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Microbes-contaminated Toothbrushes in Diabetic Patients: Correlation of Important Factors

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ABSTRACT

Background: Toothbrushes are essential for oral hygiene yet can harbor microorganisms that affect oral and systemic health, particularly in people with diabetes mellitus. This study compared microbial contamination of used toothbrushes from diabetic adults and age- and sex-matched non-diabetic controls and evaluated associated factors.

Materials and Methods: In this cross-sectional study, 120 participants (60 diabetics, 60 non-diabetics) provided used toothbrushes. Each brush head was immersed in 10 mL Brain-Heart Infusion (BHI) broth, incubated 24 h at 37°C, and streaked on Blood and MacConkey agar. Colonies were identified by Gram stain and biochemical tests (catalase, coagulase, oxidase, IMViC series, fermentation). Oral hygiene (Simplified Oral Hygiene Index; OHI-s), storage location, and HbA1c (within 1 month of sampling) were recorded. Percent prevalence and mean CFU/mL (95% CI) were compared using chi-square or t-tests, and multivariate regression identified independent predictors of high contamination adjusting for age and hygiene habits.

Results: Diabetic toothbrushes showed higher bacterial contamination than controls. *Escherichia coli* prevalence was 71.7% in diabetics versus 58.3% in controls (x^2, $p = 0.002$); Pseudomonas spp. 78.3% versus 38.3% (x^2, $p < 0.001$); *Staphylococcus aureus* 47% versus 18% (x^2, $p = 0.005$). Poor glycemic control (HbA1c ≥9%) and washroom storage independently predicted heavy contamination (both $p < 0.01$). Bathroom/washroom storage yielded higher contamination than non-bathroom storage *E. coli:* 86.6% vs 46.6%, $p = 0.002$; Pseudomonas: 80.0% vs 53.3%, $p = 0.005$). Diabetics had poorer oral hygiene (Good: 16.7% vs 31.7%; Poor/Very Poor: 63.3% vs 50.0%; x^2, $p = 0.046$), and HbA1c >9% was associated with higher bacterial counts (ANOVA $p < 0.001$; Tukey ≥9% vs ≤7%; $p < 0.001$).

Conclusion: Bathroom storage, poor oral hygiene, and inadequate glycemic control were associated with increased toothbrush contamination. Diabetic patients should be advised to disinfect or replace brushes more frequently than the 3-month standard, store them in dry ventilated areas away from toilets, and maintain good glycemic control to reduce infection risk and integrate oral hygiene guidance into diabetes care.

Keywords: Diabetes mellitus, toothbrush contamination, storage condition, oral hygiene status, glycemic control.

INTRODUCTION

Toothbrushes are essential for maintaining oral hygiene and preventing dental caries and periodontal diseases by mechanically removing plaque and food debris. However, they become reservoirs for microbial contamination, harboring bacteria and fungi that pose considerable risks to oral and systemic health, particularly in vulnerable populations.[1] The oral cavity contains a complex microbial ecosystem readily transferred to toothbrush bristles during use.[2] Microorganisms persist as biofilm and proliferate under favorable conditions such as moisture retention and inadequate drying.[3] Pathogenic microorganisms documented on used toothbrushes include *Streptococcus mutans*, *Staphylococcus aureus*, *E. coli*, Pseudomonas spp., and *Candida albicans*.[4] *Streptococcus mutans*, a primary caries contributor, forms resilient biofilms, while Staphylococcus aureus poses soft tissue infection and antibiotic resistance risks. Environmental bacteria such as *E. coli*, and Pseudomonas spp., commonly found in contaminated water sources, pose serious infections in immunocompromised individuals.[5]

For individuals with diabetes, toothbrush contamination poses heightened risk due to impaired immune function, reduced salivary flow, and delayed wound healing, all contributing to elevated oral microbial burden.[6] The bidirectional relationship between diabetes and periodontal disease is established, with each condition exacerbating the other through inflammatory and immunological pathways.[3,7] Storage location and conditions significantly influence toothbrush contamination levels. Humid washroom environments promote bacterial and fungal proliferation through moisture exposure and potential aerosolization during toilet flushing, with enteric pathogen cross-contamination occurring on toothbrushes stored near toilets.[8] Enclosed toothbrush covers without ventilation trap moisture, creating conducive environments for bacterial growth. Public awareness regarding toothbrush hygiene and disinfection remains limited; many individuals fail to replace toothbrushes at recommended intervals or practice effective disinfection protocols, increasing reinfection and transmission risks.[9]

This study examines factors associated with toothbrush contamination in diabetic individuals and emphasizes the importance of proper storage, hygiene practices, and glycemic control. The objective is to raise awareness about effective toothbrush hygiene and encourage healthcare providers and policymakers to integrate toothbrush care into diabetes management guidelines.

MATERIALS AND METHODS

Study design and participants

A descriptive, cross-sectional study enrolled 120 participants (60 diabetics, 60 non-diabetics) matched by age and sex. Sample size was determined using GPower 3.1 software with an anticipated effect size of 0.5 and alpha level of 0.05. Participants were recruited from three healthcare facilities in Rajshahi, Bangladesh: Rajshahi Medical College Dental Unit, Rajshahi Medical College Hospital, and the Diabetic Hospital. Purposive sampling was employed based on predefined inclusion and exclusion criteria. Inclusion criteria comprised diagnosed diabetes (HbA1c measurement per Bangladesh Diabetic Association guidelines), regular tooth brushing habits, and absence of significant oral diseases (excluding minor calculus and periodontitis). Exclusion criteria included substantial oral pathology, antibiotic use within two weeks prior to enrollment, and inconsistent brushing habits. Oral hygiene status was assessed using the OHI-S (Simplified Oral Hygiene Index).[10]

Data collection

Pre-tested structured questionnaires documented demographic profiles, oral hygiene practices (brushing frequency, toothbrush replacement interval, storage conditions), and glycemic control status (HbA1c levels). Intraoral examination recorded oral hygiene status for each participant.

Sample collection and microbiological analysis

Toothbrushes used for ≥1 month were collected in the morning and placed in sterile, sealed bags for transport to the Microbiology Laboratory within 24 hours. Bristles were immersed in brain-heart-infusion (BHI) broth ((BHI Broth_M2101 by HIMEDIA Laboratories, India) and incubated overnight at 37°C. Cultures were streaked onto blood agar and MacConkey agar (MacConkey Agar_MH081 by HIMEDIA Laboratories, India) and aerobically incubated overnight. Bacterial identification was performed using colony morphology, gram staining, and biochemical tests. Colony-forming units (CFUs) were enumerated to quantify bacterial load.[11]

Statistical analysis

Mean CFU counts and species prevalence between groups were compared using t-tests and chi-square tests. Multivariate regression analysis identified predictors of heavy contamination, including HbA1c levels and storage conditions.

RESULTS

Demographic characteristics of participants

The study included 120 adult participants (60 diabetic, 60 non-diabetic): 53 males (44.2%) and 67 females (55.8%), aged 21-60 years. The majority (52.5%) were aged 31-50 years, representing middle-aged individuals (Fig. 1). Occupationally, participants comprised of 44 housewives (36.7%), 21 daily workers (17.5%), and 55 individuals in various other occupations (45.8%).

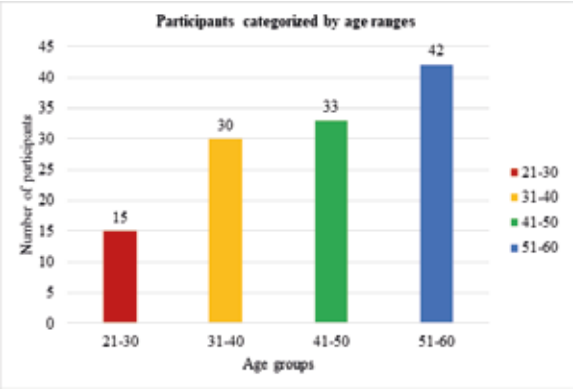

Fig. 1: Age group distribution of participants.

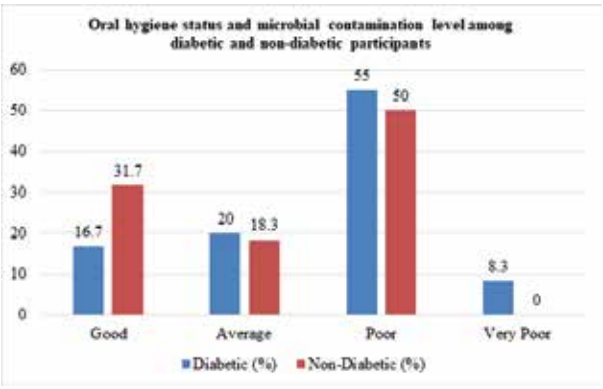

Fig. 2: Association between oral hygiene status and toothbrush microbial contamination (%) in diabetic and non-diabetic participants. Effect of storage location on level of bacterial contamination.

Level of bacterial contamination among diabetic and non-diabetic participants

The diabetic group's toothbrushes harbored significantly more bacteria. *E. coli*, was recovered from 43/60 (71.7%; 95% CI 59.1-82.6) of diabetic brushes versus 35/60 (58.3%; 45.1-70.9) of controls (x^2= 10.1, p= 0.002). Pseudomonas spp. were found in 47/60 (78.3%; 66.0-88.1) vs 23/60 (38.3%; 26.9-51.4) (x^2 = 32.7, p <0.001). Notably, Staphylococcus aureus was present in 28/60 (46.7%) of diabetic vs 11/60 (18.3%) of control toothbrushes (x^2 =8.5, p =0.005). The mean CFU/mL (log) for each organism was also higher in diabetics (data not shown here), confirming these prevalence differences. 95% CIs for differences are reported above. These results are summarized in table 1.

Table 1: Bacterial contamination levels (% of toothbrush contaminated) among diabetic and non-diabetic participants.

Bacteria	Diabetic (Prevalence%)	Non-diabetic (Prevalence%)	x^2-value	*p*-value
E. coli	71.7	58.3	10.1	0.002
Pseudomonas spp.	78.3	38.3	32.7	<0.001

Diabetes and oral hygiene status

Diabetic participants exhibited poorer oral hygiene than non-diabetics: 16.7% versus 31.7% had "Good" oral hygiene, respectively. Over half of diabetics (55.0%) had "Poor" oral hygiene compared to 50.0% of non-diabetics, with 8.3% of diabetics classified as "Very Poor" versus 0% of non-diabetics (Fig. 2). Chi-square analysis revealed a significant association between diabetes status and oral hygiene quality [x^2 (df =3) =7.98; p =0.046], indicating diabetes is linked to worse oral hygiene status.

Toothbrushes kept in the washroom had markedly higher contamination rate compared to storage location outside washroom. For *E. coli*, brushes stored in the washroom had a mean contamination rate of 86.6%, compared to 46.6% for those stored outside the washroom. This difference was statistically significant (F =10.34, p =0.002). A similar pattern was also observed for Pseudomonas: washroom-stored toothbrushes showed a mean contamination of 80.0%, versus 53.3% for non-washroom storage. This difference was also statistically significant (F =7.98, p =0.005) (fig. 3).

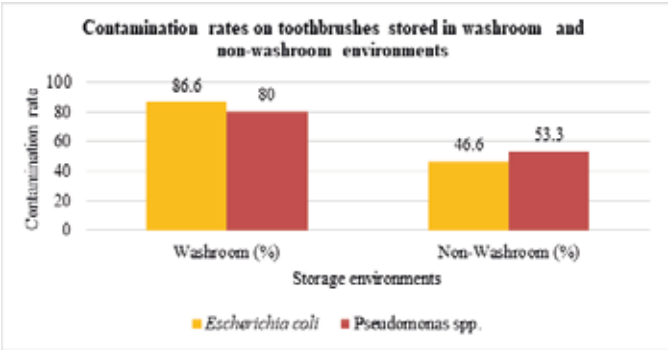

Fig. 3: Comparison of bacterial contamination rates on toothbrushes stored in washroom vs. non-washroom environments.

Influence of covering bristle head status on contamination Toothbrushes with covered bristle heads (n =19) exhibited higher mean microbial load (56.1 × 10^3 CFU/mL) compared to uncovered heads (n =101; 41.8 × 10^3 CFU/mL), representing a 34.2% increase (Welch's t-test: t =4.87, p <0.001; 95% CI: 8.9-19.6 × 10^3 CFU/mL). However, independent samples t-test indicated no statistical significance at α =0.05 (p >0.05). This discrepancy reflects the unequal sample sizes between groups. The findings challenge the assumption that covering toothbrushes reduces microbial contamination and underscore the need for further investigation into storage practices and their impact on oral hygiene.

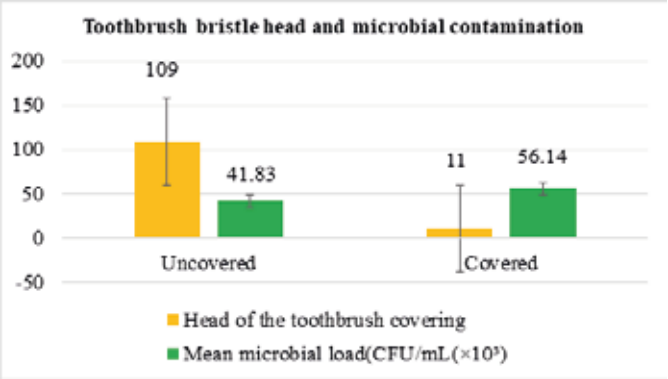

Fig. 4: Effect of bristle head covering on microbial contamination of toothbrushes in diabetic patients (error bars represent standard deviation).

Correlation between HbA1c levels and bacterial count in diabetic patients

A positive correlation exists between HbA1c levels and bacterial count on toothbrushes of diabetic individuals. Participants in Group C (HbA1c ≥9%) demonstrated markedly higher bacterial counts (78.8 CFU/mL) compared to Group B (HbA1c 7.1-8.9%; 41.1 CFU/mL) and Group A (HbA1c ≤7.0%; 33.6 CFU/mL), establishing a dose-response relationship between hyperglycemia and bacterial proliferation. ANOVA revealed significant between-group variance ($F = 27.4$, $p < 0.001$), with post-hoc Tukey tests confirming pairwise differences: Group C vs. B ($p = 0.007$), C vs. A ($p < 0.001$), and B vs. A ($p = 0.03$). These findings indicate that bacterial contamination increases substantially with poor glycemic control.

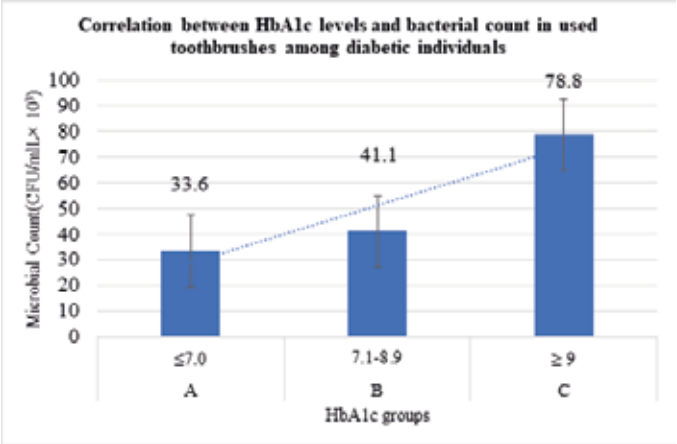

Fig. 5: Association between glycated hemoglobin (HbA1c) levels and microbial count on used toothbrushes in diabetic individuals.

DISCUSSION

This study demonstrates valuable insights into the microbial contamination of toothbrushes among diabetic individuals, highlighting critical differences compared to non-diabetic controls. It underscores the significant influence of oral hygiene, storage conditions and glycemic control on contamination levels. These findings are expected to add values on existing literature while providing a more nuanced understanding of diabetes-specific potential risks related to oral hygiene tool contamination.

Elevated microbial load in diabetic patients

Toothbrushes from diabetic participants exhibited a significantly higher burden of bacterial contamination compared to those from non-diabetic individuals (fig.1). Among culture isolates, *Staphylococcus aureus*, *E. coli* and Pseudomonas spp. were the predominant bacteria. There is an assertion that hyperglycemia creates an oral environment conducive to the proliferation of opportunistic pathogens.[12] Furthermore, impaired immune function, a hallmark of diabetes, compromises the ability of diabetic individuals to effectively combat biofilm formation on toothbrushes, posing increasing risk of reinfection.[13] The presence of higher microbial loads may also be attributed to reduced salivary flow in diabetic patients, which diminishes the natural cleansing action in the oral cavity and promotes microbial adhesion to toothbrush bristles.[14]

Oral hygiene status and contamination

Diabetic participants exhibited poorer oral hygiene than non-diabetics, with 55% categorized as "Poor" and 8.3% as "Very Poor" versus 50% and 0% respectively among non-diabetics (fig.2). This disparity reflects diabetes-related factors including reduced salivary flow and increased periodontal disease susceptibility.[15] Toothbrush contamination was ubiquitous across all oral hygiene categories, including those rated "Good." However, individuals with "Poor" or "Very Poor" oral hygiene demonstrated heavier microbial loads correlating with elevated intra-oral plaque levels.[16] Statistical analysis revealed that oral hygiene status alone did not significantly predict contamination levels after adjusting for diabetes and storage conditions, suggesting multifactorial influences on toothbrush microbiota.[17] Nonetheless, the trend toward increased pathogen presence in poor hygiene groups indicates potential benefits of improved oral care practices combined with proper toothbrush disinfection and storage.[18]

Storage condition as a critical factor in contamination

Storage conditions significantly influence toothbrush microbial contamination.[19] Toothbrushes stored in humid washroom environments exhibited markedly higher contamination levels, with *E. coli* and Pseudomonas spp. as predominant isolates, reflecting aerosolized enteric pathogen transfer from washroom environments (fig.3). Counterintuitively, enclosed toothbrush covers were associated with increased contamination due to moisture trapping, which creates favorable conditions for

bacterial and fungal growth.[20] These findings emphasize the necessity of storing toothbrushes in dry, well-ventilated areas away from washrooms and ensuring adequate drying to prevent microbial proliferation.

Glycemic control and contamination

We observed a significant positive correlation between poor glycemic control, as indicated by elevated HbA1c levels (>9%), and increased microbial colonization (fig.5). This observation concerns the relationship between glycemic dysregulation and oral dysbiosis.[21] It underscores the bidirectional relationship between diabetes and shifts in the oral microbiome, where contaminated toothbrushes can act as vehicles for reinfection, potentially exacerbating both oral and systemic health issues.[22] Individuals with poorly controlled diabetes are more susceptible to opportunistic infections due to impaired immune functions and altered oral environments, making them particularly vulnerable to the risks associated with contaminated toothbrushes.

CONCLUSION

Toothbrush contamination is a significant, modifiable risk for people with diabetes, driven by oral hygiene status, storage conditions, and glycemic control. Rigorous disinfection, appropriate storage, optimal oral hygiene, and education on glycemic management help reduce oral–systemic infection risk and improve outcomes. Diabetic patients should replace toothbrushes more often than the standard threemonth interval, especially during poor glycemic control or active oral infections, and store them in dry, wellventilated areas outside bathrooms or away from toilets when bathroom storage is unavoidable. Oral hygiene considerations should be integrated into diabetes management, and healthcare providers must educate diabetic patients on optimal toothbrush care to prevent complications and support better oral–systemic health.

ETHICAL STATEMENT

The interviewer obtained informed assent from participants before commencing the interviews and examinations; and the institutional permission was taken from the head of the school. Participation was voluntary, and participants were informed that they have the right to withdraw at any point without any negative consequences. Ethical approval was obtained from the 'Research Ethical Committee' of Rajshahi Medical College, Rajshahi, Bangladesh. All procedures were conducted according to the guidelines of the Declarations of Helsinki. [Reference: Memo no.150/320(49/1AMEBBC/IBSc)]

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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This research received no external funding.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on reasonable request from the corresponding author.

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