

Retrospective Review of Oral Probiotic Therapy

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Purpose: There have been many in vitro studies reporting on the efficacy of probiotic bacteria in inhibiting pathogens, and there have been published studies reporting on the inhibitor effects of probiotic bacteria on the salivary levels of bacterial pathogens. However, there have not been but a few studies on the clinical benefits of oral probiotic therapy. **Study design:** Dental records of 60 patients that were enrolled in an Institutional Review Board approved study were reviewed as to current caries activity status with measurement of the Decayed Missing Filled Teeth index and by Caries Management By Risk Assessment (CAMBRA) determination. The current oral health status was compared to the prior-to-study enrollment status and then analyzed in respect to published national norms.

The data (without any identifiers) had a statistical analysis by a blinded biostatistician. The data was subjected to statistical analysis (Statsgraphic) before and after the probiotic therapy. **Results:** Of the 53 subjects available for follow up, only 4 had remained caries active with a grand total of 27 carious lesions being detected and subsequently restored in this group. Of the original total of 60 patients with 292 initial carious lesions, after probiotic therapy and dental restoration, 78 total restorations were placed in the subject group over the following three years. Approximately half of these restorations were required in teeth that had initially presented with smaller lesions and had been placed in a “watch” category. Two of the patients that developed further carious lesions had been randomly assigned to the probiotic PerioBalance, while the other two caries active patients were assigned EvoraKids probiotic.

Of the original group of caries active patients, 24 did not present with any further carious involvement. Another 25 could be categorized as caries static, as the restorations required were substantially less than before probiotic therapy had been begun. The F-ratio, which in this case equals 51.3313, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 4 variables at the 95.0% confidence level. **Conclusion:** The tested probiotic supplements had a statistically significant effect on the caries experience of the enrolled subjects.

INTRODUCTION

Dental caries in the primary teeth of children ages 2 to 11 declined from the early 1970's until the mid 1990's. From the mid 1990's until the 1999-2004 National Health and Nutrition Examination Survey, this trend has reversed. A small, but significant, increase in primary tooth decay was found. This trend reversal was reportedly more severe in younger children¹. Dental caries remains the most common disease of childhood in spite of many highly touted dental prevention innovations². The addition of fluoride to water supplies and to toothpaste was heralded as a cure for dental decay. In addition, the application of pit and fissure sealants also was optimistically trumpeted as another preventative procedure of significant stature. Unfortunately, fluoride addition to water supplies only reduces decay by up to 25% and the additive effect of fluoride toothpaste is only 10-23%³⁻⁶. In fact, by age 65, 98% of all US citizens have experienced dental decay^{7,8}. The concept that fluoride simply delays carious involvement has been suggested and all practitioners have seen previously sealed molars eventually need restorations⁹⁻¹¹. Therein lies the rub, if decay is

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delayed long enough, “short” five or even ten-year public health studies will show a positive effect, even when there is no actual long term benefit. However, patients appear to be keeping their dentition healthy and longer, as the number of full and partial dentures have significantly decreased over time. But at the same time, more implants are being placed along with an increase in the number of endodontically treated teeth which leads to fewer extractions being treatment planned¹.

Streptococcus mutans remains the main pathogen in the initiation of dental decay, although other microorganisms have now been implicated¹²⁻¹⁹.

To date there have been no long-term studies of any potential benefits from probiotic therapy in improving oral health. There have been many in vitro studies reported on the efficacy of probiotic bacteria on inhibiting pathogens. Also there have been a number of studies reported on the inhibitor effects of probiotic bacteria on the salivary levels of pathogens. Still, there have not been but a few studies on the clinical benefits of probiotic therapy²⁰⁻²⁵. Most published research includes the use of probiotics in cheese, milk or yogurt²⁶⁻³². A study of children with a low caries rate demonstrated a significant decrease in new carious lesions, with a reduction from 0.8 new carious lesions to 0.2. This was a surprising result, as the subjects already were at low caries risk, which should indicate a “normal” microbiome³³. The caveat being that perhaps any carious lesions indicate an unhealthy situation, in which case, virtually none of us have a healthy microbiome. Interestingly, research studies have revealed that even avulsed teeth will have a periodontal ligament that survives better in a probiotic solution^{34 and 35}. Many such similar studies suggest that a healthy microbiome create a healthy patient. This present study was to determine what clinical effect, if any, a probiotic course had on long term caries rate of high caries risk subjects. A retrospective Institutional Review Board permission was obtained to look at the blinded data from a previously approved IRB study, three years later, and the current oral health of the subjects. The subjects had been randomly assigned to either the PerioBalance Group- *Lactobacillus reuteri* Prodentis®/™ (*L. reuteri* DSM 17938 and *L. reuteri* ATCC PTA 5289) or the EvoraKids Group- Evora-Plus, Oragenics, now ProBiora Health (ProBiora3 is a proprietary blend of three naturally occurring strains of beneficial bacteria, including *Streptococcus oralis* KJ3®, *Streptococcus uberis* KJ2®, and *Streptococcus rattus* JH145®). Thirty subjects were randomly assigned by randomizer software to each group.

MATERIALS AND METHOD

Dental records of 60 patients that were enrolled in the Institutional Review Board approved study, “A clinical trial to evaluate the effectiveness of DNA-PCR and CRT at measuring the salivary level of bacteria in caries prone children with PerioBalance or EvoraKids Plus therapy” were reviewed as to current caries activity status with measurement of the Decayed Missing Filled Teeth index and Caries Management By Risk Assessment (CAMBRA) determination. The current oral health status was compared to the prior-to-study enrollment status and then analyzed in respect to published national norms.

The dental records of patients that were enrolled in the original study were reviewed three years since they last participated. The dental charts were analyzed and data collected on the number of

teeth that had been treated since the subjects first enrolled. The data collected was compared to published national norms to determine if the patients remained cavity prone, became more cavity prone or developed fewer cavities than average for their age, race and gender. The data (without any identifiers) had a statistical analysis by a biostatistician. The data was subjected to the ANOVA (analysis of variance) to determine if there is any statistical difference before and after the probiotic therapy. The difference between the two probiotic groups was also analyzed with the Wilcoxon Two Sample test and Kruskal Wallis comparison. Of the 64 original probiotic enrolled subjects, clinical data existed for follow up on 60 subjects. The remaining 4 original subjects did participate in the probiotic regimen, but did not complete the salivary sampling. Out of the 60 remaining, fifty-three patients were available for follow up at the three-year review.

RESULTS

Of the 53 subjects available for follow up, only 4 had remained caries active with a grand total of 27 carious lesions being detected and subsequently restored in this group. Of the original 64 patients with 628 initial carious lesions, after probiotic therapy and dental restoration, 78 total restorations were placed in the subject group over the following three years. Approximately half of these restorations were required in teeth that had initially presented with smaller lesions and had been placed in a “watch” category. It is very important to note that for I.R.B. approval, only carious lesions that were clearly involving dentin could be restored. Hence, a number of carious lesions that penetrated to the Dentin Enamel Junction were placed in a “watch” category. Two of the patients that developed further carious lesions had been randomly assigned to the probiotic PerioBalance, while the other two caries active patients were assigned EvoraKids probiotic.

Of the original group of caries active patients, 24 did not present with any further carious involvement. Another 25 could be categorized as caries static, as the restorations required were substantially less than before probiotic therapy had been begun.

Table 1. Caries active, Caries resistant and Caries static patients.

| | Caries Active | Caries Resistant | Caries Static |
|--------------|---------------|------------------|---------------|
| PerioBalance | 2 | 11 | 13 |
| EvoraKids | 2 | 13 | 12 |
| Caries Count | 27 | 0 | 51 |

Table 2. Caries History Compared to Nationally Reported Values.

| Caries Experience | Pre Probiotic | National Average | Post Probiotic |
|----------------------|---------------|------------------|----------------|
| Per patient- 3 years | 5.51 | 1.84 | 0.75 |

Statistical Analysis

Kruskal-Wallis Test: Test statistic = 59.9423 P-Value = 0 The StatAdvisor

The Kruskal-Wallis test tests the null hypothesis that the median within each of the 4 columns is the same. The data from all the columns is first combined and ranked from smallest to largest. The average rank is then computed for the data in each

column. Since the P-value is less than 0.05, there is a statistically significant difference among each median at the 95.0% confidence level.

Summary Statistics

ANOVA Table

| Source | Sum of Squares | Df | Mean Square | F-Ratio | P-Value |
|----------------|----------------|----|-------------|---------|---------|
| Between groups | 52.6751 | 3 | 17.5584 | 51.33 | 0.0000 |
| Within groups | 28.733 | 84 | 0.34206 | | |
| Total (Corr.) | 81.4082 | 87 | | | |

The ANOVA table decomposes the variance of the data into two components: a between-group component and a within-group component. The F-ratio, which in this case equals 51.3313, is a ratio of the between-group estimate to the within-group estimate. Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of the 4 variables at the 95.0% confidence level.

DISCUSSION

There has been a genuine concern that probiotic therapy is at best effective for a short time and would require substantial investment in resources, time and commitment to be a long term therapeutic. This argument assumes that, all things considered, that the microbiome is rather stagnant and fails to respond to an outside stimulus. This has been demonstrated to be completely untrue, with research proving that any use of antibiotics, anti-microbials, change in diet or location, will quickly influence the microbiome^{36,38}. Another concern is that probiotic therapy won't have any long-term effect that can be measured in a patient population. The results of this study would indicate that probiotic therapy was of benefit in managing caries active patients and reducing future risk of dental disease after use for just one month. More importantly, this study proves that the effects were measurable even three years later. This means that the oral microbiome was shifted to being less pathogenic, and that probiotic therapy may indeed be more economical than other, less effective measures. It is very important to note that at least half of the carious lesions that were restored in the subjects were present at the first evaluation but had been arbitrarily placed in the "watch" category as a condition of the Institutional Review Board approval. Only very obvious carious lesions penetrating well into dentin were initially restored. This essentially means that the probiotics both arrested a number of carious lesions, or greatly slowed their progression. This would not be unlike the reported effect of fluoride, and the end result would be that many carious primary teeth could be allowed to exfoliate without needing restorative treatment³⁹. **Indeed, probiotics simply reduce the pathogens that modern, high sugar diets, feed.** Ideally, the clinician should determine the status of the oral microbiome by appropriate testing, institute a preventive protocol with polyols and probiotics, then re-test at perhaps an annual session, depending on patient compliance. Oral disease is totally preventable, and oral disease leads to systemic disease.

Although there have been numerous research publications with probiotic therapy demonstrated as effective and safe, there has been a great reluctance amongst many health care professionals to adopt probiotic protocols into their daily practice. Over one hundred articles on probiotics are published every month, in many of the most prestigious journals, and yet there is still this reluctance⁴⁰. Unfortunately, some of this reluctance is due to simple economics, as there is no insurance nor governmental reimbursement for probiotic therapy. Indeed, a number of practitioners have expressed concern that if probiotics work and their use becomes commonplace, their economic position would deteriorate. In addition, there is an occasional article/ research publication that denigrates the use of probiotics to preserve health. Review of these articles often disclose a flawed protocol, or even more basic, a misunderstanding of the mechanisms that probiotics employ for obtaining health. For instance, two articles recently published in the New England Journal of Medicine reported that a probiotic "disappointed" in the treatment of acute gastroenteritis of pediatric subjects seen in Emergency Departments⁴¹⁻⁴². Unfortunately, these studies were designed to fail as probiotics are not a treatment for a viral acute gastroenteritis, but instead they boost the immune system to prevent the disease or to ameliorate the symptoms⁴³⁻⁴⁵. Sick children that are seen in ED's are not the best subjects, they are already ill and it was totally unclear that anything was done to insure the probiotics survival. Most likely the child was on a diet of clear soda and crackers, void of any prebiotics necessary to aid in the probiotics success. Research into the mechanisms of probiotic actions would seem to suggest that at least 28 days would be required to develop the appropriate immune response. Certainly 5 days of probiotic use in an already ill pediatric subject would not be sufficient, so the results were very predictable⁴⁶. On the other hand, it may be stated that a historical precedent for use of a viral "probiotic" would be the cowpox inoculation by Edward Jenner to prevent the mortality seen with the scourge of smallpox⁴⁷. In this sense, cowpox may be considered a probiotic as it contributed to the health (actually even survivability) of the individual.

The importance of re-establishing a normal microbiome cannot be overemphasized. The connection of oral health to systemic health is now well established. Indeed, there is no real disease such as periodontal disease, it is simply a symptom of a global disease, that may best be described as Neural Arterial Gingival Simplex (or NAGS). *Porphyromonas gingivalis*, has been found to be a causative agent of periodontal disease, arteriosclerosis and inflammatory Alzheimer's⁴⁸. *Porphyromonas gingivalis* has been long linked in numerous published studies to both periodontal disease and atherosclerosis. Because there is a sole pathogen associated with all these pathologies, it is most logical to describe all the pathologies associated with it as a singular disease but caused by several pathogens with *Porphyromonas gingivalis* being foremost^{49 and 50}. Such is the case for any other disease, for instance, viral acute gastroenteritis due to rotavirus may cause fever, chills, muscle aches, fatigue and nausea, each component is not considered a separate disease^{51 and 52}. Changing the microbiome may very well become the preventive technique of choice. For example, oral and systemic preventive protocols may include probiotic supplementation with possibly overlapping beneficial bacterial, archaeon, viral or yeast probiotics.

CONCLUSION

Probiotics significantly reduced the caries rate of high caries prone pediatric subjects without any reported side effects. The reduction was not only significant statistically, it was very significant clinically with only four subjects (out of fifty-three) remaining caries prone. Dental professionals should adopt probiotic therapy as one of the most effective caries preventive measures in children.

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