Journal of Pioneering Medical Sciences
Received: June 21, 2026 | Accepted: October 03, 2026 | Published: November 05, 2026
Volume 15, Issue 10, Pages 57-64
DOI https://doi.org/10.47310/jpms2026151005



From Oral Health to Systemic Impact: Assessing Saudi Dental Professionals' Knowledge of the Nitrate–Nitric Oxide Pathway and Implications for Dental Practice

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Abstract **Background:** Nitric oxide (NO) is a multi-functional gaso-transmitter which plays a crucial role in the maintenance of oral and systemic (especially cardiovascular) health. Routine use of antiseptic mouthwash may disrupt the oral microbiome that reduces dietary nitrate (NO_3^-) to nitrite (NO_2^-), impairing NO production. This study aimed to assess Saudi dental professionals' knowledge of NO, it's microbial pathway, and the impact of mouthwash, while also examining knowledge differences across demographic and professional groups. **Method:** This descriptive, cross-sectional study used an online face-validated questionnaire comprising 22 items to assess Saudi dental professionals' general knowledge of the NO_3^-–NO_2^-–NO pathway and specific knowledge of oral bacteria and mouthwash effects on NO production. **Results:** A total of 248 participants completed the questionnaire. Most dental professionals taking part in the survey had previously heard of NO (75.4%). Among those, 50% either confused NO with nitrous oxide used in anaesthesia or were unsure. Only 17.7% knew about the NO3-NO2--NO pathway. The mean general knowledge score was 2.3±1.9 out of 7; only 6.85% scored >70%. The mean specific knowledge score was 0.9±1.1 out of 4; only 3.6% scored >70%. About 1/3 of participants (35.1%) linked mouthwash to cardiovascular risk and 60.1% didn't know about toothpaste effects on $NO3^-$– $NO2^-$– NO pathway. Knowledge scores did not significantly differ between demographic groups. **Conclusion:** The findings reveal notable gaps in dental professionals' understanding of NO physiology and its relationship to oral microbiota and mouthwash use, suggesting a need for improved education to support informed practice and patient guidance.

Key Words Antiseptic Mouthwash, Nitric Oxide, Nitrate, Dental Health Professionals

INTRODUCTION

Maintaining proper oral hygiene is crucial for maintenance of overall oral health [1]. The oral cavity is the first entry point to the body, so the oral microbiome is constantly being influenced by daily activities such as eating, brushing, friction, and habits like smoking or drinking alcohol, as well as, antiseptic mouthwash use [2]. Recent data suggest that the global mouthwash market is projected to experience steady growth, with an estimated compound annual growth rate (CAGR) of 4.6% between 2025 and 2030. This reflects the widespread use of mouthwash as part of daily oral care routines [3-6]. Several recent observational surveys from Saudi Arabia have reported mouthwash use among ranging from 30 to 80% of the population as part of common oral hygiene practices [7-9]. Healthy individuals often use mouthwash to freshen their breath or as part of their general health and wellness routine [10]. In addition, antiseptic mouthwash is widely used among hospitalized

patients to support the maintenance, or improvement, of their oral health [11,12]. However, emerging evidence suggests that routine use of antiseptic mouthwash may have detrimental systemic effects by disrupting the oral microbiome responsible for nitrate (NO_3) reduction and nitric oxide (NO) production [13,14].

NO is a signaling molecule that plays a vital role in numerous biological processes, such as regulating vascular tone, supporting neurotransmission, and maintaining muscle energy metabolism [15]. Sufficient NO production is crucial for the maintenance of a healthy vascular system [16]. NO can be generated endogenously through the classic NO synthase-dependent pathway, which synthesize NO from l-arginine and oxygen in presence several co-factors. In addition, NO can be generated through the alternative pathway by the enzyme-independent entero-salivary circulation [16]. This exogenous pathway is called nitrate-nitrite-NO (NO_{3^-} – NO_{2^-} – NO) pathway. Dietary NO_3^-, which is abundant in beetroot and green leafy vegetables, serves as a significant source of NO through the non-enzymatic pathway, where oral bacteria first reduce NO_3^- to NO_2 , which is then further converted to NO under acidic and low-oxygen conditions [17]. Subsequently, the remaining NO_2^- pass to the intestines and are systemically absorbed, where it can be further reduced to NO. Given that a substantial proportion of circulating NO_2^- originates from dietary intake, the combination of dietary NO_3^- and oral bacterial activity plays a critical role in maintaining NO homeostasis and may serve as a vital defense against conditions linked to NO deficiency [17].

Some publications discuss and support the use of antiseptic mouthwash [18] such as chlorhexidine, including studies from Saudi Arabia [19], to enhance oral hygiene. However, these papers often overlook potential systemic effects. The use of a 0.12% chlorhexidine antiseptic mouthwash has been shown to substantially impair dietary $NO3^-$ metabolism [20]. Research indicates that such mouthwash can eliminate up to 94% of the oral

bacteria involved in $NO3^-$ reduction, leading to an approximate 85% reduction in the conversion of $NO3^-$- to $NO2^-$ [21]. This disruption significantly compromises NO production and limits the health benefits usually associated with dietary $NO3^-$ intake such as lowering blood pressure [22]. Furthermore, recent findings also show that $NO3^-$ intake can beneficially modulate the oral microbiome by enriching $NO3^-$reducing bacteria, further supporting NO bioavailability and contributing to local anti-inflammatory effects in the oral cavity [23].

Therefore, the widespread promotion of mouthwash use without proper clarification of its possible systemic implications may unintentionally contribute to public health risks, particularly concerning vascular function and blood pressure regulation.

Given the essential role of oral bacteria in maintaining systemic NO levels and supporting cardiovascular health, awareness of these mechanisms among dental healthcare professionals is crucial. A recent cross-sectional study conducted among dental practitioners in the Qassim region, Saudi Arabia found that a large proportion of practitioners (46.7%) routinely recommended the use of mouthwash twice daily, with 71.7% advising its use after toothbrushing [24]. Therefore, the current study aimed to evaluate Saudi dental professionals' and dental students' knowledge about the importance of NO and its derivatives and the role of oral bacteria and the use of antiseptic mouthwash in NO production. Additionally, the study examined whether knowledge levels differed based on participants' gender, age, years of experience, professional qualification, and region of practice within Saudi Arabia. Understanding these potential adverse effects is essential for dental professionals to make informed decisions when recommending mouthwash products to their patients.

METHOD

Study Design and Participants

A cross-sectional study was undertaken across various regions in Saudi Arabia to assess the knowledge of Saudi dental professionals and students regarding the significance of NO and its derivatives, as well as the role of oral bacteria and antiseptic mouthwash use in NO production. Participants were recruited using a convenience sampling method and included dental students and practitioners recruited from the public and private dental sectors, as well as dental professionals working in academic settings.

Questionnaire Development and Validation

The questionnaire was initially drafted by the principal investigator and refined with input two subject-matter experts to ensure clarity and content validity. Face validation involved six experts in dentistry and nutrition, who assessed the questionnaire for clarity, logic, and relevance. A pilot study with 12 respondents was then conducted to evaluate the clarity and comprehension of the questions. Based on this preliminary testing, necessary revisions were made to improve the suitability and understandability of the survey instrument. A summary of these steps is provided in Figure1.

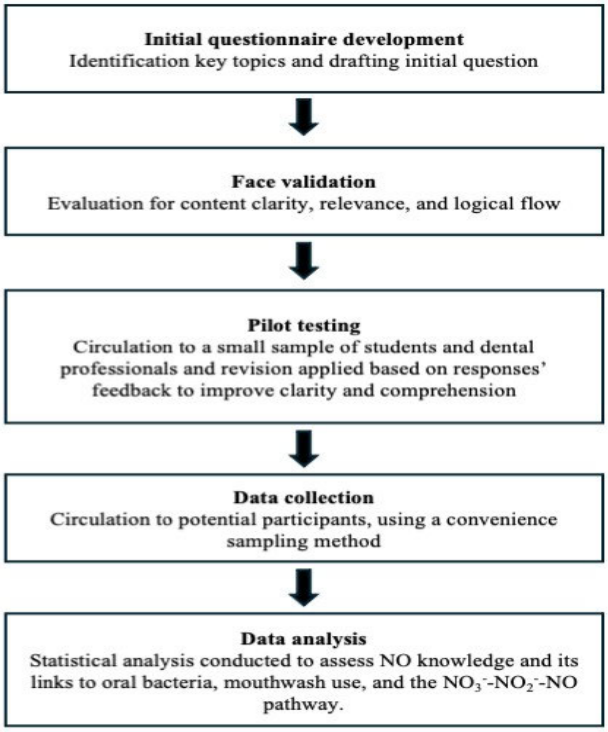


Figure: 1 Overview of Questionnaire Development and Analysis Process

The questions focused on knowledge and understanding among dental professionals and dental students about the importance of NO in health, as well as the effects of oral bacteria and using antiseptic mouthwash on dietary $NO3^-$ metabolism and NO pathway. The questionnaire consisted of three sections: (1) demographic information (gender, age, education level, years of experience, work setting, and region of practice); (2) general knowledge about NO, assessed by seven questions covering awareness, physiological roles, and dietary sources of NO; and (3) specific knowledge regarding the role of oral bacteria, the effects of antiseptic mouthwash, and the NO_3^- – NO_2^-–NO pathway, assessed by four questions respectively.

The general knowledge section began with an introductory question regarding participants' prior awareness of NO, whether participants had heard about NO. This was followed by 7 scored questions that assessed basic knowledge about NO including potential confusion with nitrous oxide, endogenous production of NO, its physiological roles in the human body, effects on cardiovascular health and immune function, association with periodontal disease, and the ability of the body to synthesize NO from dietary sources. An additional unscored question assessed familiarity with the $NO3^-$ – $NO2^-$ – NO pathway and its relevance to health.

The specific knowledge section explored participants' understanding of the role of oral bacteria in NO production, the impact of antibacterial mouthwash on NO levels. Additionally, two questions were included that focused on participants' opinions regarding the link between antibacterial mouthwash and cardiovascular disease, and on whether antibacterial toothpaste has a different effect on NO production compared to mouthwash. Questions were multiple-choice or yes/no/I don't know.

Each correct answer scored 1 point; incorrect or "I don't know" responses scored 0. The final questionnaire was distributed online, in English, between January 1st and April 24th, 2025. Recruitment utilized professional association mailing lists, university networks, and social media platforms. Respondents were informed that their participation is voluntary. An electronic consent form preceded the survey, and only consenting participants could proceed. Data were collected anonymously and stored securely to ensure participant confidentiality.

Ethics Approval and Consent to Participate

The ethical approval for this study was obtained from the Umm Al-Qura University, Faculty of Medicine, Scientific Research Ethics Committee (HAPO-02-K-012-2024-10-2302). Electronic informed consent was obtained prior to survey completion. Participants were required to read an information page and indicate their consent by selecting the 'I agree' option before accessing the questionnaire.

Statistical Analysis

Data collection and analysis were conducted using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA) and IBM SPSS version 29 (IBM Corp., Armonk, NY, USA). Descriptive statistics were generated for all variables, including counts, percentages, means±standard deviations (SD). The total scores for general NO knowledge and specific knowledge about oral bacteria, mouthwash, and NO were tested against different demographic variables using t-tests, ANOVA, and linear regression. P-value of less than 0.05 was considered significant.

RESULTS

A total of 248 dental professionals, including 104 students, participated in the study. The mean age of participants was 28.5±8.1 years. The mean years of professional experience was 3.9±6.2. Participants were recruited from 12 cities across Saudi Arabia including Riyadh, Makkah, Jeddah, Madinah, Taif, Buraidah,Dammam, Khobar, Qatif, Tabuk, Al-Makhwah, and Abha. Participants' demographic data are summarized in Table 1 Among the participants, 187 (75.4%) reported having heard about NO, while the remaining 61 (24.6%) had not. Among participants who reported having heard about NO (n = 187, 75.4%), 57 (30.5%) mistakenly believed that NO is the gas used as local anesthesia (nitrous oxide), and 22 (11.8%) indicated that they were unsure about this distinction. There were only 44 (17.7%) who reported they were familiar with the $NO3^-$ – $NO2^-$ – NO pathway and its role in human health. Participants answered seven general questions regarding basic information about NO, as shown in Table 2. They also answered four questions regarding specific information about oral bacteria, mouthwash and NO pathway (Table 3).

When participants were asked whether they believed there is a connection between the use of antibacterial mouthwash and the development or progression of cardiovascular diseases, 87 (35.1%) answered "Yes," 41 (16.5%) answered "No," and 120 (48.4%) responded "I do not know." Similarly, regarding whether antibacterial toothpastes have a different effect on NO production compared to antibacterial mouthwash, 58 (23.4%) answered "Yes," 41 (16.5%) answered "No," and 149 (60.1%) responded "I do not know."

Table 1: Demographic Characteristics of the Study Participants

Variable	Number	Percentage
Gender		
Male	68	27.4
Female	180	72.6
Qualification		
Student	104	41.9
Intern	17	6.9
General Dentistry	42	16.9
Specialist	47	18.9
Consultant	38	15.3
Work Setting		
Educational (academic)	149	60.1
Private sector	35	14.1
Governmental sector	64	25.2
Saudi Region of Practice		
Western	139	56.1
Central	78	31.5
Eastern	18	7.3
Southern	5	2.0
Northern	8	3.2

Table 2: Participants' Responses to General Knowledge Questions about NO

Question	Option	Number	Percentage
In dental practice, NO is used as a local anesthesia	Yes	60	24.2
	No*	124	50
	I do not know	64	25.8
NO is a naturally compound produced in the human body	Yes*	78	31.5
	No	41	16.5
	I do not know	129	52
NO has a significant impact on cardiovascular health	Yes*	110	44.4
	No	30	12.1
	I do not know	108	43.6
NO causes blood vessel constriction	Yes	69	27.8
	No*	57	22.9
	I do not know	122	49.2
NO plays a role in modulating immune response and inflammation	Yes*	54	21.8
	No	50	20.2
	I do not know	144	58.1
The body can produce NO from dietary sources	Yes*	72	29
	No	32	12.9
	I do not know	144	58.1
NO levels in the oral cavity can be affected by periodontal disease	Yes*	67	27
	No	33	13.3
	I do not know	148	59.7

*Indicate the correct answers. NO: Nitric oxide, NO_3 – NO_2 – NO: Nitrate- Nitrite- Nitric oxide

Table 3: Participants' Responses to Specific Questions on Oral Bacteria, Mouthwash Use, and NO

Question	Option	Number	Percentage
Consuming high amounts of leafy green vegetables and beetroot that are rich in dietary NO_3 - can leadto ------ NO levels in the bloodstream	Decrease	24	9.7
	Increase*	80	32.3
	Has no impact on	30	12.1
	I do not know	114	45.9
How do presence oral bacteria influence NO levels in the body?	Oral bacteria enhance NO levels*	37	14.9
	Oral bacteria inhibit NO levels	36	14.5
	Oral bacteria have no effect on NO levels	14	5.7
	I do not know	161	64.9
The use of antibacterial mouthwash ---------NO production	Decrease*	59	23.8
	Increase	29	11.7
	Has no impact on	18	7.3
	I do not know	142	57.3
Which of the following mouthwash has a potent effect on NO production?	Chlorhexidine *	53	21.4
	Alcohol-based Mouthwash	19	7.7
	All types of antibacterial mouthwash equally affect NO production	22	8.9
	I do not know	154	62.1

*Indicate the correct answers. CVD: Cardiovascular diseases, NO: Nitric Oxide

Regarding the general knowledge questions about NO, participants achieved a mean score of 2.3±1.9 out of a possible score of 7. Only 17 (6.9%) correctly answered more than 70% of these questions (i.e., more than 5 out of 7). Similarly, for the specific questions related to oral bacteria, mouthwash, and NO, the mean score was 0.9±1.2 out of a possible 4. Only 9 (3.6%) correctly answered more than 70% of these questions (i.e., more than 3 questions out of 4).

Differences in Knowledge between Demographic Groups

The total scores for general NO knowledge and specific knowledge regarding oral bacteria, mouthwash, and NO were analyzed in relation to against gender, years of experience, qualification, region, and work setting using simple linear regression, t-tests and ANOVA. None of these demographic variables were found to be statistically significant predictors of knowledge scores ($p > 0.05$), as shown in Table 4. However, both age and years of practical experience emerged as significant predictors of general NO knowledge. Age had a small but statistically significant effect ($\beta = 0.128$, $p = 0.043$, $R^2 = 0.016$), and years of practical experience also showed a significant association ($\beta = 0.146$, $p = 0.021$, $R^2 = 0.021$). In contrast, neither age ($p = 0.955$) nor years of practical experience ($p = .700$) were significantly associated with specific knowledge as shown in Table 4. Additionally, participants who had previously heard of NO scored significantly higher in both general NO knowledge and specific knowledge about oral bacteria, mouthwash, and NO compared to those who had not ($p<0.001$for both comparisons, t-test), as detailed in Table 4

Table 4: Demographic Variables, against General Knowledge, and Specific Knowledge

Variable		General Knowledge			Specific Knowledge		
		Mean	SD	P-value	Mean	SD	p-value
Age*		-	-	0.04	-	-	0.96
Years of experience*		-	-	0.02	-	-	0.70
Gender**	Male	2.5	1.9	0.27	0.8	1.1	0.32
	Female	2.2	1.9		0.9	1.2	
Qualification***	Student/ intern	2.1	1.9	0.28	0.9	1.2	0.98
	Working dentist	2.4	1.9		0.9	1.2	
Setting of work***	Educational (academic)	2.2	1.7	0.72	0.9	1.2	0.34
	Private sector	2.2	2.0		1.1	1.1	
	Governmental sector	2.5	2.0		0.9	1.2	
Region***	Western	2.4	1.9	0.52	1.0	1.3	0.67
	Central	2.1	2.0		0.8	1.1	
	Western	2.0	1.6		0.7	1.0	
	Southern	2.4	1.1		0.2	0.5	
	Northern	2.6	1.7		1.1	1.3	
Have you heard about	Yes	2.7	1.9	<0.001	1.1	1.2	<0.001
nitric oxide (NO)?**	No	1.0	1.5		0.5	1.0	

*Linear regression, **t-test, ***ANOVA

DISCUSSION

This study evaluated the knowledge of Saudi dental professionals and students regarding the NO pathway, the role of oral bacteria, and the impact of antiseptic mouthwash use on NO production. It also compared knowledge levels across different demographic and professional groups. A key finding was that three-quarters of participants reported having heard about NO; however, nearly one-third mistakenly believed that NO is the same as nitrous oxide, the gas commonly used for local anesthesia. This highlights a significant misconception among dental professionals regarding the basic identity and function of NO. Alternatively, these responses could suggest a lack of careful attention while completing the questionnaire. Overall, participants showed limited knowledge of both general concepts related to NO and specific information concerning oral bacteria and the effects of mouthwash on NO production. These results highlight notable gaps in knowledge among Saudi dental professionals in an area directly relevant to oral and systemic health.

NO is a key signaling molecule that is produced endogenously in the human body via an enzymatic pathway which depends on NOS and the substrate L-arginine [16]. NO has a well-established role in vascular homeostasis including vasodilation [25], regulation of blood pressure [26] and overall cardiovascular health. It also contributes to host defense by modulating inflammation and immune responses [27]. Despite the significance of NO in maintaining systemic health, our findings revealed considerable knowledge gaps among dental professionals in these aspects. Less than one-third of our participants were aware that NO is naturally synthesized in the body, and less than half of them identified its importance in cardiovascular health. However, a substantial portion of respondents were uncertain about the vasodilatory effect of NO (49%), with only 27.8% identifying this function correctly. Knowledge of NO's role in modulating immune response and inflammation was even more limited, with the majority of participants lacking clarity on this relationship. This illustrates the limited understanding of the systemic functions of NO among dental professionals. These findings suggest that the limited knowledge observed may be attributed to gaps in dental education, insufficient interdisciplinary training, and a lack of emphasis on NO's systemic roles in professional development resources.

Dietary $NO3^-$, primarily from vegetables such as green leafy vegetables and beetroot, is considered a secondary but essential source of NO in the body through entero-salivary circulation pathway [16]. However, our findings indicate that this concept is not well understood among dental professionals as the majority of respondents expressed uncertainty about the nutritional contribution to NO production. This limited understanding was further reflected in responses to a related item in the specific knowledge section, where only about one third of respondents recognized that consuming $NO3^-$-rich foods such as leafy greens and beetroot can increase NO levels in the bloodstream. Nearly half of the respondents were uncertain about the role of dietary NO_3^- in enhancing NO levels through consumption of foods like leafy greens and beetroot. While it may be understandable that dental professionals are less familiar with the nutritional aspects of NO metabolism, these findings highlight an important interdisciplinary gap. Given that $NO3^-$-reducing oral bacteria play a key role in converting dietary $NO3^-$to NO, a process that can be disrupted by antiseptic mouthwash, thus basic understanding of this pathway is becoming increasingly relevant to dental practice. This gap was clearly demonstrated in our results; when participants were asked about the role of oral bacteria in influencing NO levels, only 14.9% of participants correctly recognized that oral bacteria enhance NO production, while nearly two-thirds indicated that they did not know. In addition, over a half of our participants were uncertain if using antiseptic mouthwash can affect NO production.

Studies reported that antiseptic mouthwash, particularly chlorhexidine mouthwash, can substantially affecting dietary NO_3^-metabolism. Woessner *et al*, assessed the effect

of different strengths of mouthwash including chlorhexidine, Listerine antiseptic mouthwash and Cepacol antibacterial mouthwash against control water, on salivary and plasma $NO_3^-$and $NO2^-$concentrations, after ingestion of beetroot juice (a common NO_3^- donor). Chlorhexidine appeared to have the strongest effect on elimination any conversion of salivary NO_3^- to $NO2^-$, and subsequently reduced NO production [28]. In our study, the majority of participants were unsure about which mouthwash has the greatest impact on NO production, with only about one-fifth correctly identifying chlorhexidine as the most potent inhibitor. In addition, several studies showed that using Chlorhexidine abolished the blood pressure-lowering benefits of dietary NO_3^-, with some studies reporting increases of 3.5 mmHg or more in healthy individuals [29-31]. A recent meta-analysis found that regular use of antiseptic mouthwash is associated with a slightly but significantly increased risk of developing hypertension [32]. Furthermore, some epidemiological studies reported that there is also an association between regular use of antiseptic mouthwash and increase the risk of diabetes [14]. This growing evidence was not reflected in participants responses with half of them stating that they are unsure if there is a link between antibacterial mouthwash use and the development or progression of future cardiovascular disease. The lack of such knowledge may lead to underestimating the broader health implications of mouthwash use beyond oral hygiene, particularly its potential impact on vascular and metabolic functions. Recommending frequent antiseptic mouthwash use without consideration of such systemic effects could unintentionally contribute to negative health outcomes, especially in vulnerable populations.

While the potential systemic effects of antiseptic mouthwash on NO production are becoming more evident, less is known about the role of antibacterial toothpastes. When participants were asked whether antibacterial toothpastes might affect NO production similarly to mouthwash, most of them were uncertain. In 2012, Bondonno and colleagues examined the effect of antibacterial toothpastes on NO_3^- reduction and circulating NO, and they found that it did not affect either of them [33]. The authors explained that triclosan, which is a common antibacterial ingredient in toothpaste, is unable to effectively penetrate the biofilm within deep clefts where oral NO_3^- -reducing bacteria are primarily located [33]. While descriptive analysis indicated no significant differences in knowledge across demographic groups, regression analysis revealed that both age and years of practical experience were significant predictors of general knowledge, though the effect sizes were modest (age: β = .128, p = 0.04; experience β = 0.146, p = 0.02). In contrast, specific knowledge related to the NO_3^--NO2 –NO pathway, oral bacteria, and mouthwash use did not differ significantly by age (p = 0.96) or experience (p = 0.700). These findings suggest that while overall awareness may slightly improve with professional experience, detailed understanding remains consistently low across the sample.

This study provides novel insights into knowledge of the dietary NO_3^--NO2 –NO pathway and the impact of mouthwash use on it among dental professionals in Saudi Arabia, via using a newly developed questionnaire. This tool may also be applicable for assessing similar knowledge among dental professionals in other countries, tracking knowledge in the same group of individuals over time, or within the general population. On the other hand, our study has certain limitations. The sample size was relatively small and self-selected, which may limit the generalizability of the results to the larger population of dental professionals in Saudi Arabia. Although efforts were made to reach a large audience by distributing the questionnaire across various professional networks, dental institutions, and regions, it is possible that not all relevant subgroups within the dental community were adequately represented. In addition, our study aimed to include participants from various regions of Saudi Arabia, the distribution of the responses though was uneven, with most of them coming from western and central regions. This likely reflects the recruitment strategy which relied on institutional and community networks primarily based in the Western region. Although this may limit the representativeness, the data still shows valuable insights into awareness among dental professionals from different cities in Saudi Arabia. Another limitation was that we administered a relatively small number of closed-ended questions. This was a deliberate design feature to maximize response rate. Future follow-up qualitative research incorporating open-ended questions or interviews could be valuable in the future to assess the depth of participants' understanding. Finally, it cannot be ruled out that the low level of knowledge displayed in some individuals could reflect a lack of attention (e.g., mistaking NO for nitrous oxide), rather than a genuine lack of knowledge.

CONCLUSION

This study provides novel insights into general knowledge of NO and understanding of impact of oral bacteria and antiseptic mouthwash on NO production among Saudi dental professionals. Our findings highlight significant gaps in understanding the physiological role of NO and how oral care practices may influence its availability. Despite the growing body of evidence linking the oral microbiome, antiseptic mouthwash and dietary NO_3^- to systemic health outcomes, many participants were unaware of the role of oral bacteria and antiseptic mouthwash in NO_3^- reduction and NO production. Notably, confusion between NO and nitrous oxide was also apparent. These results underscore the need to enhance dental education by integrating updated knowledge about NO, its dietary sources, and the broader connection between oral and systemic health. Given the observed gaps in knowledge regarding NO functions and its derivatives, we recommend integrating more comprehensive content related to NO and its precursors into dental education and continuing professional development programs. Emphasizing the role of NO_3^- and NO in cardiovascular health, immune modulation, and inflammatory responses may enhance interdisciplinary understanding and

promote more holistic patient care among dental professionals. Evidence-based guidelines on the use of antiseptic mouthwashes should be developed, balancing oral health benefits with potential systemic risks, especially in patients with cardiovascular or metabolic vulnerabilities.

REFERENCES

[1] Dong L. *et al.* "The global burden of migraine: A 30-year trend review and future projections by age, sex, country, and region." *Pain and Therapy*, vol. 14, no. 1, 2025, pp. 297–315. https://doi.org/10.1007/s40122-024-00690-7

[2] Wijeratne T. *et al.* "Global, regional, and national burden of headache disorders, 1990–2021, with forecasts to 2050: A Global Burden of Disease study 2021." *Cell Reports Medicine*, vol. 6, no. 10, 2025, article 102348. https://doi.org/10.1016/j.xcrm.2025.102348

[3] Zhao H. *et al.* "Global, regional, and national burdens and trends of migraine among males aged 10–59 years from 1990 to 2021: Insights from the Global Burden of Disease study 2021." *Frontiers in Neurology*, vol. 16, 2025, article 1585512. https://doi.org/10.3389/fneur.2025.1585512

[4] Rossi M.F. *et al.* "Sex and gender differences in migraines: A narrative review." *Neurological Sciences*, vol. 43, no. 9, 2022, pp. 5729–5734. https://doi.org/10.1007/s10072-022-06178-6

[5] Husøy A.K. *et al.* "Global, regional, and national burden of headache disorders, 1990–2023: A systematic analysis for the Global Burden of Disease Study 2023." *The Lancet Neurology*, vol. 24, no. 12, 2025, pp. 1005–1015. https://doi.org/10.1016/S1474-4422(25)00402-8

[6] Shen L. *et al.* "Global, regional, and national burden of headache disorders, 1990–2021, and projections to 2050: A comprehensive analysis of the Global Burden of Disease study 2021." *Frontiers in Neurology*, vol. 16, 2025, article 1674946. https://doi.org/10.3389/fneur.2025.1674946

[7] Headache Classification Committee of the International Headache Society (IHS). "The International Classification of Headache Disorders, 3rd edition." *Cephalalgia*, vol. 38, no. 1, 2018, pp. 1–211. https://doi.org/10.1177/0333102417738202

[8] Grodzka O. *et al.* "Migraine with and without aura—two distinct entities? A narrative review." *The Journal of Headache and Pain*, vol. 26, no. 1, 2025, article 77. https://doi.org/10.1186/s10194-025-01998-1

[9] Noseda R. and Burstein R. "Migraine pathophysiology: Anatomy of the trigeminovascular pathway and associated neurological symptoms, cortical spreading depression, sensitization, and modulation of pain." *Pain*, vol. 154, Supplement 1, 2013, pp. S44–S53. https://doi.org/10.1016/j.pain.2013.07.021

[10] Cegielska J. *et al.* "Migraine and its association with hyperactivity of cell membranes in the course of latent magnesium deficiency—preliminary study of the importance of the latent tetany presence in the migraine pathogenesis." *Nutrients*, vol. 13, no. 8, 2021, article 2701. https://doi.org/10.3390/nu13082701

[11] Kurşun O. *et al.* "Migraine and neuroinflammation: The inflammasome perspective." *The Journal of Headache and Pain*, vol. 22, no. 1, 2021, article 55. https://doi.org/10.1186/s10194-021-01271-1

[12] Christensen R.H. *et al.* "The vessel-to-neuron trigeminovascular hypothesis of migraine pathogenesis – the 'pro' argument." *The Journal of Headache and Pain*, vol. 26, no. 1, 2025, article 248. https://doi.org/10.1186/s10194-025-02130-z

[13] Biscetti L. *et al.* "The putative role of neuroinflammation in the complex pathophysiology of migraine: From bench to bedside." *Neurobiology of Disease*, vol. 180, 2023, article 106072. https://doi.org/10.1016/j.nbd.2023.106072

[14] Spekker E. *et al.* "TRP channels: Recent development in translational research and potential therapeutic targets in migraine." *International Journal of Molecular Sciences*, vol. 24, no. 1, 2023, article 700. https://doi.org/10.3390/ijms24010700

[15] Fiorentini D. *et al.* "Magnesium: Biochemistry, nutrition, detection, and social impact of diseases linked to its deficiency." *Nutrients*, vol. 13, no. 4, 2021, article 1136. https://doi.org/10.3390/nu13041136

[16] Matek Sarić M. *et al.* "Magnesium: Health effects, deficiency burden, and future public health directions." *Nutrients*, vol. 17, no. 22, 2025, article 3626. https://doi.org/10.3390/nu17223626

[17] Dolati S. *et al.* "The role of magnesium in pathophysiology and migraine treatment." *Biological Trace Element Research*, vol. 196, no. 2, 2020, pp. 375–383. https://doi.org/10.1007/s12011-019-01931-z

[18] Mathew A.A. and Panonnummal R. "'Magnesium'—the master cation—as a drug: Possibilities and evidences." *BioMetals*, vol. 34, no. 5, 2021, pp. 955–986. https://doi.org/10.1007/s10534-021-00328-7

[19] Spasić S. *et al.* "Magnesium ions as modulators of voltage-gated and ligand-gated ion channels in central neurons." *International Journal of Molecular Sciences*, vol. 26, no. 24, 2025, article 12152. https://doi.org/10.3390/ijms262412152

[20] Marzell B. *et al.* "The impact of low serum and dialysate magnesium levels on clinical outcomes in peritoneal dialysis patients: A literature review." *BMC Nephrology*, vol. 26, no. 1, 2025, article 560. https://doi.org/10.1186/s12882-025-04494-5

[21] Razzaque M.S. "Magnesium: Are we consuming enough?" *Nutrients*, vol. 10, no. 12, 2018, article 1863. https://doi.org/10.3390/nu10121863

[22] Kebir N.E. and Zahzeh T. "Magnesium deficiency associated with stress, systemic inflammation, and insulin resistance in diabetes mellitus: A review." *Egyptian Academic Journal of Biological Sciences, C: Physiology and Molecular Biology*, vol. 14, no. 1, 2022, pp. 31–46. https://doi.org/10.21608/eajbsc.2022.213962

[23] Domitrz I. and Cegielska J. "Magnesium as an important factor in the pathogenesis and treatment of migraine—from theory to practice." *Nutrients*, vol. 14, no. 5, 2022, article 1089. https://doi.org/10.3390/nu14051089

[24] Talandashti M.K. *et al.* "Effects of selected dietary supplements on migraine prophylaxis: A systematic review and dose–response meta-analysis of randomized controlled trials." *Neurological Sciences*, vol. 46, no. 2, 2025, pp. 651–670. https://doi.org/10.1007/s10072-024-07794-0

[25] Patel P.S. and Minen M.T. "Complementary and integrative health treatments for migraine." *Journal of Neuro-Ophthalmology*, vol. 39, no. 3, 2019, pp. 360–369. https://doi.org/10.1097/WNO.0000000000000841

[26] Chiu H.-Y. *et al.* "Effects of intravenous and oral magnesium on reducing migraine: A meta-analysis of randomized controlled trials." *Pain Physician*, vol. 19, 2016, pp. E97–E112.

[27] Peikert A. *et al.* "Prophylaxis of migraine with oral magnesium: Results from a prospective, multi-center, placebo-controlled and double-blind randomized study." *Cephalalgia*, vol. 16, no. 4, 1996, pp. 257–263. https://doi.org/10.1046/j.1468-2982.1996.1604257.x

[28] Yaşin S. and Altunışık E. "Decreased serum magnesium levels in patients with migraine: A case-control study." *International Archives of Medical Research*, vol. 15, no. 1, 2023, pp. 26–32. https://doi.org/10.56484/iamr.1217558

[29] Talebi M. *et al.* "Relation between serum magnesium level and migraine attacks." *Neurosciences (Riyadh)*, vol. 16, no. 4, 2011, pp. 320–323. http://www.ncbi.nlm.nih.gov/pubmed/21983373

[30] ShyamSundar M. *et al.* "Study of serum magnesium levels in migraine patients during the ictal and interictal phases." *IAR Journal of Medical and Surgical Research*, vol. 4, no. 5, 2023, pp. 4–7. https://doi.org/10.47310/iarjmsr.2023.V04i05.02

[31] Ebrahim Abdelwahab E.M. *et al.* "The potential role of serum magnesium and vitamin D levels in patients with migraine." *International Journal of Medical Arts*, published online April 18, 2025. https://doi.org/10.21608/ijma.2025.364023.2138

[32] Elsayed D.A. *et al.* "Elucidation of the levels of vitamin D, calcium, and magnesium in the serum of Egyptian migraine patients: A case-control study." *The Egyptian Journal of Neurology, Psychiatry and Neurosurgery*, vol. 56, no. 1, 2020, article 43. https://doi.org/10.1186/s41983-020-00174-3

[33] Karim M.R. *et al.* "Relation between serum magnesium level and migraine." *Mymensingh Medical Journal*, vol. 30, no. 2, 2021, pp. 301–306. http://www.ncbi.nlm.nih.gov/pubmed/33830106

[34] Sunda B. *et al.* "Serum magnesium levels and clinical correlation with migraine attack frequency." *Indian Journal of Medical Research*, vol. 145, no. 3, 2017, pp. 311–318.

[35] Samaie A. *et al.* "Blood magnesium levels in migraineurs within and between the headache attacks: A case-control study." *Pan African Medical Journal*, vol. 11, 2012, article 46. http://www.ncbi.nlm.nih.gov/pubmed/22593782

[36] Mahajan R. *et al.* "Serum magnesium levels during the ictal and interictal phase in patients of migraine: A prospective observational study." *Neurology India*, vol. 70, no. 5, 2022, pp. 1852–1855. https://doi.org/10.4103/0028-3886.359247

[37] Page M.J. *et al.* "The PRISMA 2020 statement: An updated guideline for reporting systematic reviews." *BMJ*, 2021, article n71. https://doi.org/10.1136/bmj.n71

[38] Page M.J. *et al.* "PRISMA 2020 explanation and elaboration: Updated guidance and exemplars for reporting systematic reviews." *BMJ*, 2021, article n160. https://doi.org/10.1136/bmj.n160

[39] Hayden J.A. *et al.* "Assessing bias in studies of prognostic factors." *Annals of Internal Medicine*, vol. 158, no. 4, 2013, pp. 280–286. https://doi.org/10.7326/0003-4819-158-4-201302190-00009

[40] Assarzadegan F. *et al.* "Measuring serum level of ionized magnesium in patients with migraine." *Iranian Journal of Child Neurology*, vol. 9, no. 3, 2015, pp. 13–16. http://www.ncbi.nlm.nih.gov/pubmed/26401148

[41] Jabbar A.A. *et al.* "Efficacy of serum magnesium levels in migraine attack frequency and intensity: A clinical cross-sectional study." *International Journal of Neurology Research*, vol. 7, no. 1, 2025, pp. 11–16. https://doi.org/10.33545/2664908X.2025.v7.i1a.28

[42] Khan M.J. *et al.* "Serum vitamin D, calcium, and magnesium levels in patients with migraine presenting to PEMH, Pakistan: A case-control study." *Journal of Health and Rehabilitation Research*, vol. 4, no. 3, 2024, pp. 1–5. https://doi.org/10.61919/jhrr.v4i3.1077

[43] Assarzadegan F. *et al.* "Serum concentration of magnesium as an independent risk factor in migraine attacks." *International Clinical Psychopharmacology*, vol. 31, no. 5, 2016, pp. 287–292. https://doi.org/10.1097/YIC.0000000000000130

[44] Jagunmolu H. *et al.* "Efficacy of magnesium supplementation in the prophylaxis of migraine: A systematic review of clinical trials comparing oral magnesium to placebo or standard care." *The Egyptian Journal of Neurology, Psychiatry and Neurosurgery*, vol. 62, no. 1, 2026, article 81. https://doi.org/10.1186/s41983-026-01150-z