ISSN: 2581 – 3633
PubMed - National Library of Medicine - ID: 101745081

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at: www.ijmacr.com

Volume – 3, Issue – 2, March - April - 2020, Page No. : 66 - 72

A study to identify the presence of Candida Albicans in Primary Root Canal Infections in a Rural Hospital.

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Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Introduction: Candidal organisms are commensals of the oral cavity and their opportunistic infection depends on the interplay of local and systemic factors. Endodontic treatment failure has been associated with the persistence of microbial flora following therapy in the root canal system, and this includes fungi like Candida, which are resistant to conventional root canal irrigants.

Materials and Methods: A file and paper point following a disinfection protocol have been collected for fifty root canal samples from primary endodontic infections. In Sabouraud dextrose agar, the samples were inoculated and incubated for a period of 2-3 days. The microorganism was compared by using Gram staining and germ tube test.

Results: The C. albicans was present in 5 samples. There were significant differences between males and females regarding the presence of C. albicans in root canal.

Conclusion: Candidal contamination of the root canals could be the cause of endodontic treatment failure and thus emphasizes the use of irrigants with antifungal properties for sufficient duration during treatment.

Keyword: Candida, Albicans, Primary, Root, Canal

Introduction

Endodontic problems lead to dental dysfunction as much as 30 – 40%. The most common reason for endodontic therapy failure is micro species survival even after the completion of treatment.[1] The major cause of endodontic contamination by microbiota was checked by a study by Kakehashi et al. [2]The recent trend in dentistry focuses rather than simply on the way the disease process is understood.

Understanding the root canal microbiota involved in endodontic pathology has tremendously improved the success of root canal therapy. Due to the vast number of microorganisms in the root canal environment a paradigm shift from conservative to broad spectrum treatment has taken over. In fact, experiments have been carried out on a broad scale to investigate the features and techniques of destroying the "most" resistant organism. [3]

The endodontic infections are polymicrobial in origin. For primary infections, compelling anaerobic bacteria are known to dominate the system of the canal [4]. However, a variety of other microorganisms have been neglected as a cause of endodontic infection due to their low prevalence in primary infections. "One of these species is fungi."

Different studies have found fungus in endodontic infections. [5] The most frequently isolated, possibly infectious, yeast from contaminated rooted canals is *Candida albicans* [6,7].

Candida is considered to be more prevalent in secondary/persistent endodontic infections than primary infections[8]. Candida possesses virulence factors that may play a role in the onset of endodontic pathologies. These yeasts can adapt to a variety of environmental conditions andadhere to many surfaces including dentin

and root filling materials[9]; moreover,*C.albicans* can produce hydrolytic enzymes, undergo morphologic transition, form bio-film, and evade and modulate the host defense[8].

Grossman initially reported their presence in the range of 1%–17%.[10] Not many studies focused on *C. albicans* as the main cause of endodontic diseases. Thus, the present study aimed to recognize *C. albicans* in primary infections of root canal.

Methods

Patients with endodontic diseases visiting the Department of Conservative Dentistry and Endodontics, MMU, Mullana, Ambala were randomly selected for the research in the months of November 2019- February 2020.

50 patients were selected on the basis of case history, clinical examination and radiographic review for primary endodontic infection with irreversible pulpitis / pulp necrosis / pulpal and periapical abscess. In order to prevent the confounding factor, patients with reinfection or systemic fungal or antifungal medication were not included in the study.

The written informed consent was taken before including the patient in study. The demographic for the research were gender-based categorized into 3 age groups: 20–40 years, 40–60 years and over 60 years. Strict aseptic precaution was followed while collecting the microbial samples from the root canal.

The isolation of tooth was done by using Rubber Dam. Using 30 percent hydrogen peroxide for 1 minute, the tooth surface and the adjacent fields were cleaned. Operational field disinfection was conducted with a concentration of 30% hydrogen peroxide, 5% iodine stain, and 5% sodium thiosulfate was used to neutralize the study field. Samples were taken from the main canal in single rooted teeth and canal with per apicalradiolucency in multirooted teeth. Early caries or restoration wasremoved with a sterilized bur (SS White).

Sterile access opening bur (MANI) was used to reach the root canal and the root canal was irrigated with sterile saline before the collection of the sample. From each canal two samples had been taken.The first sample was taken with the use of a sterile K-file up to a provisional working length using the preoperative x-ray radiograph. The second sample was taken with a paper point inserted for 1 minute in the root canal.

The sample was transported to the Department of Microbiology, immediately (within 4 h) in a sterile container. The sample was streaked on the Sabouraud's Dextrose Agar plate using a sterile heated platinum wire loop. The culture plates were incubated at 37^0 C for 3-4 days. White colored, elevated, smooth and butyrous colonies were suggestive of fungal growth. Gram staining was done to confirm the presence of Candida. In case of positive candida growth, germ tube test was done to differentiate between *C. albicans* and non-albicans species.

In order to assess the difference between male and female and prevalence of C. albicans in various age groups, Chi square tests was used using SPSS software version 22.0 (SPSS Inc., Chicago, USA). The significance level was set at $p \leq 0.05$.

Results

The study was conducted on 50 patients with endodontic infectious root canals in the Department of Conservative Dentistry and Endodontics, MMU, Mullana, Ambala to determine the incidence of *C.albicans*. *Candida albicans* was positive in 1 patient aged 20-40 years, in the second group of 40–60 years, 3 cases were positive and in the third group patients aged above 60 years 1 case was positive.[Table 1]. Each group was also divided according

to gender in which Candida albicans was seen in 1 male and 4 females. [Table 2]

The fungus was identified in 9 patients from the sample of 50 patients. Out of the 9 fungus, 5 samples were identified as Candida by Gram staining. They were confirmed as *Candida albicans* by positive germ tube test. Therefore, Candida tested positive for five out of the fifty overall tests (10% of the samples).

Discussion

Infection of the root canal occurs through the interaction of microorganisms which is thus a mixed infection. Apart from Gram positive cocci and anaerobic bacteria, fungi were also isolated from infected tooth's root canal. They are one of the causes of endodontic failure because of its ability to enter infected root canals [11]. Grossman published in 1952 the first study on the existence of fungi [12]. It is not included however in the main infections of the root canal.

Within the fungi kingdom, the species belonging to the Candida genus may possess the properties of a potential endodontic pathogen. Members of genera Candida are the most commonly identified forms of opportunistic fungal pathogens in humans. Candida has shown to be a primary microorganism, even in the case of chronic or refractory periradicular diseases. In more than 50% of population, Candida is found in the oral cavity. [13] It is in non-pathogenic phase. However, the disease is caused by the Candida species when they become infective and invade the host tissues.[14] A shift in predisposition conditions that triggers a number of virulence factors seems to respond to the transformation of a Candida from a healthy commensal to a pathogenic organism. [6]

Our analysis has shown that 10% of Candida albicans was widespread in predominant untreated root canal infections. It corresponds to the literature that states that yeasts exist between 1 and 17 percent in polluted root canals. [15, 16]

In the recent study, the cumulative prevalence of Candidaspp. in endodontic infections was 8.20% (95% CI, 5.56%–11.21%). They stated that among the studies conducted “geographic area” proved to have a significant effect, with studies from Africa reporting a higher combined prevalence (24.82%) compared with studies from Asia, Europe, North America, and South America.[17]

In apical thirds in two out of nine biopsies, Nair etal. [5]detected yeast colonies. NajzarFleger et al. [18] has observed that 55% of the root canals produce Candida cells after examining the occurrence of Candida bacteria in different oral locations.

In this study, *C. albicans* were greater in age group 40–60 years but the difference was not statistically significant.This result is comparable to the result of study by Magare and Awasthi that has shown that in oral cavities of immunocompromised patients between 41 and 80 years the prevalence of Candida was higher. [19] Pinho and Zarembaetal have carried out similar studies and they reached to conclusion that ages ranging from 60 to 80 years the isolation rates for Candida species were high. [20, 21]

There were 27 male and 23 female in the study. There were significant difference between males and females. 4 tests were positive in female (17.39%) and 1 in male (3.70%) was positive. Candida organisms had a 4.70:1 ratio of prevalence between female and male. The interaction was shown to be 2.55:1 in another similar study. This was also based on a study carried out by Berdicevskyetal. [19] who reported similar findings.

Recent trials show increased prevalence of 21% in the molecular detection by polymerase chain reaction (PCR) [18, 24] Low numbers of yeasts at the start of root canal treatment have also been reported in the study which

eventually reach far greater proportions during conventional therapies.[8]

Sabourauds Dextrose agar has been used in this study as a culture medium that provides specific media for the growth of candida organisms. Germ tube testwas used for confirming the *C. albicans.*

The variations in isolation protocols, microbiological (cultural / molecular) research and geographical variance of the patients could be a possible explanation for the different outcomes. Molecular techniques such as PCR are more sensitive than culture methods. Using molecular detection methods, greater numbers of microbial species have been identified. [25]

Nevertheless, molecular methods cannot substitute the culture process entirely because they cannot include details on the organism's viability. This can result in the entity being overestimated and produce false results. [26]

The objective of the research is to determine the method chosen. The gold standard of culture technique is effective in therapy planning. [6]

The findings of our research showed the need to use both antibacterial and antifungal intracanal medications and irrigants. But only an initial view of the endodontic microbiology is provided by the small sample size with only microbial culture analysis. However, there is ample room for better detection and treatment of microorganisms through the use of advanced technologies.

Conclusion

This study confirmed that *Candida albicans*in present in primary endodontic infections. Though not noticeable in the existence of bacteria, *C. albicans* is playing a vital part in triggering root canal infections in primary root canal infections and in refractory periodontitis, the existence of *Candida albicans* requires testing and clinical evaluation of drugs for antifungal susceptibility to decrease endodontic failure.

Table 1: Distribution of samples according to age

Age (years)	Present	Absent	Chi Square value	P vlaue
20-40	1	12		
40-60	3	28	3.67	0.23 (NS)
>60	1	10		

Table 2: Distribution of samples according to Gender

Gender	Present	Absent	Chi Square value	P value
Male	1	26		
Female	4	23	14.25	0.01 (S)

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Legend Figure

Figure 1: Bleeding on access opening

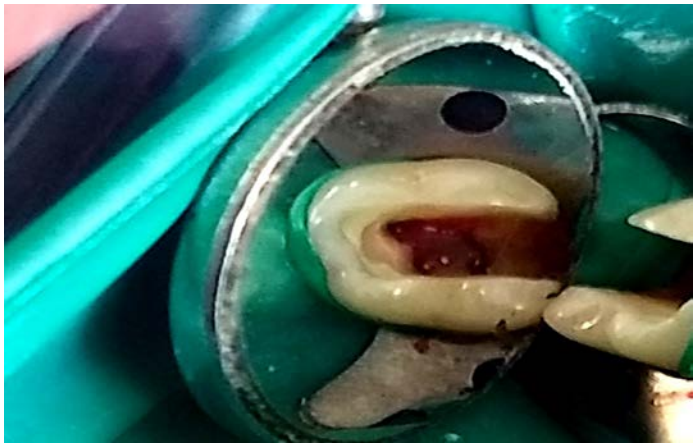

Figure 2: Orifice visible on opening the pulp chamber

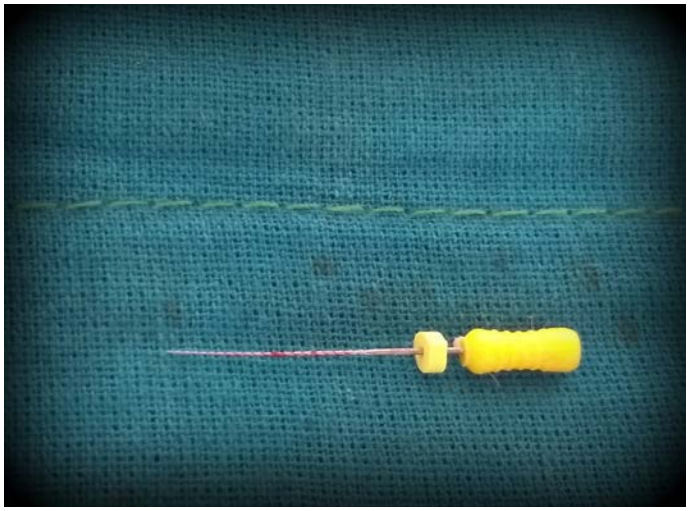

Figure 3: Method of collection of sample using K-file

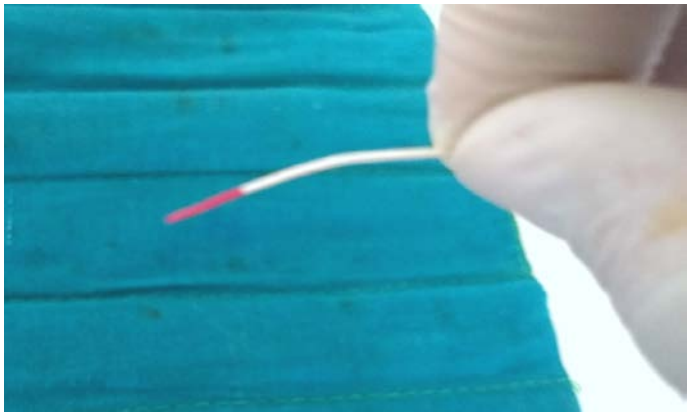

Figure 4: Method of collection of sample using paper point

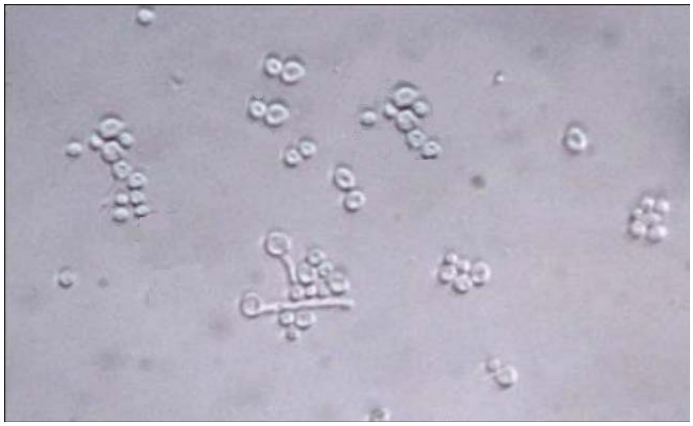

Figure 5: Candida Albicans in Germ tube test

Figure 6: Candida Albicans on Sabouraud Dextrose Agar

How to citation this article: Kartik Sharma, Kanishtha Sharma,“ A study to identify the presence of Candida Albicans in Primary Root Canal Infections in a Rural

Hospital.", IJMACR- March - April - 2020, Vol – 3, Issue -2, P. No. 66 – 72.