vol.3, Iss.4, Jul-Sep 2014. Pg.no 148-152.