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# EFFECT OF ADDING PROBIOTICS TO DAIRY PRODUCTS ON MUTANS STREPTOCOCCUS SALIVARY COUNT IN A GROUP OF SPECIAL HEALTH CARE NEEDS CHILDREN

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## ABSTRACT

**Background:** Caries prevention is better than cure, especially in patients with disabilities, to avoid the problems associated with the disease & the need for operative treatment. Bacteriotherapy in the form of probiotic bacteria is a promising concept, especially in childhood. The aim of the study was to evaluate the effect of different probiotic strains added to dairy product on salivary Mutans Streptococcus count in a group of children with special health care needs.

**Methods:** Sixty cerebral palsied children, 3 to 6 years old were enrolled in three groups all are following oral hygiene measures; one group used fermented milk containing probiotics, another one used full cream pasteurized cow milk & and the third group oral hygiene only for two weeks. Oral examination was performed before and after the study and after a 2 weeks post-treatment period for dental caries experience and oral hygiene status, and counts of salivary *Streptococcus mutans*.

**Results:** Probiotic milk showed non-statistically significant reduction ( $p\text{-value} \leq 0.005$ ) in salivary mutans streptococci levels. While with cow milk showed non-statistically significant increase ( $p\text{-value} \leq 0.005$ ) in salivary mutans streptococci levels.

**Conclusion:** Fermented milk containing probiotics may be more beneficial to cerebral palsied patients than cow's milk.

**Key Words** Functional food, Bacteriotherapy, Probiotics, Cerebral Palsy, Dental Caries, Mutans Streptococci

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## INTRODUCTION

Oral disease is a major health problem for patients with disabilities, who have a higher prevalence and severity of dental caries and periodontal disease when compared to the general population, so the most important aim of dental care for this group of children is to prevent dental disease, thus avoiding the problems associated with the disease & the need for operative treatment<sup>1</sup>. Caries prevention is better than cure, in the last decade; Research has demonstrated that nutrition plays a crucial role in the prevention of chronic diseases, as most of them can be related to diet. Functional food enters the concept of considering food not only necessary for living but also as a source of mental and physical well-being, contributing to the prevention and reduction of risk factors for several diseases or enhancing certain physiological functions<sup>2</sup>. In dental caries the major etiological players are the two  $\alpha$ -hemolytic Mutans Streptococci: *S. mutans* and *S. sobrinus*. They colonize dental plaque, adhere tenaciously to glucan-coated surfaces, produce large amounts of extracellular polysaccharides, and are highly acidogenic and acid tolerant<sup>3</sup>. The concept of microbial ecological change as a mechanism for preventing dental diseases "Bacteriotherapy" is alternative and promising way to prevent dental caries by using harmless bacteria to displace oral pathogens "Mutans streptococci" especially in children. Probiotics are one of these new agents which are widely used for their therapeutic action<sup>4</sup>. United Nations Food and Agriculture Organization and the World Health Organization<sup>5</sup>. defined probiotics as living microorganisms, principally bacteria, that are safe for human consumption and, when ingested in sufficient quantities, have beneficial effects on human health, beyond basic nutrition. Several mechanisms have been proposed to explain how probiotics work: a) Bacteria secrete various antimicrobial substances<sup>6</sup>. b) Bacteria compete with pathogenic agents for adhesion sites<sup>7</sup>. c) Probiotics can also modify the surrounding environment by

modulating the pH and/or the oxidation-reduction potential. d) Probiotics may provide beneficial effects by stimulating nonspecific immunity and modulating the humoral and cellular immune response<sup>8</sup>. A combination of probiotic strains is often used to increase these beneficial effects<sup>9</sup>. The aim of the study was to evaluate the effect of different probiotic strains added to dairy product on salivary Mutans Streptococcus count in a group of children with special health care needs.

## MATERIALS AND METHODS

**I. Subjects:** 60 children aged from 3- 6 years old were selected from Wahat Noor Alhyat foundation in Kobry Al kobba – Cairo and Faculty of Physical Therapy - Cairo University.

### • Selection criteria:

1. Age ranged from three to six years.
2. Having full set of deciduous dentition.
3. Having deciduous dentition only, patient was excluded if he has any erupted permanent tooth.
4. Children with no history of recent antibiotics administration (At least one month)<sup>10</sup>.

• **Grouping:** Patients were enrolled into 3 groups, all following oral hygiene measures (teeth brushing 3 times a day without tooth paste); Group I: 20 children taking fermented milk containing probiotics (Test group), Group II: 20 children taking full cream pasteurized cow milk (positive control group), Group III: 20 children taking no milk (negative control group).

**II. Clinical examination:** All children was examined for:

1) **Dental caries experience:** Using dft index: decayed and filled primary teeth. Dental caries was assessed according to WHO recommendations, 1987.

2) **Oral hygiene status :** Using modified Oral Hygiene Index simplified (OHI- s)

**III. Microbiological Examination:** Quantitative microbiological laboratory cultivation method of salivary Mutans Streptococci was carried out for each child at baseline and after 2 weeks study period and after additional two weeks to find out the residual effect of probiotics.

#### IV. Materials used:

1. Fermented milk containing three probiotics strains: *Bifidobacterium lactis*  $2 \times 10^7$  CFU/ml, *Streptococcus thermophilus*  $18 \times 10^7$  CFU/ml, and *Lactobacillus Acidophilus*  $14 \times 10^7$  CFU/ml bacteria.

1. Full cream pasteurized cow milk.

**V. Milk Regimen:** Patients were supplied with the milk supplements according to the group he was allocated in; the daily dose of 180 ml was taken divided on three times, 60 ml each. The guardians were instructed to give their children the milk after meals and after brushing of teeth and to drink the milk slowly, unheated and without any additions.

**VI. Statistical methods:** One-way ANOVA was used to compare between the three groups. Repeated measures ANOVA were used to study the changes after treatment in each group. Tukey's post-hoc test was used for pair-wise comparisons between the groups when ANOVA test is significant. For

non-parametric data, Kruskal-Wallis test was used to compare between the three groups. This test is the non-parametric alternative to one-way ANOVA. Mann-Whitney U test was used for pair-wise comparisons between the groups when Kruskal-Wallis test is significant. Friedman's test was used to study the changes after treatment in each group. Wilcoxon signed-rank test was used for pair-wise comparisons between the groups when Friedman's test is significant. Chi-square test ( $\chi^2$ ) was used to compare between gender distributions in the two groups. The significance level was set at  $P \leq 0.05$ . Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

**VII. Ethical aspects:** Ethics Committee for research, Faculty of Oral and Dental Medicine – Cairo University had approved the study protocol. The subject's parents received both oral and written information about the study, and signed their informed consent.

## RESULTS

**1. Mutans Streptococci:** Table (1) show the following: Pre-test, post-test and at follow up; there was no statistically significant difference between mean Log10 CFU of Streptococcus species in the three groups (One-way ANOVA test).

TABLE (1): The mean, standard deviation (SD) values for the comparison between Log10 CFU of Mutans Streptococci in the three groups

Group Period	Probiotics		Positive control		Negative control		P-value
	Mean Log10	±SD	Mean Log10	±SD	Mean Log10	±SD	
Pre-test	7.57	0.3	7.50	0.5	7.75	0.4	0.221
Post-test	7.55	0.3	7.62	0.4	7.81	0.5	0.163
Follow up	7.72	0.4	7.76	0.4	7.83	0.4	0.690

\*: Significant at  $P \leq 0.05$

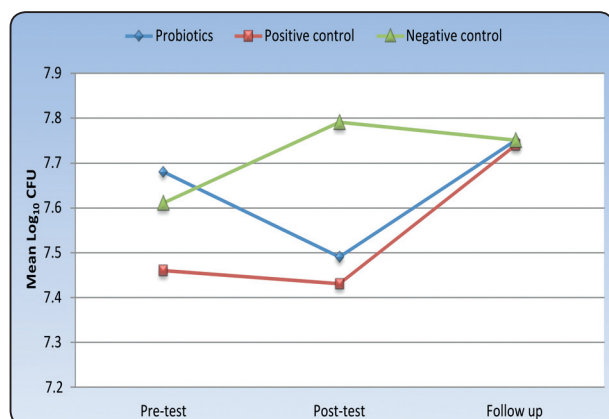


Fig. (1): Changes in mean Log10 CFU of *S. mutans* values of each group.

**2. *S. mutans* counts:** Figure (1) show the following:

In all groups; there was no statistically significant change in mean Log10 CFU of *S. mutans* post-test or at follow up (ANOVA test).

**3. *S. sobrinus* counts:** Table (2) show the following:

Pre-test, post-test and at follow up; there was no statistically significant difference between mean Log10 CFU of *S. sobrinus* in the three groups - (one-way ANOVA test).

## DISCUSSION

Bacteriotherapy is a novel and promising concept for preventing dental caries<sup>11</sup>. In several studies was reported that children with cerebral palsy are receiving little attention dental care, due to the difficulty in handling these patients and non satisfactory treatment results. Children with cerebral palsy had high levels of gingivitis and of caries experience, and that indicate the need for early intervention and preventive programs<sup>12</sup>. In this study mean age of 4, 3.8 and 4 for group I, group II and group III respectively. This matches age chosen by Näsé *et al.*<sup>13</sup> and Seki *et al.*<sup>14</sup>, who assessed the effect of probiotics on mutans streptococci in preschool children where oral flora has not yet matured and could be easily affected. Modified oral hygiene to study protocol. The probiotic product contains combination of 3 probiotic strains: *Bifidobacteria*  $2 \times 10^7$  cfu/ml, *Lactobacillus acidophilus*  $14 \times 10^7$  cfu/ml and *Streptococcus thermophilus*  $18 \times 10^7$  cfu/ml. These probiotics concentrations are compatible with probiotics concentrations in the studies of Çağlar *et al.*<sup>4,10</sup>. Milk regimen was 3 times a day to ensure frequent exposure to probiotics this is compatible with the study of Çağlar *et al.*<sup>10</sup>. Näsé *et al.*<sup>13</sup> have reported the beneficial effects of long-term (seven months) consumption of probiotics milk on dental caries. Our interest therefore was to see if a

TABLE (2): The mean, standard deviation (SD) values for the comparison between Log10 CFU of *S. sobrinus* in the three groups.

Group Period	Probiotics		Positive control		Negative control		P-value
	Mean Log10	±SD	Mean Log10	±SD	Mean Log10	±SD	
Pre-test	7.47	0.5	7.55	0.6	7.89	0.6	0.062
Post-test	7.62	0.5	7.81	0.7	7.84	0.6	0.491
Follow up	7.69	0.6	7.78	0.4	7.92	0.5	0.411

\*: Significant at  $P \leq 0.05$

short-term probiotic intervention could modify the cariogenic flora; we also wanted to study the effect of probiotics on salivary microbial counts. The results showed that Mutans Streptococcus count in the two weeks study period remain constant in group I while showed non significant increase in group II may be as a result of milk fermentation due to prolonged retention in the oral cavity because of impaired swallowing function in these children; which represent situation similar to that of nursing caries.

The results also showed that there was no statistically significant difference in Mutans Streptococci counts during the intervention period between the three groups, this agree with Ahola *et al.*<sup>15</sup> and Montalto *et al.*<sup>16</sup>, who stated that no statistically significant differences in Mutans Streptococci counts were found between the study and control group during the intervention. Similar to our study, both studies used combination of more than one probiotic type, as interaction between different probiotic strains is not clear enough they claimed that combination of different probiotic strains result in less effect because each strain may act differently against cariogenic micro-organisms. In contrary Çaglar *et al.*<sup>4</sup> and Cildir *et al.*<sup>17</sup>, all used one probiotic strain and their results showed statistically significant reduction of Mutans Streptococci.

## CONCLUSIONS

1. Fermented milk containing probiotics may be more beneficial to cerebral palsied patients than full cream pasteurized cow milk where their defective swallowing function results in prolonged retention and fermentation of cow's milk in children's mouth, and then increase in Mutans Streptococcus count.
2. Use of combination of different probiotic strains result in less effect because each strain may act differently against cariogenic micro-organisms.

## RECOMMENDATIONS

1. Bacteriotherapy can be recommended as dental caries preventive measure for cerebral palsied patients and those who are special health needs children; it's not expensive, easily applied and effective.
2. Interaction between different probiotic strains regarding their mechanism of action is a point that needs more studies.

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