INTRODUCTION
Bacteremia refers to the presence of viable bacteria in the bloodstream [1]. It is likely to occur during dental procedures and common daily activities, such as tooth brushing, dental flossing, and mastication [2]. Primary bacteremia is temporary and clinically benign, and the host's immunity is capable of removing the bacteria from the bloodstream. However, bacteremia is likely to cause infection and sepsis when the immune mechanisms fail due to anatomical problems, turbulent blood flow, foreign bodies, or compromised immunity conditions such as inherited or acquired immunodeficiency conditions (e.g. AIDS) [3]. Bacteremia is likely to cause infective endocarditis in individuals with predisposing heart conditions [4]. These conditions include having an artificial heart valve, a positive history of endocarditis, congenital heart diseases (CHD) including un repaired cyanotic
CHD, first six months after CHD repair, and repaired CHD with some remaining defects, as well as heart transplant recipients suffering from a heart valve disease [5]. In all orthodontic procedures, the placement and removal of orthodontic bands are regarded as the most important factors damaging the gingival margin. A previous study indicated that placement of orthodontic separators is likely to be the main source of bacteremia [6]. In modern orthodontics, anchorage plays a key role in yielding favorable treatment results [7]. Thus, significant attempts have been made to achieve good anchorage results by applying chin caps, headgears, and multiple brackets [8]. However, all these techniques have their own disadvantages. For example, extra-oral anchorage requires patient cooperation [8]. Also, intra-oral anchorage, especially with dental support, does not provide optimal anchorage results, and requires a sufficient number of teeth [9,10]. In contrast, temporary anchorage devices provide skeletal anchorage [11] and supply maximum anchorage without requiring full-time patient cooperation [12]. Miniscrews, considering their numerous advantages including low cost and easy placement and removal, are the most commonly used temporary anchorage devices. The proper small size of miniscrews allows their application in numerous anatomical areas, such as the inter-dental region [13].

According to previous studies, antibiotic prophylaxis is required in high-risk procedures, such as dental extraction (40%), periodontal surgery (58%) [14,15], and intraligamentary injections (97%) [16]. However, even after dental procedures with no bleeding, bacteremia may be detected; hence, it is argued that bleeding is not necessary for the occurrence of bacteremia [17]. Considering the increased usage of miniscrews in modern orthodontics and uncertainties associated with the incidence of bacteremia after the placement of miniscrews, this study aimed to investigate the possible occurrence of bacteremia, and the need to consider antibiotic prophylaxis for patients at high risk of endocarditis.

**MATERIALS AND METHODS**

The present quasi-experimental study was conducted after obtaining ethical approval from the Committee of Research Ethics of Islamic Azad University of Medical Sciences, Isfahan, Iran (IR.IAU.KHUISF.REC.1398.198). All the laboratory tests were performed at Saadi Hospital Laboratory, Isfahan, Iran. The sample size was calculated according to a similar study conducted by Livas et al, [18] using the G*power software version 3.0.10 (Franz Faul, Universität Kiel, Germany). Twenty-six participants were enrolled according to the McNemar test considering alpha=0.05, power of 80%, and odds ratio of 5. After considering 4 extra samples for the potential loss to follow-up, 30 patients were selected for this study.

The patients were selected by convenience sampling using the following inclusion criteria: healthy patients with no systemic disease who received oral hygiene instructions. Also, patients had good plaque control with an O’Leary’s plaque index and gingival index <1 [19], which were investigated by visual inspection. None of the patients, according to their own statements, had any problems in their past blood tests. Also, the patients were between 12-40 years. Patients with congenital or acquired heart disease, history of rheumatic fever or some other medically compromised conditions, infection in the past month, or history of antibiotic therapy within the past 3 months were excluded from the study.

The study sample comprised of 30 volunteers, 11 males (36.7%) and 19 females (63.3%) with a mean age of 23.67 ± 4.87 years (range 14 to 39 years), who were referred to the Orthodontics Department of the Faculty of Dentistry, Islamic Azad University of Isfahan. They received fixed orthodontic treatment and required miniscrews based on their treatment plan. Written informed consent was obtained from all patients for participation in the study. Right before the placement of miniscrews, an expert phlebotomist took 20mL of blood sample (the optimal blood volume for adult culture [12]) from the patients’ right antecubital vein using a sterile
disposable Venoject (Butterfly Becton, Dickinson, BD, USA) with an aseptic technique. Direct transfer of the samples to the vials was made possible by creating a vacuum in the vials using a BD Vacutainer Safety-Lok blood collection set. The skin and the tube's cap were disinfected with 70% alcohol; 30 seconds of time was allowed for the alcohol to evaporate. The skin was then disinfected with Povidone-iodine. After one minute, venous access was provided without touching the site, and the blood sample was transferred into special vials (aerobic plus culture vial and anaerobic plus vial; BD BACTEC; Becton–Dickinson, USA), 10 mL to each, according to the manufacturer's instructions (Fig. 1).

After taking the blood, the site was cleaned with alcohol. Following local anesthesia administration using lidocaine, autoclave-sterilized miniscrews (1.6 mm in diameter, 8 mm in length; 16-G2-8; Jeil Medical Corporation, Korea) were placed in the attached gingiva between the maxillary first molar and second premolar. To increase the accuracy of the results, all screws were placed by a trained postgraduate student of orthodontics using the same insertion technique to minimize the differences arising from different placement techniques. The next 20mL blood sample was taken 30-60 seconds after the placement of miniscrews using the previous protocol from the opposite hand. In patients who needed two or more miniscrews, the blood sample was taken before and after the placement of the first miniscrew. The samples were incubated in an automated blood culture machine (BACTEC; Becton–Dickinson Diagnostic Instrument Systems, BD, USA) for five days (Fig. 2) to find the positive and negative samples.

To determine the type of bacteria found in the positive samples, blood culture was conducted in chocolate agar (Bio-Rad, Marnes-la-Coquette, France), blood agar (Bio-Rad, Marnes-la-Coquette, France), and MacConkey agar (Bio-Rad, Marnes-la-Coquette, France). After 24 hours, coagulase, catalase, and Gram tests were performed. The patients were recommended to contact the postgraduate student of orthodontics in case of having fever or feeling lethargic within 48 hours after blood collection. If they did not contact him, the postgraduate student himself would contact the patients within 48 hours after blood collection to check the aforementioned symptoms. Upon observing the bacteremia symptoms, a sub-culture would be conducted.

Data analysis was performed using the McNemar test, and the significance level was set at 0.05. Data analysis was conducted both descriptively and inferentially. The mean, standard deviation, frequency distribution tables, and statistical diagrams were used for descriptive analysis. The McNemar test was used to compare the presence of aerobic and anaerobic bacteria before and after miniscrew placement.
RESULTS
This study aimed to investigate the presence of aerobic and anaerobic bacteria before and after the placement of miniscrews. The patients were divided into three age categories of 12-19, 20-29, and 30-40 years (Table 1). The highest number of samples was observed in the 20-29-year-old category (56.7%).

Table 1. Frequency distribution of participants

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>N</th>
<th>%</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-19</td>
<td>8</td>
<td>26.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-29</td>
<td>17</td>
<td>56.7</td>
<td>2367</td>
<td>4.87</td>
</tr>
<tr>
<td>30-40</td>
<td>5</td>
<td>16.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SD: standard deviation

According to the results, no aerobic or anaerobic bacteria were observed in any of the samples before the placement of miniscrews (Table 2). As observed in Table 2, among 30 patients investigated after the placement of miniscrews, bacteria (Staphylococcus aureus) were observed in the aerobic and anaerobic cultures of only one patient’s blood sample (3.3%). The bacteremia test was negative for the remaining 29 patients (96.7%). In terms of presence of bacteria, the McNemar test did not indicate a significant difference in bacteremia before and after the placement of miniscrews (P=1.00).

DISCUSSION
Endocarditis may occur following bacteremia. It has a very low prevalence; however, it is likely to turn into a life-threatening condition in susceptible individuals. Although it is difficult to identify all the risk factors leading to bacterial endocarditis, a damaged organ, such as a damaged endocardium, and high volumes of bacteria in the blood are among the important risk factors. Microorganisms involved in 90% of endocarditis cases include staphylococci, streptococci, and enterococci. They are likely to enter the bloodstream because of being a component of microbial dental plaque. However, most bacteremia cases are transient and self-limiting, not causing any other systemic complication [21]. According to Mang-de la Rosa et al, [21] approximately 40% of patients suffering from bacterial endocarditis had no apparent heart valve disease. Thus, given the possibility of being involved without a history of heart valve disease, and the significance of this condition, the present study aimed to investigate the possibility of bacteremia following the placement of orthodontic miniscrews.

In this study, blood samples were taken from the patients by an expert phlebotomist to minimize the risk of blood infection and lower the costs by preventing repetition of sampling [22]. Blood sampling was conducted twice, once before the placement of miniscrews to ensure that the patients did not have bacteremia arisen from a previous infection and once after the placement of miniscrews, i.e., the intervention factor. In both blood collection phases, aerobic and anaerobic cultures were performed.

Table 2. Frequency distribution of the samples in terms of presence of aerobic and anaerobic bacteria before and after miniscrew insertion

<table>
<thead>
<tr>
<th>Bacteremia incidence</th>
<th>Aerobic culture</th>
<th>Anaerobic culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Percentage</td>
</tr>
<tr>
<td>Before miniscrew insertion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
<tr>
<td>After miniscrew insertion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>1</td>
<td>3.3</td>
</tr>
<tr>
<td>Negative</td>
<td>29</td>
<td>96.7</td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>100</td>
</tr>
</tbody>
</table>
For every bottle of blood culture, 10 mL of blood was taken. Taking this volume of blood is associated with the fact that for every 1 mL of the extra blood taken for culture in adults, there is a higher chance of identifying microorganisms by 3% [23]. Failure to identify any aerobic or anaerobic bacteria in the blood may question the accuracy of the results [20]. Although microorganisms of almost all blood samples are detected within 3 days of incubation, 2.4% of the microorganisms may remain undetected. Thus, in the present study, incubation was performed for 5 days according to the manufacturer’s instructions to increase the accuracy of the results [24].

Burden et al. [25] investigated the prevalence of bacteremia after the removal of fixed orthodontic appliances. They concluded that the prevalence of bacteremia after debonding was too low to require antibiotic prophylaxis. Gurel et al. [26] noted the significant possibility of bacteremia after removing a bonded maxillary expansion appliance. Therefore, orthodontists must consider antibiotic prophylaxis for patients at high risk of endocarditis. Livas et al. [18] investigated 30 patients for presence of bacteremia after the removal of orthodontic mini-implants. Microbiological analyses reported no case of bacteremia before and after the culture. Akbulut et al. [27] recommended antibiotic prophylaxis for high risk patients after the debonding procedures. In another study, Azeem et al. [28] investigated the bacteremia incidence following micro-osteoperforation in 28 patients. No bacteremia cases were reported in patients before and 60 seconds after sampling. Thus, micro-osteoperforation was not shown to be associated with transient bacteremia.

In the present study, bacteremia was assessed after the placement of miniscrews; however, in the study conducted by Livas et al. [18] it was measured after the removal of mini-implants. Bacteremia assessment after miniscrew placement is of high significance. Perforation of gingival tissue or the oral mucosa is a high-risk procedure in patients with infective endocarditis, requiring antibiotic prophylaxis [21]. This perforation typically occurs during miniscrew placement. Hence, bacteremia assessment after the placement of miniscrews is more important than that following the removal of miniscrews. In this study, the secondary blood sample was taken 30-60 seconds after the placement of miniscrews. Bacteremia falls into three categories of transient, intermittent, and persistent [23,29]. In transient bacteremia, bacteria are present in the bloodstream from some minutes to a few hours before being cleared from the blood; furthermore, it usually occurs when an intervention is made in non-sterile parts of the body (such as dental interventions). Intermittent bacteremia occurs when a single type of microorganism is proliferated due to a continuous cycle in the blood, such as an infectious abscess. Persistent bacteremia is characterized by the continuous presence of bacteria in the bloodstream, associated with infections, such as infective endocarditis [30]. It is known that presence of bacteria in the blood is not always persistent. It seems that the incidence of transient bacteremia is sufficient for the spread of infection to distant tissues [4]. In transient bacteremia, the blood sample taken within less than one minute after the intervention is positive; whereas, no bacteremia could be detected in the blood sample taken 30 minutes after the intervention [20] since the reticuloendothelial system of a healthy person destroys the bacteria within 20 minutes [31]. Based on the data, the peak of bacteremia occurs between 30 and 60 seconds after dental extraction [32]; thus, blood samples were taken 30-60 seconds after the placement of miniscrews in this study. To ensure no bacteremia occurred after blood sampling, the patients were contacted within 48 hours after the sampling and were asked about fever and lethargy after the test. None of the patients reported the aforementioned symptoms. No more blood samples were taken after the test because bacteremia is likely to occur after daily activities, including mastication and toothbrushing [21], and this would have
Bacteremia After Orthodontic Miniscrew

nothing to do with our intervention. Infection with Staphylococcus aureus was detected in the positive sample of one patient after the blood culture and coagulate, catalase, and Gram tests, as well as the blood culture in chocolate agar, blood agar, and MacConkey agar environments. Staphylococcus is a gram-positive and facultative anaerobic microorganism, the most pathogenic form of which is Staphylococcus aureus. This bacterium is likely to be present in healthy individuals’ nose or skin [33]; however, when it enters the bloodstream, it can replicate and disseminate to many different organs, causing severe disease manifestations such as sepsis, infective endocarditis, and deep-seated abscesses in virtually every organ tissue [34]. Compared with patients with no positive blood cultures, those with Staphylococcus aureus or enterococcus bacteremia have the highest risk of death [35]. The low incidence of bacteremia in this study could be due to the strict criteria for patient selection, according to which patients with optimal oral hygiene status were selected.

CONCLUSION
The present study did not show any signs of bacteremia in the primary blood samples. However, the aerobic and anaerobic blood cultures of the secondary blood sample were positive for Staphylococcus aureus in one patient. In terms of the presence of bacteremia, no significant difference was observed before and after the placement of miniscrews. Thus, miniscrew placement was not associated with bacteremia.

ACKNOWLEDGMENTS
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CONFLICT OF INTEREST STATEMENT
None declared.

REFERENCES