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Metagenome 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: subjects selected randomly and categorized into two groups within the age range of 25 to 40 years diagnosed with and without type ii diabetes based on their hba1c values. The samples of buccal mucosa biofilms are collected in sterile swabs and stored in bacterial lysis buffer which was later subjected to quantification of dna followed by 16s rrna amplification and sequencing.

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. **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

Diabetes mellitus, a pan- metabolic disorder is characterized by chronic hyperglycemia. Diabetes a syndrome, rather than a disease entity, is classified according to whether hyperglycemia is the primary feature or is a part of some other disorder, such as those of pancreas, endocrine system or some well defined genetic syndromes. The primary category of diabetes is presently differentiated into type 1 and type

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. **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.

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 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

Sequence analysis

To determine the bacterial species present in the buccal swab samples of patients with and without type ii diabetes, the dna extracted from the buccal swabs of five uncontrolled diabetic patients and five non diabetic controls were first amplified by pcr with universal bacterial primers that are capable of amplifying the 16s rrna gene of all bacterial species in any given sample.

Discussion

Alcaeus of cappadocia coined the term diabetes, literally meaning “siphon”, characteristic of polyuria in uncontrolled disease. In the recent times, diabetes has taken over the title, “ captain of the men of death”, from tuberculosis and syphilis so much so, that the debartolo dialectologist is considered the last of internists, a physician who needs to have intimate knowledge of the function of each organ of the body in order to effectively treat his patient.

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 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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