Cover Page

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A STUDY ON THE IMMUNOMODULATION EFFECT OF PROBIOTIC CURD IN OBESE SUBJECTS

[1]Sumalata G, [2]Madhavi G, [3]Chathyushya K B and [4]Shiva Prakash M
[1, 2]Research Scholar (ICMR-SRF), [3]Research Scholar (DBT-SRF) and [4]Former Scientist
[1,2&3]Clinical Epidemiology and [4]Department of Microbiology
[1,2,3&4]National Institute of Nutrition (ICMR), Jamai-Osmania
P.O. Tarnaka, Hyderabad, Telangana, India

Abstract

Immunological imbalance is one of the key factors associated with the metabolic disturbances in obese individuals. The present study is taken up to assess the effect of supplementation of Probiotic curd on immunity biomarker, plasma Adenosine deaminase (ADA) in obese subjects. About 30 obese subjects were recruited and categorised as experimental (Group A, n = 18) and control (Group B, n =12) groups. The group A was further subdivided into below normal (<15 U/L), normal (15-25 U/L) and above normal (>25 U/L) based on initial plasma ADA levels. Further the Probiotic curd fermented with strains of Lactobacillus bulgaricus and Streptococcus thermophilus was supplemented to group A for 15 days and the study had been followed up to 30 days. Plasma ADA levels were analyzed for all the subjects before and after supplementation in all three groups along with the control group. The data obtained was tabulated for the mean values and statistically analysed using independent sample t-test. The results depicted significant changes in ADA levels after supplementation of curds i.e. in below normal and above normal obese subjects the plasma ADA reverted to normal levels. The study concluded that the developed Probiotic curd of specific bacterial strains i.e., Lactobacillus bulgaricus and Streptococcus thermophilus in a definite proportion helped to improve immune status in obese subjects.

Keywords: Obesity, Immune Response, Probiotics, Lactobacillus, Adenosine Deaminase, Probiotic Curd.

Introduction

Over the past decade there has been an increased interest in natural food supplements. Probiotics is one of the categories of food supplements that received great attention during recent times. Probiotic strains viz. Lactobacillus acidophilus and bifidobacteria have been used for many years in food products for their health benefits including therapeutic nature [1], safety [2] and immune enhancing attributes [3, 4].

Obesity constitutes a risk factor in a number of chronic diseases viz diabetes, cardio vascular diseases, and cancers etc., and it is reported that compared to lean people these subjects are more susceptible to infections [5]. The epidemiological and clinical data has proven that the severity and incidence of some specific illnesses like bacteraemia, are found to be higher in obese when compared to lean individuals in addition to poor antibody responses to antigens [6]. It is well known that the only and better treatment for obesity is weight reduction by dieting or exercise. However recently it is more emphasized to include probiotics in the treatment of obesity. Earlier studies have revealed that such treatment with probiotics may help to improve immune functions. Therefore, such studies may be needed to understand the role of probiotics on immune response in obese individuals.

Adenosine deaminase (ADA) is considered as an enzyme involved in the metabolism of purine which catalyses in the deamination of adenosine to inosine and 2'deoxyadenosine to 2'deoxyinosine. It is found that this ADA is present in human tissues and shows its activity in lymphoid tissue particularly those containing high proportion of T lymphocytes [7]. As a known marker of cellular immunity, the plasma activity of ADA is observed to be more in some diseases where there is a cell-mediated immune response [8][9]. Therefore, the present study has been focused on the immunomodulation effect of the developed probiotic curd using ADA as an immunoenzyme marker in obese individuals.

Cover Page

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Methodology

Preparation of Probiotic Curd:

The probiotic curd was prepared by using standard procedure as described below in the form of flow chart:

Pasteurization (for 15 min.) of double Toned Milk

↓

Cooled milk to 40-50 °C and dispensed into 200ml steel cups

↓

Inoculation with probiotic starter cultures (L. bulgaricus + S. thermophilus)

↓

Incubation at 37 °C for 12-24 hrs.

↓

Formation of Probiotic curd

↓

Storage at 4°C

After development of the product, Sensory attributes were evaluated using Hedonic 5-point scale scoring system and also tested for viability of probiotics upon storage at 4^0c to 8^0c during 1, 2, 3, and 4 weeks.

Selection of subjects

A total of about Thirty (30) individuals with Body Mass Index (BMI) 27.5 and above (Asian classification of BMI) considered as obese of the age group between 25-55 years were recruited from some of the selected areas in Hyderabad city, Telangana.

The Institutional ethical committee approval and informed consent from the participant subjects was obtained before commencement of the study.

Inclusion and exclusion criteria: The subjects with secondary complications of overweight related problems like diabetes, thyroid disorder, and cardiovascular disease, etc. were excluded from study. Participant subjects should not be on antibiotic treatment during the study.

Supplementation

The participant subjects were categorised into two groups, Group A treated as Experimental, and Group B as control group. Group A consisted of 18 individuals were supplemented with freshly prepared probiotic curd of about 200 gms/ per day prepared by fermentation of skimmed milk with bacterial strains viz. Lactobacillus bulgaricus and Streptococcus thermophilus. During the study Experimental group (Group A) was advised to stop taking their homemade curds, however, the control group (Group B) were allowed to take their homemade curds along with the regular diets. Blood samples were drawn for analysis of ADA before, during and after supplementation (i.e., 0 day, 15^{th}day and 30th day). The ADA levels were estimated in plasma by the method of Guisti G [10]. In the experimental group, the subjects were divided as normal, below normal and above normal based on their initial levels of ADA and were supplemented with aforementioned probiotic curd for 15 days and the study followed up to 30 days.

The results obtained were tabulated for mean ± SD values and statistically analysed using independent sample t-test.

Results

The demographic data of the subjects recruited for the study is presented in table1.The ADA values observed in the experimental group, before and after supplementation i.e., at different time points along with control group ADA values are presented in table 2.

In the experimental group the subgroup with low ADA levels were found to be significantly elevated to normal levels from day 0 (10.05 ± 3.14) today 15 (17.83 ± 2.73), and the same normal levels was maintained even up to day 30 (18.08 ± 2.51). Whereas, in high

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ADA group, it is observed that the levels decreased significantly to normal from day 0 (33.82 ± 9.40) to day 15 (18.34 ± 7.11) and the same normal levels was maintained up to day 30 (23.71 ± 6.26). This may be a clear indication of immunoregulatory effect of probiotic supplementation in these subjects.

In the normal ADA subgroup of experimental group, there is no significant difference in the ADA levels upon supplementation i.e., from day 0 (19.90 ± 3.11) to 15 day (18.43 ± 3.61) and from day 15 to day 30 (23.06 ± 5.49) and ADA values were in the normal levels. In the controls i.e., non-supplemented group, there is no significant difference in the ADA levels at 0 day (23.02 ± 4.44) as compared to that of day 15 (20.7 ± 2.65) and day 30 (22.14 ± 3.85).

TABLE: 1
Characteristics of the obese subjects studied

S. No.	Characteristics	Experimental group	Control group
1	**Number of subjects**	18	12
	Males	10	6
	Females	8	6
2	**Age**	39.45 ± 5.2	37.26 ± 5.6
3	**Weight (kgs)**	86.16 ± 0.73	87.56 ± 14.23
4	**Height (cms)**	167.66 ± 10.23	165.8 ± 11.07
5	**BMI**	33.64 ± 2.31	32.20 ± 2.80

TABLE: 2
Adenosine Deaminase Levels of the Subjects Before & After Supplementation

Category	Number of Subjects	ADA Levels (Mean ± SD)		
		0 Day	15 Day	30 Day
Group A (Supplemented Test Group)	6 (Low ADA)	10.05 ± 3.14[a]	17.83 ± 2.73[b]	18.08 ± 2.51[b]
	7 (High ADA)	33.82 ± 9.40[b]	18.34 ± 7.11[a]	23.71 ± 6.26[a]
	5 (Normal ADA)	19.90 ± 3.11[a]	18.43 ± 3.61[a]	23.06 ± 5.49[a]
Group B (Non supplemented Control group)	12	23.02 ± 4.44[a]	20.7 ± 2.65[a]	22.14 ± 3.85[a]

Normal Values for ADA: 15-25 U/L
* Means with different subscripts in rows indicates statistically significance at P<0.05

Discussion

The results obtained in the present study clearly indicated the immuno-stimulating activity of probiotics by increasing or decreasing the ADA values in low ADA and high ADA groups respectively. At the same point of time, the immuno-modulating activity of probiotics could also be perceived by observing the maintained values of ADA in normal group during the whole study. Moreover, the ADA values on day 30 in all the subgroups of experimental group showed the sustainable and promising role of probiotics on immunomodulation effect in obese subjects.

Our human immune system is considered as a constellation of responses to attack from outside the body and a number of these can change to optimize the response towards these unwanted intrusions [11]. There are numerous reports which suggest the potential immuno-stimulating and immuno-modulating activity of lactic acid bacteria and bifidobacteria in children, adults and the elderly [12]. It is found that though the immunomodulation activity of probiotics is promising, the mechanisms are not well understood [13]. However, it was observed in an earlier study that species of Lactobacillus and Bifidobacterium in the capsules form, has enhanced the peripheral B lymphocyte cells in elderly population. [14].

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ADA deficiency is found to be one of the most severe forms of immuno deficiencies in humans which is mainly associated with severe depletion of all the three major categories of lymphocytes, namely B cells, T cells and NK cells [15]. ADA is found to be essentially needed for proliferation and differentiation of T-lymphocytes. ADA deficiency is considered to affect lymphocytes activation and therefore cell mediated immunity [16]. The increase in ADA levels in the experimental subjects might be due to better availability of substrate in thymus and spleen for the metabolic synthesis of ADA with the presence of probiotic lactobacilli and bifidobacteria in the gut.

Earlier it was demonstrated that yoghurt and fermented milks enhance the immune response [17]. Further it has been observed the effect of feeding yoghurt on the small and large intestine associated lymphoid cells in mice [18]. The role of dietary supplementation of probiotics on immune response was also studied in poultry [19]. The present results in the study are in agreement with our earlier observations where it was reported the effect of supplementation of lactobacilli on immunity and morbidity status of preschool children [20].

Conclusion

It may be concluded from the present study, that probiotic curd supplementation eventually maintained the levels of ADA and thus imparting immuno-modulatory activity. The data also suggests that an increasing effort should be made to decipher the cellular and molecular mechanisms behind the role played by probiotic organisms for identifying their other benefits. The detailed studies in this area will definitely bring newer dimensions to the projected area of safe and natural probiotic microflora.

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Conflicts of Interest: No conflicts of interest.

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Cover Page

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