Implant dentistry has progressed with regard to predictable osseointegration, but mechanical and biological complications still occur with consequences for both dentist and patient. The abutment screw is usually the weakest link in the prosthetic chain, and screw loosening is one of the most significant implant complications. Screw loosening can lead to other problems such as marginal gap, microbial leakage, peri-implantitis, retention problems, crown loosening, screw fracture, patient discomfort, and financial consequences. According to the review by Goodacre et al., the mean incidence of abutment screw loosening was 8% and as high as 45% in single crowns. Tightening the abutment screw to the manufacturer’s recommended torque results in a preload or clamping force that keeps the screw joint stable. Factors that affect this holding force include applied torque, screw mechanics, and the component material and the friction between them.

In clinical practice, surface contamination may be caused during laboratory or clinical procedures. The implant-abutment connection may be contaminated with different fluids including blood (G. H. Ommati Shabestari, unpublished data, 2012), saliva, fluoridated artificial saliva, or chlorhexidine from surgery to restoration delivery. This contamination may affect the preload and consequently increase the risk of screw loosening. Higher preload has been reported in the presence of saliva at implant-abutment connections, and Duarte et al. also reported that fluoridated artificial saliva solutions increased RTVs. Al Rafiee et al. reported no significant effects of saliva on the tensile strength of gold prosthetic screws, and Micarelli et al. showed no significant effects of chlorhexidine gel on the RTVs of abutment screws.
Although Gumus et al\textsuperscript{12} reported lower RTVs after chlorhexidine, saliva, and blood contamination compared with the control group, the subject remains controversial. Studies are scarce and have reported various results.\textsuperscript{14,18-20,22,23} Therefore, the purpose of this in vitro study was to assess the effect of blood, saliva, fluoride, and chlorhexidine contaminations on the RTVs of abutment screws. The null hypothesis was that no statistically significant difference would be found in the RTVs of implant groups contaminated with different fluids at implant-abutment connections.

**MATERIAL AND METHODS**

In this in vitro study, 45 dental implants (grade 4 titanium, 4.5×10-mm bone-level implants, Implantium; Dentium Co) were divided into 5 groups (n=9). The implants were embedded in 2.5×2.5 cm acrylic resin blocks (Meliodent Rapid Repair; Kulzer GmbH), and the blocks were fixed by a metal holding device. The implant-abutment connection was conical with an internal hexagon. The screw access holes of the fixture bodies were contaminated by using a pipette with chlorhexidine (CG), saliva (SC), blood (BG), and fluoride (FG) until they were filled. An additional group served as a control group with no contamination (NC). Chlorhexidine was used in the form of a mouthwash (Chlorhexidine Gluconate 0.2%; Ecolab Co); saliva was obtained from a single healthy female donor (S.T.), and the Dental Ethical Committee’s approval was obtained for this and the blood donation. In BG, capillary blood was obtained from the same donor and used before coagulation. For fluoride contamination, sodium fluoride 0.2% mouthwash was applied (Fluorine; Fanda Pharma Co). The abutments were inserted into the fixture screw holes, and the manufacturer’s recommended initial torque value (ITV, 25 Ncm) was applied with the digital torque meter (TQ-8800.RS-232/USB; Lutron Electronic Enterprise Co). Fifteen minutes later, a second torque of 25 Ncm was applied according to the manufacturer’s recommendation to compensate for the settling effect (Fig. 1). After abutment tightening, the specimens were thermocycled (5 °C to 55 °C, 1500 cycles, 60-second dwell time) (TC300; Vafaei Industrial Co). Finally, the RTVs were recorded with the same digital torque meter. Homogeneity of variances was evaluated by the Levene test ($P= .396$). Differences in RTV among the groups were assessed using 1-way ANOVA. The Tukey honestly significant difference (HSD) test was applied to adjust for multiple comparisons ($\alpha=.05$).

**RESULTS**

One-way ANOVA revealed significant differences among the tested groups ($P<.001$). A statistically significant difference was found in the RTVs of the groups contaminated with different fluids at their implant-abutment connections (Table 1). For all groups, the RTVs were below the tightening torque (25 Ncm). The greatest decrease in RTVs was seen in the SG (13.65 ±0.91 Ncm) with a significant difference with NC (16.55 ±1.82 Ncm) ($P=.005$). The highest RTVs were detected in CG (19.74 ±1.79 Ncm), which was significantly higher than in the control group ($P=.002$), and no statistically significant difference was found for BG (16.30 ±1.66 Ncm) or FG (15.92 ±1.82 Ncm) compared with the control group ($P>.05$).

**DISCUSSION**

In this study, the effect of 4 possible contaminants on the amount of RTV at implant-abutment connections was evaluated. The results showed that RTVs were higher in the CG group when compared with the NC group; therefore, the null hypothesis was rejected. Gumus et al\textsuperscript{12} reported no significant difference between CG and NC. In a previous study, CHX concentration was 0.3% diluted to 0.1%, but in the present study, CHX 0.2% was applied and could be the cause of the different findings. That CHX concentration might affect the results is supported by Kozlovsky et al,\textsuperscript{16} who reported that CHX adhesion to Ti was influenced by Ti surface roughness and CHX concentration. According to their study, CHX adsorption and desorption to and from titanium disks were higher

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**Clinical Implications**

Saliva contamination of the fixture screw hole may increase future screw loosening; however, applying chlorhexidine may decrease screw loosening.
with 0.2% CHX than with 0.1% CHX. Micarelli et al\textsuperscript{21} reported no significant effect when applying CHX. However, the concentration of the CHX gel and how the specimens were contaminated were not mentioned in this study nor was the different geometry of the implant-abutment connection, which might explain the different findings. According to the results of the present study, in addition to its antibacterial role, applying CHX 0.2% to the fixture screw hole before tightening the abutment may decrease screw loosening; however, in vivo studies are recommended to confirm this finding.

Blood contamination did not show a significant difference with the NC group, consistent with the findings by (G. H. Ommati Shabestari, unpublished data, 2012) although the abutments were tightened 2.5 minutes after contamination, which indicates that coagulation had occurred. According to Gumus et al\textsuperscript{12} blood contamination may result in greater screw loosening in clinical practice because of significantly decreased RTVs in this group. They reported that the high protein content and presence of macromolecules such as fibrinogen and platelets, in addition to blood viscosity, may contribute to the reduction of RTVs. They applied venous blood, which has a higher viscosity and different composition compared with the capillary blood used in this study.

In the current study, saliva contamination resulted in the lowest RTVs. Gumus et al\textsuperscript{12} reported no significant difference among SG, CG, and NC. They applied fresh human saliva from a male donor, while, in the present study, the saliva of a female in rest status was obtained. Ghanbarzadeh et al\textsuperscript{19} demonstrated that saliva contamination whether in the form of abutment screw impregnation or fixture screw hole filling both increased the amount of RTV compared with the non-contaminated joint. Apparently, they used artificial saliva, which lacks proteins. (G. H. Ommati Shabestari, unpublished data, 2012, reported the difference among SG, BG, and NC was not significant. They applied mucosal saliva from a male donor after 20 minutes of exercise, which is different in terms of density and viscosity from the saliva collected from a resting female in the current study). After exercise, the resultant saliva would be thick and sticky\textsuperscript{24} and have a higher viscosity,\textsuperscript{25} which would decrease its flow between surfaces and consequently its effect on friction. Nigro et al\textsuperscript{22} concluded that a loss of initial torque always occurred when removal torque was measured in both dry specimens and specimens exposed to artificial saliva; however, the wet group had higher mean RTVs than the dry group. Al Raee et al\textsuperscript{20} suggested that the use of human saliva as a lubricant during tightening had no apparent effect on the ultimate tensile strength of the screw, and Tzenakis et al\textsuperscript{18} in a similar study reported that higher preload was achieved after the repeated use of saliva-contaminated gold prosthetic retaining screws. Both studies used gold screws, whereas in the present study, titanium screws were used. Both recent studies used repeated tightening at different times and measured preload according to ultimate tensile strength, whereas in the present study, the RTVs were measured. Also consistent with the results of the current study, Norton et al\textsuperscript{23} reported decreased RTVs for abutment screws with saliva contamination. Following saliva leakage into microgaps, microorganisms and glycoproteins will settle into the joint, acting as a lubricant and decreasing friction.\textsuperscript{13} The difference in salivary density and mucosity in different individuals leads to a range of results in that thick, high mucin content, saliva is more cohesive than thin watery saliva. Also, high mucosity results in increased distance between the surfaces because of the increased film thickness which decreases the interfacial forces.\textsuperscript{26} Therefore, avoiding saliva contamination of screw holes during final tightening may reduce future screw loosening; however, studies under oral conditions are necessary to prove this.

Duarte et al\textsuperscript{14} reported that the increased amounts of RTVs after immersing specimens in fluoridated artificial saliva were due to the formation of a corrosion layer between metallic surfaces. The composition of saliva was again different in this study. Moreover, the specimens were immersed in saliva, while in the current study, the fluids were placed in the fixture screw holes with a pipette. Unlike the CPTi used in the study by Duarte et al\textsuperscript{14}, Ti alloy grade 4, which is resistant to galvanic current and corrosion, was used in the present study. Studies evaluating the relationship between RTV and fluoride are sparse.\textsuperscript{14,15} Roselino Ribeiro et al\textsuperscript{15} exposed dental implants to a simulated 5 years of fluoride by immersing the specimens in sodium fluoride. SEM analysis revealed signs of corrosion on the surface of the specimens, and the screws fractured before attaining the predetermined number of cycles. The present study found no significant difference between the FG and NC groups. Because of the limited exposure to fluoride ions, further studies are recommended to simulate the oral condition better by immersing the specimens in the medium and prolonging the fluoride contact time to evaluate its effect on the mechanical properties of implant-abutment assembly. In all groups, RTVs were

### Table 1. Removal torque values of all groups (Ncm)

<table>
<thead>
<tr>
<th>Group</th>
<th>Min</th>
<th>Max</th>
<th>Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>13.00</td>
<td>18.20</td>
<td>16.55 ±1.82*</td>
</tr>
<tr>
<td>CG</td>
<td>17.40</td>
<td>23.10</td>
<td>19.74 ±1.79p</td>
</tr>
<tr>
<td>BG</td>
<td>13.00</td>
<td>18.10</td>
<td>16.30 ±1.66c</td>
</tr>
<tr>
<td>FG</td>
<td>12.90</td>
<td>18.40</td>
<td>15.92 ±1.84p</td>
</tr>
<tr>
<td>SG</td>
<td>12.00</td>
<td>15.00</td>
<td>13.65 ±0.91b</td>
</tr>
</tbody>
</table>

BG, contaminated with blood; CG, contaminated with chlorhexidine; FG, contaminated with fluoride; NC, noncontaminated; SD, standard deviation; SG, contaminated with saliva. Same superscript letters show mean values with no statistically significant difference between groups (P > 0.05).
lower than initial torque values, consistent with previous studies.\textsuperscript{4,8,11,12,17}

Limitations of the present study include that it was in vitro and the specimens were not aged with cyclic loading, which would have replicated the introraol environment better. Other possible contaminants such as topical anesthetic gel should be evaluated. Additionally, the implant-abutment connection may be contaminated with more than 1 substance in the oral environment. A combination of contaminants should also be evaluated in future studies.

**CONCLUSIONS**

Based on the findings of this in vitro study, the following conclusions were drawn:

1. Reverse torque values were lower than initial torque values in all groups.
2. Reverse torque values declined significantly after saliva contamination in all groups.
3. The highest reverse torque values were detected in the chlorhexidine-contaminated group.
4. No statistically significant difference was reported among the blood, fluoride, and not contaminated groups.

**REFERENCES**


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