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Original Article

Isolation of *Enterococcus faecalis* in the saliva samples of patient candidates for liver transplantation

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ABSTRACT

Background: Liver transplantation has turn into a standard management for chronic liver failure (CLF), and the number of recipients increased during the last few years. Enterococci are progressively related to nosocomial and opportunistic infections. Oral cavity may act as a reservoir for this species, especially in cases with oral infection. Immunocompromised patients are prone to serious enterococci-related disease. This study investigated the prevalence of *Enterococcus faecalis* in the saliva samples of patient candidates for liver transplantation.

Materials and Methods: In this cross-sectional study the saliva samples of 100 patient candidates for liver transplantation and 100 age- and sex-matched healthy control group were collected. Bacterial DNA was extracted from the samples and *E. faecalis* was detected using polymerase chain reaction test. Fisher's exact test and Mann–Whitney test were used to correlate the positive and negative cases with the disease. Statistically, a significant difference was considered when $P < 0.05$.

Results: There was no significant difference between both groups for the presence of *E. faecalis*. This bacterium isolated from the saliva of two cases in the study group and only one healthy control. The higher rate of carious teeth were detected in the oral cavity of CLF cases than control group ($P = 0.001$).

Conclusion: Patients with chronic liver diseases assessed in this research showed a higher incidence of poor oral health and caries compared with the healthy controls, but there was no statistical difference in the presence of *E. faecalis* in saliva samples of each group. Complete oral examination, dental treatment, and oral hygiene instruction are necessary for all these cases before liver transplantation.

Key Words: *Enterococcus faecalis,* liver transplantation, saliva

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INTRODUCTION

Several liver diseases may cause chronic or ongoing liver inflammation, and the most usual etiologies of end-stage liver failure are chronic viral hepatitis, alcoholic liver cirrhosis, autoimmune liver disease, primary sclerosing cholangitis, primary biliary cirrhosis, steatohepatitis, inherited liver disorders, and drug-induced hepatic failure.[1] The number of

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recipients of liver transplants has increased quickly in the recent few years and is expected to remain to do so in the next. The dentist should be ready to see participants who are planning or have had liver transplants. The main aim of dental intervention, before and after liver transplantation, is the inhibition of bacteremia originates from oral cavity that may lead to general infection. Furthermore, pre- and posttransplant medical problems that should be mentioned are inadequate drug metabolism, bleeding tendency, inadequate wound healing, immunosuppressive drugs, and higher rate of infections.[2,3]

Infective disorders are the main factors that can cause morbidity and mortality in end-stage liver damage and transplant recipient.[4] Oral cavity infection is estimated as a major source for general infection in a large group of liver transplant candidates and recipients.[4]

The salivary bacteria play a major role in producing oral disorders and have an interaction with other microbiota of the human body, especially the intestinal tract, but there is little information's about the similarity of them.[5,6] Relatively, most of studies focused on oral pathogenic bacteria that cause periodontal problems and caries, and human salivary microbiota did not consider perfectly.[7,8]

In the past *Enterococcus faecalis* classified as a portion of D *Streptococcus*. It is a Gram-positive, commensal bacterium of humans' gastrointestinal tracts. As well as other types in the genus *Enterococcus*, *E. faecalis* isolated from healthy participants but can initiate serious infections in individuals, particularly in the nosocomial environment.[9]

A study showed that *E. faecalis* was more frequently detected in the subgingival microbiota of HIV-positive than HIV-negative participants with periodontal disease,[10] and another documents established that *E. faecalis* is commonly accompanying with necrotizing gingival disorders in the HIV-positive cases.[11] In another study, 60% of diabetic patients showed oral *E. faecalis and Enterococcus faecium* in the mouth.[12] To the best of our knowledge, there are no available data about the presence of this bacterium in the saliva of Iranian population, especially end-stage liver disease patients. Owing to the reservoir activity of the oral cavity for several pathogens related to systemic infections, this study was conducted to determine the prevalence of *E. faecalis* as noncommensal pathogenic bacteria in the saliva samples of patient candidates for liver transplantation.

MATERIALS AND METHODS

Ethical statement

This cross-sectional study was carried out in accordance with the guidelines of the Declaration of Helsinki as revised in Edinburgh (1975). The study protocol was approved by the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran. The written informed consents were obtained from participants for sample collection, and in unable cases, verbal consent was obtained. Patients were informed about the nature of the study.

Participants

In a cross-sectional study, 100 dentate patient candidates for liver transplantation who referred to the Clinic of Emam Reza (Medical Clinic in Shiraz, Iran) for dental examination between December 2017 and April 2018 were collected. The study group comprised 63 males and 37 females. A control group consists of 100 age- and sex-matched participants that referred for routine checkups between the same times. The exclusion criteria were the patients with diabetes, pregnancy, smoking, using antibiotics within the past 2 months, and candidate for other organ transplantation. All patients in both groups were examined radiographically (panoramic X-ray) and clinically by oral medicine specialist as an examiner. The number of decayed teeth and gingival status of patients was recorded according to the Modified Gingival Index also by examiner [Table 1].

Saliva sampling

Participants were prohibited from eating and washing their mouth 1 h before sampling. Each individual will be sampled using the oral rinse technique[13] with 10 mL of 0.9% sterile saline for 60 s, and the mouthwashes will be collected in sterile 50-mL polypropylene falcons. Samples freeze (−20°C) until polymerase chain reaction (PCR) amplification is performed.

Table 1: Modified gingival index criteria

0=Absence of inflammation
1=Mild inflammation: Slight change in color; little change in texture of any portion of the marginal or papillary gingival unit
2=Moderate inflammation: Glazing, redness, edema, and/or hypertrophy of the marginal or papillary gingival unit
3=Severe inflammation: Marked redness, edema, and/or hypertrophy of the marginal or papillary gingival unit, spontaneous bleeding, congestion, or ulceration

DNA extraction

Bacterial DNA was extracted from all saliva samples, using the GeneAll DNA extraction mini-kit (GeneAll, Seoul, Korea), in accordance with the manufacturer's instructions. DNA quality and quantity were checked by a spectrophotometric method (BioPhotometer, Eppendorf, England). The extracted DNA was stored at −20°C for further use.

Molecular detection of *Enterococcus faecalis*

Molecular diagnosis of *E. faecalis* was performed by gene amplification of primers *ddl*. The primers listed in Table 2 were used to amplify the genes applied for detection *E. faecalis*. As a first step, PCR was performed with a standard protocol. Each step was carried out using a 25-μL mixture containing 12.5 μL of ×10 buffer (supplied with Taq polymerase), 10 pmol of each primer, and 70 ng of genomic DNA. The PCR was performed with Bio-Rad T100 thermal cycler (USA) with microtubes under the following conditions: denaturation for 5 min at 95°C; 35 cycles of 45s at 95°C, 45s at 48°C, and 1 min at 72°C; and a final extension step of 10 min at 72°C.

Statistical analysis

Fisher's exact test and Mann–Whitney test with odds ratio (95% confidence interval) were used to correlate the positive and negative cases with the disease. Statistically, a significant difference was considered when $P < 0.05$.

RESULTS

The age in case group ranged from 10 to 67 years (mean age: 40.84 years) and in control group from 10 to 77 years (mean age: 39.14 years).

There was no significant difference between both groups for the presence of *E. faecalis* ($P > 0.005$). This bacterium isolated from the saliva of two cases and only one control [Figure 1]. The higher rate of carious teeth was detected in the oral cavity of chronic liver failure (CLF) cases (3.23) than control group (1.84) ($P = 0.001$) [Table 3].

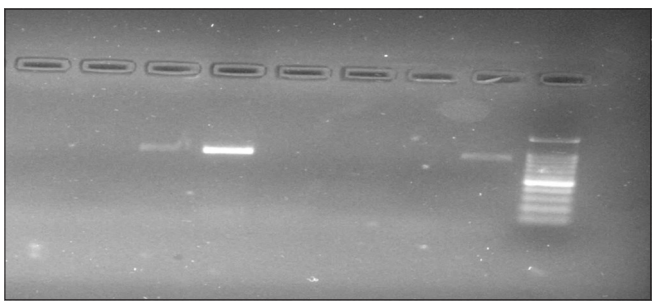

Figure 1: Agarose gel electrophoresis for *ddl* gene of *Enterococcus faecalis.*

A 45-year-old male with 14 carious teeth and moderate gingivitis and a 26-year-old female with good oral hygiene demonstrated *E. faecalis* in the saliva. In the healthy controls, a saliva sample of a 77-year-old male with severe gingival index was positive for bacterial DNA. About 10% of study group patients were positive for HBV and 1% was positive for HCV, and four patients had Wilson's disease.

DISCUSSION

In the past few years, many researches focused on the interaction between serious systemic diseases and oral microflora.[14] On the other hand, numerous studies showed that normal oral microflora may be changed due to systemic diseases or oral condition alteration. In the present study, the difference in *E. faecalis* in the saliva of both CLF and healthy participants has been compared. Although CLF cases were unable to adequately maintain the oral health, the presence of this bacterium did not differ significantly. For proper bacterial pathogenicity, the microorganism should

Table 2: List of oligonucleotide primer used for detection bacteria by polymerase chain reaction

Primer	Sequence (5'->3')	Ta	Product length
ddl $_{E. faecalis}$-F	ATCAAGTACAGTTAGTCT	48°C	941 bp
ddl $_{E. faecalis}$-R	ACGATTCAAAGCTAACTG		

E. faecalis: *Enterococcus faecalis*

Table 3: Demographic and clinical parameters of participants

	Case group	Control group
Mean age	40.84	39.14
Number of carious teeth	3.23	1.84
Gingival index		
0	54	52
1	8	12
2	15	30
3	23	6
Presence of *Enterococcus faecalis*	2	1
Type of liver disease (%)		
B hepatitis	10	
PSC	9	
Autoimmune	4	
Vinson	4	
Tumor	4	
Other causes	69	

PCS: Primary sclerosing cholangitis

be able to adhere to, grow on, and invade the host. Furthermore, it should be capable to overcome the host defense mechanisms.[15]

The previous study reported that improper oral hygiene, dry mouth, limited jaw movement, hospitalization, and immune system suppression can improve noncommensally bacterial colonization.[14] One of the major side effects of liver transplant cases is infections.[16] An increase in susceptibility to rejection resulted from oral infection was seen in numerous transplant recipients.[17]

Animal-based research showed that a different liver disturbance such as fatty liver disease, cirrhosis, and hepatocellular carcinoma may develop in cases with periodontitis; liver transplantation also affected by such problem.[18] Bajaj *et al.*[19] reported that there is an interdependence between the saliva and stool microbiota of cirrhotic patients. Inflammatory process, inadequate immunologic response, and hospitalization may accelerate this phenomenon.[14]

In cirrhotic cases, especially in those with encephalopathy, a massive circulatory inflammation associated with Th1 and Th17 is seen.[20] The saliva of CLF cases showed an increased level of interleukin (IL)-1β and IL-6 concentration and a prominent rise in secretory IgA. This procedure resulted in diminished innate local defenses and decreased histatins 1 and 5 and lysozyme. Higher levels of fecal secretory IgA in the saliva were also seen. This phenomenon may explain the entire initiation of systemic inflammation, possibly through interdependence between the intestine and the oral cavity.[19]

E. faecalis is a pathogenic bacteria that can own the fundamentals to establish an oral infection and preserve an inflammatory response that can be harmful to the host.[15]

To the best of our knowledge, there are no available data regarding the presence of *E. faecalis* in the saliva of patient candidates for liver transplant, so comparing the current research with other investigations is limited. A study demonstrated a meaningfully higher occurrence of *E. faecalis* in the saliva (40.5%) and subgingival biofilm (47.8%) of samples obtained from cases with periodontitis in comparison to healthy controls.[14] On opposite, Rams *et al.*[21] identified *E. faecalis* in only 1% of initial onset periodontitis and 5.1% of long-lasting periodontitis cases. This finding is similar to the current study result that showed only two positive samples for *E. faecalis* in participants with moderate-to-severe gingival index.

Definitely, oral enterococci yield virulence elements of potential importance to the pathogenesis of periodontitis, as well as aggregation materials, superficial adhesins, lipoteichoic acid, extracellular superoxide products, lytic enzymes such as gelatinase, hyaluronidase, and elastase, the toxin cytolysin, and hemolysins able to cause neutrophil impairment.[15]

In addition, *E. faecalis* may improve pathogenicity in complex infections with anaerobic microorganisms and is able to make experimental apical periodontitis after combination with other mouth microflora. Enterococci have long term concerned in chronic root canal infections and are the main type in failed endodontic-treated teeth. The origin of the bacteria is still unclear, as enterococci do not relate to the common oral microflora.[22]

Assessment of oral rinse of 100 dentistry students and 100 cases that had root canal-treated tooth revealed that 1% of the students and 11% of the second group showed *E. faecalis* in the samples. Genetic examination displayed that the isolates from the treated teeth were not linked to those from the usual gastrointestinal microbiota. The saliva samples of none of these cases exhibit enterococci.[23]

It seems that dissimilarities in isolating of *E. faecalis* can resulted from variations in the study groups, cultural and religious factors, nutrition, oral or systemic condition of the participants, count of the samples, approaches of the study geographical variations, and personal hygiene maintenance. Besides, saliva is extensively open and complex environment; a microflora in the saliva varied at all periods.

Moreover, it should be recognized that cross-sectional studies have a restricted time which can single observed the oral microbiota. The transitory oral microflora existing in a complicated active environment might arise in another time.[24]

CONCLUSION

Patients with chronic liver diseases assessed in this research showed a higher incidence of poor oral health and caries compared with the healthy controls, but there was no statistical difference in the presence of *E. faecalis* in both groups. Complete oral examination, dental treatment, and oral hygiene

instruction are necessary for all these cases before liver transplantation.

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Conflicts of interest
The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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