

COMPARATIVE CLINICAL AND MICROBIOLOGICAL STUDY OF PROBIOTIC STRAIN LACTOBACILLUS ON THE COUNT OF STREPTOCOCCUS MUTANS IN SALIVA

Eman Mahmoud Qeshta ¹, Wael Mahmoud Abd AlKhalek ², Sahar Zakaria Mohamed ³

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KEYWORDS

Dental plaque - *Lactobacillus*
- Probiotics - *Streptococcus mutans*.

• E-mail address:
eman_keshta@yahoo.com

1. Postgraduate student at Periodontics Department, Faculty of Dentistry, Suez Canal University
2. Associate Professor of Pediatric and Preventive dentistry and Dental Public Health, Faculty of Dentistry - Zagzigo University
3. Professor of Microbiology and Immunology, Faculty of Medicine - Suez Canal University

ABSTRACT

Introduction: The concept of microbial ecological changes and probiotic approaches is a promising concept for preventing dental disease and decreasing of oral cavity pathogens. **Aim:** to evaluate the effect of probiotic strain *Lactobacillus* on the count of *Streptococcus mutans* and *Lactobacillus* in saliva and the plaque formation rate. **Materials and methods:** This randomized clinical trial included 30 normal apparently healthy and cooperative children aged 6 to 11 years and selected from the Outpatient Dental Clinic of Pediatric dentistry Department, Faculty of Dentistry, Suez Canal University. Children were randomly divided into 2 groups as follows: Group **I:** included (15) children who brushed their teeth two times a day with fluoro toothpastes and Group **II:** included (15) children who instructed to take Periobalance G.U.M lozenges once daily at the evening immediately following brushing and flossing for 60 days. Clinical evaluation was performed by measuring plaque indices scores at all evaluation periods. Microbiological evaluation was performed by direct examination of bacterial cultures. **Results:** Probiotics showed a significant reduction in the mean *Streptococcus mutans* counts and increase the count levels of *Lactobacilli* but did not show a significant difference increase. There was decrease in the plaque index, with the lowest value after 60 days in both groups. **Conclusion:** Probiotic is a promising concept of preventing dental diseases.

INTRODUCTION

Dental caries is considered a chronic disease that commonly affects adult persons and school children. It is a multifactorial disease of bacterial origin, which causes demineralization of tooth enamel. In this disease, there is an increase in bacteria that produce acid (acidogenic) and tolerate acidic environment including mutans *Streptococci* and *Lactobacilli*. Altered homeostasis of the mouth can increase bacterial biofilm formation (specially mutans) from the *Streptococcus* group ⁽¹⁾.

Developed countries showed a big interest to overcome the problem of fluoride-enriched water and personal hygiene products. Until the moment in clinical practice, dental caries is treated symptomatically although intensive focus on preventive strategies took place in many studies. These preventive measures either change or modify the factors associated with caries which include dietary, host, salivary and bacterial factors ^(2,3).

Many anti-plaque agents had been tested for the capability of interference with biofilm proliferation or metabolism. But, going along with the increasing antibacterial resistance resulted in a real search of alternative agents because of numerous adverse events of such agents ⁽⁴⁾.

Probiotic bacteria showed a great influence on host immunity via many mechanisms. Although they are still unclear, these mechanisms might involve one of the following; 1-Modification of the pH of gastrointestinal tract. 2- Antagonizing microbes into production of antibacterial compounds. 3- Competing pathogens' binding receptors, nutrients as well as growth factors. 4- Initiating immune modulatory cells. 5- Production of lactase ⁽⁵⁾.

Dairy foods (yogurt, cheese, kefir and milk) as well as ice cream and chocolate are valuable sources of probiotics, the best administration method has not been identified until the moment. The delivery of probiotics have to be appropriate for entire ages particularly young children as the early exposure in life could facilitate long-lasting acquirement of strains that promotes health ⁽⁶⁾.

Probiotics assumed to possess following properties as well as functions; adherence to the host's epithelium, resisting acids, bile tolerance, eradication of the microbes or decreased pathogenic adherence, acids formation, hydrogen peroxide and bacteriocins, antagonistic to the microbial growth, safety, non-pathogenic, non-carcinogenic and resulted in improved gut microflora ^(7,8).

Safety issue got a notable concern over the last decade because of enhanced supplementation of probiotics in various food products. From the safety view, probiotic bacteria proposed not to be pathogenic nor have any entire growth-inducing effects onto microbes that cause diarrhea, also it must not have any capacity of transferring the antimicrobial resistance genes. On the other hand, probiotics

must have the ability of achieving genetic stability through microflora in the mouth ⁽⁹⁾.

Current study was designed to evaluate effect of probiotic strain *Lactobacillus* on both count of the *Streptococcus mutans* and *Lactobacillus* in saliva and plaque formation rate.

MATERIAL AND METHODS

The current work included thirty children of both genders from outpatient Clinic of Pedodontic Department – Suez Canal University. An informed consent of the children's parents was taken.

Inclusion criteria for selection of sample:

1. Apparently thirty healthy children with no history of systemic disease.
2. The age group of 6-11 years.
3. Permanent first molars and central incisors were fully erupted.

Exclusion criteria:

1. Children having decayed/ untreated carious teeth.
2. Children receiving any antibiotics
3. Children receiving any other probiotic supplementation.
4. Children using any xylitol products during the study ⁽¹⁰⁾.

The children were given numbers to be divided to two groups from one to thirty randomly. We used the sealed envelope method to distribute them into the two groups:

- Group I (control group): included (15) children who brushed their teeth two times per day with fluoro toothpastes (Eva- cosmetics).
- Group II (probiotic group): included (15) children who instructed to take Periobalance G.U.M lozenges once daily at the evening immediately following brushing with fluoro toothpaste. The

lozenge must dissolve in the mouth completely which takes about ten minutes. The children instructed not to brush their teeth and not rinse with antibacterial mouthwash immediately after the use of the lozenge for approximately thirty minutes with no dietary restrictions^(11, 12). Also they were instructed not to use any other probiotics supplements nor xylitol products for 21 days prior to and during our study⁽¹⁰⁾. All the children were given toothbrushes and toothpastes to brush their own teeth during the study twice a day.

Clinical examination:

1. Information was collected from caregivers about each child's general health, dental habits, dietary habits, quality of their snacks between meals, tooth brushing habits and medications.
2. Full dental examination using (def) for primary teeth; (d: decayed tooth necessitating filling, e: decayed tooth necessitating extraction due to caries, f: filled teeth due to caries) and (DMFT) for permanent teeth; (D: Decayed tooth, M: Missed tooth, F: Filled tooth).
3. Plaque Index for measuring plaque formation rate was used⁽¹³⁾.
4. In case of present decayed teeth full mouth treatment was done.

Bacteriological examination:

A-On the first visit:

1. Children were advised to avoid eating and drinking at least one hour prior to samples' collection.
2. Each child was instructed to spit in a sterile plastic wide-mouthed test tube at least one milliliter.
3. Salivary samples were investigated to determine bacterial count of both *SM.* and *LB.*
4. The child's name, number and date of sample collection were written and stucked on the tube⁽¹⁰⁾.

B-Follow-up period:

1. Clinical and bacteriological assessment was done for each child at three visits: (on day 0, 30 days and 60 days) to collect the necessary data including plaque index and assessment of the bacterial count in the salivary samples.
2. Bacteriological investigation of the salivary samples was done in the Microbiology and Immunology Department, Faculty of Medicine, Suez Canal University.

Materials:

The materials, brand names, compositions, manufacturers and lot number are illustrated in the table (1).

Table (1) Materials, brand names, compositions, manufacturers and lot number.

Material	Brand name	Composition	Manufacturer	Lot no.
Prodentis Probiotic lozenges	PerioBalance G.U.M	<i>Lactobacillus Reuteri</i> DSM 17938, ATCC PTA 5289). Hydrogenated Palm Oil, Isomalt (filler), Sucrose, Esters of Fatty Acid, Peppermint Flavor, Peppermint Oil, Menthol Flavor, Sucralose (sweetener).	Sunstar Americas	5LSA005
Mitis Salivarius Agar	TMMEDIA	Sucrose, Tryptone, Agar, Peptone meat, Dextrose, Dipotassium phosphate, Crystal violet Trypan blue.	Titan Biotech Ltd, India	M2C11001
Rogosa Agar	LAB M	Mixed peptones, Yeast extract, Beef extract, Glucose, Dipotassium phosphate, Triammonium citrate, Sodium acetate, Magnesium sulphate, Tween 80, Manganese, Agar.	A Neogen Company, UK	129426/267

PerioBalance G.U.M:

In form of mint-flavored lozenges. One lozenge consists at least 200 million live *Lactobacillus reuteri* Prodentis^(11,12).

Microbiological media:

A-Mitis Salivarius Agar

Mitis salivarius agar (Titan Biotech Ltd, India) was used to culture *Streptococcus mutans* in the saliva selectively, the Agar ingredients used in this study are shown in table (2). To increase the selectivity of the medium to *Streptococcus mutans*, mitis-salivarius agar was formulated to contain 0.2 units/ml bacitracin, 1% potassium tellurite solution, and 20% sucrose. The MSB medium was prepared according to the manufacturer instructions as follows:

1. Ninety grams mitis salivarius agar and 150 grams sucrose underwent dissolution in one liter of deionized water (the medium contains 50 gm/ml sucrose).
2. The medium was heated up to boiling point to achieve dissolution of the components and underwent autoclaving at 121°C for 15 min.
3. The medium was left to cool down until 45°C after which 1 ml of 1% potassium tellurite and 1 ml of 200 units/ml bacitracin were added, sterilization of potassium tellurite and bacitracin was done via filtration.
4. The medium poured in sterile petri dishes, allowed to harden at room temperature and underwent storage in the refrigerator at 4°C till use as shown in figure (1).

B- Rogosa Agar

Rogosa agar (Lab M limited, UK) was used to enumerate lactobacilli in the saliva selectively⁽¹⁴⁾,

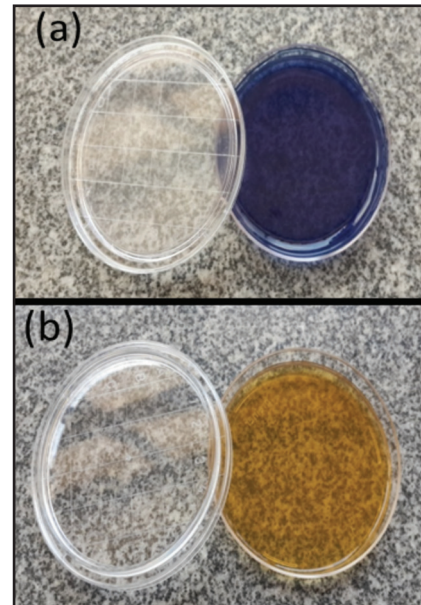


Fig. (1) Photographs showing both (a) Mitis Salivarius Agar plate, (b) Rogosa Agar

ingredients are shown in table (2). To increase the selectivity of a medium to lactobacilli, glacial acetic acid was added to the medium. The medium underwent preparation based on the manufacturer guidelines as follow:

1. Seventy grams of medium were suspended in one liter of deionized water and heated up to boiling point till completely dissolution.
2. The medium underwent autoclaving at 121°C for 15 min.
3. After cooling down to 45°C, 1.32 ml glacial acetic acid was added followed by thorough mixing.
4. The medium was poured in sterile petri dishes, allowed to harden at room temperature and underwent storage in the refrigerator at 4°C until used as presented in figure (1).

Table (2) Ingredients of *Mitis Salivarius* Agar and *Rogosa* Agar (in gm/L) :

Mitis Salivarius Agar		Rogosa Agar	
Sucrose	50	Mixed peptones	10
Tryptone	15	Yeast extract	5
Agar	15	Beef extract	10
Peptone meat	5	Glucose	20
Dipotassium phosphate	4	Dipotassium phosphate	2
Dextrose	1	Sodium acetate	5
Trypan blue	0.075	Triammonium citrate	2
Crystal violet	0.0008	Magnesium sulphate	0.2
pH	7.0 ± 2.0 at 25°C	Tween 80	1.08
		Manganese	0.05
		Agar	15

Processing of the specimen:

1. Saliva specimens were shaken well to mix them thoroughly.
2. Sterile disposable calibrated loops 1/100 and 1/1000 ml were used to transfer and streak saliva specimens into MSB and Rogosa Agar plates. Dilution 1:100 or 1:1000 with phosphate buffered saline (PBS) was done.
3. The plates of MSB agar and Rogosa Agar were incubated anaerobically using (Oxoid Ltd, England) gas pack anaerobic system at 37 °C for 72 hours.
4. After incubation, a colony counter with magnifying glass (Gallenkamp, England) was used to count number of colonies and expressed as colony forming units per ml saliva (CFU/ml) ⁽⁵⁾. By multiplying the actual colony count by 10² or 10³ (according to calibrated loop used inside this study) quantification numbers of colonies was done as illustrated in figure (2).

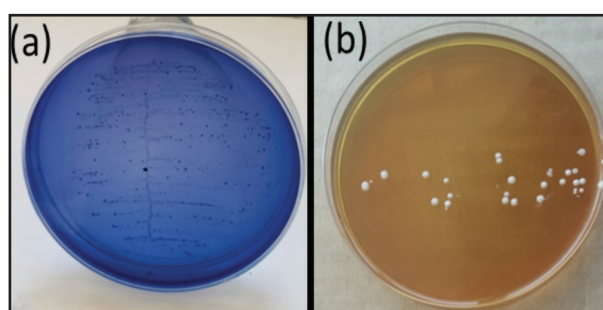


Fig. (2) Photographs showing both (a) *S. Mutans* on Streptococcus Mitis Bacitracin Agar plate, (b) *Lactobacilli* on Rogosa Agar plate.

Plaque Index (PI) ⁽¹³⁾:

The PI as described by (Loe and Silness 1964) evaluated the thickness of plaque at cervical tooth margin. Four regions, 'distal, facial or buccal, mesial, and lingual' were evaluated. Specific teeth were examined and scored which were the maxillary right-first molar, maxillary left-central incisor, mandibular left-first molar and mandibular right-central incisor.

Each patient had a complete mouth scaling and appropriate teeth cleaning using rubber cups and polishing paste till almost zero PI scores were achieved. The tooth was evaluated by a mirror and periodontal explorer. The explorer underwent passage over the cervical third in order to assess the plaque existence. From the establishment of zero PI score, the following scores were recorded after 30 days and 60 days.

Four different scores can be obtained:-

- 0:** Indicates no plaque in gingival areas.
- 1:** Plaque film adhering with free gum margin plus tooth nearby region, plaque could only be identified using a probe across tooth surfaces.
- 2:** Represents moderate accumulation of the soft deposits within gum margin and/ or adjacent tooth surface which could be observed using naked eye.
- 3:** Represents abundance of the soft matter in gingival pocket and /or on gingival margin and adjacent tooth surfaces.

Each area of all teeth was assigned with a score from 0 to 3. Scores of every tooth was totaled then divided by all 4 surfaces scored. To obtain a total PI, these scores was totaled then divided by the number of examined teeth. Four ratings were assigned: 0 = excellent, 0.1-0.9 = good, 1.0-1.9 = fair and 2.0-3.0 = poor.

Statistical analysis

A-Sample size calculations:

The sample size calculation of the entire study equals 15 for each group according to the value of statistical differences of means and standard deviation as applied to the following equation.

This approach is obtained using Minitab analysis software as plotted in figure 3 of entire values in table 3.

Table (3) The sample size calculations of each group

Difference	Sample Size	Target power	Actual power
0.55	15	0.95	0.952895

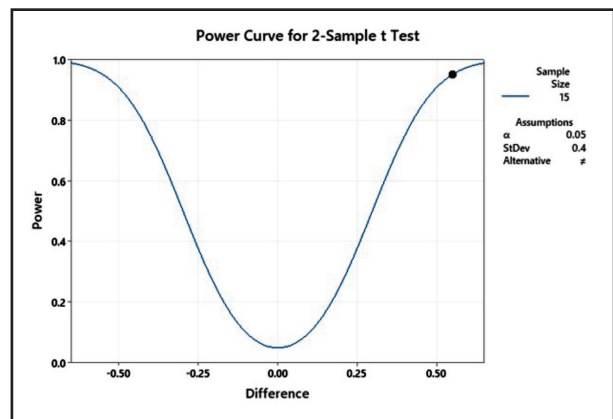


Fig. (3) Sample size calculations of entire study

B- Statistics of the results:

All data were collected, tabulated and statistically analyzed in order to compare between the control plus probiotic groups and to evaluate the change by time within the same group. Data was presented as mean ± standard deviation for numerical variables. The numbers of colonies were presented as millions. The significance level was set as $P \leq 0.05$. T-paired test was used to compare changes between two groups according to time intervals. Data were tabulated, coded then analyzed using the computer program SPSS version 16.

RESULTS

A total of thirty children of both sexes were investigated in this study. The children were given numbers so that they were divided into two different groups from one to thirty randomly, (15 for each group). The following results were obtained.

SM counts:-

A- Comparison between control and probiotic groups:

The mean, standard deviation (SD) and significance P-values between both groups of SM were calculated and plotted in table (4). Noted that when P value ≤ 0.05 is significant.

Table (4) The mean, standard deviation (SD) and significance P-values between the two groups of SM.

	Control		Probiotic		Sig. P-value
	Mean	SD	Mean	SD	
Baseline	4.2	1.35	4.15	1.215	0.916
30 days	4	0.9	4.1	1.23	0.801
60 days	4.4	1.1	4.45	1.127	0.903

From table (4) and figure (4): At baseline, after 30 days and after 60 days, statistical analysis shows no significant difference between mean log SM

counts in both groups (P= 0.896, 0.254 and 0.087 respectively).

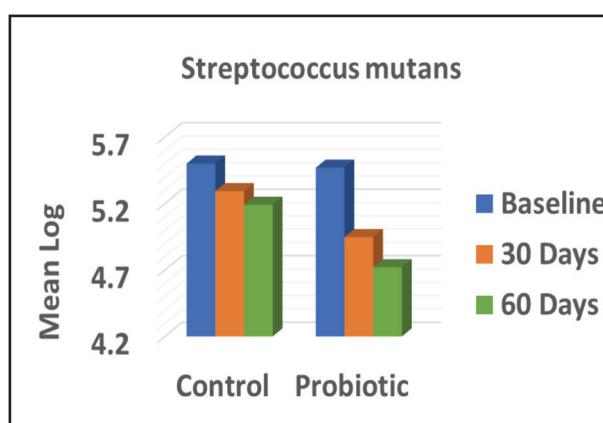


Fig. (4) Mean log SM counts at Baseline, 30 days and 60 days.

B- Changes by time in SM group:

The mean difference and significance P-values showing changes by time in log SM counts are illustrated in table (5), the following observation concluded from table data;

- Group I: Control group shows no significant reduction between baseline as well as after 30 days (P=0.458), also there was no significant difference between 30 days and 60 days with P-value (0.727) and between baseline and 60 days with P-value (0.273).

Table (5) The mean difference and significance P-values showing changes by time in log SM counts.

Control	Probiotic	
	Mean difference	Sig.P-value
Baseline vs. 30 days	0.207	0.458
30 days vs. 60 days	0.103	0.727
Baseline vs.60 days	0.31	0.273

- Group II: Probiotic group shows a significant reduction occurred between baseline and following 30 days with P-value (0.05), but no significant difference observed between 30 days and 60 days with P-value (0.407), also a significant reduction obtained between baseline and 60 days with P-value (0.002).

LB counts:-

A. Comparison between control and probiotic groups:

The mean, standard deviation (SD) and significance P-values between both groups of LB are illustrated in table (6). The Mean log LB counts at Baseline, 30 and 60 days were plotted in figure (5). At baseline, after 30 days and after 60 days, statistical analysis showed non-significant difference between mean log LB counts in both groups (P= 0.916, 0.801 and 0.903 respectively).

Table (6) The mean, standard deviation (SD) and significance P-values between the two groups of LB.

	Control		Probiotic		Sig.P-value
	Mean	SD	Mean	SD	
Baseline	4.2	1.35	4.15	1.215	0.916
30 days	4	0.9	4.1	1.23	0.801
60 days	4.4	1.1	4.45	1.127	0.903

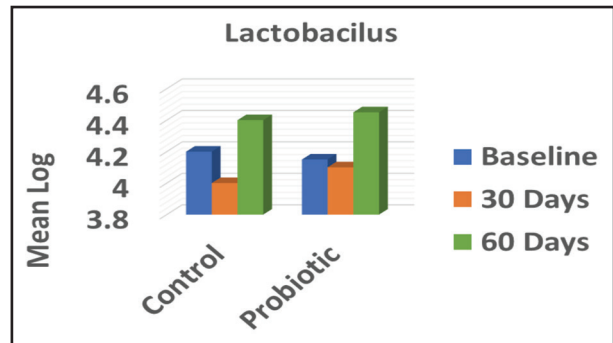


Fig. (5) Mean log LB counts at Baseline, 30 days and 60 days.

B. Changes by time in LB group:

The mean difference and significance P-values showing changes by time in log LB counts are illustrated in table (7). From table data the following are observed;

- Group I: Control group shows that there is no significant difference appeared between baseline and after 30 days with P-value (0.637), also there was a non-significant difference between 30 days and 60 days with P-value (0.286) and between baseline and 60 days with P-value (0.660).
- Group II: Probiotic group shows that there is no significant difference reported between baseline and after 30 days (P=0.912), also there is no significant difference observed between 30 days and 60 days with P-value (0.424) and between baseline and 60 days with P-value (0.489).

Table (7) The mean difference and significance P-values showing changes by time in log LB counts.

	Control		Probiotic	
	Mean difference	Sig.P-value	Mean difference	Sig.P-value
Baseline vs. 30 days	0.200	0.637	0.050	0.912
30 days vs. 60 days	-0.400	0.286	-0.350	0.424
Baseline vs.60 days	-0.200	0.660	-0.300	0.489

Plaque in probiotic and control groups:

Table (8) shows a comparison between the plaque index scores in probiotic in addition to control groups. Also a bar chart representing mean Plaque index scores in probiotic plus control groups is presented in figure (6). Figure (7) represents linear chart of mean Plaque index scores in probiotic besides control groups.

They demonstrated that there was no statistically significant difference between both groups at baseline as regard mean plaque index (P=0.775). Also it was found that statistically significant difference obtained between both groups after 30 and 60 days with P=0.031 and 0.002, respectively.

- Group I: Control group shows that there is no significant difference illustrated between baseline and after 30 days (P=0.317), also there was a non-significant difference between 30 days and 60 days with P-value (0.055) and between baseline and 60 days with P-value (0.061).
- Group II: Probiotic group shows that there was a significant reduction between baseline and following 30 days (P=0.003), there was a significant difference between 30 days and 60 days (P=0.001), also there was a significant reduction between baseline and 60 days with P-value (0.001).

Table (8) Comparison between the plaque index scores in probiotic and control groups.

Plaque index			Groups				T-Test	
			Control group		Probiotic group		t	P-value
Baseline	Range		1.25	- 1.875	1.25	- 1.875	0.288	0.775
	Mean ±SD		1.575	± 0.230	1.550	± 0.245		
After 30 Days	Range		1.25	- 1.875	1.25	- 1.625	2.276	0.031*
	Mean ±SD		1.483	± 0.188	1.350	± 0.127		
After 60 Days	Range		1	- 1.625	1	- 1.375	3.353	0.002*
	Mean ±SD		1.400	± 0.207	1.183	± 0.141		
B-30D	Differences	Mean ±SD	0.092	± 0.342	0.200	± 0.188		
	Paired Test	P-value	0.317		0.003*			
B-60D	Differences	Mean ±SD	0.175	± 0.333	0.367	± 0.277		
	Paired Test	P-value	0.061		0.001*			
30-60D	Differences	Mean ±SD	0.083	± 0.154	0.167	± 0.161		
	Paired Test	P-value	0.055		0.001*			

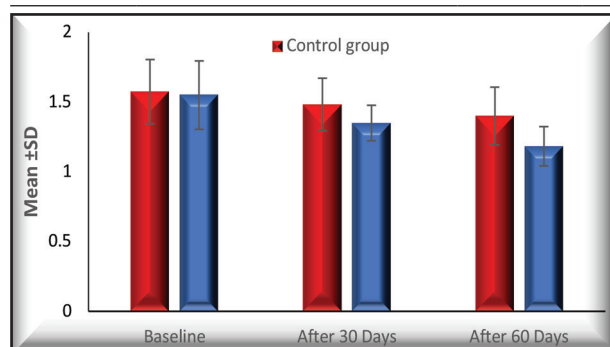


Fig. (6) Bar chart representing mean Plaque index scores in probiotic and control groups.

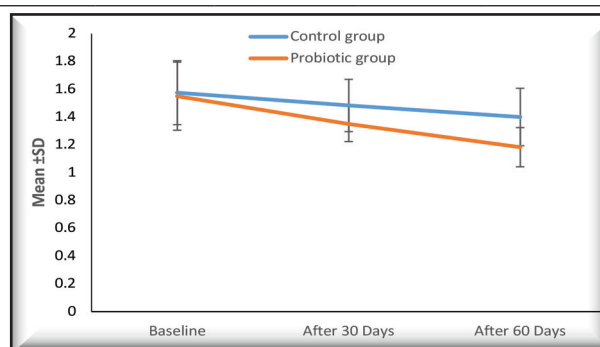


Fig. (7) Linear chart representing mean Plaque index scores in probiotic and control groups.

DISCUSSION

Caries is increasing in its incidence among pediatric and young adult persons who consume refined carbohydrates and junk foods. Probiotic bacteria is useful for oral health. For better effects, such bacteria must adhere to tooth structure and displace SM, also it must produce low acid levels while existing in oral biofilms⁽¹⁵⁾.

The probiotics beneficial therapeutic effects of oral conditions including caries, gingivitis, or periodontitis have been established in vitro. Several in vivo researches also studied short- and long-term effects of probiotic bacteria in the mouth. Various vehicles including yogurt, curd, ice creams, non-dairy products including drops, lozenges, straws, tablets and candies were reported in many researches^(16,17).

PerioBalance G.U.M (marketed by Sunstar) contains some patented combination including 2 strains of specially selected *L.reuteri* for the synergistic effects which fight cariogenic microorganisms as well as periodontal bacteria. Lozenge each dose contains no less than 2×10^8 CFU living cells of entire *L.reuteri* Prodentis. Users are recommended to utilize daily lozenge either following meals or in evening just following teeth brushing in order to permit probiotics spread in mouth and reach different dental surfaces^(11,12).

Questions about the medical history were asked, as several general conditions can affect the caries process directly or indirectly, either through influencing salivary production and composition or through the caries inducing dietary patterns or through medications. The children were not allowed to use xylitol - containing products because of their known effect on cariogenic micro-organisms and did not receive any antibiotic treatment two weeks before the study to avoid any external factors affecting the bacterial level in their saliva.

Clinical and bacteriological assessment was done for each child at three visits: (on day 0, 30 and 60) to collect the necessary data including plaque index and assessment of the SM and LB count in the salivary samples. Mitis Salivaris agar and Rogosa agar were chosen because of their highly accurate results in showing counts of bacterial colonies in salivary sample, this finding agreed with previous studies⁽¹⁸⁾.

In this study, probiotics were introduced in form of mint-flavored lozenges. The probiotics introduction in form of lozenges vehicle was compatible with previous study⁽¹⁹⁾ which assessed the effects of daily *Lactobacillus brevis* 'CD2 lozenges' in children. In accordance to this study, significant reduction in the salivary SM was found in the study after the probiotic lozenges consumption.

Also, these results came in agreements with Dawson, et al.⁽²⁰⁾ who reported that *Lactobacilli* proved to be of value into decreasing salivary SM level thus reducing caries. Chinnappa, and colleagues⁽²¹⁾ also revealed that the probiotic ice-cream caused significant decrease of SM level.

Significant reduction into salivary SM ($P \leq 0.05$) was reported after probiotic lozenges consumption, which was consistent with Bhalla, et al.⁽²²⁾ and Lin, et al.⁽²³⁾ results as they reported that the probiotic bacteria caused significant decrease of SM count. Likewise, Caglar, et al.⁽¹⁷⁾ whose study proved that the daily chewing gums including probiotics decreased SM count significantly.

In agreement with these results, Chaturvedi, et al.⁽²⁴⁾ evaluated the effect of probiotic lozenge application on SM count in the plaque of orthodontic individuals. It was found that daily short term *Lactobacilli brevis* ingestion derived probiotic into a lozenge may decrease SM count in plaque surrounding orthodontic brackets.

On the contrary with these results, Montalto, et al. ⁽²⁵⁾ reported that SM populations were not significantly modified when lactobacilli were given in liquid form and in capsules to detect the role of direct contact of oral cavity.

Also in disagreement with these results, Cildir, et al. ⁽²⁶⁾ reported non-statistically significant decrease of SM in saliva following 25 days of application of drops containing *L. reuteri*. The drop might not decrease SM in saliva in cleft lip/palate children. These results may be clarified by the various administration methods of probiotics and different sample sizes and various follow up periods.

This study obtained that, tendency for probiotics to increase lactobacilli count, but did not show a significant difference increase.

In agreement with these results various studies proved that tendency for probiotics to increase lactobacilli levels ^(17, 19-21, 24).

Montalto, et al. ⁽²⁵⁾ found that the probiotic supplement demonstrated a statistically significant rise of Lactobacilli salivary count when lactobacilli were given in liquid form and in capsules in disagreement with this study that did not show a significant difference increase.

Also in contrast to this study, Hasslöf and colleagues ⁽²⁷⁾ concluded that an early intervention with *Lactobacillus paracasei* F19 did not disclose any effect of exposure to probiotics on MS and LB levels. Such conflicting results should be clarified using various intervals between probiotics intake and microbial analysis in the oral cavity.

This study showed a decrease in the plaque index, with the lowest value after 60 days in both groups. Statistically significant difference recognized between effect of probiotics group and controls on plaque formation rate. These results came in agreements with Krasse, et al. ⁽²⁸⁾ who found that

Lactobacillus reuteri caused noticeable reduction for both plaque and gingivitis.

These results are in agreement with Vivekananda and co-workers ⁽²⁹⁾ who carried out a split mouth trial in which 2 quadrants were treated with scaling and root planning while the other 2 were left untreated. The persons got probiotic lozenges for 14 days then, scores of plaque and gingival reduced significantly.

It could be hypothesized that plaque scores partly reduced as a result of anti-inflammatory effect of *L. reuteri*. As *L. reuteri* suppresses IL-8 and regulates nerve growth factor ⁽³⁰⁾. Also, reuterin and reutericyclin presence contributes toward antibacterial effects of *L. reuteri*. Eventually, some reduction might be observed because daily chewing action in which overall oral clearance increases.

Bacterial probiotics Limitations: ⁽³¹⁾

1. Regulations for dietary supplementation are non-existent in several countries, or much less strict than those that apply for prescription medications.
2. At present, no claims approved by (FDA) that relate probiotics to disease reduction risks. The regulatory of probiotic status as a component in food cannot established through international basis. Only few countries, regulatory procedures are taken in place or sufficiently improved to enable probiotic products to describe specific health benefits.
3. Factors influencing viability during storage including temperature, moisture and air should be considered.
4. Oxygen toxicity represents another main challenge regarding probiotic bacteria survival inside dairy foods. Oxygen high levels obtained into product are detrimental of anaerobic bacteria viability. Probiotic bacteria viability of retention presents a major technological

challenge and marketing for probiotic cultures applications in functional food.

CONCLUSIONS

- The result of probiotic group showed significant reduction occurred in the mean SM counts and there was a tendency for probiotic intervention to enhance LB levels, but did not demonstrate a significant difference rise.
- There was reduction in plaque index, with the lowest value after 60 days in both groups. Statistically significant difference observed between probiotic and controls on plaque formation rate.
- A daily short-term ingestion of LB - derived probiotics delivered by lozenges decreased concentrations of Streptococcus mutans in children's saliva.

Limitations in this study:

1. Collection of samples: The saliva samples were transported within two hours in ice box to the laboratory of Microbiology and Immunology Department, Faculty of Medicine. Transportation was done in fast way to avoid multiplying of bacteria in samples.
2. Difficulty for parents / children to obey instructions for a long time:
 - Children instructed to take lozenges once daily immediately following brushing and flossing.
 - Children instructed not to brush the teeth and not rinse with antibacterial mouthwash immediately after usage of the lozenge for approximately thirty minutes.
 - Children instructed to avoid eating and drinking at least one hour prior to collecting samples.
3. Antibiotic treatment: during course study would affect the results.

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