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Analysis Of Buccal Mucosal Biofilms To Identify Bacterial Prevalence In Subjects With And Without Type II Diabetes

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Abstract

Aim: To Quantify The Two Most Prevalent Bacteria Among Type Ii Diabetic Individuals And Controls From The Buccal Mucosal Biofilms Using Molecular Methods.

Objective: To Compare The Percent Prevalence Of Veillonella And Granulicetella Bacteria In Uncontrolled Type Ii Diabetic Individuals With A Control Group.

Materials And Methods: The Sequence Obtained Is Then Surveyed Using BLAST Analysis To Define The Bacterial Flora And Two Bacteria Namely Veillonella And Granulicatella Are Selected For Further Amplification And Quantification By Real-Time PCR To Express The Bacteria In Copy Numbers.

Results: From The Collected Buccal Mucosal Biofilm Samples (N=24) Which Was Categorized Into Type II Diabetes (12) And Non-Diabetic (12). The Sequence Subjected To BLAST Analysis Gave A List Of Bacteria From Which Veillonella Sp. And Granulicatella Sp. Were Selected And Administered To Real-Time PCR For Amplification And Quantification Which Revealed An Increased Bacterial Prevalence In Type II Diabetic Subjects To Non-Diabetic Subjects Which Was Also Proved Statistically.

Conclusion: Based On The Results Obtained There Is A Significant Prevalence Of Bacterial Content In Type Ii Diabetic Subjects Compared To Non-Diabetic Subjects.

Keywords: 16srrna, Veillonella, Granulicatella, Type Ii Diabetes.

Introduction

Amidst Worldwide Chronic Diseases, Diabetes And Periodontitis Seem To Prevail The Most With A Bidirectional Relationship Including Their Underlying Regulatory Mechanism. Hyperglycemic Status Has A Direct Effect On The Microbial Composition, Collagen Metabolism And Cellular Function. By Development Of Advanced Glycation End-Products (Ages) Contributing To The Modification Of The Extracellular Matrix With The Binding To Cellular Receptor Further Exaggerating Inflammation[1,3].

The Bacterial Microbes Are Site-Specific Relating To The Vast Surfaces Present In The Oral Cavity, Coated With A Plethora Of Bacteria, The Customary Of Bacterial Biofilm, Whereas The Bacteria Veillonella And Granulicatella Are Most Commonly Evidenced Bacterial Microbe Present In All Sites Of The Oral Cavity. Hence These Both Bacteria Were Selected For Our Study[2]. In

Materials And Methods

Selection Criteria

Twenty Four Subjects(N=24) Were Included In This Study, Comprising Of Both Male And Female Genders, With An Age Group Ranging From 25 Years To 40years. They Were Divided Into Twelve Uncontrolled Types II Diabetic Subjects And Twelve Non-Diabetic Subjects As A Control Based On Their Hba1c Value. Subjects Included Should Not Suffer From Any Oral Lesions And Having Less Than 5mm Deep Periodontal Pockets Evaluated Using The CPITN Index And Subjects Presented With Neither Redness Nor Inflammation Of The Gums Were Selected. Subjects With Caries On The Teeth Were Excluded Based On Their DMFT Index. The Subjects Under Any Other Drugs Except Antidiabetic Medications For The Last 6 Months Are Excluded In This Study.

Sample Collection And DNA Extraction

Buccal Mucosal Samples Were Collected With Sterile Swabs In Universal Bacterial Lysis Buffer Containing 2% SDS (SIGMA-ALDRICH, Cat# 71736) And 10% Triton-X100 (SRL Fine Chemicals, Cat#64518). Bacterial Cells Were Lysed By Heating The Samples At 95°C For 10 Minutes.

Quantification Of DNA

The DNA Extracted From Samples Were Quantified By QUBIT Fluor meter To Determine The Total DNA Concentration.

16S Rrna Amplification And Sequencing

The PCR Amplified Products Were Cleaned With QIA Quick PCR Purification Kit (Cat# 28104) To Remove Primer Dimers, Which Otherwise May Interfere With Sequencing Reactions. The Purified PCR Amplicons Were Then Subjected To Sanger Sequencing (Also Called Direct Sequencing) To Determine The Bacterial Species Present In The Samples (Table 1).

Blast Analysis: The Nucleotide Sequences Identified By Sanger Sequencing Were Compared To The Oral Micro biome Database To Determine The Presence Of Bacterial Species That Are Known To Be Present In The Oral Cavity. The Oral Micro biome Database Is A Publicly Accessible Free Database And Is Available At Http://Www.Homd.Org/.

Amplification And Quantitation Of Veillonella And Granulicatella By Real-Time PCR

To Identify The Quantitative Presence Of The Above Two Bacteria (Table 2 & Table 3), The DNA Samples Obtained From The Patients Were Subjected To Real-Time PCR Analysis To Determine Their Quantitative Presence. 2ng Of Total DNA Was Subjected To Polymerase Chain Reaction (PCR) Amplification With Species-Specific Primers In Rotor Gene Q Real-Time PCR Unit. The Following Set Of Primers That Are

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Present Within The 16S Rrna Gene Were Used For Each Of The Species:

Statistical Analysis

The Descriptive Statistical Analysis Obtained For Veillonella And Granulicatella Bacteria In Both Type II Diabetic And Control Groups Is Demonstrated In The (Table 4 & Table 5). The Paired T-Test Obtained For Both Veillonella And Granulicetella Bacteria In Both Diabetic And Control Groups Is Demonstrated In The (Table 6 & Table 7). Based On The Statistical Analysis Obtained We Reject The Null Hypothesis

The Result Shows Us A Significant Increase In Bacterial Content Of Type II Diabetic Subjects Oral Cavity In Comparison To Non-Diabetic Subjects With A P-Value <0.05.

Sequence Analysis

The PCR Amplicons Thus Obtained Were Subjected To Sanger Sequencing. Of The Above Samples, All Diabetic Samples Showed Interpretable Sequence Outputs Whereas Only One From The Control Group Showed Interpretable Sequence Output. This Could Be Because The Amounts Of Bacterial DNA In These Controls Were Either Absent Or Were Present Below Detectable Limits Of The Present Amplification Protocol. Sequence Analysis By Comparison Of The Identified Sequence With Those In The Oral Micro biome Database (Http://Www.Homd.Org/) Indicated The Presence Of Granulicatella In Most Of The Uncontrolled Diabetic Samples. One Sample Showed Veillonella As The Top Hit Followed By Granulicatella During Data Analysis. The One Control Sample Showed None Of The Above Two Bacteria But Had Mostly Streptococcus Species.

Real-Time PCR Analysis

Analysis Of Melt Curve Showed A Sharp Peak For Veillonella At 82.5°C And Granulicatella At 86°C. Those Samples That Showed Similar Peaks Were Included For Analysis, While Those That Deviated From These Control Peaks Were Excluded From Further Analysis As These Indicated Primer-Dimers Or Non-Specific Amplification. The Data Analysis Was Done After Taking The Average Of Indicated Copy Numbers For Each Of The Bacteria. This Showed Veillonella To Be Present 3.5 Fold More In The Diabetes Samples Than The Controls, While Granulicatella Was Present 1.5 Fold More In The Diabetes Samples Than Controls.

Discussion

The main virulence factor of the microorganisms involved in periodontitis is lipo, 125 Polysaccharides (LPSs) an end toxin, the pathogenesis is activated by identification of pathogen-associated molecular structures from Toll-like receptors (TLRs), release of ROS from defending cells by correspondingly inducing oxidative stress, proinflammatory cytokines, and immunoregulatory complexes through the NF-kB pathway[3].

J. Michael Janda and Sharon L. Abbott in their literature showed that the exposed taxa are directly in debt to ease the role of 16S rRNA gene sequence disputed to the more incommodious administration of DNA-DNA hybridization investigations. DNA-DNA hybridization is the emphatically gold standard for recommended unfamiliar species and for the precise stint of a strain with obscure properties to correct taxonomic unit[5].

Conclusion

This study demonstrates the efficacy of metagenomic analysis of 16S rRNA for defining the bacterial flora and also adding on to the previous kinds of literature, projects the abundance of bacterial quantity in Type II Diabetic subjects to non-diabetic subjects with statistical

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correlation. It is fundamental to have a thorough knowledge on the bacterial diversity, the impact of diabetes on periodontal and vice-versa, for executing appropriate management of oral infections in diabetic patients with specific antibiotic therapy to avert antibiotic resistance, an upcoming global treat.

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