MOLECULAR CHARACTERIZATION OF BACTERIA IN GENETICALLY DISORDERED PATIENTS WITH NON-VITAL UNEXPOSED PULPS (IN VIVO STUDY)

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DEDICATION

I dedicate this work to my family, Mohamed, and my friends who really supported and helped me to accomplish this work

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1. Introduction

The surveys of many terrestrial and aquatic ecosystems indicated that more than 99% of microorganisms living on earth resisted cultivation under laboratory conditions and could only be identified by the use of molecular genetic methods; furhermore about 50% of oral bacteria had not yet been cultivated.

The vital pulp tissue is a sterile tissue as any other connective tissue in the body. Pulpal infection occurred only through any exogenous source of infection caries, impact trauma, or defected tooth structure. When pulpal damage is so severe, pulp inflammation and consequently necrosis occurs.

As aforementioned, in healthy individuals, teeth can undergo pulp necrosis due to many causes; the genetically disordered patients have also the same chance to have affected pulp. Genetic disorders such as Ectodermal dysplasia, and Enamel hypoplasia exhibited several aspects of common clinical, radiographic and histopathotogical features related to each other. Dental abscess formation is considered one of the main symptoms associated with these disorders which might be the main reason for extraction of the affected teeth.

The knowledge of endodontic infections had increased significantly during last 30 years, but in the same time different species of bacteria in the diseased pulp were not cultivated on artificial media and their pathogenicity was not definitely known. Epidemiological studies reveal that more than 200 different microbial species could be found in

infected root canals usually in combination of four to seven species per canal. Theoretically, any one of these species would have the potential to be an endodontic pathogen. The endodontic infections had polymicrobial origins. A restricted group of bacterial species could predominate and produced disease. The anaerobic bacteria were the predominant microorganisms in endodontic infections especially in primary infections such as genera *Bacteroids, Porphyromonas, Prevotella, Fusobacterium, Peptostreptococcus, and Eubacterium.* The bacterial species also played an important role in the expression of the symptomatology of the cases or being asymptomatic from the start.

To date, no study had specifically detected the type of bacteria that might be present in genetically affected teeth, and Whether there is any difference between those existing in normally developed teeth with affected pulp due to caries or trauma.

2. Review of Literature

2.1 Culture Method for Microbiologic Study of Necrotic Pulps:

Any exogenous source could easily affect tooth and might lead to pulp necrosis. Huge number of pathogens is responsible of root canal infection. Bacterial culture methods can be used for the detection and identification of wide variety of root canal microorganisms. Different specific media are used for aerobic and anaerobic organisms. This method is usually directed to microorganisms which could be easily cultured by the ordinary bacterial cultured methods.

Macdonald et al (1957)[(1)] studied the bacteriologic status of the pulp chambers in forty-six traumatized non-vital intact teeth. Radiographically, the periapical area in nineteen samples appeared normal. To ensure aseptic sampling uptake, the tooth was initially isolated with rubber dam, disinfected with iodine; and the access preparation was made using sterile round bur. Sterile paper points were introduced into two test tubes one of aerobic and the other for anaerobic media. The samples were incubated at 37^{o}C on sheep blood agar, for 48 hours aerobically and 96 hours anaerobically. The obtained growth was then examined by gram stain and dark field microscopy. Seventy-one stains were isolated from the samples taken. The three main isolated bacteria in a descending order were the anaerobic cocci 32.3%, then the aerobic streptococci 28.1%, and finally, the micrococci 21.1%. The authors assumed that the bacteria might have reached the pulp from oral cavity via lymphatics and blood vessels of the periodontium leading to pulp necrosis.

Brown and Rudolph (1957)[2] isolated and identified microorganisms from seventy canals of pulp-involved unexposed teeth. The aseptic conditions for sample taking were followed. Sterile paper points were introduced into the canal until saturation then placed into specific broth for spirochaetes or specific broth for aerobic and anaerobic bacteria. Different specific culture media were used to facilitate growth of different bacteria requiring different amount for growth and incubated aerobically and anaerobically at 37ºC for 48 hours. Later, the bacterial colonies were studied by dark-field, phase-contrast and light-microscopy. The percentage of isolated facultative anaerobes, anaerobes, aerobes and 10% CO_2 were 51.3, 23.9, 23.9 and 0.8%,. respectively. The four most predominating types in a descending order were *Streptococcus* (32%), *Cornybacterium* (28%), *Micrococcus* (19%), *Treponema* (14%), while the least present types were *Neisseria* (3%), *Fusobacterium* (3%), *Diplococcus* (1%), *Aerobacter* (1%), *Pseudomonas* (1%) and unidentified 12% (Cocci 7% and Rods 5%). The authors assumed that it was important to develop different and specific media for culture methods which might help in the identification of more isolated bacteria from root-canals.

Hampp (1957)[3] identified spirochetes obtained from unexposed canals of thirty-eight pulp-involved teeth. Specific media were used for the cultivation and growth of spirochaetes. Microscopic examination of the cultured plates exhibited small numbers of spirochetes which appeared to be the smaller oral treponema types. Only fourteen spirochaetes positive cultures were obtained. Ten pure isolates out of the fourteen spirochetal containing cultures were all of the smaller treponeme type, two were definite gas producers. The author concluded that many

studies should be done for facilitate detection of spirocheates from primary root canal infections.

Grossman et al (1962)[4] isolated gas-producing organisms from root canals. The three hundred samples taken from infected root canals were divided into two groups 100 (from 12 cases) and 200 (from 11 cases) at two different times and carried out by two different technicians. During sample taking, full aseptic conditions had been followed. The paper points were placed into the canals and then introduced into 15 ml brain-heart infusion broth +0.1% Bacto-agar. The tubes were incubated at 37°C for 48 hours and examined for gas production. In the first 100 samples, the bacteria found were streptococci (2/100), diphtheroids (2/100), yeasts (2/100), and only 1/100 each of *Escherichia coli*, *Serratia marcescens*, *Staphylococus aureus, and Pseudomonas*. In the second 200 samples, *streptococci* and *diphtheroids* were (7/200), yeasts (3/200), and *pseudomonas*, *lactobacillus*, and *aerobacter* were only 1/200. The results denoted that there was no relationship between the gas-producers found and any specific pulp disease, or disease of the periapical tissues.

Kantz and Henry (1974)[5] isolated and classified anaerobic bacteria of twenty-four non-vital intact teeth in man. For sampling, complete aseptic conditions were followed. A sterile bur was used for the access cavity preparation, the canal was instrumented with H-file and sterile paper points were placed until saturation then placed into a thioglycollate test-tube and incubated anaerobically for 35^{o}C for ten days. Each sample was examined by dark field microscope. A total of 377 bacterial colonies were isolated. Hundred and four (27.2%) of the isolates were strictly anaerobes; the seven identified bacteria in descending order were *Fusobacterium* (n=41), *Actinomyces Israelii*