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RESEARCH ARTICLE

FECAL ENZYMES OF PROBIOTIC SIGNIFICANCE. A COMPARATIVE STUDY ON HEALTHY BREAST FED AND FORMULA FED IN A TERTIARY CARE HOSPITAL

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ABSTRACT

Background: Our study was designed to study the fecal enzyme analysis from healthy infants from Full Term Normal Delivery (FTND).
Methods: The fecal samples were studied for fecal enzyme activity of β-glucuronidase α -galactosidase and β-glucosidase
Results: It was observed during the study that β-glucosidase and β-glucuronidase activity was dependent upon age and type of feed i.e. breast fed infants had an increased level of β-glucosidase activity as compared to formula fed which increased with increase in age.
Conclusion: The fecal enzyme activity was directly linked to the age and diet of the infants.

INTRODUCTION

The intestinal tract of mammals is sterile at birth and the first colonization in the gut is that from the anal and genital tracts of the mother and the environment during the delivery. An adult microflora comprises of more than 400 different bacterial species which work to prevent colonization of the invading pathogens by competing for available substrates and living space within the gut and also by producing bacteriocins[1]. The metabolic activity of flora provides energy and nutrients from fermented complex carbohydrates escaping digestion and also from the secretions of bile or sloughed off mucosal cells. The gut flora colonization depends primarily upon the nutrition type. The children receiving breast milk as their sole food have a predominance of Bifidobacteria, while formula fed have an accumulation of Bacteroides, Streptococci and Clostridia along with Bifidobacteria. Introduction of solid foods and an adult type diet shifts the colonization towards more facultative bacteria, Enterococci, Bacteroides, Clostridia and anaerobic Streptococci[1, 2].

Metabolic activities of the intestinal flora can be studied using fecal samples, intestinal contents and isolated bacteria [3]. Enzymes produced by bacteria can be assessed in fecal samples and they are believed to reflect the metabolic activity of the colonic flora as believed that the composition of fecal samples is similar to that of contents in the distal segment of the colon, the recto-sigmoid region. In absence of disease and antimicrobial therapy the intestinal flora is believed to remain stable over time. The composition of flora is influenced by the type of food intake thereby affecting the enzyme parameters. The bacterial enzyme most commonly studied are those involved with the generation of toxic, mutagenic or carcinogenic metabolites like β-galactosidase, β- glucosidase and β-glucuronidase [4]. In this study we report fecal activities of β-galactosidase, β- glucosidase and β-glucuronidase in healthy breast fed infants of children from Jammu Province of J and K, India ranging in age from 3weeks to 6 months, and examine the association of the activities of these enzymes with the type of milk given to the children (breast milk v/s formula milk).

MATERIALS AND METHODS

A total of 75 fecal samples of healthy infants from Full Term Normal Delivery (FTND) were collected from the Department of Paediatrics, SMGS Hospital, Shalamar, Jammu for fecal enzyme analysis. 15 were formula fed upto six months in age, 10 were above 1 year of age (routine foods introduced) and 50 were exclusively breast fed from 3 weeks to 6 months of age (Table 1). The children with FTND who were breast fed or formula fed were included in the study while those delivered by C- Section (CS) and the child or mother on antibiotic therapy were excluded. It was ensured that both the mother and the infant had not taken any antibiotic for last 2 weeks. The fecal samples were transported to laboratory at 4°C and

weighed amounts were serially diluted in MRS (de man Rogosa Sharpe) broth and the dilutions were incubated at 37°C for 24 - 72h.

Table 1 Age distribution of healthy breast fed infants.

Age distribution (in weeks)	Total number of infants
2weeks - 8weeks	FS-1, FS-2, FS-3, FS-6, FS-7, FS-8, FS-9, FS-12, FS-16, FS- 17, FS-18, FS-19, FS-25, FS-29, FS-30, FS-31, FS-32, FS-33, FS-35, FS-39, FS-48
>8weeks - 16weeks	FS-4, FS-5, FS-10,FS-11, FS-15, FS-21, FS-37, FS-42, FS-43, FS-44, FS-49, FS-50
>16 weeks-24 weeks	FS-13, FS-14, FS-20, FS-22, FS-23, FS-24, FS-26, FS-27, FS-28, FS-34, FS-36, FS-38, FS-40, FS-41, FS-45, FS-46, FS-47

The dilutions were centrifuged and the supernatant was considered as crude enzyme and processed further.

The results were analysed in two groups; first made on the basis of age and food habits and second was time interval based, wherein all 50 fecal samples from healthy breast fed infants were studied for their enzymatic pattern.

For enzyme profile studies activities of β glucuronidase (E.C. 3.2.1.31; substrate phenolphthalein mono β glucuronic acid, Sigma; in 0.1 moll^{-1} potassium phosphate, pH 6.8) and β - glucosidase (E.C. 3.2.1.21; substrate p- nitrophenyl β - D-glucopyranoside; Sigma; in 0.1mol 1^{-1} potassium phosphate, pH 7.4) were determined at 37°C as described by Freeman[5]

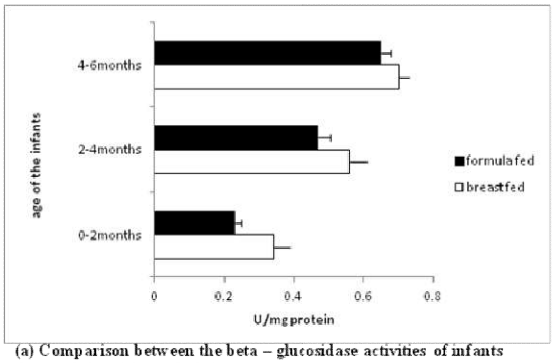
Activities of α - galactosidase enzyme was assayed according to the modified method of Donkor *et al*[6]. 50µl of the extract was mixed with 150µl 2% (w/v) *p*NPG and incubated at 37°C for 20 min. The reaction was stopped by addition of 200µl 0.1mol L^{-1} sodium carbonate solution. The amount of p-nitro phenol released was measured with a spectrophotometer at 420nm. 1 unit of α-galactosidase was defined as the amount which hydrolyzes 1 umol of ONPG to o-nitrophenol and D-galactose per min per cell [7].

To express enzyme activities as nmol substrate metabolized min^{-1} mg^{-1} protein in fecal supernatant fluids was determined in duplicate using the Lowry method [8]. BSA was used as a standard. Enzyme activities were also expressed as nmol substrate metabolized $min^{-1}g^{-1}$ fecal wet weight.

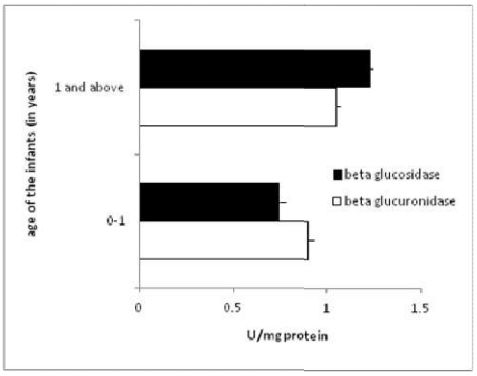
RESULTS

In the first group it was reported that β-glucuronidase activity was higher in formula fed infants as compared to the breast fed ones. The highest average activity of β-glucuronidase (0.76 mmoles/mg protein) was observed in formula fed infants (4-6 months) while the lowest activity (0.23 mmoles/mg protein) was observed in breast fed infants in the age band of 0-2 months (Fig.1 a). β - glucosidase activity analysis in breast fed and formula fed infants upto six months of age showed that breast fed infants had higher levels of β-glucosidase as compared to formula fed ones. The maximum β-glucosidase activity (0.7 mmoles/mg protein) was reported from breast fed infants in the group of 4-6 months while the formula fed infants in 0-2 months of age exhibited lowest β-glucosidase activity (0.23 mmoles/mg protein) as presented in Fig.1b. Furthermore, it was observed that activity of both the enzymes increase with increase in age of infant (Fig. 1c).

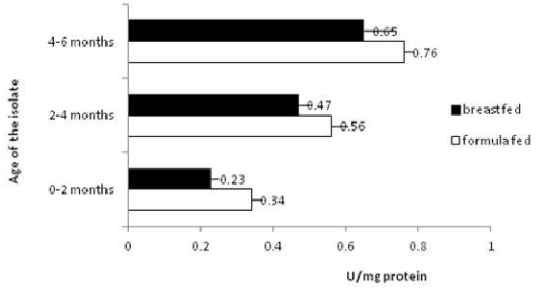
In the second group with an increase in time an increase in enzyme activity was found. All the isolates were found to have a considerable amount of β-glucosidase and α-galactosidase activity indicating probiotic bacteria. The enzymatic activity was maximum after 72h of incubation. The average activity of β-glucosidase was 1.97 mmoles/mg protein, 4.84 mmoles/mg protein and 6.83 mmoles/mg protein after 24-72h of incubation. α-galactosidase activity was reported to be 0.87 mmoles/mg protein (24h), 2.56 mmoles/mg protein (48h) and 4.48 mmoles/mg protein (72h) (Fig.2.0). As required a very low β-glucuronidase activity (0.08 mmoles/mg protein, 24h; 0.12 mmoles/mg protein, 48h and 0.13 mmoles/mg protein, 72h) was observed which was found to remain constant after 48h of incubation (1c).



(a) Comparison between the beta – glucosidase activities of infants (breast fed and formula fed) upto six months of age.



(c) Figure depicting comparison between the two fecal enzymes of infants between different age groups.



(b) Figure Depicting average beta glucuronidase activity of the infants (breast fed and formula fed) upto six months of age

Fig1 Average Enzyme Activity of infants

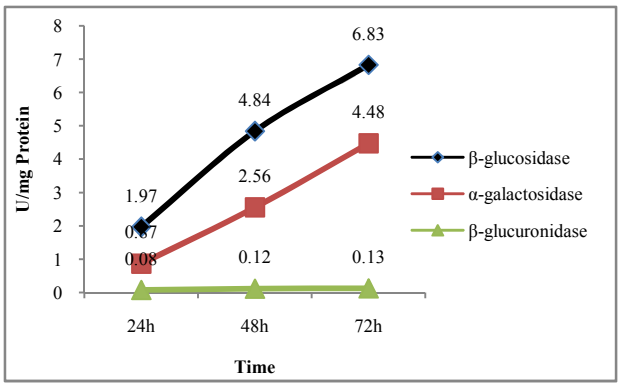


Fig 2 Average enzyme activity at different time intervals

DISCUSSION

Though, the global assessment of gut microbial functions in host is far from completion, advances have been made by studies of selected microbial enzymatic activities that are highly relevant to survival of the organism, as well as to disease in the human host. Investigation of the gut microbiota has identified β-glucuronidase as a conserved function among bacteria colonizing the human gastrointestinal tract. By uncoupling glucuronides, β-glucuronidase can deconjugate potential toxins increasing the formation of carcinogens in the bowel and promoting the enterohepatic recirculation of toxins, hormones, and various drugs in the body [9,10]. Elevated levels of β-glucuronidase may be a primary factor in the etiology of colon cancer[11], increased risk of breast cancer in postmenopausal women who have high estrogen levels [12]. β-glucosidases seems to have a more general role in the bioavailability of plant polyphenols and the extraction of energy from insoluble fibers and other indigestible carbohydrates [13]. The breast fed infants showed higher level of β-glucosidase as compared to the formula fed infants. α-galactosidase (E.C. 3.2.1.22) are known to break the galacto bonds specifically [14] and this adds a probiotic feature to the fecal flora present and feathers a beneficial effect to the host. Laclic acid bacteria with S-layer is known to cover their intracellular enzymes with it thereby rendering it more stable

The present study in general portrays variation in fecal enzyme levels of infants with respect to mode of delivery, diet and age. The results clearly indicate that the fecal enzyme levels change with an increase in age and the type of diet. Mykkanen *et al*., [15] also attributed a change in age and adoption of an adult type diet towards the variation in fecal enzyme levels. Mroczynska and Libudzisz [16] studied enzymes in LAB isolate from human feces and reported that the activity of β-glucuronidase increased with age while that of β -glucosidase decreased. The change in the number and type of microorganisms due to food habits and growing age may be responsible for a change in the fecal enzyme pattern of the infants thereby influencing the toxic and carcinogenic substances in an organism.

CONCLUSION

Analysis of fecal enzymes in stool samples of infants showed significant differences in the different groups. It was observed during the study that β-glucosidase and β-glucuronidase activity was dependent upon age and type of feed i.e. breast fed infants had an increased level of β-glucosidase activity as compared to formula fed which increased with increase in age. Moreover, it was also observed that enzyme activity depended upon diet and age of the infants. Mroczynska and libudzisc. (2010), showed that β-glucosidase activity isolated from selected bacteria strains was 32% higher in healthy children than in healthy adults. It seems that in the state of health, levels of the enzyme (at least of bacterial origin) do not correlate with age, on the contrary - they decrease. The presented analysis is the first one describing the activity of fecal enzymes from the children of Jammu region of J and K. The authors believe that further analysis of these particular enzyme may contribute to establish one perfect health indicator in infants and adults

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