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Salivary Nitric Oxide Levels in Adults with Different DMFT Scores in a Selected Iranian Population: A Cross-Sectional Study

Razina dušikova oksida u slini odraslih s različitim DMFT vrijednostima u odabranoj iranskoj populaciji: presječno istraživanje

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Abstract

Objectives: Due to the emphasis on the protective and antimicrobial role of Nitric Oxide against gastrointestinal diseases, investigating its relationship with dental caries is a right topic. Therefore, this research has investigated the amount of saliva Nitric Oxide in different volumes of DMFT indicator in adults. **Material and Methods:** In this descriptive- analytical cross-sectional study, 80 participants (20-35 years old), without a history of systemic disease and drug use participated as research samples, (53.8% of the participants in the study were women). Participants were selected from patients who had visited dental Department. The participants were divided in four groups based on DMFT (DMFT=0, 1≤DMFT≤3, 3<DMFT<10, DMFT≥10). Non-stimulating saliva was collected from all participants between 9-11 am in a calibrated tube. Saliva Nitric Oxide was measured using a Nitrous Oxide test, based on the Griess reaction. We used a correlation test to analyze quantitative variables, and t-test or ANOVA for qualitative and quantitative variables. **Results:** A significant relationship between DMFT and age was identified. At different levels of DMFT, significant relationship between DMFT and sex was not found. In different groups of DMFT, no significant relationship between Nitric Oxide and DMFT exist. **Conclusion:** The level of Nitric Oxide saliva was not affected by the amount of DMFT.

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Introduction

Tooth decay is the most common infectious disease. The prevalence of caries is declining today, but it is still a major problem. The etiology and pathogenesis of dental caries are multifactorial and acid-producing bacteria in the presence of carbohydrates can destroy tooth structure and lead to caries. When pathogens reach a level at which they can overcome the intraoral defense system, caries occurs. Thus, the host defense mechanism plays a key role in preventing tooth decay [1, 2].

Saliva is one of the basic needs of the dental system to resist tooth decay. Daily saliva production varies between 0.5 liter and 1.1 liter. Saliva is composed of 99% water and 0.01 of it is composed of salts and proteins. Salivary compounds undergo changes in the salivary duct, including selective reabsorption of sodium, chlorine, and potassium secretion. The composition of stimulating saliva is different from that of non-stimulating saliva, so that in stimulated saliva the amount of sodium and chlorine increases. On the other hand, non-stimulating saliva compounds have a more important role in combating caries than stimulated saliva [3].

Saliva plays various roles in the oral cavity including: digestion (with enzymes such as lipase and amylase ...), protection (immunoglobulin, lysozyme, lactoferrin ...), helping the sense of taste, and helping remineralization with the help of phosphate ions and calcium [4].

Several factors affect the defensive role of saliva. Due to the emphasis on the protective and antimicrobial role of nitric oxide against gastrointestinal diseases, the study of the relationship between this compound and tooth decay is valuable.

Nitric oxide (NO) is produced in two ways: chemically and enzymatically. Chemically, it refers to the metabolism of oral nitrate, and enzymatically it refers to the breakdown of L-arginine by the enzyme NO synthetize, which is produced by the salivary glands and other tissues [5,6].

Nitric oxide is a free radical that passes easily through cell membranes and has various roles such as cell death, immune system stimulation, neurotransmitter, and vasodilation. Nitric oxide synthesis has been observed during the isolation of the structure of blood vessel endothelium from structures called Endothelium Derived Relaxation Factor (EDRF), and over the years it has been concluded that NO is EDRF [7].

Nitrate of nutrients enters the bloodstream through the duodenum and upper ilium, and from there enters the saliva through active transfer. Salivary nitrate concentrations are 10 times higher than those in blood. Nitrate in the oral cavity is converted to nitrite by bacterial respiration and nitrate reductase enzymes. Microflora in the mouth, such as lactobacilli, Streptococcus mutans, Staphylococcus aureus, acidify the environment around nitrite and converts it to nitrous oxide. Nitrous oxide is unstable and spontaneously converts to nitric oxide and nitric dioxide [8, 9].

Nitric oxide is known as the strongest antibacterial compound and exerts this role in several ways such as: preventing the growth of bacteria, increasing the toxicity of macrophages. Nitric oxide easily crosses cell membranes and exerts

Uvod

Karijes je najčešća zarazna bolest. Prevalencija je danas u padu, ali je i dalje velik problem. Etiologija i patogeneza zubnog karijesa su multifaktorijalne, a bakterije koje proizvode kiselinu u prisutnosti ugljikohidrata mogu razoriti strukturu zuba i potaknuti karijes. To se događa kada patogeni dosegnu razinu na kojoj mogu nadvladati intraoralni obrambeni sustav. Zato je obrambeni mehanizam domaćina ključan u sprječavanju karijesa (1, 2).

Slina je jedna od osnovnih potreba zuba da se odupru karijesu. Njezina dnevna proizvodnja varira između 0,5 i 1,1 litre. Sastoji se od 99 % vode, a 0,01 % soli su i proteini. Spojevi sline podliježu promjenama u salivarnom kanalu, uključujući selektivnu reapsorpciju izlučenog natrija, klora i kalija. Sastav stimulirane sline drukčiji je od nestimulirane – u stimuliranoj slini povećava se količina natrija i klora. S druge strane, nestimulirani spojevi sline važniji su u borbi protiv karijesa od stimulirane sline (3).

Slina ima različite zadaće u usnoj šupljini, uključujući probavu (s enzimima kao što su lipaza i amilaza), zaštitu (imunoglobulin, lizozim, laktoferin), pomaže u osjetu okusa i remineralizaciji s pomoću fosfata iona i kalcija (4).

Nekoliko čimbenika utječe na obrambeni utjecaj sline. Zbog naglaska na zaštitnu i antimikrobnu ulogu dušikova oksida, kad je riječ o gastrointestinalnim bolestima, dragocjeno je proučavanje odnosa između toga spoja i karijesa.

Dušikov oksid (NO) proizvodi se na dva načina – kemijski i enzimski. Kemijski se odnosi na metabolizam oralnoga nitrata, a enzimski na razgradnju L-arginina s pomoću enzima NO sintetaze, koji proizvode žlijezde slinovnice i druga tkiva (5, 6).

Dušikov oksid je slobodni radikal koji lako prolazi kroz stanične membrane i ima različite zadaće kao što su stanična smrt, stimulacija imunosnog sustava, neurotransmiter i vazodilatacija. Sinteza dušikova oksida uočena je tijekom izolacije strukture endotela krvnih žila iz struktura koje se nazivaju faktor relaksacije izveden iz endotela (EDRF), a tijekom godina zaključeno je da je NO EDRF (7).

Nitrati iz hranjivih tvari ulaze u krvotok kroz duodenum i gornji ilium, a odatle aktivnim prijenosom u slinu. Koncentracija nitrata u slini 10 je puta veća od one u krvi. Nitrat se u usnoj šupljini pretvara u nitrit bakterijskim disanjem i enzimom nitrat-reduktazom. Mikroflora u ustima, poput laktobacila, bakterija *Streptococcus mutans i Staphylococcus aureus*, zakiseljuje okolinu oko nitrita i pretvara ga u dušikov oksid. Dušikov oksid je nestabilan i spontano prelazi u dušikov dioksid (8, 9).

Dušikov oksid poznat je kao najjači antibakterijski spoj i to čini na nekoliko načina kao što su sprječavanje rasta bakterija i povećanje toksičnosti makrofaga. Također lako prolazi kroz stanične membrane i antibakterijski djeluje preko nekoliko mehanizama kao što su oštećenje DNK inhibicijom sinteze DNK, kombinacija sa željeznim i sumpornim centrima mitohondrijskih enzima potrebnih za stanično disanje te u kombinaciji sa superoksidom koji proizvodi zamjenski dušikov oksid i aktivni hidroksid slobodnih radikala (3, 8).

its antibacterial role in several mechanisms such as: DNA damage by inhibition of DNA synthesis, combination with iron and sulfur centers of mitochondrial enzymes required for cellular respiration, and combined with superoxide which produces proxy nitrous oxide and active free radical hydroxide [3,8].

Considering that in recent years, dental caries is one of the most common oral problems in Iranian society and previous studies have shown no agreement on the effect of nitric oxide on caries, this study was conducted to investigate the role of nitric oxide on DMFT.

Material and methods

This study is a descriptive cross-sectional study. It was performed in the oral diseases department of Qazvin Dental School of Qazvin province of Iran. Participants were selected from patients who had visited dental department to receive dental treatment. Before starting data collection, information and consent form was given to patients and they entered the study voluntarily by signing the informed consent to enter the study. At first, participants were asked to fill out a questionnaire based on inclusion and exclusion criteria. Participants had to respect the inclusive criteria of being without systemic or local diseases that affect salivation, not taking any systemic or local medications in the previous two months that might affect their saliva composition and non-smokers. Participants with periodontal disease, hypertension, diabetes, a history of radiotherapy, chemotherapy were not included in the study. As a result, a total of 80 participants who were aged between 20 and 35 years were selected to participate in the study. Then the DMFT index was determined based on the WHO recommendation.

The occlusal surfaces of the participant's teeth were cleaned with a prophylactic brush and then dried, and the DMFT was calculated. Based on the number calculated from DMFT, participants were divided into two groups including: 1) Caries free group, 2) Caries active group.

The second group was divided into three subgroups, each subgroup comprised of 20 participants:

I: 1≤DMFT≤3;

II: 3 <DMFT <10;

III: DMFT ≤10

About 2.5 ml of non-stimulating saliva was collected from all participants between 9 and 11 in the morning in a tube (Falcon). Patients were asked not to have any chewing function that affects saliva for about two hours before sampling. Participants did not eat vegetables at their last meal. Participants were asked to sit in an ordinary chair other than the dental unit to prevent patient anxiety.

During sampling, the samples were stored in a refrigerator at 4° C. After sampling, all samples were sent to the laboratory. It should be noted that the samples were transferred in a 4° C flask. They were then stored at -40° C until laboratory testing.

Saliva nitric oxide was measured using a nitric oxide measuring kit made by Iranian Navand Salamat Company, based on the Griess reaction. In this reaction, the criterion

S obzirom na to da je posljednjih godina zubni karijes jedan od najčešćih oralnih problema u iranskome društvu i da dosadašnja istraživanja nisu pokazala slaganje kad je riječ o učinku dušikova oksida na karijes, ovo je istraživanje provedeno da bi se istražio utjecaj dušikova oksida na DMFT.

Materijal i metode

Ovo je istraživanje deskriptivno i presječno. Provedeno je u Zavodu za oralne bolesti Stomatološkog fakulteta Qazvin u pokrajini Qazvin u Iranu. Sudionici su odabrani među pacijentima koji su posjetili kliniku radi stomatološke terapije. Prije početka prikupljanja podataka pacijenti su dobili sve potrebne informacije i potpisali su da dobrovoljno sudjeluju u istraživanju. Na početku su zamoljeni da ispune upitnik s kriterijima za uključivanje i isključivanje iz istraživanja. Da bi sudjelovali u istraživanju morali su poštovati kriterije za uključivanje – da nisu imali sistemske ili lokalne bolesti koje utječu na salivaciju, da nisu uzimali nikakve sistemske ili lokalne lijekove u protekla dva mjeseca koji bi mogli utjecati na sastav njihove sline i da nisu pušači. Sudionici s parodontnom bolešću, hipertenzijom, dijabetesom, obavljenom radioterapijom i kemoterapijom nisu uključeni u istraživanje. Nakon toga je ukupno 80 sudionika u dobi između 20 i 35 godina odabrano za sudjelovanje u istraživanju. Zatim je određen DMFT indeks prema preporuci SZO-a.

Okluzalne površine zuba sudionika očišćene su profilaktičnom četkicom i zatim osušene te je izračunata DMFT vrijednost. Na temelju te vrijednosti podijeljeni su u dvije skupine:

1) skupinu bez karijesa, 2) skupinu s aktivnim karijesom.

Druga skupina podijeljena je u tri podskupine, a svaka se sastojala od 20 sudionika:

I: 1 ≤ DMFT ≤ 3; II: 3 < DMFT < 10; III: DMFT ≤10.

Oko 2,5 mL nestimulirane sline skupljeno je u epruvete (Falcon) od svih sudionika između 9 i 11 sati prijepodne. Pacijenti su zamoljeni da izbjegavaju žvakati oko dva sata prije uzorkovanja jer bi to moglo utjecati na slinu. Uz to nisu smjeli jesti povrće u zadnjem obroku. Zamoljeni su da sjednu na običan stolac, a ne na stomatološki kako bi se spriječila tjeskoba. Nakon uzorkovanja uzorci su pohranjeni u hladnjak na temperaturi od 4 °C, a zatim su poslani u laboratorij. Treba napomenuti da su uzorci prebačeni u tikvicu na 4 °C, a zatim su čuvani na -40 °C do laboratorijskog ispitivanja.

Dušikov oksid u slini izmjeren je priborom za mjerenje dušikova oksida koji je proizvela iranska tvrtka Navand Salamat, na temelju Griessove reakcije. U toj reakciji kriterij je bio izmjeriti količinu nitrita jer je vijek trajanja dušikova oksida vrlo kratak i zbog njegove kombinacije s kisikom kada se brzo pretvara u nitrit. Reakcija je da nitrit u kombinaciji sa sulfaniličnom kiselinom (reagens 1 u kompletu) stvara diazonijevu sol koja omogućuje reakciju s N-naftil-etilen dia-

was to measure the amount of nitrite because the lifespan of nitric oxide is very short and because of its combination with oxygen when it rapidly converts to nitrite. The reaction of the grease is that nitrite in combination with sulfanilic acid (Reagent 1 in the kit) forms a diazonium salt which allows the reaction with N-Naphthyl-ethylene diamine (Reagent 2) and produces a stable azo compound, which is read by the ELISA device at a wavelength of 570 nm.

The test was performed according to the instructions of the kit manufacturer, which are as follows:

To begin the experiment, all kit solutions should be placed at room temperature for 15 to 30 minutes to reach temperature equilibrium.

Samples should be precipitated to increase accuracy and remove protein interferes.

For this purpose, 150 µl of the sample was mixed with 80 µl of buffer A in the kit, in a 1.5ml micro tube, and after complete vortex, 80 µl of buffer B was added and vortexed. After complete mixing, it was placed in a centrifuge at 14,000 rpm for 10 minutes and then the supernatant was separated and used as a sample (refrigerated centrifuge was used in all steps).

Reagent R1 was added to all wells of microplate at 50 µl and mixed, then incubated at room temperature for 10 minutes. After incubation, 50 µl of R2 reagent was added to each well and mixed. Ten minutes after mixing the reagents, the plate was placed at 570 nm of ELISA and the nitrite level, which represents Nitric Oxide, was obtained.

Results

In the present study, 80 patients referred to Qazvin Dental School were evaluated for nitric oxide in terms of DMFT. After checking the normality, it was found that the age of the participants in the present study did not have a normal distribution.

The median age was 23 years and the mid-quarters ranged from 23 to 25 years. 53.8% of the participants in the study were women.

According to Table 1, there was no significant relationship between gender and nitric oxide with DMFT, but there was a significant relationship between the age and DMFT, so that DMFT increased with age.

minom (reagens 2) i proizvodi stabilan azo-spoj koji očitava ELISA uređaj na valnoj duljini od 570 nm.

Test je proveden prema uputama proizvođača kompleta, a one su sljedeće:

- na početku eksperimenta sve otopine iz kompleta treba držati na sobnoj temperaturi od 15 do 30 minuta kako bi se postigla toplinska ravnoteža
- uzorke treba pustiti da se slegnu kako bi se povećala točnost i uklonile interferencije proteina
- u tu svrhu 150 µL uzorka pomiješa se s 80 µL pufera A u setu u mikroepruveti od 1,5 mL te se nakon potpunog vorteksiranja doda 80 µL pufera; nakon potpunog miješanja uzorak se stavlja u centrifugu 10 minuta na 14 000 okretaja u minuti, a kada se supernatant odvoji koristi se kao uzorak (u svim koracima korištena je rashlađena centrifuga); reagens R1 dodaje se u sve jažice mikroploče u količini od 50 µL i promiješa te zatim inkubira na sobnoj temperaturi 10 minuta; nakon inkubacije dodaje se 50 µL R2 reagensa u svaku jažicu i promiješa; deset minuta poslije toga ploča je postavljena na 570 nm ELISA testa te je dobivena razina nitrita koja pokazuje količinu dušikova oksida.

Rezultati

U ovom istraživanju sudjelovalo je 80 pacijenata upućenih na Stomatološki fakultet Qazvin da bi se procijenila razina dušikova oksida, ovisno o DMFT-u. Nakon provjere normalnosti, ustanovljeno je da dob sudionika u ovom istraživanju nije imala normalnu distribuciju.

Prosječna dob bila je 23 godine, a srednje kvartile bile su u rasponu od 23 do 25 godina. Ukupno 53,8 % sudionika u istraživanju bile su žene.

Prema tablici 1. nije bilo značajne povezanosti između spola i dušikova oksida s DMFT-om, ali postojala je značajna povezanost između dobi i DMFT-a, tako da je DMFT rastao s godinama.

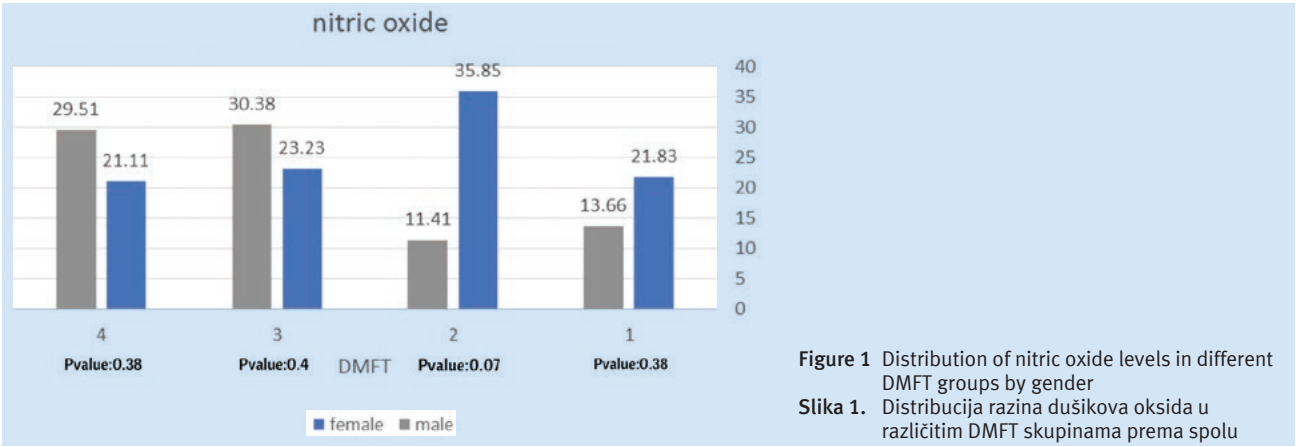

Figure 1 Distribution of nitric oxide levels in different DMFT groups by gender
Slika 1. Distribucija razina dušikova oksida u različitim DMFT skupinama prema spolu

Table 1 Comparison of age, nitric oxide and gender with DMFT index.
Tablica 1. Usporedba dobi, dušikova oksida i spola s DMFT indeksom

		DMFT				
		0	**1-3**	**4-9**	**≥10**	**Pvalue**
Sex • Spol n (%)	Female • Žene Male • Muškarci	36(45) 44(55)	52(65) 28(35)	40(50) 40(50)	43(53.8) 57(46.2)	**0.62**
Age • Dob	Median (IQR) • Medijan (IQR)	22.5(22-24)	23(21.25-23.75)	23(21.2-23.75)	24.5(25-40.75)	**0.001**
Nitric oxide Median (IQR) • Dušikov oksid –medijan (IQR)		13.69(8.2-22.7)	13.4(9.11-45.38)	30.39(15.91-36.96)	23.66(3.37-31.38)	**0.17**

According to Chart 1, there was no significant relationship between gender and nitric oxide content.at different levels of DMFT.

Discussion

In the present study, a significant relationship was not found between salivary nitric oxide level and DMFT index, as an indicator of caries activity.

In this study, the DMFT index was used to evaluate the caries status to be comparable with other studies, while in other studies such as Javadi Nejad [5], dfs was used instead of DMFT.

Saliva is equivalent to serum in terms of function in reflecting the physiological state of the body including hormonal, nutritional and metabolic changes; and because it is an accessible and non-invasive method, it has been considered to be a diagnostic tool by numerous researchers [10]. Therefore, in the present study, saliva samples were used to measure nitric oxide.

Saliva composition can change under the influence of saliva flow rate. On the other hand, saliva flow rate is a function of circadian rhythm, thus saliva sampling time should be standardized. It is best to take a saliva sample while the individual is sitting, with the head slightly bent forward and the eyes open [11]. In the present study, to prevent the effect of circadian rhythm on saliva, a sample of non-stimulating saliva was collected between 9 to 11 am.

In the current study, the use of systemic antibiotics was excluded from the study criteria for at least the past two months, as Dougal et al. reported that salivary nitrite production decreased following the use of broad-spectrum antibiotics [12].

In the study of Mobarak [3], saliva samples were taken from people who did not eat vegetables at their last meal because, as reported by Olin [13], salivary nitric oxide concentrations increased significantly three hours after consuming nitrate-rich foods. However, in the studies of Javadi Nejad [5] and Saini [9], it was only mentioned that nothing was eaten two hours before sampling, but there was no mention of consuming or not consuming vegetables in the last meal.

The age group of this study was selected for two reasons: firstly, to be comparable to Bayindir [6] and Mobarak [3]. Secondly, since DMFT is highly age-dependent, it is expected that the grouping of participants according to DMFT in this age group is more reliable than grouping in younger or older participants [3]. In the study of Javadinejad [5], children between the age of 6 and 12 years were studied.

Kako se vidi u dijagramu 1. nije bilo značajne povezanosti između spola i sadržaja dušikova oksida pri različitim vrijednostima DMFT-a.

Rasprava

U ovom istraživanju nije ustanovljena značajna povezanost između razine dušikova oksida u slini i DMFT indeksa kao pokazatelja aktivnosti karijesa.

DMFT indeks korišten je za procjenu karijesnog statusa kako bi se mogao usporediti s drugim istraživanjima, a u drugim istraživanjima, kao u onomu Javadija Nejada (5), korišten je dfs umjesto DMFT-a.

Slina je ekvivalentna serumu u smislu funkcije u odražavanju fiziološkog stanja tijela, uključujući hormonske, prehrambene i metaboličke promjene, a s obzirom na to da je pristupačna i neinvazivna metoda, mnogobrojni istraživači smatraju je korisnim dijagnostičkim sredstvom (10). Zato su u ovom istraživanju uzorci sline korišteni za mjerenje dušikova oksida.

Sastav sline može se promijeniti pod utjecajem brzine njezina protoka. No, brzina protoka sline funkcija je cirkadijalnog ritma, pa bi vrijeme uzorkovanja sline trebalo biti standardizirano. Najbolje je uzeti uzorak sline dok osoba sjedi s glavom blago pognutom naprijed i otvorenim očima (11). U ovom istraživanju, da bi se spriječio učinak cirkadijalnog ritma na slinu, uzorak nestimulirane sline uzet je između 9 i 11 sati.

U ovom istraživanju isključni je kriterij bila sistemska uporaba antibiotika barem tijekom posljednja dva mjeseca jer, kao što su izvijestili Dougal i suradnici, proizvodnja nitrita u slini smanjila se poslije uporabe antibiotika širokog spektra (12).

U svojem istraživanju Mobarak (3) je uzeo uzorke sline ljudi koji nisu jeli povrće u zadnjem obroku jer, kako je izvijestio Olin (13), koncentracija dušikova oksida u slini znatno se povećava tri sata poslije konzumiranja hrane bogate nitratima. Međutim, u istraživanjima Javadija Nejada (5) i Sainija (9) samo je spomenuto da se ništa nije jelo dva sata prije uzorkovanja, ali ne spominje se konzumiranje ili nekonzumiranje povrća u zadnjem obroku.

Dobna skupina u ovom istraživanju odabrana je iz dvaju razloga – prvi je da bi bila usporediva s onom Bayindira (6) i Mobaraka (3), a drugi je razlog to što se, zato što DMFT uvelike ovisi o dobi, očekuje da je grupiranje sudionika prema DMFT-u u ovoj dobnoj skupini pouzdanije od grupiranja mlađih ili starijih sudionika (3). U istraživanju Javadinejada (5) proučavana su djeca u dobi od 6 do 12 godina.

One of the limitations of cross-sectional studies is the lack of blinding of the samples, but in the present study this limitation has not raised because the samples were coded and then decoded before measuring the concentration of nitric oxide.

The findings of the present study are in the line with those of Mathew and Rezvi [14]; and Mobarak [3] who stated that nitric oxide has no significant relationship with caries.

Studies by Javadinejad [5] and Bayindir [6] found a significant positive relationship, in which nitric oxide acts as a defense mechanism. One of the differences between our method and Javadi Nejad method was the age of the patients; and the other difference was the method of measuring nitric oxide. In the present study, ELISA was used, while in the Javadi Nejad study, ion chromatography was used. In the Bayindir study [6], the age of the participants was almost identical to ours; however, the number of participants included 10 men and 12 women, which was less than the number of participants in the present study (80 participants).

Hedge's study [1] found a significant negative relationship so that people without caries had more saliva nitric oxide. In terms of the difference between the present study and the Hedge's study, we can point to more subgroups and an equal division of the number of participants in our study than the Hedge study with only two groups of participants.

Differences in study results may be due to methodology, so that in the study of Doel [15], which found a significant negative relationship, saliva samples were obtained using swaps, which is reported to be the least valid for saliva testing [11].

On the other hand, there are differences in terms of studied groups. For example, in the study of Javadi Nejad [5], the samples were divided into three groups based on dfs (DFS≤1, 5<DFS<10 and DFS≥10); also in the study of Hedge [1] the samples were divided into two groups based on DMFT (DMFT = 0 and DMFT> 5), while in our study the participants were divided into four groups (DMFT = 0, 1≤DMFT≤3, 3 <DMFT <10, and DMFT≥10).

Another reason for the difference in results, apart from the amount of nitrate in the food, could be the amount of nitric oxide in the exhaled air [9].

Anaerobic bacteria use nitrate instead of oxygen as an electron receptor under hypoxia and produce adenosine triphosphate. This energy is used to oxidize carbon compounds and produce nitrate reductase. This enzyme converts nitrate to nitrite. This nitrite is converted to nitrous oxide in an acidic environment, which is very unstable and is rapidly converted to nitric oxide and nitrogen dioxide [16].

Some studies, such as Bayindir [6], have used dental plaque as a source of nitric oxide. They thought that an increase in thickness of the plaque reduces the amount of oxygen in the plaque depth, which leads to the predominance of forced anaerobic bacteria. These bacteria produce more nitric oxide by producing nitrate reductase, a belief that justifies higher levels of nitric oxide in people with poor health, but it may not necessarily be linked to caries activity. In fact, poor oral hygiene produces more nitric oxide, but its high

Jedno od ograničenja presječnih istraživanja jest nedostatak zaslijepljivanja uzoraka, ali u ovom istraživanju to ograničenje nije istaknuto jer su uzorci bili kodirani, a zatim dekodirani prije mjerenja koncentracije dušikova oksida.

Nalazi u ovom istraživanju u skladu su s onima Mathewa i Rezvija (14) te Mobaraka (3) koji su izjavili da dušikov oksid nema značajnu vezu s karijesom.

U istraživanjima koja su proveli Javadi Nejad (5) i Bayindir (6) ustanovljen je značajan pozitivan odnos u kojemu dušikov oksid djeluje kao obrambeni mehanizam. Jedna od razlika između naše metode i metode Javadija Nejada bila je dob pacijenata, a druga razlika bila je metoda mjerenja dušikova oksida. U ovom istraživanju korištena je ELISA, a u istraživanju Javadija Nejada primijenjena je ionska kromatografija. U istraživanju Bayindira (6) dob sudionika bila je gotovo identična našoj, no broj sudionika uključivao je 10 muškaraca i 12 žena, što je manje od broja sudionika u ovom istraživanju (80 sudionika).

U Hedgeovu istraživanju (1) utvrđen je značajan negativan odnos tako da ljudi bez karijesa imaju više dušikova oksida u slini. Kad je riječ o razlici između ovoga i Hedgeova istraživanja, možemo istaknuti više podskupina i jednaku podjelu broja sudionika u našem istraživanju u usporedbi s Hedgeovim sa samo dvije skupine sudionika.

Razlike u rezultatima istraživanja mogu biti posljedica metodologije, tako da su u istraživanju Doela (15) koji je utvrdio značajan negativan odnos, uzorci sline dobiveni korištenjem zamjena, što je navodno najmanje valjano za testiranje sline (11).

S druge strane, postoje razlike u vezi s proučavanim skupinama. Na primjer, u istraživanju Javadija Nejada (5) uzorci su podijeljeni u tri skupine na temelju dfs-a (DFS ≤ 1, 5 < DFS < 10 i DFS ≥ 10); u istraživanju Hedgea [1] uzorci su također podijeljeni u dvije skupine na temelju DMFT-a (DMFT 0 i DMFT > 5), a u našem istraživanju sudionici su podijeljeni u četiri skupine (DMFT = 0, 1 ≤ DMFT ≤ 3). , 3 < DMFT < 10 i DMFT ≥ 10).

Drugi razlog za razliku u rezultatima, osim količine nitrata u hrani, mogla bi biti količina dušikova oksida u izdahnutom zraku (9).

Anaerobne bakterije koriste se nitratom umjesto kisikom kao receptorom elektrona pod hipoksijom i proizvode adenozin-trifosfat. Ta se energija koristi za oksidaciju ugljikovih spojeva i proizvodnju nitratne reduktaze. Taj enzim pretvara nitrat u nitrit. Taj se nitrit u kiseloj sredini pretvara u dušikov oksid koji je vrlo nestabilan i brzo se pretvara u dušikov dioksid (16).

U nekim istraživanjima, kao što je Bayindirovo (6), autori su se koristili zubnim plakom kao izvorom dušikova oksida. Smatrali su da povećanje debljine plaka smanjuje količinu kisika u dubini plaka, što rezultira prevlašću anaerobnih bakterija. Te bakterije stvaraju više dušikova oksida proizvodnjom nitratne reduktaze, što opravdava višu razinu dušikova oksida kod ljudi lošega zdravlja, ali ne mora nužno biti povezano s karijesom. Zapravo, loša oralna higijena uzrokuje je za više dušikova oksida, ali visoka stopa karijesa znači da visoka razina dušikova oksida ne može spriječiti karijes zbog plaka (5).

caries rate means that high levels of nitric oxide cannot prevent plaque caries [5].

Subramanian [17] investigated the relationship between salivary nitric oxide in children with Down syndrome and caries and he found no association between the two. Also Dharmadhikari [2] found a significant negative relationship between salivary nitric oxide in children with Down syndrome and caries. It should be noted that several factors in children with Down syndrome can play a role in caries including: delayed tooth growth, high PH of saliva and higher levels of bicarbonate, interdental space and microdontia [18].

It is difficult to evaluate any anti-caries agent in saliva as an effective unit alone, because saliva changes all compounds in different ways [15]. In addition, antimicrobial proteins can interact with each other and weaken or enhance these effects, and no antibacterial compound alone is sufficient to determine the likelihood of tooth decay [5].

Further studies are needed to investigate the role of nitric oxide in caries. In future studies, the use of reactive nitrogen species such as dinitrogen trioxide may be useful. The stimulus in this regard is also continuously provided by the enormous possibility offered by the currently available tests in the planning and analysis of interleukins and inflammatory factors present in the saliva and at the level of the crevicular fluid present in the implant and periodontal sulcus [19-22]. Furthermore, it is essential to consider the long-term importance in the primary prevention of carious lesions in order to obtain greater safety margins, particularly in the treatment of orthodontic patients undergoing fixed therapy, with devices that risk predisposing to the onset of caries [23].

Dinitrogen trioxide reacts with thiol to form S-nitrosothiol [24], which has a longer lifespan and stability and biological activity similar to nitric oxide [25].

Conclusions

In this study, nitric oxide level in adult saliva was not affected by DMFT. However, further studies are needed to confirm the role of nitric oxide in caries.

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Informed Consent Statement

Written informed consent has been obtained from the patient(s) to publish this paper.

Conflicts of Interest

The authors declare no conflict of interest.

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Subramanian (17) je istraživao odnos između dušikova oksida u slini djece s Downovim sindromom i karijesa te nije pronašao povezanost između to dvoje. Dharmadhikari (2) je također pronašao značajnu negativnu korelaciju između dušikova oksida u slini djece s Downovim sindromom i karijesa. Treba napomenuti da nekoliko čimbenika kod djece s Downovim sindromom može biti važno za pojavu karijesa, uključujući zakašnjeli rast zuba, visoki PH sline i više razine bikarbonata, interdentalni prostor i mikrodonciju (18).

Teško je izdvojiti samo jedan antikarijesni agens u slini kao jedinu učinkovitu jedinicu jer slina mijenja spojeve na različite načine (15). Uz to, antimikrobni proteini mogu uzajamno komunicirati i oslabiti ili pojačati te učinke, a nijedan antibakterijski spoj sam po sebi nije dovoljan za određivanje vjerojatnosti karijesa (5).

Potrebna su daljnja istraživanja da bi se istražio utjecaj dušikova oksida u karijesu. U budućim istraživanjima može biti korisno korištenje reaktivnih vrsta dušika kao što je dušikov trioksid. Poticaj u tom smislu kontinuirano pruža i golema mogućnost koju nude trenutačno dostupni testovi u planiranju i analizi interleukina i upalnih čimbenika u slini i na razini krevikularne tekućine prisutne u implantatu i parodontalnom sulkusu (19 – 22). Nadalje, bitno je razmotriti dugoročnu važnost primarne prevencije karijesnih lezija da bi se postigle veće sigurnosne margine, osobito u liječenju ortodontskih pacijenata koji su podvrgnuti fiksnoj terapiji s napravama koje su rizične za nastanak karijesa (23).

Dinitrogen-trioksid reagira s tiolom da bi se stvorio S-nitrozotiol (24) koji ima dulji vijek trajanja, stabilnost i biološku aktivnost sličnu dušikovu oksidu (25).

Zaključak

U ovom istraživanju DMFT nije utjecao na razinu dušikova oksida u slini odrasle osobe. No potrebna su daljnja istraživanja kako bi se potvrdila uloga dušikova oksida u karijesu.

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Doprinos autora: **M. E., Z. K., L. T.** – koncept; **M. E., Z. K., M. T., R. R., F. P.** – metodologija; **L. T.** – validacija; **M. M.** – formalna analiza; **M. T., R. R.**- istraživanje i izvori; **M. E.** – obrada podataka; **M. E. , Z. K. M. T.** – pisanje i priprema izvornog teksta; **R. R., L. T.** – pisanje te pregled i uređivanje; M. T. – vizualizacija; **M. E, L. T.** – nadzor. Svi su autori pročitali tekst i složili se s objavljenom verzijom.

Sažetak

Svrha istraživanja: Zbog zaštitnog i antimikrobnog djelovanja dušikova oksida kad je riječ o gastrointestinalnim bolestima, istraživanje njegove povezanosti sa zubnim karijesom važna je tema. Zato je u ovom istraživanju analizirana količina dušikova oksida u slini s različitim vrijednostima DMFT indeksa kod odraslih osoba. **Materijal i metode:** U ovom deskriptivno-analitičkom presječnom istraživanju sudjelovalo je 80 ispitanika a (20 – 35 godina) bez sistemskih bolesti i uzimanja lijekova u anamnezi (53,8 % sudionika bile su žene). Odabrani su među pacijentima koji su došli na Odjel dentalne medicine. Bili su podijeljeni u četiri skupine na temelju DMFT vrijednosti (DMFT = 0, 1 ≤ DMFT ≤ 3, 3 < DMFT < 10, DMFT ≥ 10). Nestimulirana slina skupljena je od svih sudionika između 9 i 11 sati prijepodne u kalibriranu epruvetu. Dušikov oksid u slini mjeren je s pomoću testa dušikova oksida temeljenog na Griessovoj reakciji. Za analizu kvantitativnih varijabli upotrijebljen je korelacijski test, a za kvalitativne i kvantitativne varijable t-test ili ANOVA. **Rezultati:** Ustanovljena je značajna korelacija između DMFT-a i dobi. Na različitim razinama DMFT-a nije pronađena značajna povezanost između DMFT-a i spola. U različitim skupinama DMFT-a nije postojala značajna povezanost između dušikova oksida i DMFT-a. **Zaključak:** DMFT vrijednost nije utjecala na razinu dušikova oksida u slini.

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