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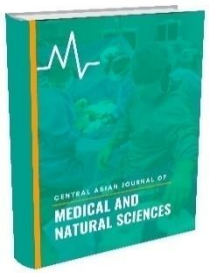

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The Effect of Probiotic Administration on the Modulation of Subgingival Bacteria in Patients with Gingivitis

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Abstract:

Aim: To evaluate the efficacy of a probiotic consumption on gingivitis and to assess its impact on the subgingival microbiota.

Material and Methods: the study is a randomized clinical trial that conducted on 30 subjects divided two groups: 15 patients test group suffered from gingivitis who administered probiotic two oral tablets per day containing different probiotic bacterial strains for 20 days and 15 individual control group administered a placebo tab that resemble the probiotic tab. Clinical and microbiological examinations were carried out for 20 days at three stages: (0 day 10 days and 20 days). clinical examination included measurement of gingival (GI) &plaque (PI) indices microbiological examination by counting sub-gingival bacteria at the three stages follow up, the study was approved by the Research Ethics Committee of College of Dentistry University of Mosul with the reference number UoM. Dent/H.OM.61/22. Subjects attending the Faculty of periodontal disease, University of Mosul, College of Dentistry (Mosul, Iraq), from February to November 2022 were screened.

Result: Fredmans showed that there was no significant difference between the mean plaque & gingival indices (0.557, 0.078) respectively for control group conversely for test group who received probiotic supplement showed significant difference between the mean plaque & gingival indices (0.000, 0.000) after the follow up stages.

Key words: Probiotic, Sub gingival bacteria, Gingivitis, plaque index, gingival index.

Mann-Whitney U & Wilcoxon W test showed no significant differences for the mean Plaque& gingival Indices for zero day (0.170, 0.724) respectively ,while significant difference (0.000,0.000) for

the mean Plaque& gingival Indices at 20 day respectivel. Pairwise comparisons, ANOVA & Duncun test showed significant effect of probiotic on the mean total count of sub gingival anaerobic bacteria (strict & facultative)for three stages follow up,. that the total number of strict anaerobic bacteria on 0 day , 10 day and 20 day are decreased significantly compare with control group which showed no significant effect.

Conclusion: Probiotic admiration improved the effect clinical and microbiological diagnostic parameters.

Introduction

Periodontal disorders are characterized as long-term inflammation of the tissues that support teeth, which causes the structures that support teeth to gradually deteriorate. . Sender *etal*,(2016) Approximately 20–50% of individuals worldwide are affected with periodontal disease, and the prevalence of the condition is anticipated to rise in the coming years, especially as the world's population ages. Nazir *et al*,(2020) . The reversible type of periodontal disease known as gingivitis is brought on by microbial assault, which irritates the gingival tissues with chemicals that are produced from subgingival microbial plaque deposits. Murakami etal,(2018). A wide variety of microbial communities may be found in the subgingival crevice. After the development of gingivitis and periodontitis, which are regarded to be consecutive phases of periodontal health degradation, modifications in the composition of these communities take place. These diseases are characterized by inflammation of the gums and the tissue that surrounds and supports the teeth.It is unclear, however, to what degree microbiota associated with health and gingivitis are protective, or if these communities support the sequential expansion of periodontitis-related taxa. Moreover, it is unknown whether these communities play a role in the progression of periodontitis.This research is one of the unique ways employing probiotic to control the microbial community. Its purpose is to increase our understanding of the dynamics of the microbial sachem community disturbances both before and after gingival inflammation, as well as to examine the impact of various treatment options. Abusleme *et* al,(2021). Controlling gingival inflammation may be accomplished by mechanical or chemical means. Gingivitis treatment is very important for avoiding the progression of gingivitis to the irreversible type of periodontal disease. Singh *etal*,(2018), Chapple *etal*,(2015). Different microbial species populate the human skin as well as all mucosal surfaces, which has led to the realization that host-microbiota interactions are an inherent and necessary component of human physiology. This realization has helped to contribute to the field of microbiome research. Araos *etal*,(2019) . Even the most current evidence-based recommendations for the treatment of caries and periodontal disease continue to be dominated by therapeutic approaches that are centered on the removal of oral bacterial biofilms without regard to the kind of bacteria that are present. Fan *et* al,(2021), Schwendicke *etal*,(2020). Microorganisms that have a Positive Influence on the Health of Their Host and Are Referred to as Probiotics. Microorganisms are referred to be probiotics when they impart a health benefit on the host when they are taken in sufficient quantities. Examples of these include several strains of the bacteria *Lactobacillus spp*., *Streptococcus spp.*, *Bifidobacterium spp*., and *Bacillus spp*., as well as the yeast *Saccharomyces spp. boulardii* Funieru *etal*,(2017). Interfering with the metabolism and/or environmental circumstances of other microbes that are fighting for the same space and/or substrates enables probiotics to suppress the growth of those other microorganisms. This may take place as a result of a number of different processes, such as a shift in the pH level of the surrounding environment or an increase in the concentration of reactive oxygen or nitrogen species (ROS; RN), which is caused by the creation of metabolites such as organic acids, peroxide, or nitric oxide. Fukuda *etal*,(2011), Rosier *etal*,(2020)

MATERIALS AND METHODS

Study Population

The research was carried out in gingivitis patients using a clinical trial strategy that included of randomization, double blinding, placebo control, and parallel groups. The Research Ethics Committee of the College of Dentistry at the University of Mosul gave their stamp of approval to the protocol, informed consent, and subject information sheets by assigning them the reference number UoM. Dent/H.OM.61/22.. Subjects attending the Faculty of periodontal disease , University of Mosul, College of Dentistry (Mosul , Iraq), from February to November 2022 were screened. 30 patients (17 female and 13 male) aged between 18 and 55 years were included in the study (Alkaya, B., et al,2017) patients suffering from gingivitis were divided into two groups: The test group 15 patients that will give probiotic supplement as chewable tablets for 20 days(Chandran, et al, 2023), and the control group 15 patients, also suffered from gingivitis when taking a placebo in the form of lozenges that were shaped like probiotic pills. nevertheless, the lozenges did not contain any probiotics. During the course of the research, the participants were kept in the dark about both the contents of the pill and their specific therapy.. (Borrel,etal 2021) The patients were selected according to the following inclusion and exclusion criteria: **Inclusion Criteria** included patients range age 18-55 years (Montero, Eduardo, et al.), have clinical sign and symptoms of Chronic gingivitis , the presence of gingival inflammation, as determined by BoP, in more than thirty percent of sites, with probing depths of less than three millimeters, without radiographic bone loss and evident attachment loss owing to periodontitis, did not get probiotic supplements, did not have antibiotics during the last month, and did not have gingivitis. (Montero, Eduardo,*et al*(2017), Standardized oral hygiene instructions, consisting on the use the same type of brush, the same tooth paste without invasive ingredient and for standard brushing time Nadkerny, *et al*(2015) , female should not be pregnant or breast feeding ,patients didn't suffering from any other oral infection/inflammatory disease or systemic disease, didn't undergo any periodontal treatment the last six months ago **Exclusion Criteria**: Any patient don't follow the inclusion criteria will be excluded from the study, use antibiotics Systemic disease &other oral disease,pregnant women, history smoking habit or any allergy(Eduardo,*et al*2017).

Probiotic supplement

The probiotic pills, which are manufactured by NatureWise in the United States and sold under the brand name Oral Health Chewable Probiotics, include 3 billion CFU each tablet (110 mg), and one item has a total of 50 tablets. BLISM and BLIS K12TM are registered trademarks of Blis Technologies Limited, and the technology behind them is protected by United States patent no. 6772912. BLIS Technologies Limited is the owner of the trademarks BLIST and BLIS M18TM, which are also the subject of United States patent no. 7226590. **Composition**: *Lactobacillus casei subsp. casei, Lactobacillus paracasei Lpc-37, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus salivarius, Bifidobacterium lactis BI-04, Lactobacillus rhamnosus GG, Bifidobacterium breve Bb-03, Bifidobacterium infantis, Streptococcus thermophilus, Streptococcus salivarius BLIS-K-12, Streptococcus salivarius BLIS-M-18*; **Other Ingredients**: Isomalt, inulin, microcrystalline cellulose, glyceryl behenate, dicalcium phosphate, natural peppermint flavor, natural spearmint flavor, stevia leaf extract. Contains no wheat, soy, gluten, eggs, tree nuts, peanuts, fish, or shellfish.

Patients Clinical Examination

Under the illumination of artificial light, a dental examination was carried out on the dental chair located at the Periodontics Department. Under the direction of the periodontist, one examiner was responsible for both the evaluation and collection of clinical characteristics.. The clinical parameters that had been used in this study to assess patient's periodontal conditions, included the plaque index(Silness and Löe, 1964) &gingival index (Löe and Silnes, 1963)

Probiotic consumption:

Recruitment and selection of participants attending the Teaching Hospital/ College of Dentistry/ University of Mosul, College of Pharmacy/ University of Mosul. After brushing teeth, chew or dissolve twice tablet daily, according to the instruction of product leaflet . The samples were collected in three phases the zero phase this stage is before the patient takes the chewable tablets , the second phase samples were taken on the tenth day 10 after probiotic consumption, and the third phase samples were taken on the day 20 (Chandran, et al, 2023) .

Sample Collection

Sub-gingival plaque sample was taken from the affected site , supra gingival plaque was removed by cotton pledgets ,the site was isolated well to prevent salivary contamination & dried from saliva using a cotton roll , the sample was taken by inserting single sterile paper point size 45 (Alph-Dent Company, USA)for 30 seconds into the sub gingival area.

Microbiological work

1. Serial dilution

Dilution was carried out using 5ml sterile thioglycholate broth tubes to adjust the dilution to be hundred dilution 50 microliter of the broth was withdrawn from each tube using the micropipette, four dilutions tubes were used, the tubes were sterilized in autoclave at 121C for 15 min. The dilution series was carried out by adding 50 microliters from each stock patient sample by micropipette to the first tube in the dilution, then to the second ,third and fourth tube, 100 microliters from each dilution tube were transported by micropipette to inoculate blood agar plate for each dilution tube two blood agar plates were inoculated one for facultative aerobic growth in candle jar for 48hrs at 37 Cº and the other for strict anaerobic growth incubated under by adding a gas pak and incubating for 7 days (Göncziet al 2021) at a temperature of 37C

2. Counting

After the incubation period the bacterial growth were observed on the blood agar plates and comparing the growth with the dilutions, it turned out to be more clear and easy to count and isolate and differentiate between the forms of colonies on the surface of the media. It is dilution No. 10-2, that is, the second tube after the stock, as it was often relied upon in counting bacteria, where the number is between 30-300 A colony and also in the ease of isolating different types of bacteria, where all types of bacteria were counted in general and the number was multiplied by the reciprocal of dilution, i.e. multiplied by 100, and we got the CFU for each sample

Bacteria/ml = Number of colony (CFU)* 1/Diluent

In addition to the counting of bacteria, there was also the isolation of colonies that appeared to be very different from one another, as well as the microscopic examination of multiple colonies. The smear preparation was given a gram stain, and then it was examined under a light microscope with the assistance of an oil immersion lens in order to identify the various shapes of bacteria.

Statistical analysis

The data were processed with the SPSS® version 24. statistical and subjected to Fredmans(nonparametric) , Mann-Whitney U & Wilcoxon W, one-way analysis of variance One way ANOVA -Test, Duncan test and Pairwise Comparisons . $P \leq 0.05$ consider statistically significant .

Results

The Effect of probiotics supplements on Mean Gingival Index and Plaque Index by Fredmans test.

Samples were distributed into three stages first stage 0 day , second stage10 day and third stage20 day after taking probiotic supplements Table (1) Fredmans test showed that there was no significant effect (0.557, 0.078) for plaque index & gingival index respectively for control group , for test group who received probiotic supplement showed significant effect on the Plaque Index(P< 0.000) , the difference between 3ed-1st stage for the test group (P=0.03) , between 3ed-2nd stage (P=0.036) and between 2nd-1st(P=0.03)

significant effect on the gingival index (P= 0.000) , the difference between 3ed-1st stage for the test group (P=0.000) , between 3ed-2nd stage (P=0.006) and between 2nd-1st(P=0.006)

Table (1) The Effect of probiotics supplements on Mean Gingival Index and Plaque Index by Fredmans test.

Index	No. samples	Sig*	Test groups -Time		
PI Control group	45	0.557			
PI Test groups	45	0.000*	3^{ed}-1^{st}	3^{ed}-2^{nd}	2^{nd}-1^{st}
			0.000	0.036	0.03
GI Control group	45	0.078			
GI Test groups	45	0.000*	3^{ed}-1^{st}	3^{ed}-2^{nd}	2^{nd}-1^{st}
			0.000	0,006	0.006

The Effect of probiotics supplements on Mean Gingival and Plaque Indices by Mann-Whitney U & Wilcoxon W

Comparing the mean plaque and gingival indices records for test group at the three stages follow up. The plaque index showed no significant effect at the 0 day (170), the plaque index showed significant effect at the 10 day (0.006)and the plaque index showed significant effect at the 20 day (0.000).The gingival index plaque index showed no significant effect at the 0 day (0.724), showed significant effect at the 10 day (0.000), showed significant effect at the 20 day (0.000).show in table (2)

Table (2) The Effect of probiotics supplements on Mean Gingival and Plaque Indices by Mann-Whitney U & Wilcoxon W

Index	No.	Mann-Whitney U	Wilcoxon W	P Value
PI 0 day	15	79.500	199.500	.170
PI 10 day	15	47.500	167.500	.006
PI 20day	15	10.500	130.500	.000
GI 0 day	15	104.000	224.000	.724
GI 10 day	15	20.000	140.000	.000
GI 20 day	15	1.000	121.000	.000

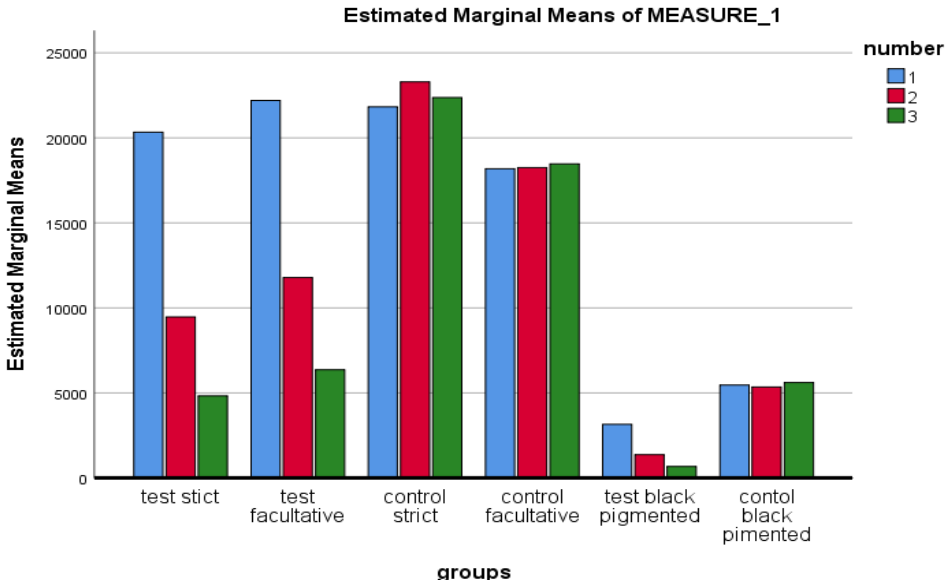
The Effect of probiotics on the mean total count sub gingival Anaerobic Bacteria for three stages follow up Descriptive analysis

Table (3) and the means total count of anaerobic bacteria (C.F.U/ml) for test and control groups at different stages follow up and showed the differences in figure(1) . The total count of the strict anaerobic sub gingival bacteria for test group the means were (23313.507, 11353.866 , 7189.252) for the (Day 0, Day 10, Day 20) respectively showed the differences on blood agar in figure (2), the total count of the facultative anaerobic sub gingival bacteria for test group the means were (25180.173,13673.866 ,8722.586) for the (Day 0, Day 10, Day 20) respectively showed the differences on blood agar in figure(3), The total count of the strict anaerobic sub gingival bacteria for Control group the means were (24806.840 ,25180.533 ,24722.586) for the (Day 0, Day 10, Day 20) respectively showed the differences on blood agar figure(4), the total count of the facultative anaerobic sub gingival bacteria for Control group the means were (21166.840, 20140.533, 20820.786) for the (Day 0, Day 10, Day 20) respectively showed the differences on blood agar figure(5), the total count of the black pigmented anaerobic sub gingival bacteria for test group the means were (6140.173, 3260.533, 3029.252) for the (Day 0, Day 10, Day 20) respectively and the total count of the black pigmented anaerobic sub gingival bacteria for Control group the means were (8440.173, 7240.533, 7969.25) for the (Day 0, Day 10, Day 20) respectively. figure (1) showed the differences all stages &groups between as on histogram

Table (3) The mean of Sub gingival An aerobic Bacteria for three stages follow up Descriptive analysis

	Groups	Time	Mean	SD*	Sample No.
a.	Strict An aerobic total count for test gp.	Day 0	±23313.507	17353.160	15
		Day 10	11353.866 ±	7579.467	15
		Day 20	7189.252 ±	2477.414	15
b.	Test Facultative An aerobic	Day 0	25180.173 ±	19219.827	15
		Day 10	13673.866 ±	9899.467	15
		Day 20	8722.586 ±	4010.748	15
c.	Control Strict An aerobic	Day 0	24806.840 ±	18846.493	15
		Day 10	25180.533 ±	21406.134	15
		Day 20	24722.586±	20010.748	15
d.	Control Facultative An aerobic	Day 0	21166.840±	15206.493	15
		Day 10	20140.533±	16366.134	15
		Day 20	20820.786±	16108.948	15
e.	Test black pigmented	Day 0	6140.173±	179.827	15
		Day 10	3260.533±	-513.866	15
		Day 20	3029.252±	-1682.586	15
f.	Control black pigmented	Day 0	8440.173±	2479.827	15
		Day 10	7240.533±	3466.134	15
		Day 20	7969.25±2	3257.414	

*SD=stander division



Fig(1) Show Effect of probiotics on the mean of Total Count of sub gingival Anaerobic Bacteria for three stages

The Effect of probiotics on the mean of Total Count of sub gingival Anaerobic Bacteria by One way ANOVA -Test

in table (4) One way ANOVA show that there was significant difference(P= 0.000) between the mean total count of test strict anaerobic group for the three stages follow up , showed significant difference(P= .000) between the mean total count of test facultative anaerobic group for the three stages follow up, no significant difference(P= .0 .838) between the mean total count of control strict anaerobic group for the three stages follow up, no significant difference(P= .0 . 993) between the mean total count of control facultative anaerobic group for the three stages follow up, showed significant difference(P= .000) between the mean total count of test black pigmented anaerobic group for the three stages follow up and showed no significant difference(P= .000) between the mean total count of control black pigmented anaerobic group for the three stages follow up.

Table (4) The Effect of probiotics on the mean of Total Count of sub gingival Anaerobic Bacteria by ANOVA -Test

		Sum of Squares	df	Mean Square	F	Sig.
Test strict	Between Groups	1879344444.444	2	939672222.222	44.269	.000
	Within Groups	891510468.667	42	21226439.730		
	Total	2770854913.111	44			
Test facultative	Between Groups	1942541777.778	2	971270888.889	72.993	.000
	Within Groups	558870666.667	42	13306444.444		
	Total	2501412444.444	44			
Control strict	Between Groups	16507111.111	2	8253555.556	.178	.838
	Within Groups	1947092000.000	42	46359333.333		
	Total	1963599111.111	44			
Control facultative	Between Groups	632930.178	2	316465.089	.007	.993
	Within Groups	1871415680.400	42	44557516.200		
	Total	1872048610.578	44			
Test black pigmented	Between Groups	49328444.444	2	24664222.222	20.187	.000
	Within Groups	51314666.667	42	1221777.778		

	Total	100643111.111	44			
Control black pigmented	Between Groups	512444.444	2	256222.222	.024	.976
	Within Groups	445470666.667	42	10606444.444		
	Total	445983111.111	44			

The Effect of probiotics on the mean of Total Count of sub gingival Anaerobic Bacteria by Duncan test

Table (5) Duncun test showed that the mean of test strict anaerobic bacteria on 0 day , 10 day and 20 day are decreased significantly after taking probiotics supplements and to compare with control strict anaerobic bacteria is not show significant , test facultative anaerobic bacteria show significantly effect after taking probiotics supplements against the control facultative anaerobic bacteria and test black pigmented bacterial groups not show significant effects on 0& 10 day like control black pigmented bacterial groups but on 20 day show significantly effect

Table (5) The Effect of probiotics on the mean of Total Count of sub gingival Anaerobic Bacteria by Duncan test

Time / groups	No.	Subset for alpha = 0.05		
		1	2	3
test strict3ed stage	15	4833.33		
test strict 2nd stage	15		9800.00	
test strict 1st stage	15			20333.33
test facultative 3ed stage	15	6366.6667		
test facultative 2nd stage	15		11786.6667	
test facultative1st stage	15			22200.0000
control strict3ed stage	15	22366.6667		
control strict 2nd stage	15	23293.3333		
control strict 1st stage	15	21826.6667		
control facultative 3ed stage	15	18464.8667		
Control facultative 2nd stage	15	18253.3333		
Control facultative1st stage	15	18186.6667		
test black pig. 3ed stage	15	673.3333		
test black pig 2nd stage	15	1373.3333		
test black pig 1st stage	15			3160.0000
control black pig. t3ed stage	15	5613.3333		
control black pig 2nd stage	15	5353.3333		
control black pig 1st stage	15	5460.0000		

The Effect of probiotics on the Mean Total Count of sub gingival Anaerobic Bacteria by Pairwise Comparisons

Results of pairwise multiple comparison table (6) showed the mean difference between (test &control groups) for the three stages follow up, on 0 day showed no significant intergroup difference between test strict & control strict Anaerobic Bacteria (p=1.000), for test facultative and control facultative groups (p=0.926) and for test black pigmented Bacteria and control black pigmented Bacteria

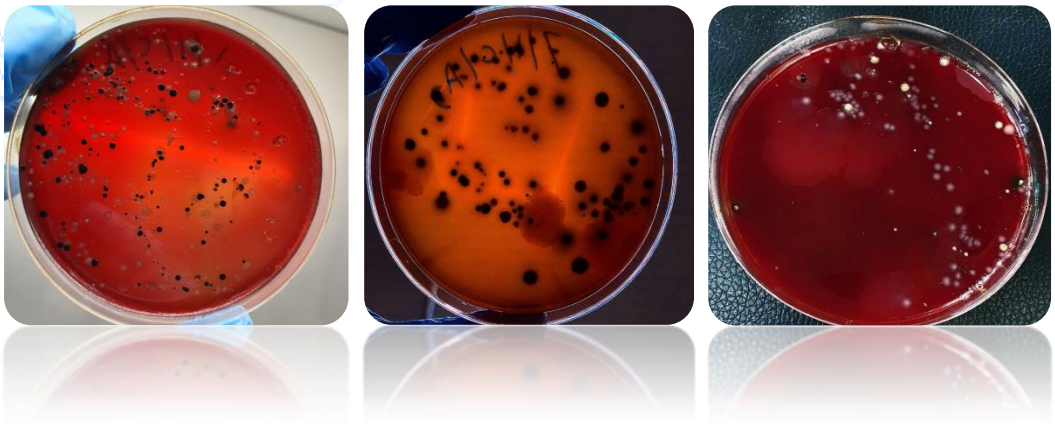
(p=1.000) , on 10 day showed significant intergroup difference between test strict & control strict Anaerobic Bacteria (p=0.000), for test facultative and control facultative groups (p=0.000) and for test black pigmented Bacteria and control black pigmented Bacteria (p=0.59) and on 20 day showed significant intergroup difference between test strict & control strict Anaerobic Bacteria (p=0.000), for test facultative and control facultative groups (p=0.000) and for test black pigmented Bacteria and control black pigmented Bacteria (p=0.62)

Table (6) The Effect of probiotics on the Mean Total Count of sub gingival Anaerobic Bacteria by Pairwise Comparisons

Time	Group (I)	Group(J)	Mean Difference (I-J)	Std. Error	Sig.*
Day 0	test stict	control strict	-1493.333	2119.371	1.000
	test facultative	control facultative	4013.333	2119.371	.926
	test black pigmented	contol black pigmented	-2300.000	2119.371	1.000
Day 10	test stict	control strict	-13826.667*	1342.095	.000*
	test facultative	control facultative	-6466.667*	1342.095	.000*
	test black pigmented	contol black pigmented	-3980.000	1342.095	.059
Day 20	test stict	control strict	-17533.333	1675.428	.000*
	test facultative	control facultative	-12098.200*	1675.428	.000*
	test black pigmented	contol black pigmented	4940.000	1675.428	.062

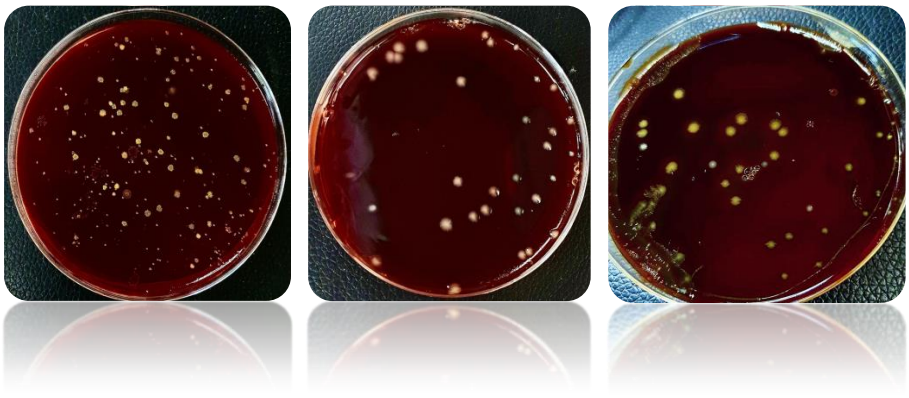
Based on estimated marginal mean

*The mean difference is significant at the 0.05 level.b. Adjustment for multiple comparisons: Bonferroni



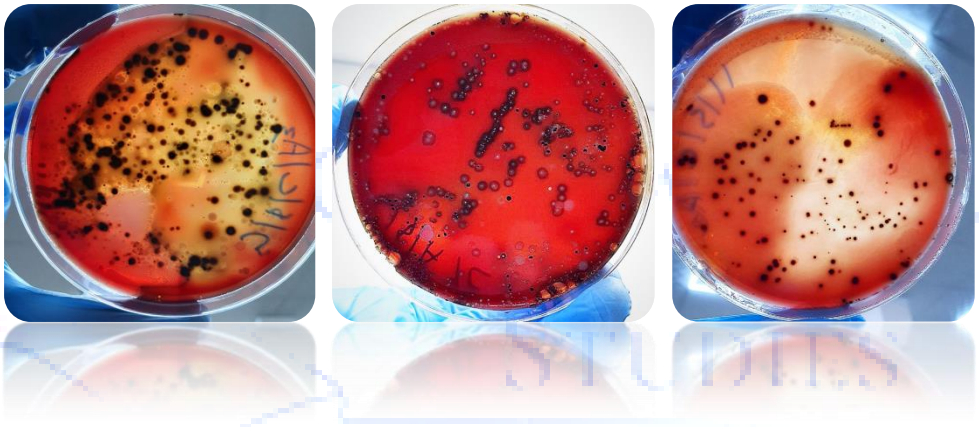
a **b** **c**

Fig(2) Result for The Effect of probiotics on media culture for the Total Count of Anaerobic Bacteria to the test group for strict anaerobic three phases : a.First phase =0 day b.Second phase =10 day c.Third phase = 20 day



a b c

Fig(3) Result for The Effect of probiotics on the Total Count of Facultative An aerobic for test group for facultative anaerobic on three phases : a.First phase =0 day b.Second phase =10 day c.Third phase = 20 day



a b c

Fig(4) Result for The Effect of placebo on the Total Count of Anaerobic Bacteria for controll group for strict anaerobic on three phases: a.First phase =0 day b.Second phase =10 day c.Third phase = 20 day

a b c

Fig(5) Result for The Effect of placebo on the Total Count of Facultative An aerobic bacteria for controll group for facultative anaerobic on three phases : a.First phase =0 day b.Second phase =10 day c.Third phase = 20 day

Discussion

In recent years, research on the use of probiotics for the prevention of dental and periodontal diseases has progressed considerably, with advances in the characterization of specific probiotic strains, and of the quantities and frequency of administration needed to secure beneficial effects in certain oral disorders (Iniesta et al.2020) Different studies used probiotic bacterial strains (Sinkiewicz et al.,2019)and (Iniesta et al.2020) used combination of L. reuteri ATCC 55730 and ATCC PTA 5289. In our study we used the (*Lactobacillus casei subsp. casei, Lactobacillus paracasei Lpc-37, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus salivarius, Bifidobacterium lactis BI-04, Lactobacillus rhamnosus GG, Bifidobacterium breve Bb-03, Bifidobacterium infantis, Streptococcus thermophilus, Streptococcus salivarius BLIS-K-12, Streptococcus salivarius BLIS-M-18*).Our study showed that plaque and gingival Index were significantly decreased in test group patients who administered probiotics for 10 day and 20 days ,with no significant reduction for control groups (patients who administered a placebo) analyzed by Mann-Whitney U and Wilcoxon W statically (de Almeida Silva Levi *etal* 2023). (Penala *etal*, 2012) Researchers who examined the impact of a probiotic mouthwash containing *Lactobacillus salivarius* and *Lactobacillus reuteri* on the periodontal indices of patients with chronic periodontitis found that the application of probiotics in the mouthwash and sub-gingival form along with scaling and root planning (SRP) treatment for 15 days (Ranjith,et al 2022) could significantly reduce plaque index and bleeding on probing (BOP). (de Almeida ,*etal* 2023) This double-blind, randomized, placebo-controlled clinical trial evaluated the adjuvant effects of *Bifidobacterium lactis* HN019 on the treatment of plaque-induced gingivitis. The effects of professional prophylaxis leading to reduced gingival bleeding were observed in both the test and control groups at 2 weeks. From 2 to 8 weeks, gingival hemorrhage continued to decrease substantially in the experimental group, but not in the control group. It is possible to hypothesize that the probiotic strain employed promotes immunomodulation, one of the principal mechanisms of action of probiotics. (Monteagudo-Mera A,2019. Within-groups statistically significant differences in GI scores were analyzed using Friedman test (de Almeida Silva Levi 2023). In our study fredmann statistical analysis test that compared within stages of test group , showed high significant effect (0.000) of Plaque Index between third stage (20 day), first stage (0 day) and third stage compared with second stage (0.036) and compared second and first also were significant (0.03). For gingival Index this test compared between stages, third stage(20 day) with first stage (0 day) that showed high significance(0.000),third stage(20days) compared with second stage(10days) showed significantly (0.006) and compared second(10day and first (0day) stages showed also significant reduction (0.006). So probiotics chewable tablets revealed significant effect on plaque and gingival Indices that reduced after 0 day , 10 and 20 days follow up. (Alanzi, *etal* 2018) significant reduction ($P<0.001$) was noted in the total salivary bacterial counts of the test group. The short-term daily consumption of LGG and BB-12 probiotic lozenges improved the gingival health in adolescents and decreased the microbial counts of *A. actinomycetemcomitans*, and *P. gingivalis*. Hence probiotic supplements may serve as a simple adjunct to standard oral care for promoting the oral health in adolescents. Alanzi *et al*, (2018).

This study showed reduction in the mean number of total count of microbial sub gingival plaque for test group on 10 day and 20 day ,with concomitant reduction in mean number of total count of black pigmented bacteria in sub gingival plaque compared with control group were no significant effect noted in the total counts of sub-gingival microbiota and along for black pigmented pathogens after follow up . in our study the time of probiotics admiration wasn't exceeding 20 day to assure founding lactobacillus during 20 days(Chandran, et al, 2023). The study of (Morales *etal*, 2018) the evaluating the clinical and microbiological effects of *L. rhamnosus* administered once a day for three months and azithromycin indicated that the adjunctive use of *L. rhamnosus* and azithromycin during initial therapy resulted in comparable periodontal clinical improvements compared with mechanical therapy alone. At the microbiological level, the total cultivable microbiota decreased substantially in the probiotic+ SRP

and antibiotic+SRP groups, while the percentage of P. gingivalis decreased in all groups compared to baseline (P 0.05). In the placebo(SRP) group, the prevalence of black-pigmented P. gingivalis (P. gingivalis) decreased. There were no significant differences between the groups, however. in another stud the three-month follow-up , Streptococcus-containing probiotics significantly decreased the CFU/ml of T. forsythia, P. gingivalis, A. actinomycetemcomitans, Fusobacterium nucleatum, and Prevotella intermedia in the probiotic and placebo groups, but there were no significant differences between the groups.in another studyThere was a decrease in the number of CFU/ml of *P. gingivalis, A. actinomycetemcomitans, and P. intermedia* in the quadrants of patients who took probiotics (*L. reuteri DSM-17938 + ATCC PTA 528*) in the study by Vivekananda et al. (2010). in another studyOur research focused on the quantitative change in periodontal pathogen (counts) throughout periodontal illnesses and covered different types of plaque-induced gingivitis. To provide definitive findings, we also included research using several probiotic strains. in another studyBecause of its antibacterial components, the most popular probiotic, L. casei.subp.casei, was said to have the ability to defeat pathogenic microorganisms. It was also said to have an immunomodulatory impact on the host (Britton, 2017).

Recommendation

long-term studies are needed in larger population across all age ranges and ethnic groups to obtain more consistent clinical results, and to determine if probiotics need to colonize the oral cavity to cause a beneficial effect.

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Ethical approval

The study was approved by the local Ethics Committee (registration number UoM. Dent/H.OM.61/22

subject's information sheets were approved by the Research Ethics Committee of College of Dentistry University of Mosul with the reference number UoM. Dent/H.OM.61/22. Subjects attending the Faculty of periodontal disease , University of Mosul, College of Dentistry (Mosul , Iraq), from February to November 2022 were screened

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