Uniwersytet Medyczny w Białymstoku

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ROZPRAWA DOKTORSKA

Zmiana składu śliny palaczy papierosów tradycyjnych, elektronicznych oraz heat-not-burn products.

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Spis treści

Zestawienie publikacji 5
Wykaz publikacji stanowiących rozprawę doktorską 6
Wykaz stosowanych skrótów i oznaczeń 7
Wstęp 10
Omówienie prac wchodzących w skład rozprawy doktorskiej 16
Piśmiennictwo 30
Publikacje stanowiące rozprawę doktorską 34
Streszczenie w języku polskim 56
Streszczenie w języku angielskim 77
Informacja o charakterze udziału współautorów w publikacjach wraz z szacunkowym określeniem procentowego wkładu 79
Oświadczenia współautorów 82
Uchwały Komisji Bioetycznej 99

Zestawienie publikacji

Rodzaj publikacji	Liczba	Impact Factor	Punktacja MNiSW
Prace włączone do rozprawy doktorskiej	3	9,4	340
Prace nie włączone do rozprawy doktorskiej	8	32,636	850
Polskie streszczenia zjazdowe	10	-	-
Zagraniczne streszczenia zjazdowe	2	-	-
Razem	23	42,036	1190

Wykaz publikacji stanowiących rozprawę doktorską

Prace oryginalne:

1. **Zięba S**., Błachnio-Zabielska A., Maciejczyk M., Pogodzińska K., Szuta M., Lo Giudice G., Lo Giudice R., Zalewska A.: Impact of Smoking on Salivary Lipid Profile and Oxidative Stress in Young Adults: A Comparative Analysis between Traditional Cigarettes, E-Cigarettes, and Heat-Not-Burn Products. **Medical Science Monitor**. 2024: 30, 12 pp. doi:10.12659/msm.942507. **IF=2,200, MNiSW=140.**

2. **Zięba S.,** Maciejczyk M., Antonowicz B., Porydzaj A., Szuta M., Lo Giudice G., Lo Giudice R., Krokosz S., Zalewska A.: Comparison of smoking traditional, heat not burn and electronic cigarettes on salivary cytokine, chemokine and growth factor profile in healthy young adults - pilot study. **Frontiers in Physiology.** 2024: 15, 10 pp. doi:10.3389/fphys.2024.1404944. **IF=3,200, MNiSW=100.**

Prace przeglądowe:

1. **Zięba S.**, Maciejczyk M., Zalewska A.: Ethanol- and cigarette smoke-related alternations in oral redox homeostasis. **Frontiers in Physiology**. 2022: 12, 19 pp. doi:10.3389/fphys.2021.793028. **IF=4,00, MNiSW=100.**

Wykaz stosowanych skrótów i oznaczeń

4-HNE – ang. 4-hydroxynonenal; 4-hydroksynonenal

8-OHdG – ang. 8-hydroxyguanosine; 8-hydroksyguanozyna

API – ang. aproximal plaque index; aproksymalny wskaźnik płytki

BMI – ang. body mass index

CAT – ang. catalase; katalaza

Cer (C14:0, C16:0, C18:1, C18:0, C20:0, C22:0, C24:1, C24:0) – ceramid (C14:0, C16:0, C18:1, C18:0, C20:0, C22:0, C24:1, C24:0)

FGF – ang. fibroblast growth factor; czynnik wzrostu fibroblastów

GM-CSF – ang. granulocyte-macrophage colony-stimulating factor; czynnik stymulujący tworzenie kolonii granulocytów i makrofagów

Gro-α – ang. growth-regulated protein alpha; białko alfa regulujące wzrost

GSH – ang. glutathione; glutation

HGF – ang. hepatocyte growth factor; czynnik wzrostu hepatocytów

IFN-γ - interferon-gamma

IL-10 - ang. interleukin-10; interleukina-10

IL-1β - ang. interleukin-1 beta; interleukina-1 beta

IL-16 – ang. interleukin- 16; interleukina- 16

IL-18 – ang. interleukin-18; interleukina- 18

IL-1RA – ang. interleukin-1RA; interleukina-1RA

IL-2 - ang. interleukin-2; interleukina-2

IL-4 - ang. interleukin-4; interleukina-4

IL-6 - ang. interleukin-6; interleukina-6

IL-8 - ang. interleukin-8: interleukina-8

IP-10 – ang. interferon gamma-induced protein 10; białko indukowane interferonem gamma 10

MCP-1 – ang. monocyte chemoattractant protein-1; białko chemoatrakcyjne monocytów-1

M-CSF – ang. macrophage colony-stimulating factor; czynnik stymulujący kolonie makrofagów

MDA – ang. malondialdehyde; dialdehyd malonowy

MIF – ang. macrophage migration inhibitory factor; czynnik hamujący migrację makrofagów

MIG – ang. monokine induced by gamma interferon; monokina indukowana przez interferon gamma

MIP-1α – ang. macrophage inflammatory protein-1 alpha; białko zapalne makrofagów-1 alfa

NWS – ang. non-stimulated whole saliva; ślina niestymulowana

SWS – ang. stimulated whole saliva; ślina stymulowana

PBI – ang. papilla bleeding index; wskaźnik krwawienia z brodawek dziąsłowych

PPD – ang. pocket probing depths; wskaźnik oceny głębokości kieszonek przyzębnych

PUWZ – zębowy wskaźnik próchnicy dla uzębienia stałego

Px – ang. peroxidase; peroksydaza

ROS – ang. reactive oxygen species; reaktywne formy tlenu

S1P – ang. sfingozyno-1-fosforan

SCF – ang. stem cell factor; czynnik komórek macierzystych

SOD - ang. superoxide dismutase; dysmutaza ponadtlenkowa

SPA – ang. sphinganine; sfinganina

Sph –ang. sphingosine; spingozyna

TAC – ang. total antioxidant capacity; całkowita zdolność oksydacyjna

TBARS - ang. thiobarbituric acid reactive substances; substancje reagujące z kwasem tiobarbiturowym

TNF-α – ang. tumor necrosis factor alpha; czynnik martwicy nowotworów alfa

TOS – ang. total oxidative stress; całkowity stres oksydacyjny

TRAIL – ang. TNF-related apoptosis-inducing ligand; ligand indukujący apoptozę związany z TNF

UA – ang. uric acid; kwas moczowy

Wstęp

Pomimo wdrażania programów antynikotynowych, palenie papierosów pozostaje nadal poważnym problemem zdrowia publicznego [1]. Dym papierosowy zawiera w swoim składzie ponad 5000 tysięcy szkodliwych substancji, z których ponad 400, między innymi benzen, benzopiren, formaldehyd, akrylonitryl uważane są za kancerogenne [2, 3]. Dym tytoniowy wpływa negatywnie na niemal każdy organ w ludzkim organizmie i zaliczany jest do głównych przyczyn wielu stanów chorobowych [4]. Do najczęstszych schorzeń związanych z paleniem tytoniu należą: choroby nowotworowe (ponad 85% przypadków raka płuc jest bezpośrednio związanych z dymem tytoniowym), udary mózgu, zawały serca czy też przewlekła obturacyjna choroba płuc [3, 5, 6]. Znacząca liczba zgonów i przypadków niepełnosprawności związanych z paleniem tytoniu podkreśla konieczność intensywnej kontroli tego nałogu jako priorytetu zdrowotnego na całym świecie. Wzrost uzależnienia od tytoniu obserwuje się, zwłaszcza wśród młodzieży, a nałóg ten prowadzi do śmierci połowy uzależnionych. Szacuje się, że w ubiegłym stuleciu palenie było przyczyną ponad 100 milionów zgonów. Jeśli nie zostaną wprowadzone bardziej efektywne środki mające na celu zahamowanie epidemii tytoniowej, w obecnym stuleciu liczba ta może przekroczyć miliard w obecnym stuleciu liczba ta może przekroczyć miliard [6].

Rozwiązaniem problemów zdrowotnych wynikających z omawianego uzależnienia miało być wprowadzenie nowoczesnych urządzeń dostarczających do organizmu nikotynę: e-papierosów oraz tzw. „heat-not-burn products”. Urządzenia te w opinii publicznej powszechnie uważane są za mniej szkodliwe [7, 8].

Elektroniczne systemy dozowania nikotyny znane jako e-papierosy, symulują palenie tytoniu, dostarczając użytkownikowi aerozol poprzez

podgrzewanie cieczy, znanej jako e-liquid [9]. Zasadniczo składają się z pojemnika wypełnionego e-płynem, elementu grzewczego/atomizera, który podgrzewa e-płyn przekształcając go w parę do inhalacji przez ustnik, oraz akumulatora [9, 10]. Zarówno urządzenia elektroniczne, jak i różne warianty smakowe e-liquidów są łatwo dostępne do nabycia w sklepach stacjonarnych i internetowych, często bez odpowiedniej weryfikacji wiekowej [11].

Liczne kampanie marketingowe i lobbingowe, przedstawiające powyższe urządzenia jako mniej szkodliwe (a wręcz pomocne w rzuceniu nałogu palenia tradycyjnych wyrobów tytoniowych) doprowadziły do „renormalizacji" wzorców związanych z paleniem, zwłaszcza wśród młodych osób dorosłych [12]. Statystyki podają, że użytkowanie e-papierosów było najwyższe wśród dorosłych w wieku 18-24 lat (9,3%), przy czym ponad połowa (56,0%) tych osób zgłosiła, że nigdy nie paliła tradycyjnych papierosów [13]. E-płyn zazwyczaj zawiera substancje utrzymujące wilgoć oraz aromaty (obecnie na rynku konsumenckim dostępnych jest około 400 wariantów smakowych), z dodatkiem nikotyny lub bez niej [14, 15]. Ponieważ e-papierosy nie wymagają spalania, które jest źródłem produktów tytonio-pochodnych, powszechnie zakłada się, że konsumpcja e-papierosów, czyli "vaping", jest bezpieczniejsza niż konwencjonalne palenie [9]. Niemniej jednak, ostatnie doniesienia wskazują, że proces podgrzewania może prowadzić do powstawania nowych, potencjalnie niebezpiecznych związków chemicznych [16, 17]. Chociaż glikol propylenowy i glicerol są głównymi składnikami e-liquidów, w badaniu z wykorzystaniem chromatografii cieczowej, po podgrzaniu liquid'u wykryto wiele związków z których prawie połowa nie została wcześniej zidentyfikowana [17]. Analiza e-liquidu ujawniła w nim: formaldehyd, acetaldehyd i akroleinę, tj. trzy związki karbonylowe o znanej wysokiej toksyczności [17]. Autorzy obliczyli, że jedno zaciągnięcie się może spowodować narażenie na akroleinę w wysokości 0,003-0,015 μg/ml. Przy założeniu, że typowy użytkownik e-papierosów średnio zaciąga się ok.

400 do 500 razy na wkład, użytkownicy mogą być narażeni na działanie do 300 μg akroleiny (dla porównania, palacze tradycyjni narażeni są na 18,3-98,2 μg akroleiny w zależności od ilości wypalanych dziennie papierosów) [17, 18]. Z kolei w moczu użytkowników e-papierosów, wykryto znacznie wyższe stężenia metabolitów akrylonitrylu, akroleiny, tlenku propylenu, akrylamidu i aldehydu krotonowego w porównaniu do osób niepalących (osiągając nawet dwukrotnie wyższe wartości) [19–22]. Do ujemnych skutków wapowania zalicza się również uszkodzenie komórek śródbłonka naczyń oraz pogorszenie jego funkcji, co związane jest ze zmniejszeniem biodostępności tlenku azotu (wywołanego stresem oksydacyjnym i następczym rozwojem stanu zapalnego) [20, 22, 23]. Podczas gdy większość badań dotyczących wpływu e-papierosów na zdrowie ludzi skupiała się na samych składnikach e-płynów i aerozolach powstałych w wyniku ich podgrzewania, kilka badań dotyczyło składu materiałów z jakich wykonane są składowe e-papierosa oraz ich potencjalnych konsekwencji zdrowotnych. W szczególności zwracano uwagę na obecność metali, takich jak cząsteczki miedzi, niklu czy srebra w e-płynach i aerozolach, które mogą pochodzić z włókien drutów oraz atomizera [24, 25]. Inne istotne składniki aerozoli obejmują cząsteczki krzemianów pochodzące z knotów z włókna szklanego lub silikonu [24, 25]. Wiadomo, że wiele z tych produktów wywołuje nieprawidłowości w funkcjonowaniu układu oddechowego prowadząc m.in. do limfocytarnego zapalenia pęcherzyków płucnych, przerostu pneumocytów typu II czy też zwiększonej produkcji fosfolipidów w obrębie płuc [26–28]. Co ciekawe, napięcie wyjściowe baterii również wydaje się wpływać na cytotoksyczność oparów aerozolu. E-liquidy podgrzewane przy wyższym napięciu wyjściowym baterii wykazują większą toksyczność w stosunku do komórek A549 (linia niedrobnokomórkowego raka płuc, wykorzystywana jako model do badania i opracowywania terapii lekowych) w porównaniu do urządzeń o niższym napięciu wyjściowym [29].

Podobnie jak e-papierosy, „heat-not-burn products” zyskują coraz większą popularność wśród młodzieży oraz obecnych i byłych palaczy tradycyjnych [7,29]. Przewiduje się, że całkowita sprzedaż produktów podgrzewających tytoń osiągnie prawie 68 mld USD do 2027 r., co stanowi siedmiokrotny wzrost w porównaniu do 2020 r. [31, 32]. Chociaż urządzenia podgrzewające tytoń różnią się nieznacznie między sobą, obecny rynek został zdominowany przez wprowadzony w 2014 r. produkt firmy Philip Morris - IQOS (skrót od I Quit Ordinary Smoking). Składa się on z ładowarki, uchwytu i patyczków tytoniowych (o różnych smakach) oraz zatyczek [7]. Patyczek tytoniowy jest wkładany do uchwytu, a tytoń jest podgrzewany za pomocą elektronicznie sterowanego ostrza grzewczego, które jest wkładane do zatyczki tytoniowej [7, 33]. Producent podaje, że urządzenia te podgrzewają tytoń do temperatury 350°C w celu wytworzenia wdychanego aerozolu nikotynowego, zamiast spalania go w znacznie wyższych temperaturach (około 800°C), a co za tym idzie - reklamowane są jako produkty o potencjalnie zmniejszonej szkodliwości [7, 34, 35]. Pomimo krótkiej dostępności na rynku i stosunkowo niewielkiej ilości niezależnych badań (większość dostępnych wyników sponsorowana jest przez przemysł tytoniowy) wykazano, że ‘heat-not-burn products” nie pozostają obojętne dla zdrowia [34, 36]. W wydychanym aerozolu pochodzącym z produktów „heat-not-burn” wyizolowano nitrozoaminy specyficzne dla tytoniu, substancje smoliste, tlenek węgla, aminy aromatyczne, cyjanowodór, amoniak, fenol, lotne związki organiczne, wielopierścieniowe węglowodory aromatyczne, reaktywne formy tlenu (ROS) i karbonyle [37]. W badaniu przeprowadzonym na użytkownikach „heat-not-burn products” (z okresem użytkowania min. 3 lata), którzy zaprzestali palenia tradycyjnego tytoniu na rzecz wyżej wspomnianych urządzeń, nie zaobserwowano poprawy czynności płuc, ani zmniejszenia ogólnoustrojowego markera stanu zapalnego ostrej fazy - białka C-reaktywnego w stosunku do okresu palenia tradycyjnych papierosów [38]. Inni autorzy porównali wpływ

palenia wyrobów „heat-not-burn” oraz papierosów tradycyjnych na: częstość akcji serca, ciśnienie krwi oraz prędkość fali tętna szyjno-udowego u ogólnie zdrowych palaczy w średnim wieku. Powyższe parametry oceniano przed oraz bezpośrednio po 5 minutach zaciągania się [39]. W obu grupach palaczy po 5 minutowej ekspozycji oparami tytoniu, doszło do istotnego wzrostu wartości wszystkich badanych parametrów w porównaniu do wartości uzyskanych w „czasie zero” [39]. Co istotne, nie wykazano istotnych statystycznie różnić pomiędzy grupą palaczy „heat-not-burn products” oraz palaczami tradycyjnymi.

Jama ustna jest pierwszym miejscem kontaktu wdychanego dymu tytoniowego/ aerozolu nowoczesnych urządzeń dostarczających do organizmu nikotynę z organizmem człowieka. W warunkach fizjologicznych jest stale pokryta śliną, produkowaną przez 3 pary głównych gruczołów ślinowych (przyuszne, podżuchwowe i podjęzykowe) oraz liczne mniejsze, niesparowane gruczoły ślinowe rozproszone w błonie śluzowej jamy ustnej [40, 41]. Duże ślinianki wydzielają ponad 90% całkowitej ilości śliny niestymulowanej (NWS), z czego aż 70% produkowane jest przez śliniankę podżuchwową [42]. Pobudzenie gruczołów ślinowych zmienia ten procentowy rozkład na korzyść ślinianki przyusznej, której wydzielina stanowi 70% śliny stymulowanej (SWS). 99% składu śliny to woda [43, 44]. Pozostałą część stanowią związki organiczne i nieorganiczne, które determinują właściwości śliny [44]. Uznaje się, że wydzielina ślinianek jest dla jamy ustnej tym samym, co krew dla ustroju. Do niektórych z wielu funkcji śliny zaliczamy: wstępne trawienie pokarmu, nawilżenie oraz ochronę błony śluzowej jamy ustnej, remineralizację zębów, umożliwienie percepcji smaku, udział w walce z wolnymi rodnikami [42, 45]. Dzięki zawartości białek odpowiedzi swoistej i nieswoistej zapewnia pierwszą linię obrony przed infekcjami bakteryjnymi, wirusowymi i grzybiczymi [46]. W ślinie możliwe jest monitorowanie szeregu biomarkerów, których obecność odzwierciedla nie tylko lokalny stan zdrowia jamy ustnej, ale

w również w większości przypadków stan całego ustroju [47–49]. Powyższy fakt sprawia, że jest ona idealnym materiałem do badań diagnostycznych, dzięki jej dostępności i możliwość łatwego, wielokrotnego, nieinwazyjnego pobierania próbek [50].

Wiele publikacji donosiło o negatywnym wpływie palenia tradycyjnych papierosów na wybrane ilościowe i jakościowe właściwości śliny [51–54]. Niewiele jednak wiadomo na temat wpływu nowoczesnych urządzeń dostarczających do organizmu nikotynę, na ślinę. Dostępne dane literaturowe często pozostają ze sobą sprzeczne.

Omówienie prac wchodzących w skład rozprawy doktorskiej

Zięba S., Błachnio-Zabielska A., Maciejczyk M., Pogodzińska K., Szuta M., Lo Giudice G., Lo Giudice R., Zalewska A.:

Impact of Smoking on Salivary Lipid Profile and Oxidative Stress in Young Adults: A Comparative Analysis between Traditional Cigarettes, E-Cigarettes, and Heat-Not-Burn Products.

Medical Science Monitor. 2024: 30, 12 pp.

doi:10.12659/msm.942507.

Celem powyższej publikacji była ocena wpływu palenia papierosów tradycyjnych, elektronicznych oraz systemów do podgrzewania nikotyny na stężenie wybranych sfingolipidów: sfinozyny (Sph), sfinganiny (SPA), sfingozyno-1-fosforanu (S1P), ceramidów (C14:0-Cer, C16:0-Cer, C18:1-Cer, C18:0-Cer, C20:0-Cer, C22:0-Cer, C24:1-Cer, C24:0-Cer) oraz produktów peroksydacji lipidów: 4-hydroksynonenalu (4-HNE) oraz dialdehydu malonowego (MDA) w NWS oraz SWS pobranej od zdrowych młodych dorosłych. Lipidy w ślinie stanowią jej istotny komponent [41]. Wykazano, że nieprawidłowości składu lipidowego śliny mają związek z chorobami przyzębia oraz mogą towarzyszyć wielu schorzeniom ogólnoustrojowym, takim jak zespół Sjögrena, mukowiscydoza czy choroba Alzheimera [41, 55, 56]. Sfingolipidy i ich pochodne stanowią liczną grupę bioaktywnych związków lipidowych zlokalizowanych w zewnętrznej warstwie błon komórek eukariotycznych, determinując tym samym ich kształt [57]. Oprócz funkcji strukturalnych, sfingolipidy działają jako aktywatory szlaków sygnałowych i wtórne przekaźniki sygnałów [57]. Ceramidy odgrywają główną rolę w metabolizmie sfingolipidów i są zaangażowane w regulację takich procesów komórkowych, jak proliferacja i różnicowanie, wzrost, starzenie się i śmierć komórek [58]. Co ciekawe, zdecydowana większość (około 98%) lipidów

znajdujących się w ślinie jest syntetyzowana bezpośrednio w komórkach gruczołów ślinowych. W związku z powyższym zmiany w ich stężeniu można uważać za bezpośrednie odzwierciedlenie patologii toczącej w obrębie gruczołów ślinowych [41, 56].

Materiał i metody

Badanie zostało przeprowadzone po uzyskaniu zgody Lokalnej Komisji Bioetycznej w Białymstoku (numer zezwolenia: APK.002.343.2020). Uczestnicy badania pozostawali pod stałą opieką stomatologiczną Zakładu Stomatologii Zachowawczej Uniwersytetu Medycznego w Białymstoku, gdzie regularnie uczęszczali na wizyty kontrolne. W badaniu wzięło udział 100 osób, które podzielono na cztery grupy:

1. grupa kontrola - osoby nigdy nie palące (n=25),
2. palacze papierosów tradycyjnych (n=25),
3. palacze e-papierosów (n=25),
4. palacze systemów do podgrzewania nikotyny (n=25).

Warunki włączenia do jednej z trzech powyższych grup palaczy były następujące: czas trwania nałogu wynoszący od roku do 3 lat, stosowanie wyłącznie jednej z trzech metod dostarczania do organizmu nikotyny, wiek poniżej 30 lat, brak stanów zapalnych w jamie ustnej, prawidłowy zakres BMI (ang. body mass index), sporadyczne spożywanie alkoholu, brak w wywiadzie zażywania substancji psychoaktywnych. Ponadto, uczestnicy nie byli w trakcie leczenia z wykorzystaniem aparatów stałych, retencyjnych, szyn Invisalign, protez ruchomych, stałych uzupełnień protetycznych, mini implantów ortodontycznych ani implantów tytanowych.

Grupę kontrolną stanowiły osoby młode niepalące (brak historii nałogu palenia w przeszłości), dopasowane pod względem powyższych wymagań i płci do uczestników grup badanych.

Uczestnicy zostali poproszeni o niepalenie tytoniu, niespożywanie pokarmów i napojów innych niż czysta woda oraz niewykonywanie żadnych

zabiegów higienicznych w obrębie jamy ustnej na co najmniej 2 godziny przed pobraniem śliny. W celu zminimalizowania wpływu rytmów dobowych na procesy wydzielania śliny, pobieranie materiału odbywało się między godziną 8:00 a 10:00. Próbki NWS pobierano przez 15 minut, do kalibrowanej probówki. SWS pobierano przez 5 minut, podając co 30 sekund 20 µl kwasu cytrynowego na grzbietową powierzchnię języka pacjenta. Przed odwirowaniem, zmierzono objętość wydzielin (za pomocą kalibrowanej pipety), a szybkość wydzielania śliny określono dzieląc objętość śliny w probówce przez czas potrzebny do jej uzyskania.

Bezpośrednio po pobraniu materiału diagnostycznego uczestnicy zostali poddani badaniu stomatologicznemu, które obejmowało: ocenę stanu uzębienia za pomocą zębowego wskaźnika próchnicy (PUWZ) oraz aproksymalnego wskaźnika płytki (API), jak również ocenę wskaźnika krwawienia z brodawek dziąsłowych (PBI) oraz wskaźnika głębokości kieszonek przyzębnych (PPD). Badanie przeprowadzono przy sztucznym oświetleniu z użyciem lusterka oraz sondy periodontologicznej

Stężenie Sph, SPA, S1P oraz ceramidów w ślinie zmierzono za pomocą ultrawysokosprawnej chromatografii cieczowej i tandemowej spektrometrii [59].

Stężenie MDA oznaczono kolorymetrycznie z wykorzystaniem metody substancji reagujących z kwasem tiobarbiturowym (TBARS) (Sigma-Aldrich, Saint Louis, MO, USA). Absorbancję mierzono przy 535 nm za pomocą czytnika mikropłytek ELx800 i oprogramowania Gen5 2.01 (BioTek Instruments, Winooski, VT, USA).

Stężenie 4-HNE mierzono przy użyciu komercyjnego testu immunoenzymatycznego (ELISA) zgodnie z instrukcjami producenta (Cell Biolabs Inc., San Diego, CA, USA i USCN Life Science). Absorbancję mierzono przy 405 nm za pomocą czytnika mikropłytek ELx800 i oprogramowania Gen5 2.01 (BioTek Instruments, Winooski, VT, USA).

Wyniki

Stężenie SpH było istotnie niższe w NWS oraz SWS u wszystkich palaczy („heat-not-burn products", e-papierosów, tradycyjnych papierosów) w porównaniu z grupą kontrolną (NWS odpowiednio: $p<0,0001$, $p<0,0001$, $p<0,0001$; SWS odpowiednio: $p<0,0001$, $p<0,0001$, $p<0,0001$). W grupie użytkowników systemów do podgrzewania nikotyny stężenie SpH było istotnie niższe w porównaniu z grupą palaczy e-papierosów, zarówno w NWS ($p<0,01$), jak i SWS ($p=0,03$). Ponadto grupa użytkowników „heat-not-burn products" charakteryzowała się istotnie niższym stężeniem SpH w SWS w porównaniu do grupy palaczy tradycyjnych ($p<0,0001$).

Stężenie SPA było znacząco niższe w NWS i SWS u wszystkich użytkowników nikotyny („heat-not-burn products", e-papierosów, tradycyjnych papierosów) w porównaniu z grupą kontrolną (NWS odpowiednio: $p<0,0001$, $p<0,0001$, $p<0,0001$; SWS odpowiednio: $p<0,0001$, $p=0,006$, $p<0,0001$). Użytkownicy „heat-not-burn products" charakteryzowali się istotnie niższym stężeniem SPA w NWS w porównaniu do palaczy e-papierosów ($p=0,002$). Stężenie SPA w SWS palaczy systemów do podgrzewania nikotyny było istotnie niższe w porównaniu do palaczy e-papierosów ($p<0,0001$) i palaczy tradycyjnych ($p=0,002$).

Stężenie S1P było istotnie niższe w NWS u wszystkich użytkowników nikotyny („heat-not-burn products", e-papierosów, tradycyjnych papierosów) w porównaniu z grupą kontrolną (odpowiednio: $p<0,0001$, $p<0,0001$, $p<0,0001$). Podobnie zawartość ceramidu C14 była znacznie niższa w NWS wszystkich użytkowników nikotyny („heat-not-burn products", e-papierosów, tradycyjnych papierosów) w porównaniu z grupą kontrolną (odpowiednio: $p<0,0001$, $p<0,0001$, $p=0,003$). Ponadto w NWS palaczy „heat-not-burn products" stężenie omawianego parametru było istotnie niższe w porównaniu do grupy tradycyjnych palaczy ($p=0,007$).

Z kolei stężenie ceramidu C14 w SWS palaczy „heat-not-burn products” było istotnie wyższe w porównaniu z grupami palaczy e-papierosów ($p=0,02$) i tradycyjnych papierosów ($p=0,008$).

Stężenia ceramidów C16 i C24 były istotnie niższe w NWS i SWS u wszystkich palaczy („heat-not-burn products”, e-papierosów, tradycyjnych papierosów) w porównaniu z grupą kontrolną (C16, NWS odpowiednio: $p<0,001$, $p<0,001$, $p<0,001$; C16, SWS odpowiednio: $p=0,04$, $p=0,003$, $p=0,04$; C24, NWS odpowiednio: $p<0,001$, $p<0,001$, $p<0,001$, C24, SWS odpowiednio: $p<0,0001$, $p<0,0001$, $p<0,0001$).

Stężenie ceramidu C18-1 było istotnie niższe w NWS i SWS u wszystkich użytkowników nikotyny („heat-not-burn products”, e-papierosów, tradycyjnych papierosów) w porównaniu do kontroli (NWS odpowiednio: $p<0,0001$, $p<0,0001$, $p<0,0001$; SWS odpowiednio: $p<0,0001$, $p<0,0001$, $p<0,0001$). W NWS i SWS palaczy tradycyjnych papierosów stężenie omawianego parametru było istotnie niższe w porównaniu do grupy „heat-not-burn products”, jak i e-papierosów (NWS odpowiednio: $p<0,0001$, $p<0,0001$, SWS odpowiednio: $p<0,0001$, $p<0,0001$).

Stężenie ceramidu C18 było istotnie niższe w NWS u wszystkich palaczy („heat-not-burn products”, e-papierosów, tradycyjnych papierosów) w porównaniu z grupą kontrolną (odpowiednio: $p<0,0001$, $p<0,0001$, $p<0,0001$). W NWS palaczy systemów do podgrzewania nikotyny stężenie omawianego parametru było istotnie wyższe w porównaniu zarówno z grupą e-papierosów, jak i tradycyjnych palaczy (odpowiednio: $p=0,005$, $p=0,004$). Zawartość ceramidu C18 była istotnie niższa w SWS w grupie palaczy e-papierosów i tradycyjnych papierosów w porównaniu z grupą kontrolną (odpowiednio: $p<0,0001$, $p<0,0001$). W SWS palaczy „heat-not-burn products” stężenie omawianego parametru było istotnie wyższe w porównaniu z grupą palaczy tradycyjnych papierosów ($p=0,04$).

Stężenie ceramidu C20 było istotnie niższe w NWS i SWS u wszystkich użytkowników nikotyny („heat-not-burn products", e-papierosów, tradycyjnych papierosów) w porównaniu z grupą kontrolną (NWS odpowiednio: p=0,001, p<0,0001, p<0,0001; SWS odpowiednio: p=0,03, p<0,0001, p=0,0004). W NWS i SWS palaczy tradycyjnych papierosów stężenie analizowanego parametru było istotnie niższe zarówno w porównaniu do grupy palaczy „heat-not-burn products", jak i e-papierosów (NWS odpowiednio: p<0,0001, p<0,0001; SWS odpowiednio: p<0,0001, p<0,0001).

Stężenie ceramidu C22 było istotnie niższe w NWS wszystkich palaczy („heat-not-burn products", e-papierosów, tradycyjnych papierosów) w porównaniu z grupą kontrolną (odpowiednio: p<0,0001, p<0,0001, p<0,0001). Z kolei w NWS palaczy e-papierosów, powyższy parametr było istotnie wyższy w porównaniu z użytkownikami „heat-not-burn products" (p=0,02) oraz palaczami tradycyjnymi (p=0,0006). Stężenie ceramidu C22 w SWS palaczy tradycyjnych było istotnie niższe w porównaniu z grupą kontrolną (p=0,0001) oraz grupą korzystającą z e-papierosów (p=0,004).

Stężenie ceramidu C24-1 i Total Cer było istotnie niższe w NWS i SWS u wszystkich użytkowników nikotyny („heat-not-burn products", e-papierosów, tradycyjnych papierosów) w porównaniu do kontroli (C24-1, NWS odpowiednio: p<0,0001, p<0,0001, p<0,0001, Total Cer, NWS odpowiednio: p<0,0001, p<0,0001, p<0,0001; C24-1, SWS odpowiednio p<0,0001, p<0,0001, p<0,0001, Total Cer, SWS odpowiednio: p<0,0001, p<0,0001, p<0,0001). Stężenie C24-1 w NWS osób z grupy e-papierosów było istotnie wyższe w porównaniu z grupą palaczy tradycyjnych (p<0,0001), natomiast stężenie Total Cer w NWS użytkowników „heat-not-burn products" było istotnie wyższe w porównaniu z grupą palaczy tradycyjnych (p= 0,004). Stężenie ceramidu C24-1 w SWS palaczy e-papierosów było istotnie wyższe w porównaniu z grupą palaczy „heat-not-burn products" (p=0,008) i grupą palaczy tradycyjnych (p<0,0001).

Stężenie 4-HNE i MDA w NWS było istotnie wyższe w grupie palaczy tradycyjnych papierosów w porównaniu z grupą kontrolną (NWS odpowiednio: p=0.0008, p=0.002), natomiast w SWS wykazano istotnie wyższe stężenie 4-HNE w grupie palaczy tradycyjnych papierosów vs osoby niepalące (p<00001). Co więcej, stężenia 4-HNE w SWS użytkowników „heat-not-burn products" i e-papierosów były istotnie niższe w porównaniu z grupą palącą tradycyjne papierosy (odpowiednio p=0,0002, p<0,0001). Stężenie MDA w SWS palaczy tradycyjnych papierosów była istotnie wyższa w porównaniu z grupą osób niepalących (p=0.003) i palących e-papierosy (p=0.009).

Nie zaobserwowano istotnych różnic w obrębie badanych parametrów stomatologicznych.

Wnioski

1. Spadek stężenia większości badanych sfingolipidów w NWS i SWS nie jest zależny od sposobu dostarczania nikotyny. Zmiany w stężeniu sfingolipidów w ślinie palaczy w porównaniu z grupą kontrolną są bezpośrednim odzwierciedleniem trwającej patologii w obrębie gruczołów ślinowych.
2. Istotny wzrost stężenia 4-HNE i MDA w ślinie NWS i SWS wskazuje na zaburzenie równowagi redoks w gruczołach ślinowych palaczy i nasiloną peroksydację lipidów ślinowych.
3. Uzyskane wyniki należy traktować jako zmiany zachodzące na wczesnym etapie trwania nałogu.
4. Pomimo braku istotnych różnic w charakterystyczne stomatologicznej pomiędzy grupami, prawdopodobnym jest, że zmiany w stężeniu ślinowych sfingolipidów oraz produktów peroksydacji lipidów mogą w przyszłości manifestować się klinicznie u osób z dłuższym stażem palenia.

Zięba S., Maciejczyk M., Antonowicz B., Porydzaj A., Szuta M., Lo Giudice G., Lo Giudice R., Krokosz S., Zalewska A.:

Comparison of smoking traditional, heat not burn and electronic cigarettes on salivary cytokine, chemokine and growth factor profile in healthy young adults -pilot study.

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Celem drugiej części rozprawy doktorskiej była ocena wpływu palenia papierosów tradycyjnych oraz nowoczesnych urządzeń dostarczających do organizmu nikotynę na stężenie ślinowych cytokin/chemokin/czynników wzrostu. Liczne publikacje donoszą, że długotrwałe palenie tradycyjnych papierosów prowadzi do uwalniania mediatorów stanu zapalnego i cytokin, co wiąże się z rozwojem chorób jamy ustnej, w tym stanów przedrakowych, raków i chorób przyzębia [60]. Dym papierosowy wpływa głównie na równowagę cytokin wytwarzanych przez limfocyty T pomocnicze [61]. Wykazano znacznie wyższe stężenie prozapalnych cytokin IL-1β, IL-6, IL-2, IL-4 oraz zmniejszenie stężenie przeciwzapalnej interleukiny IL-10 w ślinie palaczy papierosów [62, 63]. Co ważne, stężenie tych cytokin wzrasta wraz z czasem trwania palenia [62].

Niewiele wiemy na temat wpływu nowoczesnych urządzeń dostarczających do organizmu nikotynę na lokalny układ immunologiczny jamy ustnej. W literaturze występują doniesienia, że smak mentolowy (cieszący się największą popularnością wśród użytkowników e-papierosów i „heat-not-burn products") może wywierać immunomodulujące działanie na organizm [64].

Materiały i metody

Badanie zostało przeprowadzone po uzyskaniu zgody Lokalnej Komisji Bioetycznej w Białymstoku (numer zezwolenia: APK.002.175.2023). Do badania zakwalifikowano 75 palaczy podzielonych na 3 podgrupy:

1. palaczy papierosów tradycyjnych (n=25),
2. palaczy papierosów elektronicznych o smaku mentolowym (n=25).
3. palaczy podgrzewanych wyrobów tytoniowych o smaku mentolowym (n=25).

Każdy uczestnik mógł być użytkownikiem tylko jednej metody dostarczania nikotyny do organizmu, a okres trwania nałogu nie mógł być krótszy niż rok i dłuższy niż 3 lata.

Grupa kontrolna składała się z 25 osób niepalących (bez historii palenia w przeszłości) dopasowanych pod względem wieku i płci do grup badanych.

Wszyscy uczestnicy badania byli ogólnie zdrowymi młodymi dorosłymi (poniżej 30 roku życia), bez zmian zapalnych w jamie ustnej, z prawidłową masą ciała (BMI w zakresie normy), nie nadużywającymi alkoholu i nie przyjmującymi leków psychoaktywnych. Uczestnicy badania regularnie zgłaszali się na wizyty kontrolne do Zakładu Stomatologii Zachowawczej Uniwersytetu Medycznego w Białymstoku. W ciągu 6 miesięcy poprzedzających badanie uczestnicy z grupy badanej i kontrolnej nie przyjmowali żadnych leków wpływających na odpowiedź immunologiczną (antybiotyków, sterydów, leków przeciwhistaminowych, leków przeciwzapalnych). Uczestnicy badania nie byli leczeni ortodontycznie aparatami stałymi, retencyjnymi. szynami Invisalign, protezami ruchomymi, stałymi uzupełnieniami protetycznymi, implantami ani mini implantami ortodontycznymi.

Od pacjentów grup badanych oraz grupy kontrolnej została pobrana NWS metodą odpluwania. Protokół pobierana śliny, obróbka materiału oraz sposób przeprowadzenia badania stomatologicznego były identyczne jak w

przypadku poprzedniej publikacji składającej się na powyższą rozprawę doktorską.

Stężenia cytokin, chemokin i czynników wzrostu w ślinie analizowano przy użyciu systemu Bio-Plex® Multiplex System (Bio-Rad Laboratories, Inc., Hercules, CA, USA) zgodnie z instrukcjami producenta. Dane z reakcji zostały pozyskiwane przy użyciu dedykowanego czytnika płytek (Bio-Plex 200) i szybkiego cyfrowego procesora sygnału.

Wyniki

Palacze tradycyjnych papierosów wykazywali istotnie wyższe stężenie IFN-γ w NWS ($p=0,03$) w porównaniu z osobami niepalącymi. Osoby użytkujące „heat-not-burn products" miały istotnie niższe stężenie TNF-α, HGF, IL-1RA w NWS (odpowiednio: $p=0,0006$, $p=0,0008$, $p=0,0001$) oraz IL-8, IL-16, IL-18, MIF (odpowiednio: $p<0,0001$, $p=0,002$, $p=0,02$, $p=0,0003$) w porównaniu z osobami niepalącymi. Podobnie, grupa palaczy podgrzewających tytoń charakteryzowała się istotnie niższymi stężeniami ślinowych HGF i IL-16 (odpowiednio: $p=0,0004$, $p=0,05$) w porównaniu do palaczy tradycyjnych papierosów. Palacze papierosów elektronicznych wykazywali istotnie niższe stężenie cytokin ślinowych TNF-α, IL-1β, IL-1RA (odpowiednio $p=0,03$, $p=0,0008$, $p<0,0001$) oraz IL-8, IL-16, IL-18, MIF (odpowiednio $p<0,0001$, $p=0,0003$, $p=0,002$, $p<0,0001$) w porównaniu z grupą osób niepalących. Dodatkowo, użytkownicy e-papierosów wykazywali istotnie niższe stężenia cytokin: IL-1RA, IL-8, IL-16 i MIF (odpowiednio $p=0,0002$, $p=0,0004$, $p<0,0001$, $p<0,0001$) w NWS w porównaniu do grupy palaczy tradycyjnych.

Stężenia chemokin Gro-α, MCP-1 α, SCF, MIG, IP-10 w NWS osób niepalących były istotnie niższe w porównaniu do palaczy tradycyjnych papierosów (odpowiednio: $p<0,0001$, $p<0,0001$, $p=0,0004$, $p<0,0001$, $p=0,02$). Z kolei palacze produktów „heat-not-burn" wykazywali istotnie niższe stężenie chemokin: Gro-α, MCP-1, SCF, MIG, IP-10 (odpowiednio: $p=0,0002$, $p=0,02$,

p=0,03, p=0,008, p=0,003) w porównaniu do palaczy papierosów tradycyjnych. Stężenia Gro-α, MCP-1, MIG, IP-10 w NWS były istotnie niższe w grupie palaczy e-papierosów w porównaniu z grupą kontrolną (odpowiednio p<0,0001, p=0,0001, p<0,0001, p= 0,004). Dodatkowo, palacze e-papierosów wykazywali istotnie niższe stężenia ślinowych Gro-α, MCP-1, MIG, IP-10 (odpowiednio p=0,04, p= 0,0005, p=0,0002, p=0,0006), w porównaniu do palaczy tradycyjnych papierosów.

Osoby stosujące produkty „heat-not-burn" miały istotnie niższe stężenia G-CSF, TRAIL, M-CSF w NWS (odpowiednio: p<0,0001, p <0,0001, p=0,0003) w porównaniu z grupą osób niepalących oraz istotnie niższe stężenie ślinowych G-CSF, TRAIL (odpowiednio p=0,002, p=0,0005) w porównaniu do palaczy tradycyjnych papierosów. Stężenia G-CSF, TRAIL i M-CSF w ślinie były istotnie niższe w grupie palaczy e-papierosów w porównaniu z grupą kontrolną (odpowiednio p=0,001, p<0,0001, p=0,002). Dodatkowo, palacze e-papierosów wykazywali istotnie niższe stężenia ślinowych G-CSF, TRAIL (odpowiednio p=0,04, p<0,0001) w porównaniu do palaczy tradycyjnych papierosów.

Warto nadmienić, że pomiędzy grupą palaczy e-papierosów a grupą użytkownik „heat-not-burn products", nie wykryto statystycznie istotnych różnić w stężeniu żadnej z badanych ślinowych cytokin/chemokin/czynników wzrostu.

Ponadto, nie zaobserwowano istotnych różnic w obrębie badanych parametrów stomatologicznych.

Wnioski

1. Stosowanie obu alternatywnych urządzeń dostarczających nikotynę wydaje się wyraźnie hamować lokalną odpowiedź immunologiczną w NWS palaczy, podczas gdy palenie tradycyjnych papierosów tylko nieznacznie nasila ślinową odpowiedź zapalną w porównaniu do osób niepalących.

2. Zaobserwowany hamujący wpływ na syntezę lub uwalnianie badanych cytokin z komórek może być spowodowany wpływem mentolowego dodatku do e-papierosów i niespalania.
3. Uzyskane wyniki należy traktować jako zmiany zachodzące na wczesnym etapie trwania nałogu.
4. Pomimo braku istotnych różnic w obrębie badanych parametrów stomatologicznych, niewykluczone jest, że zachodzące zmiany w lokalnym układzie immunologicznym śliny mogą w przyszłości uzewnętrzniać się klinicznie u osób z dłuższym stażem palenia.

Szczegółowy opis planowania doświadczeń oraz otrzymanych wyników, znajduje się w publikacjach załączonych poniżej.

Ostatnią część rozprawy doktorskiej stanowi publikacja przeglądowa dotycząca wpływu palenia tytoniu na ślinę.

Zięba S., Maciejczyk M., Zalewska A.:

Ethanol- and cigarette smoke-related alternations in oral redox homeostasis.

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W artykule opublikowanym w **Frontiers in Physiology (2022: 12, 19 pp., doi:10.3389/fphys.2021.793028)** dokonano przeglądu piśmiennictwa na temat wpływu palenia tradycyjnych papierosów na homeostazę redox jamy ustnej. Składniki dymu tytoniowego odpowiedzialne są za wzrost produkcji ROS, które zaburzają równowagę oksydoredukcyjną w jamie ustnej. U nałogowych palaczy wykazano zmniejszoną aktywność enzymów antyoksydacyjnych tj. dysmutaza ponadtlenkowa (SOD), katalaza (CAT) i peroksydaza (Px) (za sprawą zaburzeń w metabolizmie pierwiastków śladowych, które są kofaktorami powyższych enzymów) oraz zmniejszone stężenie nieenzymatycznych przeciwutleniaczy: glutationu (GSH) oraz kwasu moczowego (UA). Proporcjonalnie do czasu trwania nałogu, w ślinie palaczy obserwuje się niższe wartości całkowitej zdolności antyoksydacyjnej (TAC) oraz wyższe wartości całkowitego statusu oksydacyjnego (TOS) w porównaniu do osób niepalących. Zwiększona produkcja ROS i niewydolność ślinowych układów przeciwutleniających u osób uzależnionych od tytoniu, prowadzi do wzrostu stężenia produktów oksydacyjnych modyfikacji DNA, białek oraz lipidów w ślinie. Powyższe modyfikacje mogą prowadzić do postępującej choroby przyzębia oraz powstania zmian przednowotworowych/raków jamy ustnej. Oksydacja białek przez ROS, odpowiada za uszkodzenia w obrębie cytoszkieletu fibroblastów, upośledzając ich ruchliwość, adhezję i podział.

Ponadto, wyższe stężenia markera oksydacyjnego uszkodzenia DNA - 8-hydroksyguanozyny (8-OhdG) oraz 4-HNE, MDA wykazano w ślinie palaczy, ze zdiagnozowaną zmianą przednowotworową/rakiem jamy ustnej (stężenie ww. związków było proporcjonalnie do stadium zaawansowania choroby) w porównaniu ze zdrową niepalącą kontrolną.

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Impact of Smoking on Salivary Lipid Profile and Oxidative Stress in Young Adults: A Comparative Analysis between Traditional Cigarettes, E-Cigarettes, and Heat-Not-Burn Products

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Data Collection B
Statistical Analysis C
Data Interpretation D
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Background: Smoking nicotine is considered to be one of the most harmful addictions, leading to the development of a number of health complications, including many pathologies in the oral cavity. The aim of this study was to examine the effect of smoking traditional cigarettes, e-cigarettes, and heat-not-burn products on profiles of salivary lipids and lipid peroxidation products in the unstimulated and stimulated saliva of healthy young adults with a smoking habit of up to 3 years.

Material/Methods: We enrolled 3 groups of 25 smoking patients each and a control group matched for age, gender, and oral status. In saliva collected from patients from the study groups and participants from the control group, the concentrations of sphingolipids: sphingosine, sphinganine, sphingosine-1-phosphate, ceramides, and salivary lipid peroxidation products – malondialdehyde (MDA) and 4-hydroxynonenal (HNE) – were measured. The normality of distribution was assessed using the Shapiro-Wilk test. For comparison of the results, one-way analysis of variance (ANOVA) followed by post hoc Tukey test was used.

Results: We demonstrated that each type of smoking causes a decrease in the concentration of salivary lipids, and there was an increased concentration of salivary MDA and 4-HNE.

Conclusions: Smoking in the initial period of addiction leads to an increase in the concentration of lipid peroxidation products through increased oxidative stress, leading to disturbance of the lipid balance of the oral cavity (eg, due to damage to cell membranes).

Keywords: Cigarette Smoking • Electronic Nicotine Delivery Systems • Lipid Peroxidation • Lipids • Oxidative Stress • Salivary Glands

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Background

The negative effects of smoking tobacco and e-cigarette vaping are primarily evaluated in terms of causes of development of cardiovascular diseases, lung cancer, respiratory chronic inflammatory diseases, or disorders of the gastrointestinal microbiota [1-3]. However, other tissues also succumb to toxic effects of nicotine contained in commonly available nicotine carriers, including the oral cavity and upper respiratory tract [4,5].

The oral cavity is the place of first contact with cigarette smoke in the human body. Evidence has shown that smoking is a risk factor in the development and progression of periodontal diseases, cancer, and precancerous conditions of the oral cavity area, as well as salivary gland dysfunction and disorders of saliva composition [6-9].

It is well known that smoking traditional cigarettes leads to redox imbalance. We can observe increased production of free radicals, which can cause damage to cell membranes or DNA. It has been demonstrated that long-term smoking leads to a decrease in the activity of endogenous salivary enzymatic antioxidants such as SOD, CAT, and Px, and significantly reduces the efficiency of non-enzymatic endo- and exo-antioxidant systems: GSH, UA, and vitamin C [6,10,11]. Similarly, e-cigarettes can induce oxidative stress and increase the expression of advanced glycation end products (AGEs) and their cellular receptors (RAGEs) in gingival and periodontal tissues within just 1 year of starting smoking [12-14]. Furthermore, in an in vitro study, Ganapathy et al [13] showed that a 14-day exposure of cells to e-cigarette aerosol extracts increases DNA damage in oral epithelial cells, which is expressed by increased concentrations of 8-oxo-dG levels. Long-term smoking of traditional cigarettes and e-cigarettes reduces the content of salivary components of specific and non-specific immunity, such as sIgA, peroxidase, lactoferrin, and lysozyme [6,15,16].

Moreover, saliva contains a wide variety of lipids, including cholesterol and its esters, fatty acids, triglycerides, wax esters, and polar lipids such as phosphatidylcholine, phosphatidylethanolamine, sulfides, and glycolipids, including ceramides [15,16]. Ceramides are composed of sphingosine linked by an amide bond to any fatty acid. The most common ceramides are C14: 0-Cer, C16: 0-Cer, C18: 1-Cer, C18: 0-Cer, C20: 0-Cer, C22: 0-Cer, C24: 1-Cer, and C24: 0-Cer. These lipids form cell membranes and are also precursors of more complex sphingolipids, such as sphingomyelin, ceramide-1 phosphate, and glycerosphingolipids. In addition to their structural function, ceramides determine the process of cell differentiation, proliferation, and apoptosis, and regulate the process of protein phosphorylation, which is essential in signal transduction [16,17]. Sphingolipids, on the other hand, show antimicrobial and antiviral activity in a dose-dependent manner, and induce cellular damage. Pretreatment of cells with sphingosine prevents the viral spike protein of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) from interacting with host cell receptors and inhibits the propagation of herpes simplex virus type 1 (HSV-1) in macrophages [18]. Cigarette smoke strongly activates inflammatory pathways in lungs and in myocardial and skeletal muscle cells, which increases biosynthesis of ceramide and its derivatives in these tissues [3,19,20]. High concentration of this group of lipids in response to exposure to cigarette smoke has been linked to endothelial barrier dysfunction, emphysema, inflammation, and altered myocardial mitochondrial function [21,22]. Lipidomic profiling of sputum samples showed increased levels of 28 ceramides in long-term smokers with COPD (chronic obstructive pulmonary disease) compared to long-term smokers without COPD. Differences between smokers without COPD and people who have never smoked cigarettes revealed significant changes only in the level of salivary glycosphingolipids. Interestingly, disorders in plasma sphingolipid composition were observed only in smokers of traditional cigarettes, while subjects using e-cigarettes only showed dysregulation of tricarboxylic acid cycle-related metabolites [22].

Lipids perform many important functions in the oral cavity, from structural to functional. In addition to their key role in maintaining the integrity and function of cells, they affect the processes of digestion, protection, and communication, as well as maintaining the internal balance in the oral cavity [17]. Lipids contained in saliva help to moisturize and protect the mucous membranes, facilitating eating, speaking, and other functions of the oral cavity. In the oral cavity, lipids can form a thin protective layer on the surface of the teeth and mucous membranes, which helps protect against the effects of irritants and infectious substances and prevents excessive evaporation of water from tissue surfaces [17,23].

Considering the role of saliva and its lipids in maintaining oral homeostasis, we decided to evaluate the effect of smoking traditional cigarettes, e-cigarettes, and heat-not-burn products on the concentration of selected sphingolipids (eg, sphingosine, sphinganine, and sphingosine-1-phosphate), ceramides, and the lipid peroxidation products 4-hydroxynonenal (4-HNE) and malondialdehyde (MDA) in unstimulated and stimulated saliva from healthy young adults who had been smoking for 1-3 years.

Material and Methods

Approval for the study was obtained from the Bioethics Committee in Białystok (permission number: APK.002.343.2020). Each patient signed a written consent to participate in the study, and could ask questions or withdraw any time during the experiment.

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Subjects

A group of 75 smokers was enrolled in the study group. Smokers were divided into 4 subgroups according to the type of the smoking: Group 1 was traditional cigarette smokers (n=25), Group 2 was e-cigarette smokers (n=25), and Group 3 was heated tobacco device smokers (n=25). Each patient in the study group had been smoking for 1-3 years and used only 1 of the 3 methods of delivering nicotine to the body. Participants smoked on average about 10 cigarettes a day. The control group consisted of non-smokers (n=25) matched by age and gender to the subjects from the study group. The study participants were under continuous care of the Department of Restorative Dentistry at the Medical University of Białystok, reporting regularly for follow-up visits. The number of subjects was determined according to our previous study, assuming power of the test=0.8 (α=0.05) using Fisher's formula [24]. All study subjects were young adults, under the age of 30 years, in generally good health (no chronic diseases of any kind), without any oral inflammatory lesions, with a normal BMI (within the range of 18.5-25), drinking alcohol only occasionally, and not taking psychoactive drugs. At that time, participants in the study were not using fixed orthodontic appliances or retainers, Invisalign splints, did not have removable dentures, fixed restorations, implants, or titanium implants. The subjects had not taken medicines, vitamins, or other dietary supplements within 6 months before the study. Their diet was typical, consisting of 70% carbohydrates, 20% proteins, and 10% fats.

Saliva Collection and Dental Examination

Saliva was collected by an experienced person (S. Z.) at a prior dental examination, including assessment of DMFT (decayed, missing, and filled teeth), GI (gingival index), and PPD (periodontal pocket depth). The examination was performed under artificial lighting, using a mirror, an explorer, and a periodontal probe (WHO, 621). The examiner was previously calibrated, and 20 patients were randomly examined by another dentist (A. Z.). Interrater agreement for DMFT was r=1.0, for GI: r=0.96, for PPD: r=0.9. The tested material consisted of unstimulated and stimulated saliva, collected via the spitting method between 8 and 10 a.m. Before collection of the diagnostic material, patients were instructed not to smoke or consume food or beverages other than water and not to perform any oral hygiene procedures at least 2 hours before the visit. To avoid patients' embarrassment, saliva was collected in a separate room, in a sitting position, with the head slightly inclined downwards, with minimal movement of the face and lips. Before spitting unstimulated saliva into a plastic centrifuge tube, patients rinsed their mouths 3 times with room-temperature water. Saliva collected within the first minute was discarded. Unstimulated saliva was then collected for 15 minutes into a calibrated tube. Stimulated saliva was gathered in a similar manner for 5 minutes, during which 20 µl of citric acid was spotted on the dorsal surface of the patient's tongue every 30 seconds. Prior to centrifugation, the volume of the spat secretion was measured (with a calibrated pipette) and the rate of saliva secretion was determined by dividing the volume of saliva in the tube by the time required to obtain it. The saliva was centrifuged for 20 minutes at 4°C, 10000×g, then the fluid was collected from above the sediment, frozen at -84°C, and stored until assays were performed, but no longer than 4 months.

Lipids Analysis

The concentration of sphingolipids (sphingosine (Sph), sphinganine (SPA), sphingosine-1-phosphate (S1P) and ceramides (C14: 0-Cer, C16: 0-Cer, C18: 1-Cer, C18: 0-Cer, C20: 0-Cer, C22: 0-Cer, C24: 1-Cer, C24: 0-Cer) in saliva was measured according to the method described by Blachnio-Zabielska et al via ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC/MS/MS), with minor modification [21]. Briefly, an internal standard mixture (Sph-d7, SPA-d7, S1P-d7, C15: 0-d7-Cer, C16: 0-d7-Cer, C18: 1-d7-Cer, C18: 0-d7-Cer, 17C20: 0-Cer, C24: 1-d7-Cer and C24-d7-Cer) (Avanti Polar Lipids, Alabaster, Al, USA) and an extraction mixture (isopropanol: ethyl acetate, 15: 85; v/v) (Merck, Saint Louis, MO, USA) were added to each sample (100 µL of saliva). Samples were then vortexed, sonicated, and centrifuged (5 minutes at 3000 g, 4°C). The supernatants were transferred to new vials and the pellets were re-extracted. Both supernatants were combined and evaporated under a nitrogen stream and reconstituted in solvent B (2 mM ammonium formate (Sigma-Aldrich, Saint Louis, MO, USA), 0.1% formic acid (Honeywell Fluka, Morris Township, NJ, USA) in methanol (Merck, Saint Louis, MO, USA)). Sphingolipids were analyzed with a Sciex QTRAP 6500 + triple quadrupole mass spectrometer (AB Sciex Germany GmbH, Darmstadt, Germany) using a positive ion electrospray ionization (ESI) source (except for S1P, which was analyzed in the negative mode) with multiple reaction monitoring (MRM) against standard curves constructed for each compound. The chromatographic separation was performed on a reverse-phase Zorbax SB-C8 column 2.1×150 mm, 1.8 µm (Agilent Technologies, Santa Clara, CA, USA) in binary gradient using 1 mM ammonium formate (Sigma-Aldrich, Saint Louis, MO, USA), 0.1% formic acid (Honeywell Fluka, Morris Township, NJ, USA) in water (Merck, Saint Louis, MO, USA) as solvent A, 2 mM ammonium formate (Sigma-Aldrich, Saint Louis, MO, USA) and 0.1% formic acid (Honeywell Fluka, Morris Township, NJ, USA) in methanol (Merck, Saint Louis, MO, USA) as solvent B at the flow rate of 0.4 mL/min. To acquire and process the data, we used Analyst (Software version 1.7., AB Sciex Germany GmbH, Darmstadt, Germany) and Sciex OS-Q (AB Sciex Germany GmbH, Darmstadt, Germany).

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Table 1. Clinical and stomatological characteristics of patients and control group participants.

	Non-smokers, *n*=25	Traditional smokers, *n*=25	E-cigarette smokers, *n*=25	Heat-not-burn products smokers, *n*=25	p
Age (years)	24.7±2.4	25.3±3.1	23.4±3.2	23.7±1.9	NS
BMI (kg/m^2)	20.6±1.7	21.9±1.8	21.2±1.2	20.8±1.9	NS
Duration of addiction (years)	–	2.1±0.3	2.3±0.4	2.2±0.3	NS
US (mL/min)	0.68±0.1	0.62±0.1	0.65±0.1	0.69±0.1	NS
SWS (mL/min)	0.91±0.02	0.93±0.01	0.91±0.01	0.9±0.02	NS
DMFT	17±0.23	18±0.32	17±0.31	18±0.28	NS
API	24.56±0.36	21.54±0.31	21.54±0.27	21.54±0.31	NS
PBI	0.36±0.1	0.35±0.12	0.34±0.1	0.34±0.1	NS
PPD (mm)	2.0±0.5	2.0±0.5	2.0±0.5	2.0±0.5	NS

BMI – body mass index; UWS – unstimulated whole saliva; DMFT – Decayed, Missing, Filled Teeth; API – approximal plaque index; PBI – papilla bleeding index; PPD – periodontal pocket depth; NS – statistically insignificant; SWS – stimulated whole saliva.

Oxidative Damage Assays

MDA concentration was assayed colorimetrically using the thiobarbituric acid reactive substances (TBARS) method with 1,3,3,3-tetraethoxypropane (Sigma-Aldrich, Saint Louis, MO, USA) as a standard [25]. The absorbance was measured at 535 nm with microplate reader ELx800 and Gen5 2.01 software (BioTek Instruments, Winooski, VT, USA).

4-HNE concentrations was measured using a commercial enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Cell Biolabs, Inc., San Diego, CA, USA, and USCN Life Science). The absorbance was measured at 405 nm with microplate reader ELx800 and Gen5 2.01 software (BioTek Instruments, Winooski, VT, USA).

Statistical Analyses

GraphPad Prism 8.3.0 for MacOS (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analysis. Normality of distribution was assessed using the Shapiro-Wilk test. For comparison of the quantitative variables, one-way analysis of variance (ANOVA) followed by the Tukey post hoc test was used. The statistical significance level was established at $P<0.05$

Results

Clinical and Stomatological Findings

There were no significant differences in age, BMI, duration of addiction, unstimulated and stimulated saliva flow rate, DMFT, API, PBI, and PPD among the 3 study groups and among the study groups vs the control group. Clinical and stomatological characteristics of participants are presented in **Table 1**.

Unstimulated (US) and Stimulated (S) Saliva

SpH concentration was significantly lower in US and S in all nicotine users (IQOS, e-cigarette users, CS) compared to the controls (US: $P<0.0001$, $P<0.0001$, $P<0.0001$, respectively; S: $P<0.0001$, $P<0.0001$, $P<0.0001$, respectively). In the group of IQOS users, SpH concentration was significantly lower compared to e-cigarette group, both in US ($P<0.01$) and S ($P=0.03$).

SPA concentration was significantly lower in US and S of all nicotine users (IQOS, e-cigarette users, CS) compared to the controls (US: $P<0.0001$, $P<0.0001$, $P<0.0001$, respectively; S: $P<0.0001$, $P=0.006$, $P<0.0001$, respectively). In IQOS users, SPA concentration in US was considerably lower compared to e-cigarette smokers ($P=0.002$), SPA concentration in stimulated saliva of IQOS subjects was significantly lower compared to e-cigarette smokers ($P<0.0001$) and CS ($P=0.002$).

The concentration of S1P was significantly lower in US of all nicotine users (IQOS, e-cigarette users, CS) compared to the controls ($P<0.0001$, $P<0.0001$, $P<0.0001$, respectively). S1P concentration in S did not differ significantly between the study groups.

Similarly, ceramide C14 content was considerably lower in unstimulated saliva of all nicotine users (IQOS, e-cigarette users, CS) compared to the controls ($P<0.0001$, $P<0.0001$, $P<0.003$, respectively). In IQOS users, the concentration of the parameter

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in question was significantly lower compared to the CS group (*P*=0.006). The concentration of ceramide C14 in S in the IQOS group was considerably higher compared to the e-cigarette (*P*=0.02) and CS (*P*=0.008) groups.

The concentrations of ceramides C16 and C24 were significantly lower in US and S of all nicotine users (IQOS, e-cigarette users, CS) compared to the control group (C16, US: *P*<0.001, *P*<0.001, *P*<0.001, respectively, C16, S: *P*=0.04, *P*=0.003, *P*=0.04, respectively; C24, US: *P*<0.001, *P*<0.001, *P*<0.001, respectively; C24, S: *P*<0.0001, *P*<0.0001, *P*<0.0001, respectively).

The level of ceramide C18 was considerably lower in US and S of all nicotine users (IQOS, e-cigarette users, CS) compared to the controls (US: *P*<0.0001, *P*<0.0001, *P*<0.0001, respectively; S: *P*<0.0001, *P*<0.0001, *P*<0.0001, respectively). In US and S of the CS group, the concentration of the discussed parameter was significantly lower in both the IQOS and e-cig groups (US: *P*<0.0001, *P*<0.0001, respectively, S: *P*<0.0001, *P*<0.0001, respectively).

Ceramide C18 concentration was clearly lower in US of all nicotine users (IQOS, e-cigarette users, CS) compared to the controls (*P*<0.0001, *P*<0.0001, *P*<0.0001, respectively). In the US of the IQOS group, the concentration of the parameter in question was significantly higher compared to both the e-cig and CS groups (*P*=0.005, *P*=0.004, respectively). The content of ceramide C18 was considerably lower in S of e-cigarette and CS groups compared to the controls (*P*<0.0001, *P*<0.0001, respectively). In S of the IQOS group, the concentration of the discussed parameter was significantly higher compared to the CS group (*P*=0.04).

Ceramide C20 concentration was significantly lower in US and S of all nicotine users (IQOS, e-cigarette users, CS) compared to the control group (US: *P*=0.001, *P*<0.0001, *P*<0.0001, respectively; S: *P*=0.03, *P*<0.0001, *P*=0.0004, respectively). In the US and S of the CS group, the concentration of the parameter analyzed was significantly lower compared to both the IQOS and e-cig groups (US: *P*<0.0001, *P*<0.0001, respectively; S: *P*<0.0001, *P*<0.0001, respectively).

Ceramide C22 concentration was significantly lower in US of all nicotine users (IQOS, e-cigarette users, CS) compared to the controls (*P*<0.0001, *P*<0.0001, *P*<0.0001, respectively). The content of C22 in US of CS subjects was significantly lower compared to IQOS users (*P*=0.02) as well as the CS group (*P*=0.0006). Ceramide C22 concentration in the S of CS-group participants was significantly lower compared to the control group (*P*=0.0001) as well as the e-cigarette group (*P*=0.004).

The concentration of ceramide C24 1 and total Cer was notedly lower in US and S of all nicotine users (IQOS, e-cigarette users, CS) compared to the controls (US, C 24.1: *P*<0.0001, *P*<0.0001, *P*<0. 0001, respectively, US, total Cer: *P*<0.0001, *P*<0.0001, *P*<0.0001, respectively; S, C24 1 *P*<0.0001, p<0.0001, p<0.0001, respectively, S, total Cer: *P*<0.0001, *P*<0.0001, *P*<0.0001, respectively). The concentration of C24 1 in the US of e-cigarette group subjects was significantly higher compared to the CS group (*P*=0.005), while total Cer concentration in US of IQOS users was significantly higher compared to the CS group (*P*=0.003). Ceramide C24 1 concentration in S of e-cigarette group was significantly higher compared to the IQOS group (*P*=0.008) and CS group (*P*<0.0001).

The content of ceramide C24 1 in the S of the e-cig group was significantly elevated compared to the groups: IQOS (*P*=0.008) and CS (*P*<0.0001).

The concentration of 4-HNE and MDA in US was significantly higher in the group of traditional cigarette smokers compared to the controls (4-HNE *P*=0.0022; MDA *P*=0.0008), whereas in stimulated saliva we demonstrated considerably higher 4-HNE concentration in the group of traditional cigarette smokers vs non-smokers (*P*<0.0001). Moreover, 4-HNE levels in stimulated saliva of IQOS and e-cigarette users were significantly lower compared to the traditional cigarette smoking group (*P*=0.0002, *P*<0.0001, respectively). The content of MDA in the stimulated saliva of traditional cigarette smokers was significantly higher compared to the non-smoking group as well as the e-cigarette smoking group (*P*=0.0030; *P*=0.0078, respectively).

Graphical presentation of the results is presented in **Figures 1-4**.

Discussion

Saliva is the secretion of the even-numbered salivary glands: parotid, submandibular, sublingual as well as numerous smaller glands scattered in the oral mucosa [17]. It contains a number of proteins, glycoproteins, and lipids that determine its function and properties. The secretion of the salivary glands provides, inter alia, lubrication for the surface of the teeth and mucosa, is responsible for the initial stage of food digestion, and conditions protection of oral tissues from irritating stimuli, which certainly include cigarette smoke [26-28].

Cigarette smoke contains over 4000 toxic components responsible for a number of irregularities in the body [29]. It has been proven that in long-term compulsive smokers, cigarette smoke can lead to changes in the amount of saliva secreted, as well as trigger qualitative changes in the salivary gland secretion (decreased buffering capacity, altered bacterial microflora, increased concentration of oxygen free radicals, and changes in the local immune system) [30-35]. The solution to the harmful effect of traditional smoking was supposed to

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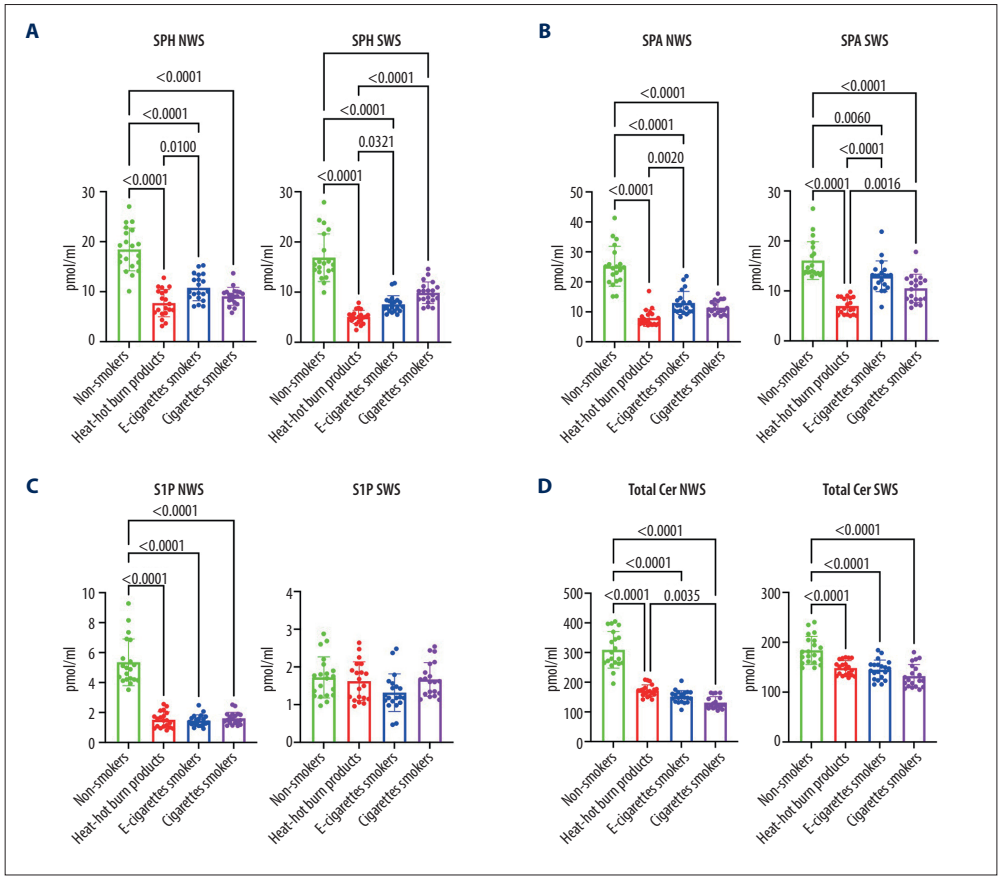

Figure 1. (**A-D**)Influence of smoking traditional cigarettes, e-cigarettes, and heat-not-burn products on the concentration of lipids in unstimulated and stimulated saliva. NWS – non-stimulated whole saliva; SPH – sphingosine; SPA – sphinganine; S1P – sphingosine-1-phosphate; SWS – stimulated whole saliva; Total Cer – total ceramide; p<0.0001. *The figure was created in GraphPad Prism.*

be new equipment that delivers nicotine to the body – e-cigarettes and heat-not-burn products.

E-cigarettes are mechanical devices that heat special solutions for inhalation, giving the user a sensation similar to ordinary smoking [36,37]. In addition to propylene glycol, flavors, and nicotine, e-cigarette liquids contain carcinogenic formaldehyde and numerous heavy metals [38,39]. Heat-not-burn products, on the other hand, can be described as a 'hybrid' of the 2 above-mentioned smoking methods. This system is based on tobacco cartridges (similar to traditional cigarettes) and an electronic device designed to heat the tobacco [40]. Heating, rather than burning, is intended to lead to less intense production and supply of harmful substances to the body. According to the literature on the subject, these devices cause adverse health effects, particularly with regard to respiratory organ complications [41,42]. Despite this fact, many people believe that the use of these devices is a "healthy" alternative to traditional cigarettes [39]. Due to their relatively short time on the consumer market, their exact mechanism on both the body and oral health has not been thoroughly evaluated, so any discoveries related to this topic are of great interest in the scientific community. Knowledge regarding the effect of smoking e-cigarettes on saliva composition is scarce, and there are no publications related to the effect of using heat-not-burn products. It is established that e-cigarettes can decrease saliva secretion, change composition of the oral microbiome, and affect the local immune response system [43-45].

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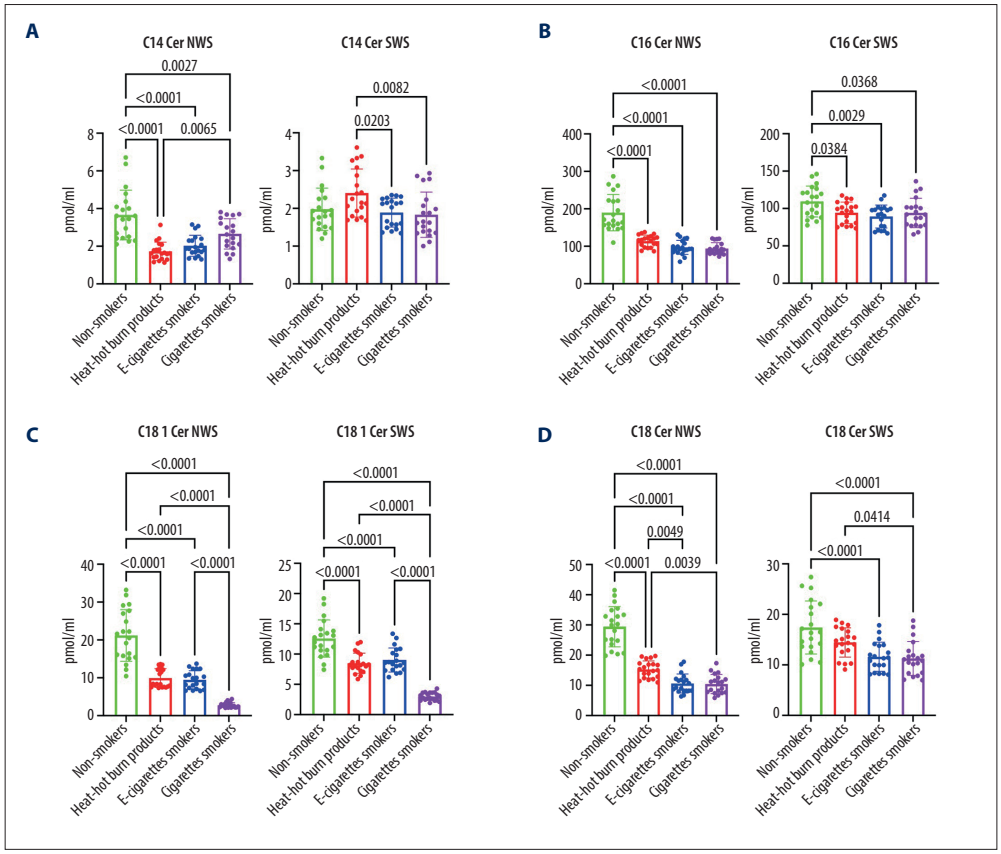

Figure 2. (**A-D** Influence of smoking traditional cigarettes, e-cigarettes, and heat-not-burn products on the concentration of lipids in unstimulated and stimulated saliva. Cer – ceramides (C14: 0-Cer, C16: 0-Cer, C18: 1-Cer, C18: 0-Cer); NWS – non-stimulated whole; SWS – stimulated whole saliva; *P*<0.0001. *The figure was created in GraphPad Prism.*

Salivary lipids are considered a very important component of saliva, as their qualitative and quantitative composition can be altered in the course of many pathological conditions [17]. It has been proven that disorders of lipid homeostasis in saliva are associated with the occurrence of periodontal diseases, and may occur in the course of a number of systemic diseases (including Sjögren's syndrome, cystic fibrosis, and Alzheimer's disease) [17,46,47].

There has been no research to evaluate the effect of using different nicotine delivery methods on the concentration of sphingolipids and ceramides in unstimulated and stimulated saliva. In addition, there have been no studies examining the processes of salivary lipid peroxidation in smokers of e-cigarettes and heat-not-burn products.

The purpose of our study was to evaluate the effect of smoking traditional cigarettes, e-cigarettes, and heat-not-burn products on the concentration of sphingolipids and lipid peroxidation products (4-HNE, MDA) in unstimulated and stimulated saliva. The participants in the study were generally healthy young adults whose had been smoking for 1-3 years. Furthermore, participants could only use 1 method of delivering nicotine to the body. The fact that young people were enrolled in the study group and the predetermined duration of the addiction are not coincidental. First, the new nicotine delivery devices have been mainly popularized among the "younger generation." Older smokers, due to the length of their addiction and the associated habit, are far less likely to decide to change their nicotine delivery method. Moreover, harmful agents accumulate in the oral cavity with age and prolonged smoking, as has already been proven.

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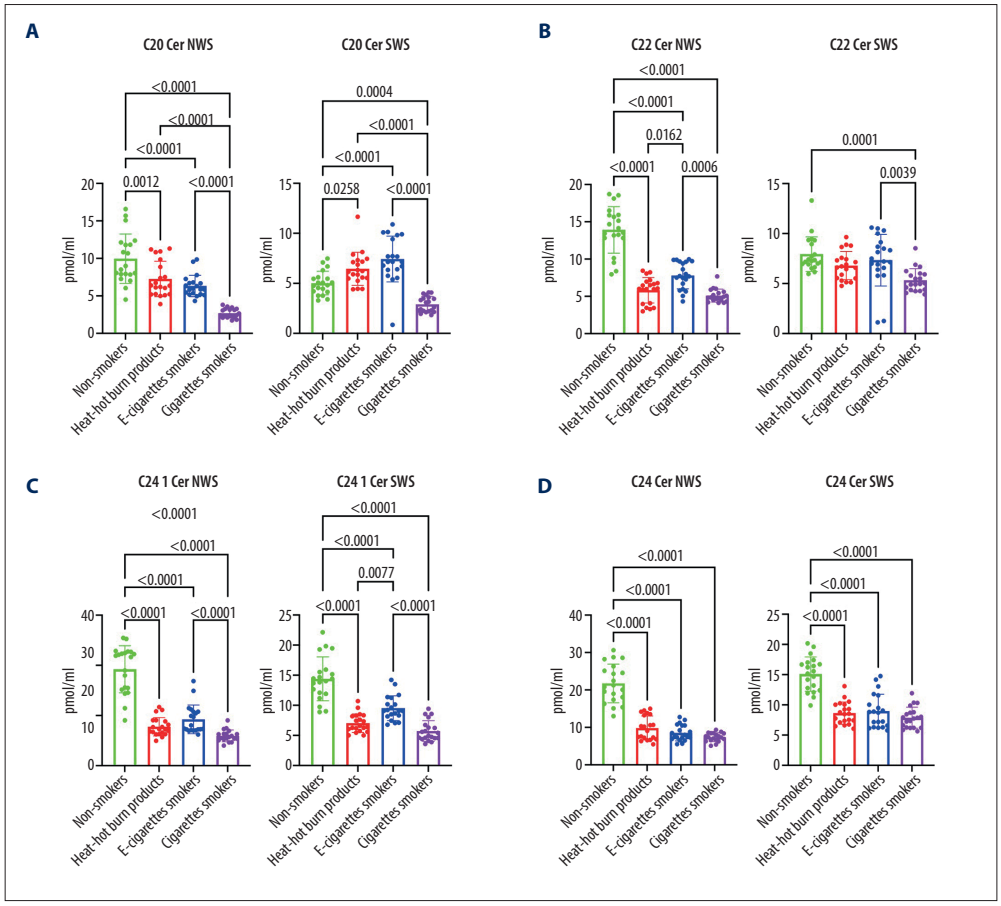

Figure 3. (**A-D**) Influence of smoking traditional cigarettes, e-cigarettes, and heat-not-burn products on the concentration of lipids in unstimulated and stimulated saliva. Cer – ceramides (C20: 0-Cer, C22: 0-Cer, C24: 1-Cer, C24: 0-Cer); NWS – non-stimulated whole; SWS – stimulated whole saliva; *P*<0.0001. *The figure was created in GraphPad Prism.*

According to the results of our study, smoking traditional cigarettes, e-cigarettes, or heat-not-burn products reduces the concentrations of all sphingolipids examined by us in the unstimulated and stimulated saliva.

To understand the likely cause of the reduction in sphingolipid levels in the unstimulated and stimulated saliva of smokers, it is necessary to briefly characterize and explain the functions and metabolic processes occurring within this group of compounds.

Sphingolipids and their derivatives constitute a numerous group of bioactive lipid compounds located in the outer layer of eukaryotic cell membranes, thus determining its shape. This group of compounds includes sphingosine, sphinganine, sphingosine-1-phosphate, and ceramides. In addition to their structural functions, sphingolipids act as activators of signaling pathways and secondary signal transducers [48]. The central molecule of the sphingolipid structure is the aliphatic amino alcohol – sphingosine. The combination of sphingosine and fatty acid residue results in the formation of ceramide [48-51]. Ceramides play a primary role in sphingolipid metabolism and are involved in the regulation of such cellular processes as proliferation and differentiation, growth, aging, and cell death [50]. They also provide the basis for the synthesis of other sphingolipids. They can be transformed, with participation of ceramidases, into sphingosine, which is phosphorylated by sphingosine kinases type 1 and 2 to sphingosine-1-phosphate [48,50].

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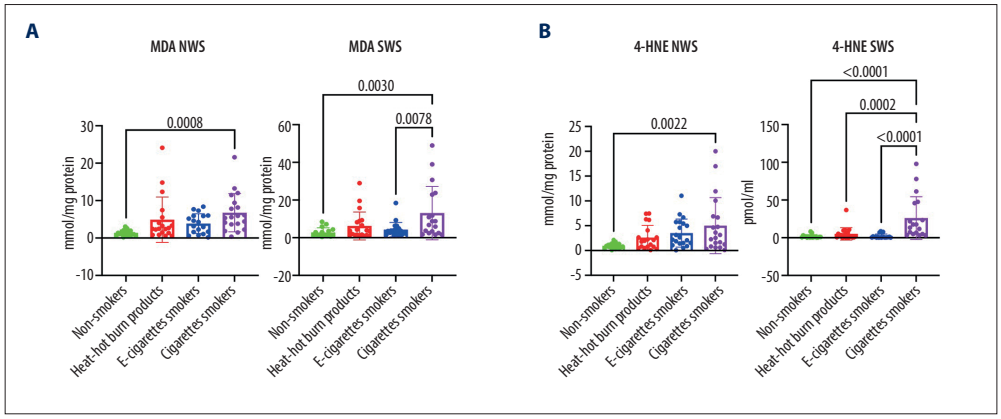

Figure 4. (**A, B**) Influence of smoking traditional cigarettes, e-cigarettes, and heat-not-burn products on the concentration of lipid peroxidation products in unstimulated and stimulated saliva. 4-HNE – 4-hydrokxynonenal; MDA – malondialdehyde; NWS – non-stimulated whole; SWS – stimulated whole saliva; *P*<0.0001. *The figure was created in GraphPad Prism.*

The signaling pathway of sphingosine-1-phosphate is one of the key regulators of cell survival, proliferation, and differentiation. Thus, sphingosine kinases are important enzymes involved in maintaining the balance among bioactive sphingolipids such as S1P, ceramide, and sphingosine [48,49].

The results of our study may seem surprising, since most studies assessing the effect of smoking on sphingolipid concentration within tissues and plasma demonstrated increased sphingolipid levels [52-54]. Although saliva predominantly constitutes the ultra-filtrate of plasma (which might appear controversial in the context of the results we obtained), the vast majority (nearly 98%) of lipids found in saliva are synthesized directly in salivary gland cells [17,47]. This suggests that the significant changes in sphingolipid content in unstimulated and stimulated saliva of smokers compared to the control group directly reflect the ongoing pathology within the salivary glands.

Toxins in cigarette smoke are responsible for DNA damage within the cells of secretory glands. In turn, sphingomyelin, ceramide – synthesized de novo in cells – and sphingosine are characterized by antiproliferative and pro-apoptotic properties. It is likely that cigarette smoke "activates" these functions of sphingolipids to lead to programmed cell death. Ceramides present in the cell are not stored but rather are transported directly to the Golgi apparatus, where they are transformed into derivative compounds. It is possible that chronic irritation of the oral cavity with tobacco smoke toxins decreases the concentration of the compounds we studied as a result of depletion of reserves of sphingolipids, which attempts to mitigate the adverse effects of cigarette toxins on secretory cells of salivary glands.

Reduced ceramide concentrations were observed in gastrointestinal cancer. Changes in the activity of enzymes responsible for the metabolism of this compound trigger an increase in glycosylation of ceramide, resulting in reduced concentration of this compound. In addition, elevated levels of S1P have been observed in many types of cancer, including colorectal cancer cells. This sphingolipid – S1P – unlike ceramide, is anti-apoptotic, enhances proliferation of tumor cells, and stimulates their angiogenesis. This contradicts the results of our study in which S1P concentrations were also reduced in the studied material. Nevertheless, cancer is an advanced form of complications of organs connected with smoking. Further studies are necessary to better understand the effects of smoking on salivary sphingolipid concentration.

Moreover, in the groups of all smokers, regardless of the source of nicotine delivery to the body, we observed an increase in 4-HNE and MDA concentrations in unstimulated and stimulated saliva compared to the controls; however, only in the group of smokers of traditional cigarettes vs the controls was this result statistically significant, suggesting that smoking traditional cigarettes is the most harmful in terms of oxidative modification of salivary lipids, whereas it is possible that a longer period of using modern nicotine delivery devices may give similar results. This is particularly important considering that lipids perform many important functions in the oral cavity, from structural to functional. They affect the processes of digestion, protection, and communication, as well as maintaining the internal balance in the oral cavity [17].

4-HNE is one of the byproducts of lipid peroxidation that the body under oxidative stress. It is mainly formed by oxidation of linoleic acid, one of the unsaturated fatty acids. 4-HNE is a

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highly reactive aldehyde that affects various physiological processes in the body; it can lead to cell membrane damage, protein degradation, enzyme inactivation, and inflammatory reactions. In addition, 4-HNE can affect mitochondrial function, introducing disorders in the process of cellular energy production. MDA, on the other hand, is a byproduct of lipid peroxidation and can damage body cells and tissues through its toxic and pro-inflammatory effects. It can also lead to the formation of free radicals, which cause further damage to cells and can contribute to development of numerous diseases, including heart disease, respiratory diseases, and cancer.

The increased concentrations of these compounds in unstimulated and stimulated saliva obtained in our study clearly indicate a redox imbalance in the salivary glands of smokers. Increased levels of free radicals lead to oxidative modification of lipids and thus damage to cell membranes. Similar results were obtained by Celec et al, who did not demonstrate any correlation between the concentrations of lipid peroxidation products in saliva and plasma, which confirms the glandular origin of lipids in saliva. Additionally, some recently discovered compounds have a significant influence on the oral environment; lysates and postbiotics can modify clinical and microbiological parameters, so these products should be considered in future trials as they could also influence saliva composition and balance [55-57].

Lipids are one of the main components of cell membranes; therefore, changes in the lipid composition of saliva can reflect changes in the composition of salivary gland cell membranes. Salivary lipids perform many functions, the disruption of which can lead to disintegration throughout the oral cavity [17]. They participate in the signal for saliva secretion, which can consequently lead to impaired salivary secretion. They also have a protective function towards the oral mucosa. Both reduced saliva secretion and weakened protective function may consequently lead to a number of abnormalities, including dry mouth, difficulties in forming a bolus of food, increased susceptibility to mechanical injuries in the oral mucosa (including during consumption or use of prosthetic devices), and an increased risk of bacterial, fungal and viral infections of the gums and oral mucosa (including increased susceptibility to HSV-1 infection and lichen planus) [17,58]. As a consequence, discomfort increases and the patient's overall well-being deteriorates. Of course, in our study, considering the age of the recruited patients and the duration of their addiction, we did not observe the above phenomena, but it cannot be ruled out that in the long-term, smokers may develop these pathologies.

Limitations

Our study has several limitations. Due to the small group size, this should be regarded as a pilot study. The people qualified for the study and control groups were considered matched, with deficiencies in systemic diseases and other factors that may directly affect the increase in oxidative stress in the body. Therefore, fewer patients were included in the study.

Because of the young age of our participants and relatively short smoking histories (up to 3 years), our findings have limited generalizability to long-term or older smokers who may experience different oral health effects. The results may not capture the full spectrum of oral health effects associated with smoking, particularly in older individuals with longer smoking durations.

Moreover, only some salivary lipids were included in our study, so the results do not reflect the overall effect of smoking on the salivary lipid profile. This study also did not compare heavy vs light smokers.

Conclusions

The results of our research clearly show that:

- Decrease the concentration of all sphingolipids in unstimulated and stimulated saliva is not dependent on the mode of delivery of nicotine.
- Significant changes in the content of sphingolipids in the unstimulated and stimulated saliva of smokers in comparison to the control group are a direct reflection of ongoing pathology within the salivary glands.
- The increased concentration of the 4-HNE and MDA in unstimulated and stimulated saliva indicates an imbalance in the redox balance in the salivary glands of smokers.

Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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Comparison of smoking traditional, heat not burn and electronic cigarettes on salivary cytokine, chemokine and growth factor profile in healthy young adults–pilot study

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Objective: Smoking is the cause of numerous oral pathologies. The aim of the study was to evaluate the effect of smoking traditional cigarettes, e-cigarettes, and heat-not-burn products on the content of salivary cytokines, chemokines, and growth factors in healthy young adults.

Design: Three groups of twenty-five smokers each as well as a control group matched in terms of age, gender, and oral status were enrolled in the study. In unstimulated saliva collected from study groups and participants from the control group, the concentrations of cytokines, chemokines, and growth factors were assessed by Bio-Plex® Multiplex System.

Results: We demonstrated that smoking traditional cigarettes is responsible for increasing the level of IFN-γ compared to non-smokers and new smoking devices users in unstimulated saliva in the initial period of addiction. Furthermore, e-cigarettes and heat-not-burn products appear to have a similar mechanism of affecting the immune response system of unstimulated saliva, leading to inhibition of the local inflammatory response in the oral cavity.

Conclusion: Smoking traditional cigarettes as well as e-cigarettes and heat-not-burn products is responsible for changes of the local immune response in saliva. Further research is necessary to fill the gap in knowledge on the effect of new smoking devices on the oral cavity immune system.

KEYWORDS

e-cigarettes, heat-not-burn products, saliva, smoking, cigarettes

1 Introduction

It has been evidenced that cigarettes contain about 400 scientifically proven carcinogens, i.e., formaldehyde, benzene or vinyl chloride; therefore, it is not surprising that cigarette smoking has been documented to be associated with an increased risk of developing cancer, as well as elevated risk of stroke, respiratory diseases, inflammation, and weakening of the body's immune function (West, 2017; Reitsma et al., 2021; Soleimani et al., 2022). For this reason, smoking is believed to be one of the causes of premature deaths, shortening the life of an addict by up to 8 years. According to statistics, smoking kills over 8 million people annually (Carter et al., 2015; Zhu et al., 2021).

Electronic cigarettes and heat-not-burn products, considered by the public to be less harmful, were supposed to become an alternative to traditional cigarettes. Electronic cigarettes are mechanical devices that heat special solutions for inhalation, giving the user a sensation similar to ordinary smoking (DeAtley et al., 2022; Harlow et al., 2022). The liquid of e-cigarettes mainly consists of odorless carriers (propylene glycol, glycerol), nicotine, and a wide range of flavorings (Leventhal et al., 2019). It has been demonstrated that menthol flavor is one of the most popular flavors among young users. However, the effects of flavoring agents added to nicotine-containing vapors and the underlying mechanisms are largely unknown. What is more, detailed studies have confirmed also the presence of formaldehyde, acrolein and numerous heavy metals in the composition of e-cigarettes (Hahn et al., 2014; Zhao et al., 2020).

On the other hand, heat-not-burn products have been available on the consumer market for a relatively short time. Heating tobacco, rather than burning it, is intended to decrease the production of tarry substances and their supply to the body (Ratajczak et al., 2020). A detailed analysis of the composition of the heat-not-burn products' inserts made it possible to isolate benzene, acrolein, tobacco-specific nitrosamines (Fried and Gardner, 2020). Tobacco sticks resembling traditional cigarettes may also contain flavors that make this type of addiction more attractive. Although the opinion that the new devices for supplying nicotine to the body are less harmful is becoming increasingly controversial, their use has already become extremely popular among young adults (Kreslake et al., 2021).

Research has additionally shown that the use of new devices delivering nicotine to the body may lead to the initiation of tobacco smoking among non-smokers and the relapse of smoking among former smokers (Gomajee et al., 2019; McMillen et al., 2019). But most sinisterly, many adolescents and young adults who have never smoked have started "vaping," becoming another population that has become addicted to nicotine through these drug delivery devices (Sayed et al., 2021).

The oral cavity is the first point of contact between the toxins contained in cigarette smoke and the human body. Long-term smoking of traditional cigarettes has been proven to lead to the release of inflammatory mediators and cytokines, which is connected with the development of various oral diseases, including precancerous conditions and periodontal disease (Zhang et al., 2019). Cigarette smoke mainly affects the balance of cytokines produced by helper T cells (Zięba et al., 2024). Importantly, the concentrations of these cytokines increase with the duration of smoking. Mokeem et al. (2018) demonstrated significantly higher levels of proinflammatory IL-1β and IL-6 in the saliva of cigarette smokers. Another study showed significant differences in the expression of salivary interleukins (↓IL-10, ↓IL-5, ↑IL-2, ↑IL-4) in traditional cigarette users (Rodríguez-Rabassa et al., 2018).

Little is known about the effects of smoking electronic cigarette and heated tobacco units on the release of inflammatory mediators in saliva. Few publications report an increase in IL-6 or PGE2 concentrations in the unstimulated saliva of e-cigarette smokers compared to non-smokers (Mokeem et al., 2018; Ye et al., 2020). There are no similar studies for heat-not-burn products.

The aim of the study presented below is to evaluate and compare the concentrations of a wide panel of cytokines, chemokines, and growth factors in unstimulated saliva samples of young smokers of traditional and electronic cigarettes as well as tobacco heating systems, with a duration of addiction of one to 3 years.

2 Materials and methods

The study was approved by the Bioethics Committee of the Medical University of Bialystok (permission number: APK.002.175.2023). It was implemented in accordance with the Declaration of Helsinki that defines procedures in human biomedical research. Prior to qualification and collection of diagnostic material, each participant had been informed with detailed information on the purpose and methodology of the study, and gave a written consent to participate in it.

2.1 Subjects

The study group consisted of 75 smokers divided into 3 subgroups: TS–25 smokers of traditional cigarettes (regular cigarettes; not light/not strong); ES–25 smokers of menthol flavor electronic cigarettes; HS–25 smokers of menthol heated tobacco products. In order to qualify for the study groups, the duration of addiction of the participants could not be less than 1 year but not more than 3 years, and each subject could be a user of only one method of delivering nicotine to the body. The control group consisted of 25 non-smokers (no history of smoking traditional cigarettes/new devices delivering nicotine to the body in the past) matched in terms of age and gender to the study groups. All of the study subjects were generally healthy young adults (under 30), without inflammatory lesions in the oral cavity, with normal body weight (BMI ranging from 18.5 to 24.9), not abusing alcohol, and not taking psychoactive drugs. Participants to the study regularly attended follow-up visits to the Department of Restorative Dentistry at the Medical University of Bialystok. The number of subjects was determined according to our previous study, assuming power of the test = 0.8 (significance level alpha = 0.05) using Fisher's formula (Jung, 2014). Within 6 months preceding our experiment, the participants from the study and control groups had not taken any medications affecting the immune response (antibiotics, steroids, antihistamines, anti-inflammatory drugs). During the study, the participants were not using fixed orthodontic appliances, and did not have Invisalign splints,

removable dentures, fixed prosthetic restorations, implants, or titanium implants.

2.2 Saliva collection and dental examination

The study material consisted of unstimulated saliva collected from study group as well as the control group via the spitting method. The participants had been asked not to smoke or consume food or beverages other than pure water and not to perform any oral hygiene procedures at least 2 h before saliva collection. In order to minimize the effect of diurnal rhythms on saliva secretion processes, unstimulated saliva was collected between 8 a.m. and 10 a.m. In order to eliminate the subjects's sense of restraint, saliva collection was performed in a separate room in a sitting position, with the head slightly inclined downward and minimized movements of the face and lips. Before spitting of unstimulated saliva into a plastic centrifuge tube, each participant rinsed his/her mouth three times with water at room temperature. Saliva collected within the first minute was discarded. Before centrifugation, the volume of the spat secretion was measured (with a calibrated pipette) and the rate of saliva secretion was determined by dividing the volume of saliva in the tube by the time necessary to collect it. The saliva was centrifuged for 20 min at 4°C, 10,000 × g, and then the supernatant fluid was collected, frozen at −84°C and stored until the assays were performed, but not longer than 4 months.

Dental examination was performed upon collection of the diagnostic material in order to avoid possible contamination of saliva with blood. The oral health of smokers and the control group was assessed by means of a dental mirror and a periodontal probe. The examination was conducted under electric light and included: assessment of the condition of the lips and the mucous membrane lining the tongue and cheeks; palpation of the parotid, submandibular and sublingual salivary glands; assessment of the oral hygiene index (Approximal Plaque Index, API), papillary bleeding index (PBI), measurement of periodontal pocket depth (PPD) and the DMFT index. The latter is used to determine the number of teeth with a primary or secondary carious lesion (D–decayed), teeth extracted due to caries (M–missing), and filled (F) teeth (T). The examination was conducted by one dentist (S. Z.) who had been trained beforehand, and an inter-rater examination by another dentist (A. Z.) was performed on 15 randomly selected study participants. Based on the dental examinations conducted, 10 smokers from the study groups (due to periodontal disease) and 6 subjects from the control group (poor oral hygiene, presence of numerous dental deposits) were excluded from the experiment.

2.3 Biochemical methods

Salivary cytokines, chemokines, and growth factors were analysed using the Bio-Plex® Multiplex System according to the manufacturer's instructions. Bio-Plex Pro Human Cytokine Assay (Bio-Rad Laboratories, Inc., Hercules, CA, United States) is a multiplex assay based on magnetic beads whose performance can be compared to a typical ELISA. The captured antibodies directed against a specific biomarker bind covalently to magnetic beads. The coupled beads then react with the sample containing the selected biomarker. A series of rinses is performed in order to remove the unbound protein, and then a biotinylated detection antibody is added to create a sandwich complex. The final complex is formed by adding streptavidin-phycoerythrin (SA-PE) conjugate. Data from the reactions are acquired using a dedicated plate reader (Bio-Plex 200) and high-speed digital signal processor.

2.4 Statistical analysis

The analysis of the obtained data was performed using GraphPad Prism 8.3.0. statistical software for MacOS (GraphPad Software, La Jolla, United States). Shapiro–Wilk test was used to assess normality of distribution, and the Levene's test was used to evaluate homogeneity of variance. A one-way Kruskal–Wallis analysis of variance (ANOVA) followed by Dunn's *post hoc* test was used to compare the quantitative variables. Multiplicity-adjusted *p*-values were also calculated. The results are presented in box plots as the median (minimum–maximum). A significance level of less than 0.05 was assumed for the statistical analyses performed.

3 Results

3.1 Clinical and stomatological findings

There were no significant differences in age, BMI, duration of addiction, unstimulated saliva flow rate, DMFT, API, PBI, and PPD between the study groups and the study groups and the control group. Clinical and stomatological characteristics were presented in Table 1.

3.2 Concentrations of cytokines/chemokines/growth factors in unstimulated saliva

The concentration of cytokines: IL-3, IL-5, IL-6, IL-12 (p40), IL-10, IL-1α, IL-2, IL-2Ra, IL-4, IL-7, IL-9, IL-12 (p70), IL-13, IL-15, IL-17, TNF-α; chemokines: CTACK, MIP-1α, B-NGF, RANTES, SCGF-B, Eotaxin, LIF, SDF-1a, and growth factors: VEGF, GM-CSF, PDGF-BB in unstimulated saliva collected from subjects was below the detection level of the assay used. Concentrations of statistically significant levels of cytokines/chemokines/growth factors in particular study groups and control group were presented in Figures 1–3 as well as in Supplementary Tables S1–S3.

3.2.1 Concentrations of cytokines

Smokers of traditional cigarettes demonstrated significantly higher levels of salivary IFN-γ (↑68%, $p = 0.0347$) compared to the non-smoking controls. Other salivary cytokines (TNF-α, HGF, IL-1β, IL-1RA, IL-8, IL-16, IL-18, MIF) did not differ significantly between the two groups.

Subjects using heat-not-burn products had significantly lower content of salivary TNF-α, HGF, IL-1RA (↓57% $p = 0.0006$, ↓69% $p = 0.0008$, ↓51% $p = 0.0001$, respectively) and IL-8, IL-16, IL-18,

10.3389/fphys.2024.1404944

TABLE 1 Clinical and dental characteristics of subjects from the study groups and the control group (BMI, body mass index; UWS, unstimulated saliva; DMFT, Decayed, Missing, Filled Teeth; API, approximal plaque index; PBI, papilla bleeding index, PPD, periodontal pocket depth; NS, not statistically significant).

	Non-smokers *n* = 25	Traditional smokers *n* = 25	E-cigarettes smokers *n* = 25	Heat-not-burn products smokers *n* = 25	*p*
Age (years)	24.7 ± 2.4	25.3 ± 3.1	23.4 ± 3.2	23.7 ± 1.9	NS
BMI (kg/m^2)	20.6 ± 1.7	21.9 ± 1.8	21.2 ± 1.2	20.8 ± 1.9	NS
Duration of addiction (years)	—	2.1 ± 0.3	2.3 ± 0.4	2.2 ± 0.3	NS
UWS (mL/min)	0.68 ± 0.1	0.62 ± 0.1	0.65 ± 0.1	0.69 ± 0.1	NS
DMFT	17 ± 0.23	18 ± 0.32	17 ± 0.31	18 ± 0.28	NS
API	24.56 ± 0.36	21.54 ± 0.31	23.32 ± 0.27	24.89 ± 0.32	NS
PBI	0.36 ± 0.1	0.35 ± 0.12	0.34 ± 0.1	0.34 ± 0.1	NS
PPD (mm)	2.0 ± 0.5	2.0 ± 0.5	2.0 ± 0.5	2.0 ± 0.5	NS

MIF (↓54% $p < 0.0001$, ↓50% $p = 0.0016$, ↓41% $p = 0.0222$, ↓51% $p = 0.0003$, respectively.) in comparison with non-smokers. The concentration of other salivary cytokines (IFN-γ, IL-1β) between the group of heat-not-burn products smokers and the group of non-smokers revealed no statistical significance. Similary, the group of heat-not-burn products smokers was characterized by significantly lower levels of salivary cytokines HGF and IL-16 (↓54% $p = 0.0004$, ↓61% $p = 0.0463$, respectively.) compared to traditional cigarette smokers. Concentrations of other detectable salivary cytokines (IFN-γ, TNF-α, IL-1β, IL-1RA, IL-8, IL-18, MIF), presented no statistical significance between the group of tobacco-heating-system smokers and traditional smokers.

Smokers of electronic cigarettes had significantly lower levels of salivary cytokines TNF-α, IL-1β, IL-1RA (↓47% $p = 0.0307$, ↓58% $p = 0.0008$, ↓79% $p < 0.0001$, respectively.) and IL-8, IL-16, IL-18, MIF (↓78% $p < 0.0001$, ↓62% $p = 0.0003$, ↓56% $p = 0.0019$, ↓85% $p < 0.0001$, respectively.) compared to the group of non-smokers. Concentrations of the other salivary cytokines (IFN-γ, HGF) between the e-cigarette smoking group and the non-smokers did not present statistical significance. Additionally, e-cigarette users demonstrated significantly lower concentrations of salivary cytokines: IL-1RA, IL-8, IL-16 and MIF (↓72% $p = 0.0002$, ↓72% $p = 0.0004$, ↓64% $p < 0.0001$, ↓81% < 0.0001, respectively.) compared to smokers of traditional cigarettes. The levels of salivary cytokines (IFN-γ, TNF-α, HGF, IL-1β, IL-18) between the groups of e-cigarette smokers and traditional cigarette smokers showed no statistical significance.

Between the group of e-cigarette smokers and the group of heat-not-burn products smokers, no significant differences were observed in the levels of the detected salivary cytokines.

3.2.2 Concentrations of chemokines

The concentrations of Gro-α, MCP-1α, SCF, MIG, IP-10 (↓68% $p < 0.0001$, ↓58% $p < 0.0001$, ↓70% $p = 0.0004$, ↓56% $p =< 0.0001$, ↓47% $p = 0.0178$, respectively.) in the saliva heat-not-burn products group were considerably lower compared to the non-smoking control group. Similary, smokers of heat-not-burn products demonstrated significantly lower levels of salivary chemokines: Gro-α, MCP-1, SCF, MIG, IP-10 (↓65% $p = 0.0002$, ↓47% $p = 0.0253$, ↓63% $p = 0.0286$, ↓48% $p = 0.0081$, ↓52% $p = 0.0027$, respectively.) compared to traditional cigarette smokers. Concentrations MIP-1α presented no statistical significance between the group of tobacco-heating-system smokers vs. non-smokers as well as tobacco-heating-system smokers and traditional smokers.

Concentrations of salivary chemokines Gro-α, MCP-1, MIG, IP-10 (↓74% $p < 0.0001$, ↓62% $p = 0.0001$, ↓73% $p < 0.0001$, ↓58% $p = 0.0039$, respectively.) were significantly lower in the group of e-cigarette smokers compared to the non-smoking controls. Additionally, e-cigarette smokers demonstrated significantly lower concentrations of Gro-α, MCP-1, MIG, IP-10 (↓51% $p = 0.0435$, ↓71% $p = 0.0005$, ↓68% $p = 0.0002$, ↓62% $p = 0.0006$, respectively.), compared to smokers of traditional cigarettes. The levels of salivary MIP-α and SCF between the groups of e-cigarette smokers and non-smokers as well as between e-cigs users and traditional cigarette smokers showed no statistical significance.

Between the group of traditional smokers vs. non-smokers as well as compared e-cigarette smokers and the group of heat-not-burn products smokers, no significant differences were observed in the levels of the detected salivary chemokines.

3.2.3 Concentrations of growth factors

Heat-not burn products users had significantly lower concentrations of the salivary G-CSF, TRAIL, M-CSF (↓62% $p < 0.0001$, ↓57% $p < 0.0001$, ↓52% $p = 0.0003$, respectively) compared to the non-smoking control group. There was no statistical significance in FGF levels between those two groups. Similary, smokers of heat-not-burn products was characterized by significantly lower levels of G-CSF, TRAIL (↓57% $p = 0.0019$, ↓55% $p = 0.0005$, respectively.) compared to traditional cigarette smokers. The levels of FGF and M-CSF presented no statistical significance between the group of tobacco-heating-system smokers and traditional smokers.

The concentrations of salivary G-CSF, TRAIL, M-CSF (↓54% $p = 0.0014$, ↓71% $p < 0.0001$, ↓53% $p = 0.0023$, respectively.) were significantly lower in the group of e-cigarette smokers compared to the non-smoking controls. Concentrations of FGF did not present statistical significance. Additionally, e-cigarette smokers demonstrated significantly lower concentrations of G-CSF, TRAIL (↓48% $p = 0.0369$, ↓70% $p < 0.0001$, respectively.) compared to smokers of traditional cigarettes. The levels of

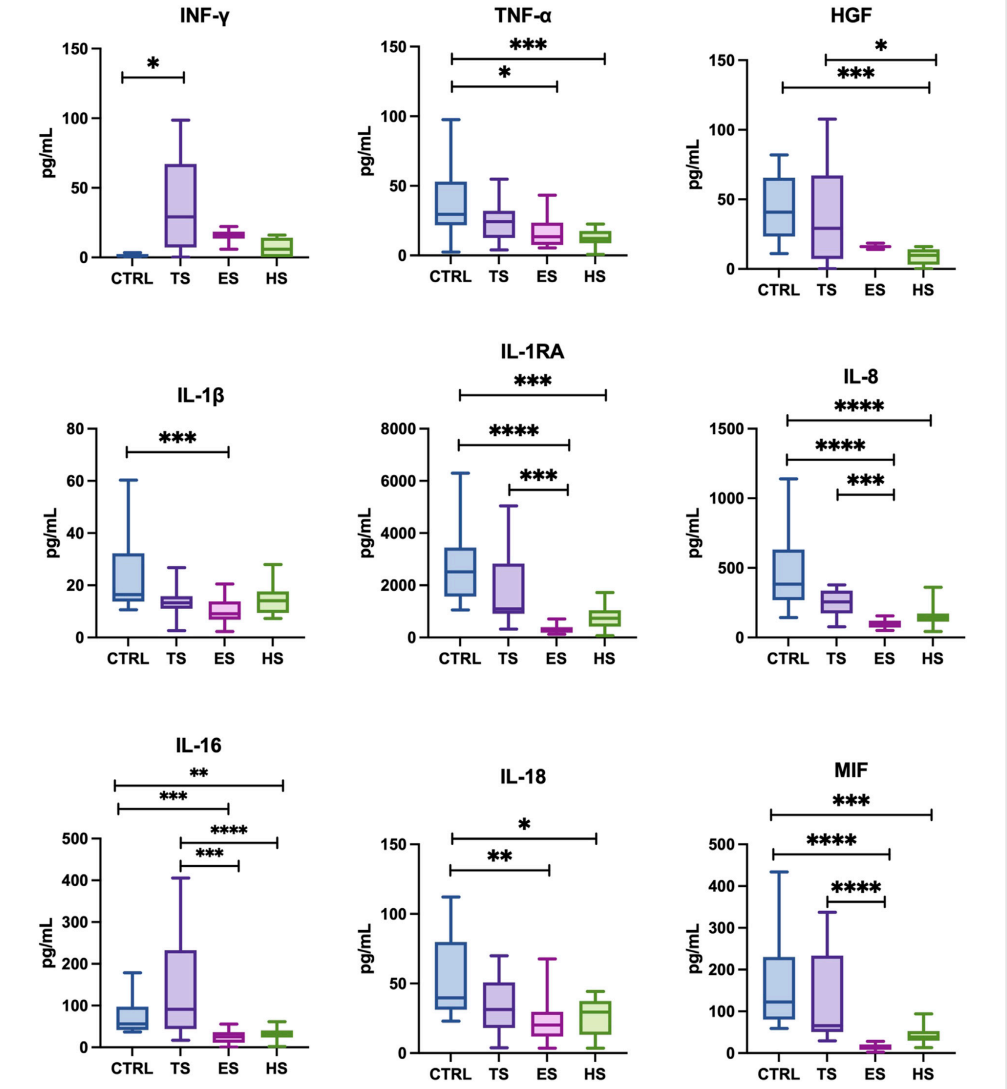

FIGURE 1
The effect of different methods of delivering nicotine to the body on the cytokine profile in unstimulated saliva: IL: 1β, 1RA, 8, 16, 18–interleukin- 1β, 1RA, 8, 16, 18; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor α; HGF, hepatocyte growth factor; MIF, macrophage migration inhibitory factor; CTRL, control; TS, traditional cigarettes smokers; ES, electronic cigarettes smokers; HS, heat-not-burn products smokers. *$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$, ****$p < 0.00005$.

salivary FGF between the groups of e-cigarette smokers and traditional cigarette smokers showed no statistical significance.

Between the group of traditional smokers vs. non-smokers as well as compared e-cigarette and heat-not-burn products smokers, no significant differences were observed in the concentrations of the salivary growth factors.

4 Discussion

Cigarette smoking is associated with numerous diseases and constitutes a serious challenge to the current healthcare system worldwide. Scientific sources report that up to 1/3 of the world's population may be affected by this problem (West, 2017; Dai et al.,

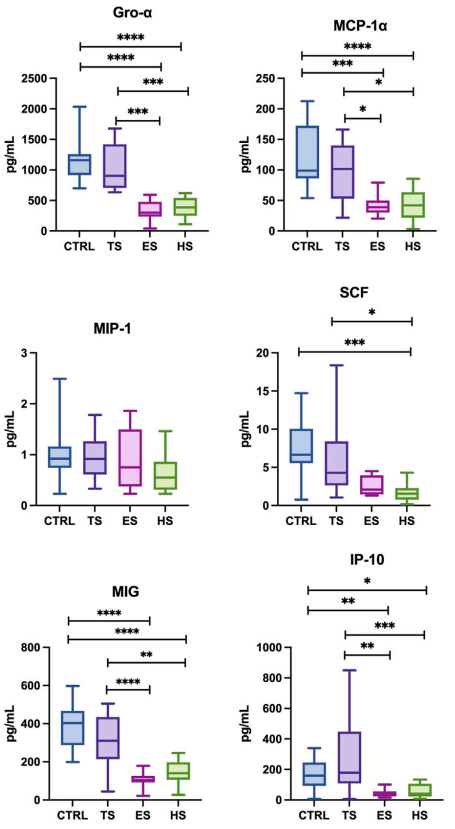

FIGURE 2
Effect of different methods of delivering nicotine to the body on the chemokine profile in unstimulated saliva: MCP-1, monocyte chemoattractant protein-1; MIP-1-α, macrophage inflammatory protein-1 alpha; GRO-α, regulated oncogene-alpha, IP-10, interferon gamma-induced protein 10; MIG, monokine induced by gamma interferon; SCF, stem cell factor; CTRL, control; TS, traditional cigarettes smokers; ES, electronic cigarettes smokers; HS, heat-not-burn products smokers. *$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$, ****$p < 0.00005$.

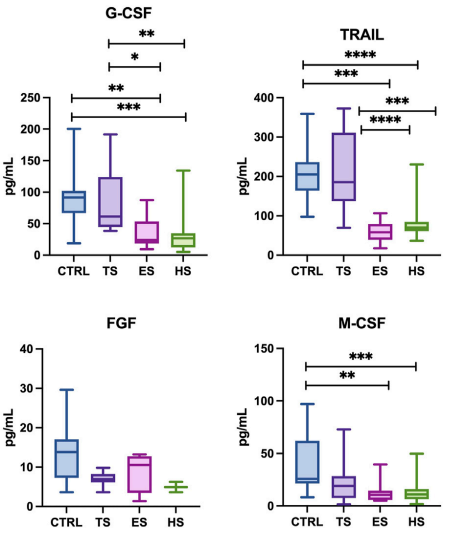

FIGURE 3
The effect of different methods of administering nicotine to the body on the profile of growth factors in unstimulated saliva: FGF, fibroblast growth factor; G-CSF, granulocyte colony-stimulating factor; M-CSF, macrophage colony-stimulating factor; TRAIL, TNF-related apoptosis-inducing ligand; CTRL, control; TS, traditional cigarettes smokers; ES, electronic cigarettes smokers; HS, heat-not-burn products smokers. *$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$, ****$p < 0.00005$.

2022a; Dai et al., 2022b). Exposure to tobacco smoke is considered an important cause of death and is connected with the development of numerous systemic disorders, including: respiratory and gastrointestinal diseases, promotion and progression of the development of cancer, as well as local effect on the oral environment (West, 2017; Zhang et al., 2022).

The purpose of this publication was to evaluate the effects of various forms of delivering nicotine to the body on the local immune defence system of the oral cavity. It should be underlined that we still know relatively little about the influence of alternatives to smoking traditional cigarettes on our health, both in the systemic and local context, and published research results often remain contradictory.

In our experiment, the study groups consisted of both traditional cigarette smokers and users of modern methods of nicotine delivery to the body: e-cigarettes and heated tobacco products. Due to numerous modifications and marketing campaigns, the above-mentioned "new" devices that supply nicotine to the body are gaining enormous popularity among young people, leading to "renormalisation" of the smoking habit (DeAtley et al., 2022; Harlow et al., 2022; Jankowski et al., 2022). Their high popularity is influenced by the fact that there are many flavors of e-cigarette liquids/cigarette sticks for heat-not-burn products on the market (over 8,000) (Xu et al., 2022). Research shows that the menthol flavor in modern devices that deliver nicotine to the body is the most common choice among young smokers (Leventhal et al., 2019). It is worth mentioning that in most countries, regulations prohibit the sale of traditional flavored cigarettes, and similar restrictions are planned for e-cigarettes and heat-not-burn products.

For the above reasons, we qualified only young, generally healthy adults (under 30 years of age) using only one source of nicotine to participate in the study (we excluded those who can be considered mixed-smokers). Smokers of modern devices that delivered nicotine to the body used the menthol flavor. The duration of the participants' addiction ranged from 1 to 3 years. Unstimulated saliva was used because its collection is easy, non-invasive, and quick. Its measurement does not require special equipment or expertise (Malamud, 2011; Dawes and Wong, 2019). To the best

of our knowledge, this experiment is the first to evaluate the behaviour of a wide range of immune markers in the unstimulated saliva of smokers of as many as 3 (currently the most popular among young people) methods of supplying the body with nicotine. The results of our study appear all the more interesting given the widespread stereotype that e-cigarettes and heat-not-burn products are "healthier" alternatives to traditional cigarettes. The use of both alternative nicotine-delivery devices seems to clearly inhibit the local immune response in the unstimulated saliva of smokers, while smoking traditional cigarettes only slightly intensifies the inflammatory response compared to non-smokers. The results obtained should be viewed as a summary of the processes occurring at early stages of immune dysfunction in young, generally healthy individuals as a result of smoking.

The negative effects of smoking traditional cigarettes on oral health have been well documented to date. It is known that long-term smoking of traditional cigarettes affects the quantitative and qualitative composition of saliva (decreased secretion/buffering capacity, altered bacterial microflora with predominance of anaerobic bacteria, imbalance in the salivary redox status and inflammation) (Voelker et al., 2013; Animireddy et al., 2014; Zięba et al., 2022, 2024).

Cigarette smoke from traditional cigarettes induces an inflammatory response in a short period of addiction, which was manifested in our experiment by elevated concentration of INF-γ observed in traditional smokers. As indicated by the study of Rahimi et al. (2018), prolonging the time of exposure to tobacco smoke intensifies the immune system response, which indicates an increase in the concentration of subsequent proinflammatory cytokines (IL-2). This theory is confirmed by the positive correlation between the duration of smoking and the content of INF-γ in the unstimulated saliva of subjects (Rahimi et al., 2018). In light of the evidence it appears that the boost of the immune system reaction is caused by components of cigarette smoke other than nicotine. According to the results of studies, cadmium present in inhaled cigarette smoke is responsible for increased concentration of proinflammatory IL-1α or COX-2, PGE2 and IL-6 in human lung cells (Martey et al., 2004; Odewumi et al., 2015; Lim et al., 2019; Paniagua et al., 2019).

As mentioned before, both e-cigarettes and heat-not-burn products weaken the local immune response of unstimulated saliva of smokers compared to non-smokers and smokers of traditional cigarettes. Smokers using heat-not-burn tobacco systems had significantly lower levels of only some of the salivary cytokines (TNF-α, HGF, IL-1RA, IL-8, IL-16, IL-18, MIF) as well as chemokines (Gro-α, MCP-1, SCF, MIG, IP-10), and growth factors (G-CSF, TRAIL/M-CSF) assayed, compared to subjects without the smoking addition. Similarly, electronic cigarette users presented a significant decrease in the levels of salivary cytokines (TNF-α, IL-1β, IL-1RA, IL-8, IL-16, IL-18, MIF), chemokines (Gro-α, MCP-1, MIG, IP-10), and growth factors (GM-CSF, TRAIL, M-CSF) compared to those who never smoked. It is also worth mentioning that we did not observe any statistically significant differences in salivary cytokine/chemokine/growth factor levels between the group of heat-not-burn product users and e-cigarette smokers, which may suggest that their mechanism of acting on the local immune response system is similar. Moreover, the group of heat-not-burn tobacco system smokers showed lower levels of salivary cytokines (IL-16, HGF), chemokines (Gro-α, MCP-1, SCF, MIG, IP-10), and growth factors (G-CSF, TRAIL) compared to traditional cigarette smokers. In the saliva of e-cigarette smokers, we also demonstrated significantly lower concentrations of cytokines (IL-1RA, IL-8, IL-1), chemokines (MCP-1, Gro-α, MIG, IP-10), and growth factors (G-CSF, TRAIL) compared to traditional cigarette smokers. The observed inhibitory effect on the synthesis or the release of the studied cytokines from cells may be caused by the influence of the menthol additive of e-cigarettes and heat-not-burn.

Some data suggest that the menthol flavor contained in modern nicotine delivery devices may modulate cytokine expression. Xu et al. (2022) showed a strong reduction in the tested inflammatory factors in the serum of smokers of menthol-flavored e-cigarettes. Similarly to our study, the concentration of M-CSF and C5 in serum was significantly reduced in the group smoking e-cigarettes containing a mixture of nicotine and menthol compared to the control group receiving carrier and the group smoking carrier + nicotine (Xu et al., 2022).

The study performed by Sayed et al. (2021) also showed lower levels of IL-1 receptor antagonist (IL-1Ra) in saliva and Gro-α in the sputum. Reduced IL-1Ra levels in saliva may indicate an early stage of gingivitis. Because IL-1Ra has an inhibitory effect on IL-1, reduced levels may play a role in the subsequent progression of pulpitis and periodontitis (Rawlinson et al., 2000; Lu et al., 2002). On the other hand–GRO-α, a member of the CXC chemokine family, is responsible for induces neutrophil chemotaxis (Meyer-Hoffert et al., 2003). Reduction of GRO-α levels in sputum may suggest increased susceptibility to respiratory infections due to the immunocompromised state.

Supplementary to data concerning changes in inflammatory cell levels, there is a discovery indicating that the use of e-cigarettes leads to alterations in the oral microbiome, affecting both microorganisms and host cells (Chopyk et al., 2021). Studies demonstrate that e-cigarette use has an adverse impact on oral health. It is observed that circulating monocytes in e-cigarette users exhibit phenotypic changes indicative of inflammation in response to reduced e-cigarette use (Sayed et al., 2021). Following stimulation with bacterial lipopolysaccharide, decreased release of IL-8 and IL-6 has been observed, suggesting a limited capacity for an effective response to bacterial infection (Sayed et al., 2021).

The cytotoxicity induced by flavoring agents used in e-cigarettes has also been assessed on cell lines and in humans. It has been found that repeated exposure to menthol significantly reduces cell viability (Rickard et al., 2021). Therefore, further research is necessary to understand the mechanisms of toxicity of flavoring agents and chemical combinations present in modern nicotine-delivering devices.

Perhaps not insignificant is the concentration of nicotine, which is considerably higher in e-cigarettes and heat-not-burn products than in traditional cigarettes (Morean et al., 2016; Romberg et al., 2019).

Nicotine is a low-lipid molecular protein that affects cell function via nicotinic acetylcholine receptors (nAChRs). Nicotine has been shown to bind to nAChR, thus reducing the expression of TNF-α through an α7 nAChR/MyD88/NF-κB pathway in HBE16 human epithelial cell line (Churg et al., 2002; Demirjian et al., 2006). The nAChR receptor has been proven to regulate the immune response primarily via nerve X, which is referred to as the

"cholinergic anti-inflammatory pathway." Nerve X releases acetylcholine which is a cholinergic agonist of the aforementioned receptor (Shytle et al., 2004; de Jonge and Ulloa, 2007). However, as shown in numerous studies, nicotine is much more potent in reducing proinflammatory factors and inflammatory signals (Demirjian et al., 2006; Li et al., 2011). Naturally, the concentration of nicotine must be high enough to displace acetylcholine from its receptor. Nicotine activates the α7 receptor of the nAChR subunit, thereby inhibiting the expression of inducible nitric oxide synthase and nitric oxide via the mitogen-activated protein kinase (MAPK)/NF-κB signalling pathway (Carlisle et al., 2007). Moreover, nicotine has been shown to reduce IL-8, IL-1, and PGE 2 from human epithelial cells after stimulating the α7 subunit of nAChR. On the other hand, according to the available studies, nicotine stimulates neutrophils to produce IL-8, and induces endothelial cells to produce ICAM-1 and VCAM-1, both via nAChR activation, which, according to the authors, requires further studies (Sugano et al., 1998; Dowling et al., 2007; Li et al., 2011).

The study by Mokeem et al. (2018) suggests less harmful effects of smoking electronic cigarettes. In this experiment, the concentrations of the proinflammatory cytokines IL-1β and IL-6 in the whole saliva of smokers of traditional cigarettes were significantly higher than the levels obtained from EC (electronic cigarette) users and non-smokers. Interestingly, the levels of the tested interleukins in EC smokers reached similar values to those of non-smokers. On the other hand, Ye et al. (2020) found elevated levels of the inflammatory marker PGE2 (prostaglandin 2) in traditional smokers compared to EC users and the non-smoking group. In contrast to the above-mentioned authors, Faridoun (2019); Singh et al. (2019) showed a significant increase in IL-1β in e-cigarette smokers. However, it should be mentioned that in the said works the duration of addiction was longer, the average age of the study participants was higher, and the presence of periodontal disease was taken into account, which is not without an effect on the local immune response system.

Although in our research we did not observe significant differences in dental characteristics (Table 1) due to the young age of the participants and the pilot nature of the study, it should be emphasized that changes in the local immune system may manifest clinically in the future in people with longer history of addiction. Smoking is considered the most important determinant increasing the risk of periodontal disease (by as much as 85%), it is also responsible for the disruption of the oral immune system (Leite et al., 2018). People with a long history of smoking are characterized by deeper probing depth as well as greater loss of connective tissue attachment, bone resorption and tooth loss than non-smokers (Johnson and Hill, 2004). The diverse effects of cigarette smoking on host-pathogen interactions in the oral cavity lead to a decrease in cell-mediated and humoral immune responses, promotion of infection with microbial pathogens, they also interfere with antimicrobial therapies, and strengthen antimicrobial resistance (Sopori, 2002). It is postulated that "proper" cytokine production results in protective immunity, while "improper" cytokine production leads to tissue destruction and progression of periodontal disease (Gemmell and Seymour, 2004).

Lower concentrations of Il- 16 in gingival crevicular fluid were found in smokers with periodontal disease compared to the healthy control, and this cytokine correlates with disease severity in smokers (Tsai et al., 2005). Reduced production of the pro-inflammatory cytokine IL-18, in turn, can lead to uncontrolled bacterial and viral infections of the oral cavity (Orozco et al., 2007). In addition, being a smoker significantly affects the failure rate of implant treatment, the risk of postoperative infection and marginal bone loss as well as chronic irritation of the mucosa by components of tobacco smoke (which can lead to oral ulceration) (Zuabi et al., 1999; Naseri et al., 2020; Alade et al., 2022). The above may explain by reduced concentration of HGF in the saliva of smokers compared to the control group which we demonstrated in our research. It is well known that HGF and its receptor, MET, play a key role in promoting tissue repair, supporting osteointegration of implants and also inhibiting inflammation by improving migration and proliferation of mesenchymal stem cells (Wang et al., 2017). On the other hand, TNF-α which is a pro-inflammatory cytokine, serves as a mediator of the immune response, helping to eliminate cancer cells. Reduced levels of it may be responsible in the future, for developing precancerous lesions or oral cancers (Li et al., 2011; Singh et al., 2014).

Our research has identified several limitations. Firstly, the small size of our participant group necessitates that this study be viewed as pilot study. We ensured that participants in both the study and control groups were matched for not having systemic diseases and other pertinent factors influencing, which resulted in a smaller sample size.

Additionally, the relatively young age and short smoking histories (up to 3 years) of our participants limit the generalizability of our findings to older or long-term smokers who may experience distinct oral health ramifications. Consequently, our results might not encompass the entire spectrum of smoking-related oral health effects, especially among older individuals with prolonged smoking durations.

It is important to note that we did not differentiate between heavy and light smokers in this investigation.

5 Conclusion

1. Modern nicotine delivery devices, namely, e-cigarettes and heat-not-burn products, inhibit the local inflammatory response in the oral cavity of young adults with a smoking addiction lasting no longer than 3 years.
2. Both e-cigarettes and heat-not-burn products appear to act via a similar mechanism on the immune response system of unstimulated saliva.
3. Smoking traditional cigarettes slightly induces the local salivary immune response of young adults with a smoking addiction lasting up to 3 years.
4. Disruption of local immune response in the oral cavity, developing from smoking traditional cigarettes, e-cigarettes, or heat-not-burn products, may have a negative effect on smokers' oral health in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by the Bioethics Committee of the Medical University of Bialystok. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

SZ: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Resources, Visualization, Writing–original draft, Writing–review and editing. MM: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Software, Visualization, Writing–review and editing. BA: Data curation, Formal Analysis, Project administration, Writing–review and editing. AP: Data curation, Investigation, Project administration, Visualization, Writing–review and editing. MS: Data curation, Investigation, Project administration, Writing–review and editing. GL: Project administration, Visualization, Writing–review and editing. RL: Formal Analysis, Visualization, Writing–review and editing. SK: Data curation, Software, Visualization, Writing–review and editing. AZ: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing–original draft, Writing–review and editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2024.1404944/full#supplementary-material

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REVIEW
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Ethanol- and Cigarette Smoke-Related Alternations in Oral Redox Homeostasis

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Alcohol abuse as well as smoking cigarettes has been proven to negatively affect the oral environment. The aim of this work was to provide a systematic review of the literature on the influence of ethanol and cigarette smoking on oral redox homeostasis. A search was performed for scientific articles indexed in the PubMed, Medline and Web of Science databases. We identified 32,300 articles, of which 54 were used for the final review, including the results from 2000 to 2021. Among the publications used to write this article, *n* = 14 were related to the influence of alcohol consumption (clinical studies *n* = 6, experimental studies *n* = 8) and *n* = 40 were related to the influence of smoking (clinical studies *n* = 33, experimental studies *n* = 7) on oral redox homeostasis. The reviewed literature indicates that alcohol abusers and smokers are more likely to suffer from salivary gland dysfunction, as well as develop precancerous lesions due to DNA damage. Compared to alcohol abstainers and non-smokers, alcohol drinkers and smokers are also characterized by a deterioration in periodontal health measured by various indicators of periodontal status. In summary, alcohol abuse and smoking are associated with disrupted oral redox homeostasis, which may lead not only to tooth loss, but also contribute to various adverse effects related to mental health, digestive processes and chronic inflammation throughout the human body.

Keywords: smoking, alcohol, oxidative stress, free radicals, salivary antioxidants

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INTRODUCTION

Molecular oxygen is an essential element of life for aerobic organisms. However, its incomplete reduction leads to the formation of reactive oxygen species (ROS), such as hydrogen peroxide H_2O_2, superoxide radical anion ($O_2^{\cdot-}$), hydroperoxyl radical ($HO_2^{\cdot}$), hydroxyl radical ($^{\cdot}OH$), nitric oxide ($NO^{\cdot}$) as well as singlet oxygen ($^1O_2$), peroxyl radical ($LOO^{\cdot}$), lipid hydroperoxide (LOOH), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl) and ozone (O_3) (Birben et al., 2012). At physiological concentrations, ROS play important functions in cells, including participating in intra- and intercellular signal transduction as well as exerting toxic effects on phagocytosed bacteria, parasites and tumor cells (Gołąb et al., 2002).

Aerobic organisms have developed mechanisms protecting them against the effects of an excessive supply of ROS and their reactions with cellular components. According to the

definition: "antioxidants are compounds that, when present in low concentration compared to an oxidized substrate, can inhibit the processes of that substrate oxidation, scavenge or detoxify the already formed ROS, and repair ROS-induced damage of a target molecule" (Waddington et al., 2008). These include both low molecular weight antioxidants (uric acid, ascorbic acid, reduced glutathione (GSH), lactoferrin, amylase, thioredoxin, methionine) and enzymatic proteins (superoxide dismutase (SOD), catalase (CAT), peroxidase (Px), glutathione peroxidases (GPx), heme oxygenase, NADPH oxidoreductases).

The state in which the overproduction of ROS exceeds the body's antioxidant capacity is called oxidative stress (OS). The effect of non-neutralized ROS can be temporary or even permanent changes in the structure of DNA, RNA, proteins, lipids and glycoconjugates. It has been demonstrated that oxidation of amino acid residues of a polypeptide chain leads to its defragmentation, formation of cross-links within one or between several protein chains, and modification of the amino acid itself (Stadtman and Levine, 2003; Ponczek and Wachowicz, 2005). A thoroughly researched free radical process is the phenomenon of lipid peroxidation. The biological consequence of lipid peroxidation is damage and depolarization of cytoplasmic and mitochondrial membranes: (Kalra et al., 1988, 1990; Kalra, 1989) and cell damage, as well as generation of further free radicals in the cell (Nigam and Schewe, 2000; Kang and Hamasaki, 2003; Knaś et al., 2012). ROS reactions with DNA result in damage to single nitrogen bases, breaks in the DNA strand and formation of adducts (Williams and Jeffrey, 2000; Cadet, 2003). These ROS effects at the cellular level result in cell death, accelerated ageing, neoplastic transformation and cell proinflammatory responses. These processes constitute the cellular basis for ROS- mediated diseases (Hopkins, 2017).

The oral cavity can be defined as the site of exchange between the body and the external environment, a site characterized by high complexity of ROS exposure. These exposures can result from ionizing and ultraviolet radiation as well as air and food pollution. Other sources of ROS in the oral cavity include medications, dental materials and treatment procedures, ongoing inflammation and bacteria (Waszkiewicz et al., 2012b; Zieniewska et al., 2020). Ethanol intake and smoking cigarettes are also significant sources of ROS. The latter two sources of exogenous ROS constitute a particular global problem in both health and economic terms. According to the WHO, people over 13 years of age consume approximately 6.2 L of pure ethanol/year, which amounts to 13.5 g of ethanol/day (WHO, 2014). Alcohol has been classified by the International Agency for Research on Cancer as a human carcinogen, and its consumption has been recognized as an increased risk factor for liver, breast, colorectal and upper aerodigestive tract cancers (Stornetta et al., 2018). Moreover, it is estimated that about 6–7% of global mortality is associated with alcohol use (WHO, 2014). Similarly alarming are the data on tobacco smoking. Globally, there are approximately 1.3 billion smokers and this number is predicted to rise to 1.7 billion by 2025 (Babizhayev and Yegorov, 2011). It should be emphasized that smoking causes the deaths of 5 million adults per year, including hundreds of thousands of so-called premature death cases (Babizhayev and Yegorov, 2011).

Although smoking can disrupt oral homeostasis, there is a lack of review summarizing current knowledge on the effects of alcohol and tobacco smoke on oral redox balance. Our manuscript is the first to systematically discuss the effects of ethanol and cigarettes on the antioxidant systems, oxidative and nitrosative stress, and mitochondrial function in the oral cavity.

It has been proven that OS may lead to morphological changes in the parenchyma of the salivary glands, which results in the decrease of the salivary secretion and biochemical changes in the saliva (Zalewska et al., 2014, 2015; Knaś et al., 2016; Kołodziej et al., 2017a,b). This state can lead to the development of other oral diseases such as xerostomia, burning mouth syndrome, periodontitis and precancerous lesions. Increased concentration of protein, lipids, and DNA oxidative damage markers, as well as changes of concentrations/activity of enzymatic and non-enzymatic antioxidants, were observed in the saliva and/or gingival fluid of patients with periodontitis, diabetes, insulin resistance and dementia (Choromańska et al., 2015; Wang et al., 2015, 2017; Zalewska et al., 2015; Knaś et al., 2016; Kołodziej et al., 2017a,b). Therapy with antioxidants has been suggested as a new therapeutic option for the above-named patients, however, sources of free radicals in the oral cavity are still not known exactly.

MATERIALS AND METHODS

Search Strategy

The literature review was conducted from 2000 to January 2021 using the PubMed, Medline and Web of Science databases. The available literature was browsed based on the following keywords: [alcohol and ROS], [alcohol and salivary oxidative stress] [alcohol and periodontal tissue], [alcohol and cancer], [binge drinking], [smoking and salivary oxidative stress], [smoking and ROS], [smoking and oral cancer], [smoking and oral inflammation], [smoking and periodontal health]. The inclusion and exclusion criteria are presented in **Table 1**.

Data Extraction

The preliminary data extraction was performed based on the evaluation of titles and abstracts of manuscript independently by three researchers (SZ, AZ, MM). Then, texts of all the papers selected at the first stage of our work were reviewed and the

TABLE 1 | Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
1. Articles written in English only. 2. Publications on the effects of salivary free radicals generated through smoking and alcohol consumption on oral health 3. Results obtained from experiments participated by humans as well as experimental works, including *in vitro* studies. 4. Meta-analyses.	1. Publications written in a language other than English. 2. Publications that did not evaluate the effects of salivary free radicals generated by smoking and alcohol consumption on oral health 3. Surveys. 4. Case descriptions.

studies meeting the inclusion and exclusion criteria were used for the final analysis. Determination of the reliability level of the researchers was performed using Cohen's kappa coefficient (κ) which amounted to κ = 0.93. In order to ensure data quality, all publications were evaluated in terms of methodology and the following variables were distinguished: authors, year of publication, study design, size of the study population, inclusion and exclusion criteria, duration of the study and research results.

RESULTS

Of approximately 32,300 publications, 54 were classified as meeting the inclusion and exclusion criteria. The literature review revealed 32,331 works from the MEDLINE (PubMed) library, of which 31,651 were excluded due to the title. A total of 680 abstracts were read, 498 of which met the inclusion and exclusion criteria. Among the qualified articles, 128 turned out to be irrelevant for the subject of our review. Therefore, 54 papers were finally included (**Figure 1**).

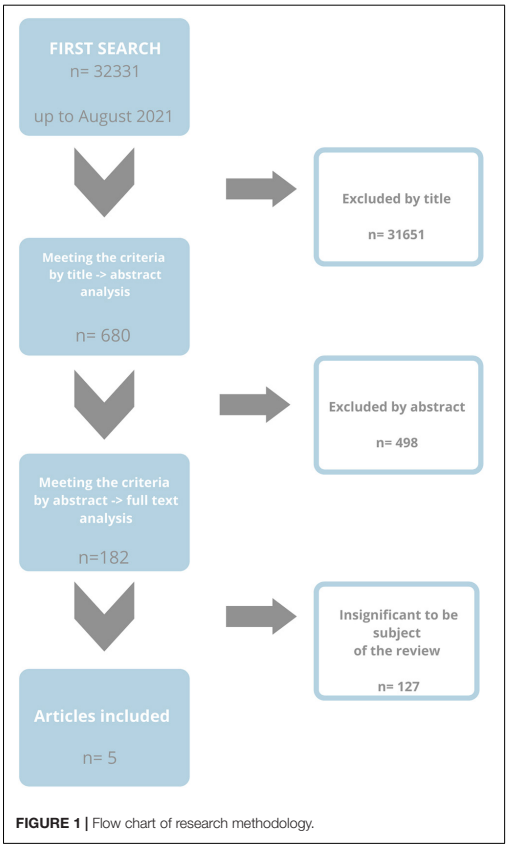

FIGURE 1 | Flow chart of research methodology.

Alcohol and Reactive Oxygen Species

Ethanol has a form of non-ionized, small and chemically inert molecules. Due to these characteristics, it diffuses very easily into saliva and oral tissues after ingestion. Post-alcoholic damage to oral tissues results either from direct effects of ethanol or indirectly via its metabolites: acetaldehyde, reactive oxygen and nitrogen species (ROS, RNS) as well as fatty acid ethyl esters. The latter are non-oxidative metabolites of ethanol (Waszkiewicz et al., 2011).

The role of ethanol in terms of ROS/RNS, and thus oxidative stress, is ambiguous. Ethanol is one of the most effective "scavengers" of $^{\cdot}OH$ which is the most reactive oxidant able to oxidatively modify all substances present in the body. The human body does not have endogenous antioxidant systems capable of neutralizing this free radical (Halliwell and Gutteridge, 2006). However, for ethanol to neutralize $^{\cdot}OH$ we would have to consume it in quantities many times exceeding the lethal dose.

In the human body, ethanol is oxidized to acetaldehyde and then to acetate. This process is called ethanol intoxication. The former reaction is catalyzed mainly by alcohol dehydrogenases (ADH). It is worth mentioning that immediately after consumption, the concentration of ethanol in saliva is higher than in plasma. Half an hour after consumption, salivary concentration of ethanol is 10 times higher compared to its plasma level (Waszkiewicz et al., 2008b). The main metabolite of ethanol, acetaldehyde, reaches 10–40 times higher concentrations in saliva than in plasma, regardless of the ethanol dose ingested (Lachenmeier and Sohnius, 2008). Such high levels of acetaldehyde are caused by the intensification of ethanol metabolism in saliva as a result of ADH activity of the oral mucosa, as well as of bacterial ADH activity (Salaspuro, 2003). In the course of chronic alcoholism or in case of consuming large amounts of ethanol at a time, ADH are supported by microsomal processes of ethanol metabolism, which is undoubtedly affected by increased NADH reoxidation. Higher level of NADPH is a defense reaction of the body aimed at accelerating ethanol oxidation and eliminating it from the body (French, 1989). In microsomes, ethanol is oxidized by cytochrome P450, particularly by its isoenzyme 2E1 (CYP 2E1) and microsomal oxidoreductase independent of cytochrome P450, consuming iron ions (Dicker and Cederbaum, 1991). Furthermore, consumption of a high dose of ethanol at a time leads to a higher than normal one-electron oxygen reduction with the formation of $O_2^{\cdot}$, which, in turn, damages mitochondria leading to hypoxia of the organ (Nordmann et al., 1992). Another enzyme that oxidizes ethanol is CAT which exhibits peroxidase properties in the presence of ethanol. Of the enzymes mentioned, only CAT – in the process of ethanol oxidation – does not produce ROS. On the contrary, it consumes H_2O_2. Alcohol dehydrogenase oxidizes ethanol with simultaneous formation of $HO_2^{\cdot}$, while the reaction catalyzed by microsomal enzymes generates the most dangerous $OH^{\cdot}$ (Bondy, 1992)! By activating cytochromes CYP2E1 and salivary cytochromes CYP1A2 and CYP3A4 (Waszkiewicz et al., 2013) as well as undergoing enzymatic (xanthine oxidase and aldehyde oxidase) conversion to acetic acid, the resulting

acetaldehyde increases the ROS pool in the form of $O_2^{\cdot}$ and H_2O_2 (Niemelä, 2007).

Furthermore, ethanol has been demonstrated to promote the release of low molecular weight iron from bound intracellular reserves, which facilitates the Fenton reaction (Rouach et al., 1990). It can also induce the conversion of xanthine dehydrogenase to xanthine oxidase (Bondy, 1992).

Binge Drinking Related Reactive Oxygen Species/Reactive Nitrogen Species and Saliva and Salivary Glands

According to the definition, 5 or more drinks for men and 4 or more for women, consumed within a 2-h period, are referred to as binge drinking. Such manner of ethanol intake results in blood concentrations of this alcohol of 0.08 g/dL or higher. Binge drinking is a major social problem as it leads to injuries, violence, chronic diseases, unwanted pregnancies, fetal alcohol spectrum disorders (FASDs) and other pregnancy complications (Waszkiewicz et al., 2008a). As demonstrated by, admittedly, few studies, this method of drinking results in salivary gland dysregulation and oral redox imbalance. Ferreira et al. (2021) investigated the effect of binge drinking during critical periods of pregnancy on the salivary glands of offspring in rats. Ethanol was administered during gestational days: GD6, GD7, GD8, i.e., during gastrulation and neurulation. The results of their study demonstrated that the parotid glands of the offspring of rats was more vulnerable to alcohol binge drinking. This greater damage was reflected in considerably intensified process of lipid peroxidation and decreased antioxidant capacity compared to the parotid glands of control group rats. In addition to boosted lipid peroxidation, the results of the ethanol-exposed group indicated a reduction in the peroxyl radical scavenging reaction in the parotid glands, which, according to the authors, appears to be one of the causes of the morphometric changes observed in the parenchyma of these salivary glands. Similar significant changes related to the phenomenon of lipid peroxidation were observed in the submandibular salivary glands, but they were approximately 1.5 times less severe than in the parotid glands. In contrast, the antioxidant capacity of the submandibular glands was not altered, which, according to the researchers, confirms the low intensity of OS in the cells of these salivary glands. The effect of episodic and intensive ethanol intoxication in a 3-day/week binge pattern on the redox balance of the parotid and submandibular glands of rats during their adolescence to young adult period showed a higher level of lipid peroxidation rate in the parotid glands of the ethanol group after 1 and 4 weeks of alcohol consumption (Fagundes et al., 2016). Submandibular glands of ethanol-treated group revealed considerably higher concentration of malondialdehyde (MDA) only after the first week of ethanol intake in a binge model. The lack of change during the further period of the experiment suggests adaptation of the glands to oxidative injury in a period of chronic binging. As suggested by the authors of both study groups, the observed differences in redox imbalance between the salivary glands of rats exposed to ethanol intake in a binge model were caused by the different morphology of the two glands as well as the fact that the parotid glands, unlike the aerobic submandibular glands, have an aerobic metabolism, which predisposes them to greater exposure and damage by ROS. In our study, we attempted to explain the effects of acute ethanol intoxication in occasional drinkers on antioxidant salivary proteins. We demonstrated that even a single ingestion of a relatively large but still tolerable dose of ethanol by young men significantly reduces the activity of peroxidase and minute secretion of lactoferrin in saliva, which undoubtedly affects the overall antioxidant capacity of the saliva (Waszkiewicz et al., 2012b). It is noteworthy that salivary peroxidase is one of the most important antioxidants synthesized by the salivary glands, although it constitutes only 0.01% of the total salivary protein content. Taking into account the antibacterial properties of peroxidase and lactoferrin, the observed reduction in their activity/minute secretion may predispose the oral mucosa to more frequent infections (Zalewska et al., 2013, 2014).

Chronic Alcohol Intoxication Related-Reactive Oxygen Species/Reactive Nitrogen Species and Oral Health

Alcoholism has been recognized as a disease by the World Health Organization, and thus is included in the International Statistical Classification of Diseases and Related Health Problems (ICD-10) and the Classification of the American Psychiatric Association (DSM-V) as one of mental and behavioral disorders caused by the use of psychoactive substances. Alcohol dependence syndrome is a group of physiological, behavioral and cognitive phenomena, among which drinking alcohol dominates over other behaviors previously more important to the person. The main symptom of alcohol dependence syndrome is a loss of control over the amount of alcohol consumed and a constant need/desire to drink it (known as alcohol craving). Approximately 2% of the population exhibits chronic alcohol dependence (Waszkiewicz et al., 2012a,b,c). Devastating effects of alcohol dependence obviously include the negative consequences on oral health, thus also the redox balance of the salivary glands, mucosa and periodontal tissues.

Ethanol- Derived Reactive Oxygen Species and Saliva and Salivary Glands

In the research of Campos et al. (2005) ethanol was applied to animals participating in the experiment in drinking water according to the model of moderate alcohol intoxication. Additionally, the authors decided to evaluate the effect of the exogenous antioxidant, α- tocopherol, in order to determine its influence on ethanol-associated salivary gland alteration. The observed changes in oxido-reductive impairment, as in the binge drinking models, depended on the type of salivary glands of rats.

The authors demonstrated increased activity of SOD and GPx in the homogenates of the parotid glands of ethanol-intoxicated rats, but no change in CAT activity. Simultaneous administration of ethanol and α-tocopherol caused these activities to drop to a level observed in the control group. Interestingly, none of the analyzed antioxidant enzymes revealed significant changes in the submandibular glands of ethanol-exposed rats as well as rodents exposed to ethanol combined with α-tocopherol

compared to the control rats. The authors conclude that the observed changes in the activity of antioxidant enzymes in the parotid glands are due to the fact that this type of salivary gland has a greater abundance of antioxidant activity vs. the submandibular gland. The observed increase in enzyme activity may also be a defensive reaction of these glands to the increasing number of ethanol-derived ROS. Unfortunately, as further results of studies indicate, increased activity of antioxidant enzymes was not enough to counterbalance ROS, since significantly higher concentrations of thiobarbituric reactive substances (TBARS, products of lipid peroxidation) and carbonyl groups (products of protein oxidation) were observed vs. the control group. Administration of α-tocopherol considerably reduced the process of lipid peroxidation, while it could not inhibit the boosted process of protein oxidation in comparison with the group consuming ethanol. The effect of α-tocopherol on the process of lipid peroxidation in ethanol-treated group is most probably caused by the participation of this antioxidant in the synthesis of prostaglandins. It has been demonstrated that ethanol reduces prostaglandin synthesis in the sublingual and submandibular glands of rats (Wu-Wang et al., 1991), which proves the self-regulatory modulation of receptor binding induced by high ethanol concentration (Wu-Wang et al., 1992). α-tocopherol antagonizes inhibitory effect of ethanol on prostaglandin synthesis, thereby reducing the negative influence of ethanol on the parotid glands. Ethanol consumption in a model of moderate ethanol dependence did not induce oxidative stress in the submandibular glands of rats, as demonstrated by the lack of changes in the measurable results of lipid peroxidation and protein oxidation. The latter results in particular show that rat parotid glands are more damaged than the submandibular glands due to ROS activity generated by chronic alcohol consumption (Campos et al., 2005).

The study by Waszkiewicz et al. (2012b) indicated a significant increase in Px activity after chronic alcohol intoxication in subjects additionally addicted to nicotine in relation to the control group. Despite the well-known fact that ethanol induces changes in mucosa permeability, which facilitates the penetration of ROS/RNS from cigarette smoke, Waszkiewicz et al. (2012b) did not observe any additive effect of nicotine on Px activity. Moreover, they demonstrated a positive correlation between Px activity and the number of days of alcohol intoxication and no such correlation between Px activity and the number of cigarettes smoked or the duration of nicotine dependence.

According to the authors, these observations prove that the increase in Px activity is a consequence of chronic OS caused by ethanol action rather than smoking. On the other hand, higher Px activity may result from an influx of lymphocytes into damaged oral mucosa, as evidenced by increased periodontal indices (Waszkiewicz et al., 2012b). Moreover, based on the performed analyses, the authors suggest that salivary Px has a high diagnostic value connected with chronic alcohol abuse, as it is helpful in differentiating between alcoholics and the control group (Waszkiewicz et al., 2012b). However, acute ethanol intoxication in chronic drinkers significantly decreases the minute secretion of lactoferrin (Waszkiewicz et al., 2008b), glutathione S-transferase and total antioxidant capacity (TAC) of saliva (Peter, 2013). Reduced parameters of the aforementioned components of the

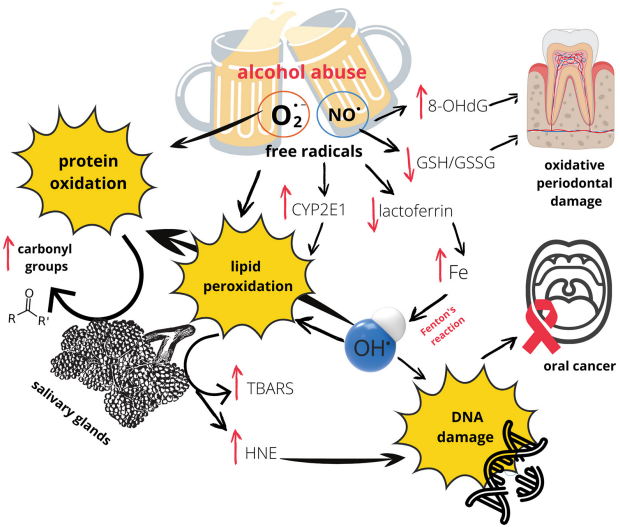

FIGURE 2 | Effects of alcohol abuse on oral redox homeostasis. 8-OHdG, 8-Hydroxy-2′-deoxyguanosine; CYP2E1, Cytochrome P450 2E1; GSH/GSSG, glutathione/glutathione disulfide; HNE, 4 hydroxynonenal; $O_2^{\cdot -}$, superoxide anion radical; $NO^{\cdot}$, nitric oxide; TBARS, thiobarbituric acid reactive substances.

antioxidant barrier prove a high increase in ROS production, which consistently utilizes antioxidants in ROS combating. Lactoferrin deficiency with accompanying inflammation may be a factor increasing iron bioavailability, which may lead to enhanced Fenton reactions and boosted hydroxyl radical production. OS process and its negative effects on the redox balance of saliva appear to be partially reversible once the source of ROS generation is removed. Peter (2013) demonstrated that only a one-month period of alcohol withdrawal significantly increases glutathione S-transferase and TAC, although not to the levels observed in the control groups.

Oral Cancer and Precancer and Ethanol-Derived Reactive Oxygen Species/Reactive Nitrogen Species

The mechanism of ethanol-induced carcinogenesis is poorly defined; therefore, studies are being conducted to investigate it (Hecht, 2003). Warnakulasuriya et al. (2007) examined the generation and distribution of protein oxidation adducts with acetaldehyde and lipid peroxidation products: MDA and hydroxynonenal (HNE), as well as ethanol-stimulated expression of CYP2E1 in the oral mucosa of the alcohol-dependent patients diagnosed with leukoplakia (pre-cancerous condition) and squamous cell carcinoma (SCC). Positive staining toward the formation of protein adducts in oral tissues was observed in 61% for acetaldehyde, 57% for MDA and 80% for HNE in the dysplastic or cancerous biopsy specimens taken from the oral cavity. When the samples were differentiated into pre-neoplastic and neoplastic, MDA protein adducts were more marked in the first group. In contrast, patients with SCC were characterized by longer duration of addiction to alcohol. The authors also noted a positive correlation between the amount of alcohol consumed and CYP2E1 expression, which in turn was reflected in the intensity of tissue accumulation of protein adducts from HNE in both groups of patients. It was evidenced that the accumulation of ethanol-induced protein adducts in tissues reveals impairment of mechanisms aimed at removal of complex protein oxidation products. The accumulation of such adducts favors the formation of mutations and may lead to malignancy, as described previously (Guéraud, 2017). The authors postulate taking their results into account in studies on ethanol-induced carcinogenesis and in the comprehensive assessment and treatment of patients who excessively consume ethanol.

The results of Carrard et al. (2013) are contradictory to the above conclusions. These researchers demonstrated that increased cell proliferation with alcohol-related mucosa damage is not directly caused by the oxidative-reductive imbalance in tongue tissue. Interestingly, the authors did not observe OS symptoms in the form of increased concentration of oxidation products, but only changes in the activity of antioxidant enzymes and only in the group of rats exposed to alcohol for 60 days (decreased SOD activity, increased CAT activity). It is not surprising that increased CAT activity coexisted with decreased expression of nuclear factor E2-related factor 2 (NrF2), which is consistent with the previously demonstrated NrF2-independent mechanism of regulating the activity of this enzyme (Hopkins, 2017). NrF2 is a factor that regulates the expression of antioxidant genes and thus the antioxidant response. When bound to a cis-regulatory element, known as an antioxidant response, the element in the promoter region of antioxidant enzyme genes boosts the transcriptional expression (Hopkins, 2017). CAT is an enzyme without an antioxidant response element in its promoter center and it is not subjected to NrF2-mediated transcriptional activation. It is interesting to note that the authors observed a decrease in the concentration of lipid peroxidation products after 14 and 60 days of exposure to ethanol, which, according to them, is evidence of positive modification of cell membranes (Carrard et al., 2009, 2013). The keratinized layer of the tongue epithelium is a tissue consisting of extremely flattened and dehydrated cells. According to the authors, it is possible that high doses of ethanol enhance this protective layer, which has previously been described as "fixative," and improve the epithelial permeability barrier (Du et al., 2000). At 120-day exposure, they observed normalization of all the evaluated OS parameters in the tongue mucosa to the control group levels. However, their findings indicate that the signal for cell proliferation, but not for an increase in alcohol-related epithelial proliferation, may result from elevated concentration of H_2O_2, which, according to them, is reflected by higher CAT activity at day 60 of exposure.

Relationship Between Periodontal Disease and Alcohol-Derived Reactive Oxygen Species

The existence of a correlation between ethanol abuse and the development and severity of periodontal disease prompted a search for the effect of ethanol-induced ROS overproduction on periodontal tissues. Irie et al. (2008) examined the effect of chronic ethanol consumption on the periodontal tissue status and OS with and without ligature-induced inflammation in a rat model. Ethanol consumption increased 8-hydroxy-2′-deoxyguanosine (8-OHdG) concentrations, simultaneously reducing GSH/GSSG ratio in the "healthy"′′ gingiva vs the control group. A similar pattern of results was obtained in the group of rats with induced periodontal disease and exposed to ethanol compared to the rat group with induced periodontal disease. It should be emphasized that although the reduction of GSH/GSSG ratio was at a comparable level in both groups exposed to ethanol, the concentration of 8-OHdG was higher in the group of ethanol-exposed rats with periodontal disease, which proves an addictive effect of this alcohol on gingival oxidative modification and destruction. This finding coincides with the evaluation of the effect of ethanol on periodontal tissue morphology. In the ethanol-exposed group with ligature-induced periodontitis vs. the group with periodontitis, only an influx of multinucleated lymphocytes into the gingival tissue was observed. Exposure of healthy rats to ethanol resulted only in apical migration of junctional epithelium, alveolar bone resorption and influx of multinucleated leukocytes into gingival tissue vs. healthy gingiva, but still more periodontal tissue destruction was observed in the ethanol plus periodontitis group

vs. ethanol group. The results of Tomofuji et al. (2008) are also interesting, even though they do not directly involve the oral cavity. The authors demonstrated that the combination of ethanol and ligature-induced periodontitis caused higher concentrations of hexanoyl-lysine (HEL, lipid hydroperoxide-modified lysine residue formed at the early stage of lipid peroxidation) and 8-OHdG in the rat liver in comparison with ethanol exposure alone. The authors believe that periodontal diseases exacerbate the effect of ethanol on oxidative liver damage, particularly related to lipid peroxidation.

Short-term ethanol (20%) consumption exerts addictive effect only on inducible nitric oxide synthase (iNOS) mRNA expression, but not on ligature-induced alveolar bone loss or on prostaglandin E2 concentration, both enhanced by periodontitis (Dantas et al., 2012). Moreover, the authors observed increased iNOS activity in the gingival tissue after short period of alcohol intake vs. healthy gingiva, which entails an increase in nitric oxide (NO) concentration in the gingiva. It was evidenced that NO promotes osteoclast maturation and enhances bone resorption induced by inflammatory cytokines (Brandi et al., 1995; Ralston et al., 2009). Additionally, ethanol-induced OS is one of the factors involved in the activation of the mammalian target of rapamycin (mTOR), which is essential to premature senescence in cementoblasts and human periodontal ligament cells (Bae et al., 2018). Therefore, the observed lack of changes in alveolar bone volume after exposure of healthy rats to ethanol is quite surprising, according to the authors. They explain their results by the short duration of the experiment and only 20% of ethanol concentration (Dantas et al., 2012). Graphic presentation of the influence of alcohol on oral redox homeostasis (**Figure 2**).

Beneficial Effects of Red Wine Consumption

Research conducted over the past few years have proven the positive effect of polyphenols contained in red wine on oral health, obviously under conditions of moderate consumption of this type of alcohol. It should be mentioned that polyphenols are also contained in many other food products (herbs, spices, fruits and vegetables like apples, carrots, broccoli) as well as in green tea and their positive effect on redox homeostasis has been summarized in many other reviews (Tarascou et al., 2010; Gaur and Agnihotri, 2014; Wojtunik-Kulesza et al., 2020). Antioxidant capacity of polyphenols contained in grapes/wine is much stronger than that of vitamins C and E. One-electron reduction potential of epigallocatechin gallate is almost the same as of α-tocopherol, 550 and 480 mV, respectively (Frei and Higdon, 2003; Maeta et al., 2007). The antioxidant properties of polyphenols result, *inter alia*, from direct inactivation of ROS, activation of antioxidant enzymes (Nagata et al., 1999), ability to chelate metal ions (Chlebda et al., 2010), inhibition of oxidases (Hanasaki et al., 1994; Ursini et al., 1994) and increase in concentrations of uric acid and other low molecular weight antioxidants (Natella et al., 2002).

Varoni et al. (2013) evaluated salivary antiradical capacity and total polyphenol content after consumption of wine (125 mL) and then after 4 weeks of total abstinence 300 mg of non-alcoholic red wine extract (minimum 95% polyphenols). The results of their study indicated that wine consumption (18% ethanol) did not significantly reduce salivary antiradical capacity, which could result from the antioxidant properties of polyphenols counteracting the pro-oxidant properties of ethanol. The authors argue that the lack of increase in salivary antioxidant capacity after wine consumption (presence of antioxidants!) is caused by the possible pro-oxidant properties of polyphenols which attach to the oral mucosa and may undergo oxidation (Varoni et al., 2013). Wine extract increased the antioxidant properties of saliva very efficiently, which was most likely related to the presence of the polyphenols themselves, although their concentration in saliva after wine intake was negligible. Varoni et al. (2013) suggest that polyphenols from the extract, but not from the wine, increase uric acid and glutathione production via unknown mechanisms. The suggestion is too far-fetched, as the authors did not determine salivary concentrations of the aforementioned antioxidants, which could have confirmed their assumption. The researchers observed higher amounts of polyphenols remaining in the oral cavity after the consumption of wine than of the extract, although it should be highlighted that polyphenols from wine were deprived of their antioxidant properties (they were used in scavenging of ethanol-induced ROS). Melatonin contained, for instance, in red wine is characterized by a rich spectrum of activities, including antioxidant and anti-inflammatory effects. It is believed that melatonin can act as a potent antioxidant enhancing mechanisms counteracting oral diseases (Balaji et al., 2015). Unfortunately, according to a study by Varoni et al. (2018), consumption of red wine with high melatonin content did not significantly increase its salivary concentration. However, the authors emphasize numerous limitations of their experiment (the need for versification of red wine melatonin isomers, need for additional measurements of daily salivary melatonin concentrations, which would help to better understand how wine can modify the circadian rhythm of endogenous melatonin) that may potentially influence the obtained results.

Red wine is rich in polyphenols, but also has a very low pH comparable to orange and apple juice, which makes it a beverage of high erosion potential. Carvalho et al. (2021), evaluating the erosion kinetics of red wine and apple as well as orange juice in the presence and absence of acquired pellicle, demonstrated that in both experiments red wine showed the highest surface reflection intensity (lowest degree of erosion of the polished enamel surface) and the highest surface hardness, followed by orange and apple juice. Moreover, the authors observed a nearly linear correlation between the polyphenol content of the tested beverages and intensity of tooth enamel erosion: the higher the polyphenol concentration, the smaller the erosive demineralization. The anti-erosive effect of red wine is most likely caused by polyphenol-induced acquired pellicle modification. According to the authors, polyphenol molecules bind with salivary proteins to form protein-polyphenol complexes that build into the acquired pellicle. Once attached to the pellicle, they form a scaffold to which other salivary proteins can easily attach. Evidence has shown that the protein content of the salivary pellicle modified by red wine consumption is approximately 10

times higher than in the control plate, making it thicker and more resistant to removal (Joiner et al., 2003, 2004; Sierpinska et al., 2013). However, the described pellicle modification occurs only in case of frequent and slow red wine drinking (the drink stays in the mouth for a long time). Such a pellicle provides better protection against low pH of red wine, as evidenced by slight changes in enamel hardness before and after its consumption. The authors underline that white wine, containing about 100 times less polyphenols than red wine, has truly high erosive potential.

NO is not only a free radical involved in pathophysiological processes in the oral cavity, as described above. As a signaling molecule, NO plays very important roles in physiological processes. It has been demonstrated that moderate consumption of nitrate-rich fruit and vegetables is a natural and low-cost way to stay healthy. According to the authors of the experiment, the increase in salivary NO (Takahama et al., 2010) obtained after mixing white wine with saliva is therefore a positive sign. It can increase eradication of microorganisms (Xu et al., 2001) and oral mucosal thickness (Björne et al., 2004), as can be observed in the stomach.

Physiological NO concentrations are also essential in the regulation of oral blood flow (Toda et al., 2012) and proper salivary gland secretion (Modin et al., 1994). It should be mentioned, that saliva is for the oral cavity the same as blood is for the whole body. The correct flow of saliva determines the hydration of the mucosa and provides nutrients and components of specific and non-specific immunity, and thus may have a preventive effect against mucositis and oral infections.

Smoking and Reactive Oxygen Species

Traditional cigarettes are a source of massive amounts of ROS/RNS. The source of the generated ROS/RNS is the cigarette smoke (CS) produced during smoking. CS consists of a main stream (inhaled by the smoker at the time of drawing on a cigarette) and a secondary stream produced while burning the tobacco. The latter poses danger both to the smoker and the environment.

The main stream can be divided into a gas phase and a solid phase, or cigarette tar (a residue that remains on a filter that traps 99.9% of particles with a diameter of over 0.1 micrometer after passing cigarette smoke through) (Valavanidis et al., 2009). The amount of cigarette tar produced depends on the quality of a given cigarette. However, it is estimated that smoking an average cigarette weighing 1 g leads to the formation of 20 mg of tar.

The quality and quantity of ROS/RNS present in the gas and solid phases differ. Tobacco tar provides huge amounts of ROS (about 10^{17} per gram of tar) as well as heavy metals and carcinogenic organic compounds. The main representatives of cigarette ROS are stable free radicals quinone, semiquinone, hydroquinone (Q/QH•/QH_2) (Pryor et al., 1990; Pyror, 1992). These compounds are in mutual balance and have a long half-life. Extraction of the tar phase in aqueous solution leads to the formation of $O_2^{\cdot -}$, H_2O_2 and, as mentioned above, the most dangerous $^{\cdot}OH$ in the following: the QH• radicals reduces O_2 into $O_2^{\cdot -}$, which can dismutate to form H_2O_2 and then with Fe2 + can generate through the Fenton reaction highly oxidizing hydroxyl radicals (Pryor et al., 1983a; Valavanidis et al., 2009). Moreover, ROS of the solid as well as gas phase are responsible for the release of iron from the endogenous enzyme ferritin, and may thus enhance the production of $^{\cdot}OH$ in the aforementioned Fenton reaction. It is interesting to note that concentrations of tar-like substances in cigarettes is similar to that in exhaust particles of diesel engines (Ross et al., 1982). The above-mentioned heavy metals included in cigarette smoke, especially cadmium play an important role in the production of oxygen free radicals (Genchi et al., 2020). Its toxic effect is mainly due to the blockage of the mitochondrial electron transfer chain by impeding the flow of electrons through mitochondrial complex III (main place of production of ROS) (Checa and Aran, 2020). Cadmium changes the activity of mitochondrial proteins by inhibiting the enzymes of the respiratory chain, which in turn leads to mitochondrial swelling and impairment of cellular respiration (Cannino et al., 2009). In addition, it can lead to the release of cytochrome c from the mitochondrion, which is synonymous with the activation of the caspase pathway and the initiation of the process of cellular apoptosis (Checa and Aran, 2020; Genchi et al., 2020). It is estimated that the gas phase of one drag of a cigarette delivers about 10^{15} RO with a low reactivity period into the body (Pryor et al., 1983b). Despite the short reactivity of individual radicals, their high concentration in cigarette smoke persists for more than 10 min. ROS/RNS in the gas phase are formed continuously, and their concentration in the oral cavity increases with the duration of the addiction to smoking. An important role in the generation of free radicals in the gas phase is played by nitric oxide (NO) (Pryor and Stone, 1993) which is responsible for the initiation of a cascade of oxidation reactions. Significant amounts of NO can be found in smoke and therefore, despite its low reactivity, it undergoes constant oxidation to the much more reactive nitrogen dioxide (NO_2). In the next stage, NO_2 reacts with other components of cigarette smoke such as isoprene to form free radicals with an unpaired electron on the carbon atom and peroxyl and alkoxy radicals.

As in the main stream, the side-stream smoke consists of a gas and a solid phase. The secondary stream of CS is estimated to contain even 170 times more ammonia, five times more carbon monoxide and ten times more hydrogen cyanide or nitrogen oxides than the CS main stream. The ROS generated by the secondary smoke are highly reactive despite their short duration period. These implications mean that the passive smoker unknowingly "smokes" several cigarettes a day and inhales massive amounts of ROS generated by the active smoker. It has been evidenced that passive smoking is responsible for serious health consequences for non-smokers, making the secondary smoke even more harmful (Valavanidis et al., 2009).

Cigarette Addiction and Oral Health

Saliva, Salivary Glands and Cigarette-Related Reactive Oxygen Species/Reactive Nitrogen Species

The oral cavity is the first place where cigarette smoke comes into contact with the human body, and this fact that has

an impact on its health. A large panel of papers evaluating the antioxidant capacity of smokers' saliva is available. The results of most studies clearly indicate that smoking cigarettes is accompanied by decreased activity of endogenous salivary enzymatic antioxidants such as SOD, CAT and Px as well as reduced concentration of non-enzymatic endo- and exo-antioxidants: GSH, UA (Nagler) and vitamin C (Klein et al., 2003; Reznick et al., 2003; Nagler, 2007; Abdolsamadi et al., 2011; Falsafi et al., 2016; Arbabi-Kalati et al., 2017; Singh et al., 2018). According to the available evidence, cigarette smoke disturbs the metabolism of trace elements which are cofactors of antioxidant enzymes (copper and zinc as cofactors of SOD, iron as cofactor of CAT), thus reducing their activity (Nobari et al., 2021). It is noteworthy that a significant reduction in salivary Px activity was observed in a group of non-smokers after smoking one cigarette (Reznick et al., 2003). Smoking one cigarette led to a greater decrease of Px activity in the non-smoking group compared to the smoking group. The authors suggest a protective role of higher concentrations of SCN^- ions in the saliva of compulsive smokers. It has been proven that the addition of exogenous SCN^- to the saliva of non-smokers "immunized" to some extent, but only temporarily, salivary Px against damaging effects of cigarette smoke, which is attributed to the extended biological half-life of the molecule (9.5 h) (Azen, 1978; Reznick et al., 2003). However, the exact mechanism of such "protection" has not been demonstrated. Reduced activity of the aforementioned antioxidant enzymes is probably the reason for the significant TAC reduction/TOS increase observed in numerous studies (Charalabopoulos et al., 2004; Abdolsamadi et al., 2011; Bakhtiari et al., 2015; Neshat et al., 2020). Importantly, it was stated in several papers that TAC was significantly lower in the saliva of passive smokers that in active smokers (Azadbakht et al., 2016; Neshat et al., 2020) as well as that TAC in passive smokers was significantly lower compared to non-smokers (Mottalebnejad et al., 2014; Nobari et al., 2021). Nobari et al. (2021) evaluated the effect of intense physical exercise, the side effect of which is extremely intense OS, on the activity of SOD, CAT and Px in groups of physically active and passive subjects exposed and not exposed to cigarettes. The researchers demonstrated that intense training significantly increases SOD, CAT and Px activity, which the authors believe is a positive response to the increasing amount of ROS. However, in the smoking groups, the activity of the said enzymes was lower than in the non-smoking groups. The authors conclude that the smoking habit attenuates the good effect of daily physical activity on acute exercise-induced OS.

It has been demonstrated that even a 3-week exogenous vitamin C supplementation is unable to elevate TAC levels in smokers in whom these levels remain similar to the status before the supplementation period (Bakhtiari et al., 2012). However, consumption of green tea improves the salivary TAC in smokers. Azimi et al. (2017) measured the salivary TAC of smokers at the baseline, then after 7 and 21 days of drinking two cups of green tea per day. At day zero, the non-smoking group had statistically significantly higher TAC. Although drinking green tea did not considerably increase salivary TAC in the smoking group to the levels observed in the control group after 7 and 21 days, there was an upward trend in both groups. Regular drinking of black tea has been shown to have an even better effect on the oral antioxidant defense system. In a study by Pal et al. (2012) smokers with a tea drinking habit were characterized by lower levels of ROS and lower DNA damage in exfoliated buccal cells. Moreover, the habit of drinking tea effectively decreased the expression of ROS-generating proteins – I_KB (I kappa B kinase), NF-$_K$B (nuclear factor kappa-light-chain-enhancer of activated B cells), DNA-associated proteins p53 (tumor protein p53) and MLH1 (gene mutS HOMOLOG 1) in tobacco users. Activation of NF-$_K$B is responsible, among others, for stimulation of osteoclasts and increased activity of metalloproteinases, resulting in overproduction of lipid peroxides, oxidized proteins and inflammatory factors, and ultimately leading to oxidative damage of tissues.

When antioxidant systems fail and the redox balance shifts toward oxidation, an increase in the concentration of salivary lipid peroxidation products generated by non-enzymatic pathways is observed (Guentsch et al., 2008; Demirtaş et al., 2014; Metgud and Bajaj, 2014; Kurku et al., 2015). In the study by Demirtaş et al. (2014), salivary MDA content in smokers was found to be significantly higher compared to the control group and the group of passive smokers, which was partially compliant with the findings of other research groups (Guentsch et al., 2008; Kurku et al., 2015). It has been proven that salivary MDA plays a pathological role in multistep oral carcinogenesis and progression. Metgud and Bajaj (2014) observed significant elevation of salivary MDA, rising progressively form healthy controls to pre-cancerous conditions and individuals with SCC, in line with a state of OS connected with SCC. In the saliva of compulsive smokers and subjects who were not smokers on a daily basis but who were exposed to smoking one cigarette for the purpose of the experiment, Reznick et al. (2003) observed significantly increased carbonylation of salivary proteins, which is a form of their oxidative damage. A similar increase in the concentration of carbonyl groups of proteins in the saliva of tobacco addicts, the so-called heavy smokers, immediately after smoking a cigarette was obtained by Nagler (2007). Proteins of great importance for maintaining not only the redox balance, but also the immunological and microbiological balance of saliva, such as amylase, acidic proline-rich proteins and lysozyme, were carbonylated. A significant reduction in bioavailable reduced disulfide groups of proteins was observed in the saliva of heavy smokers, but only directly after smoking a cigarette, compared to non-smokers (Kurku et al., 2015). This fact was associated by the authors with a significant decrease in GSH activity whose main role in the saliva is to maintain the disulfide groups of proteins in a reduced state, which determines their biological activity.

The significantly reduced gamma-glutamyltransferase activity in the saliva of smokers vs non-smokers, reported in the study by Greabu et al. (2007), may lead to the accumulation of harmful substances in the tissues of the oral cavity and salivary glands and to the development of numerous pathologies. The mentioned enzyme catalyzes the reaction of GSH with electrophilic substances. The products of the reaction are glutathione S-conjugates, and their formation is one of the

steps of removing xenobiotics, including lipid peroxidation products, from the body.

Oral Cancer and Pre-cancerous and Cigarette-Related Reactive Oxygen Species/Reactive Nitrogen Species

It should be emphasized that in a situation of reduced Px and CAT activity, intoxication with H_2O_2 in the oral cavity is significantly reduced. In the presence of metal ions, either from the parotid glands or directly from cigarette smoke, H_2O_2 initiates the previously described Fenton and Haber Weiss reactions, leading to the formation of $^{\cdot}OH$ (Nagler et al., 1997). It is worth remembering that mitochondrial DNA (mtDNA) is particularly sensitive to the effect of $^{\cdot}OH$. The amount of $^{\cdot}OH$ -related damage to mtDNA has been found to exceed the level of damage to nuclear DNA of the same cell by 10 times (Karolkiewicz, 2011). This is attributable to the presence of the respiratory chain in the mitochondria and the absence of histones, i.e., proteins that protect DNA from damage. At the same time, it has been observed that the ability to repair mtDNA and proteins associated with oxidative phosphorylation and damaged due to replication errors is limited (Pędzik et al., 2008; Ścibior-Bentkowska, 2009). Increased levels of oxidative DNA damage have been found to lead to reduced synthesis as well as activity of enzymes that remove these lesions, which is associated with increased cancer incidence (Halliwell, 2006; Greabu et al., 2009; Zaremba, 2010). Oxidative modifications of mtDNA are among the causes of oral squamous cell carcinoma (SCC) (Banerjee et al., 2017), and the incidence of oral SCC in smokers is four to seven times higher than in non-smokers. Naturally, one cigarette does not cause SCC, particularly since activity of, for instance, Px in the oral cavity returns to 90–100% of its initial level 30 min after smoking a cigarette, which is due to its continuous synthesis in the parotid glands (Reznick et al., 2003). However, according to the studies, compulsive smokers are firstly exposed to gradual accumulation of the damaging effects of damage caused by free radicals, which is most likely caused by the dysfunction of systems eliminating them (Isik et al., 2007; Caliri et al., 2021). In addition, the continuous daily deficit of H_2O_2-detoxifying enzymes in the saliva of compulsive smokers (who smoke 20 or more cigarettes per day on average) does not sufficiently protect the oral epithelium (Nagler et al., 1999). As a result, lesions are formed which are initially dysplastic, but over time may develop into a full-blown carcinoma *in situ*. These hypotheses are supported by the findings of Wu et al. (2005) who discovered that nicotine-induced DNA damage in oral cancer cell line was initiated by ROS.

Although the results quoted below were determined in the saliva, due to the fact that they are related to neoplastic conditions, we discuss them in the present subsection.

In terms of SCC development or pre-cancerous conditions in the oral cavity, the excess of NO delivered with cigarette smoke also appears to be of great significance (Preethi, 2016). It should be stressed, however, that the determination of NO in the saliva is controversial because it is a very unstable compound and is transformed into numerous nitrogen derivatives, including its most dangerous derivative – peroxynitrite. This might explain the results of Nagler who demonstrated a significant decrease in NO in the saliva of heavy smokers. Similar findings were obtained by Kanehira et al. (2006), but these authors observed a significant increase in NO concentration in the saliva of smokers immediately after smoking a cigarette, both in comparison with the control group and in the saliva of smokers before smoking a cigarette. It is believed that overproduction of NO may contribute to carcinogenesis or other pathological processes in the oral cavity in two ways: via its direct effect on biological targets (short-term effect), or indirectly – through the generation of peroxynitrite. It has been proven that peroxynitrite is involved in the pathogenesis of oral cancer through the formation of carcinogenic nitrosamines or inhibition of the DNA repair mechanism (Cortés-Ramírez et al., 2009). In addition to the above-mentioned functions, GSH is also used as a cofactor by glutathione peroxidase in the neutralization of peroxynitrite (Hopkins, 2017). The aforementioned reduction in GSH concentration in the saliva of smokers vs non-smokers may enhance OH. formation, thus increasing the ROS and peroxynitrite load (Zappacosta et al., 2002). It should be noted there are doubts concerning the source of NO in the saliva of smokers in the literature. Authors wonder whether elevated salivary NO concentrations contribute to the development of pre-cancerous lesions or pre-cancerous lesions contribute to increased levels of Preethi (2016).

The results of a study on the effect of smoking on the function of the 18 kDa translocator protein (TSPO) are also important in the terms of oral cancer development (Nagler et al., 2010). TSPO is an intracellular protein located mainly on the outer mitochondrial membrane. It is present in almost all tissues (mainly, however, in those with high energy requirements) and its presence has been found in the cell fraction of human saliva (Nagler et al., 1999). TSPO protein plays a role, *inter alia*, in the regulation of apoptosis. Interestingly, depending on the type of ligand, it can induce apoptosis as well as exhibit anti-apoptotic effects (Veenman et al., 2007, 2008). The exposure of saliva to cigarette smoke free radicals results in a three tines lower affinity of salivary TSPO to its specific ligand PK 11195. It should be mentioned that PK 11195 is responsible for cell cycle arrest in the G1/S phase and induces cell apoptosis (Mendonça-Torres and Roberts, 2013). These observations indirectly suggest that PK 11195 ligand expression decreased by TSPO may lead to the development of malignant lesions, including oral cancers, due to inhibition of apoptosis and, consequently, uncontrolled division of damaged cells.

Depletion of salivary antioxidant defense was undoubtedly not always observed when exposed to ROS increased by cigarette smoke. Kanehira et al. (2006) observed higher SOD activity in non-stimulated saliva of older smokers (65 years old or more) compared to their non-smoking peers, which agreed with Nagler's (2007) results in the saliva of older heavy smokers immediately after smoking a cigarette. According to Kanehira et al., the elevated SOD activity results from adaptive mechanisms that develop in older adults in response to dysfunction of mitochondrial respiratory chains. Mitochondrial dysfunction is one of the manifestations of aging. Mitochondria are the main cellular site of ROS generation. Studies have revealed

an age-related dysfunction of respiratory-chain complex I and increased production of O_2^-, which naturally increases the activity of dismutases (Martínez-Cisuelo et al., 2016). In both aforementioned studies, other antioxidant enzymes, CAT and Px, are not efficient in neutralizing excess H_2O_2, which consequently promotes OH^- generation. Moreover, Nagler (2007) showed that salivary antioxidant capacity (ImAnOx) of older heavy smokers was significantly higher compared to non-smokers, which was an unsuccessful attempt to neutralize ROS/RNS – unsuccessful, because, as mentioned above, the authors noted increased carbonylation of salivary proteins, which is a sign of OS.

There are reports demonstrating no significant changes in enzyme activities, TAS/TOS levels, concentrations of oxidation products in the saliva of young moderate smokers compared to the control group (Charalabopoulos et al., 2004; Mottalebnejad et al., 2014; Kurku et al., 2015; Neshat et al., 2020). The authors believe that this lack of changes is caused by great compensatory capacity of young organisms to the increasing ROS/RNS levels and the short duration of addiction of the examined subjects.

Cigarette- Born Reactive Oxygen Species/Reactive Nitrogen Species and Periodontal Diseases

Secondary to bacterial plaque, smoking cigarettes is one of the major determinants of the onset and progression of periodontal disease. The effects of periodontal diseases include not only tooth loss, social alienation, psychological injuries related to disturbed facial aesthetics and pronunciation, as well as digestive processes (Coelho et al., 2020). It is primarily a chronic inflammation that spreads throughout the body through the bloodstream. It has been proven that periodontal disease is associated with arthritis and other autoimmune diseases as well as cardiovascular disease, diabetes and obesity (Keller et al., 2015; Kaur et al., 2017; Makkar et al., 2018).

Components of tobacco smoke contribute to the dysfunction and oxidative damage of human gingival fibroblast (HGF), consequently leading to progressive failure of gingival connective tissue. In a study by Colombo et al. (2012) exposure of HGF to cigarette smoke had a lethal effect within a short period of time. Interestingly, the authors suggest that the decreased viability of HGF was not caused by cell apoptosis (microscopic examination did not reveal the presence of any apoptotic bodies typical of this process, and no condensation of cell nuclei or fragmentation of DNA/perinuclear bodies was observed).

The authors suggest that one of the possible reasons for the reduced viability of HGF is the occurrence of abnormalities in their cellular morphology. Smoking increases the concentration of intracellular ROS and carbonylation of proteins, including cytoskeletal proteins such as actin (the formation of actin filaments plays a major role in shaping and maintaining cell stability) and cofilin-1 (by mediating actin filament crossing and depolymerization/remodeling, it is responsible for cytoskeleton dynamics). Oxidative modification of cytoskeletal proteins results in impairment of the motility, adhesion and division of fibroblast cells (Hotulainen et al., 2005). In addition, a CS exposure dose-dependent decrease of reduced cellular protein thiols and intracellular GSH in HGF/PDLF (periodontal ligament fibroblast) has been reported, which partly coincides with the results of Chang et al. (2002). The noted result is not surprising in light of the above facts, as the main function of GSH is to maintain a reduced state of the thiol groups of proteins. Previously, studies showed that human gingival fibroblasts rapidly absorb and accumulate high levels of nicotine *in vitro*. The absorbed nicotine remains inside the fibroblasts, where it can affect cell metabolism or function (Hanes et al., 1991; Chang et al., 2002). To determine the mechanism of cytotoxicity of nicotine on PDLF, Chang et al. incubated cells with nicotine in medium and treated them with SOD, CAT, 2-oxothiazolidine-4-carboxylic acid (OTZ) and sulfoximine buthionine (BSO). Although SOD and CAT did not inhibit nicotine-induced cytotoxicity, the application of OTZ (a cysteine precursor that promotes GSH synthesis) led to cytotoxicity inhibition. In contrast, the addition of BSO (which is an inhibitor of cellular GSH synthesis) enhanced the cellular cytotoxicity of nicotine. Therefore, it appears that the cytotoxic effect of nicotine in PDLF culture was caused by GSH deficiency. Similarly, Tinti and Soory (2012) evaluated mechanisms for redox capacity of nicotine and GSH in HGF and human periodontal fibroblasts (HPF). This study involved a novel use of dihydrotestosterone (DHT) as an indicator of oxidative stress and wound healing (HGF and HPF cells exhibit high expression of the androgen receptor). Through the activation of androgen receptors, DHT has the ability to induce anti-apoptotic protein and to alleviate H_2O_2-induced oxidative stress. DHT shows the ability to activate CAT and suppress protein kinases (including NF-κB) (Giretti and Simoncini, 2008; Lee et al., 2008). Nicotine was shown to significantly decrease DHT activity. A similar report of nicotine dose-dependent OS, including in human periodontal ligament fibroblasts (PDLF) cells, was published by Nguyen et al. (2019). The authors demonstrated that oxidized guanine species (Ox-Gs) in PDLF lysate increased with an increase in nicotine concentration. Oxidative stress induced by *Porphyromonas gingivalis* lysate was also evaluated in this study. An interesting finding is the occurrence of significantly higher Ox-GS concentration in PDLF cultures exposed simultaneously to *P. gingivalis* and nicotine after 24 h, compared to cell cultured in serum-free medium served as a negative control. As the authors observed no differences after 2 h of exposure, they concluded that the effect of nicotine on periodontal tissues depends not only on its dose but also on the time of exposure. Another noteworthy conclusion of the study is the additive effect of nicotine and *P. gingivalis* on OS formation in periodontal tissues. Lipopolysaccharides present in *P. gingivalis* stimulate polymorphonuclear neutrophils (PMN) to phagocytosis, thus contributing to the "respiratory burst" process during which free radicals are generated. Moreover, nicotine metabolites, exerting effects similar to the activity of toxins produced by periopathogens, lead to the accumulation of free radicals and boost OS in periodontal tissues.

The expression of oxidative stress-related genes is thought to be induced particularly after exposure to compounds which react with thiols (Heikkila et al., 1982). Nicotine has been proven to be responsible for up-regulation of heme oxygenase-1 (HO-1) expression in human gingival fibroblasts (Chang et al., 2005). HO-1 acts as an oxidative enzyme by catalyzing the oxidative degradation of heme to biliverdin, which is then reduced to

bilirubin – a compound with antioxidant properties (Vile and Tyrrell, 1993). In the study by Chang et al. (2005) HGF exposure to 10 mM nicotine induced HO-1 protein expression *in vivo* in a time-dependent manner. It should be mentioned that the regulation of nicotine-induced HO-1 expression depends on intracellular GSH concentration (Chang et al., 2005). The addition of SOD and CAT did not decrease HO-1 induction. After adding NAC – a GSH precursor – the authors observed a significant decrease in HO-1 expression compared to HGF challenged only with nicotine. Furthermore, smoking-induced free radical imbalance leads to the induction of cyclooxygenase-2 (COX-2) mRNA expression in HGF (Chang et al., 2003c). COX-2 is an enzyme responsible for the synthesis of prostaglandins as well as initiation of an inflammatory cascade (Chang et al., 2003b,a). Nevertheless, it has been demonstrated that the use of OTZ resulted in an approximately 60% reduction in nicotine-induced COX-2 protein levels in HGF (Ho and Chang, 2006). Similarly, application of PD98059 (an inhibitor of protein kinase regulated by an extracellular signal) led to a significant decrease in nicotine-induced levels of COX-2 in HGF, whereas the addition of BSO resulted in approximately three times higher levels of COX-2 protein. These data suggest that the oxidative effect of nicotine mediates COX-2 induction in HGF, and thiol pools may act as an intracellular buffer against nicotine-induced COX-2 expression.

Corrêa et al. (2019) evaluated the influence of resveratrol on oxidative stress-induced experimental periodontitis in rats exposed to cigarette smoke. Resveratrol is a molecule with anti-inflammatory and antioxidant properties. Furthermore, it inhibits the synthesis of the CYP450 enzyme responsible for ROS production and is an antagonist of the receptor of aryl hydrocarbon which also leads to increased free radical generation (Leonard et al., 2003; Deng et al., 2014). This study revealed a reduction in bone mass loss in rats exposed to cigarette smoke inhalation + resveratrol (SMK + RESV) compared to the control group (cigarette smoke inhalation + placebo/SMK + PLAC). Resveratrol supplementation in the SMK + RESV group resulted in increased SOD activity and reduced NADPH oxidase activity in periodontal tissues compared to the SMK + PLAC group. Moreover, in the study by Andreou et al. (2004) resveratrol demonstrated a pronounced antagonism to aryl hydrocarbon receptor (which has an oxidative effect) in rat bone marrow cells, and Ikeda et al. (2018) observed reduced bone loss and promotion of bone healing by systemic treatment with melinjo seed extract rich in resveratrol derivatives. It can be assumed that the above correlations are also due to the antioxidant effect of resveratrol, and thus the reduction of osteoclast activity.

Tonguç et al. (2011) demonstrated the additive effect of smoking and periodontitis on OS severity and reduction of antioxidant systems. The group of smokers with periodontitis had the highest level of MDA and the lowest activity of SOD, CAT and GSH-Px in gingival tissues compared to the control group of non-smokers with healthy periodontium.

Changes in the antioxidant capacity of smokers with periodontitis can also be monitored in saliva, although the available results on this subject are contradictory. Buduneli et al. (2006) observed no significant differences in a TAC level in NWS of smokers with periodontitis. Agnihotri et al. (2009) reported a significant decrease in SOD activity in the NWS and gingival fluid of smokers with periodontal disease compared to the control group (non-smokers with periodontal disease), which is also confirmed by the results of Bizoń and Milnerowicz (2014). The reduction in SOD activity was more visible in GCF than in saliva. The authors explain this observation by higher concentration of ROS in the GCF unit, which is lower compared to the volume of saliva in the mouth (the total non-stimulated saliva flow rate is approximately 0.3–0.4 ml/min, whereas for GCF it is several microliters per hour). In addition to "resource depletion," cigarette-induced ROS may inactivate SOD through oxidative modifications of its protein chains as well as via modification of its activity caused by impaired metabolism of trace elements that are its cofactors. Yadav et al. (2020) compared metallothionein (MT) concentrations in NWS and GCF of smokers and non-smokers with chronic periodontitis as well as those with healthy periodontium. MT is an antioxidant protein rich in metal-binding cysteine. It is effective particularly against hydroxyl radicals (Thornalley and Vašák, 1985). MT content in the saliva of smokers with periodontitis was significantly higher compared to the other three groups. Furthermore, no difference in MT concentration was observed between non-smokers with periodontitis and smokers without any periodontal disease, which again proves an additive effect of cigarette radicals on periodontal disease progression. A similar correlation was observed in the study by Katsuragi et al. (1997) who demonstrated significantly higher MT concentration in the layer of gingival spinous epithelial cells in smokers with periodontitis compared to non-smokers with periodontitis. Interestingly, increased MT synthesis may down-regulate Cu^2 + /Zn^{2+} SOD expression in the course of a compensatory mechanism (Nzengue et al., 2011). This is due to free radicals-induced MT-transcriptional responses through metal regulatory transcription factor 1 (MTF-1) which in the presence of Zn ions (displaced from SOD by cadmium contained in cigarette smoke), binds to a metal-responsive element in the promoter region of the MT gene to initiate its transcription (Andrews, 2000). Alteration in the activity of salivary cigarette-formed antioxidants results in increased concentrations of biomolecule oxidative modification products in saliva. In a study by Celec et al. (2005), TBARS and MDA levels were considerably higher in smokers with periodontitis compared to healthy controls. It is noteworthy that the study presented no significant correlations between TBARS in saliva and plasma of patients with periodontopathy. Therefore, it can be concluded that systemic oxidative stress does not directly affect salivary TBARS levels, and markers of lipid peroxidation in smokers with periodontitis are valuable indicators of exposure to ROS. Ips (8-epi-PGF2α, among others) are isomers of prostaglandins, formed in the process of enzymatic peroxidation of polyunsaturated fatty acids. In the study by Wolfram et al. (2006), the concentration of 8-epi-PGF2α in the NWS of smokers with periodontitis was significantly higher compared to non-smokers with periodontitis. Varghese et al. (2020) showed higher levels of salivary 8-OhdG in the group of smokers with chronic periodontitis compared

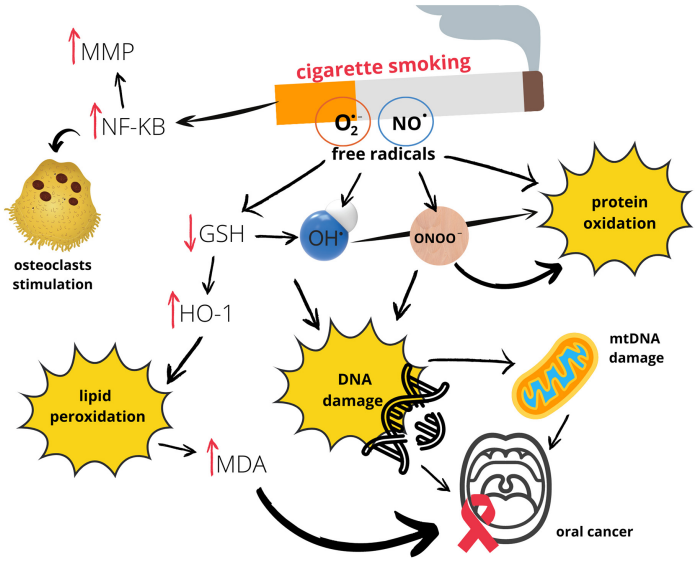

FIGURE 3 | Effects of smoking on oral redox homeostasis. GSH, glutathione; HO–1, heme oxygenase 1; MDA, malondialdehyde; MMP, metalloproteinase; mtDNA, mitochondrial DNA; NF-$_K$B, nuclear factor kappa-light-chain-enhancer of activated B cells; $NO^{\cdot}$, nitric oxide; $O_2^{\cdot -}$, superoxide anion radical; $OH^{\cdot}$, hydroxyl radical; $ONOO^-$, peroxynitrite.

to non-smoking controls with periodontitis. Salivary 8-OhdG levels correlated with clinical indicators of periodontal disease (plaque index, gingival index, pocket probing depth and clinical attachment levels). These correlations confirm that oxidative modification of bio-molecules, in this case DNA, may be one of the mechanisms of periodontal tissue destruction in the course of periodontitis. Unfortunately, non-surgical treatment consisting in scaling and root planning (SRP) performed in both groups did not lead to a reduction of 8-OhdG levels in the smoker group to the levels observed in healthy controls. A similar lack of effect of SRP on ascorbic acid levels in the NWS of smokers with periodontitis was demonstrated by Mathias et al. (2014). Contrary results were obtained Hendek et al. (2015) who determined the levels of 8-OhdG, 4-hydroxynonenal (HNE) and GPx activity in gingival fluid and saliva collected at the beginning as well as in the first and third month after SRP. The authors found that after non-surgical periodontal treatment, 8-OhdG concentration in GCF and saliva decreased significantly in both periodontitis groups (however, it was still higher in the smoking group compared to non-smokers), while the content of 4-HNE and GPx enzyme activity in GCF and saliva did not change significantly after the treatment in any of the study groups. In contrast, Guentsch et al. (2008) found that non-surgical periodontal treatment in smokers reduced MDA and GPx concentrations to levels comparable to healthy controls, both smokers and non-smokers. These results clearly indicate that periodontal therapy may be helpful in diminishing OS in smokers with periodontitis.

In light of changing trends connected with smoking, electronic cigarettes have been introduced to the market. These are devices that heat special inhalation solutions that may or may not contain nicotine (Korfei, 2018). Although most of long-term effects of e-cigarettes are still unknown due to the short presence of these devices on the consumer market, recent findings suggest that e-cigs may induce oxidative stress and increase the expression of advanced glycation end products (AGEs) and their cellular receptors (RAGE), also in gingival and periodontal tissues (Javed et al., 2017; Al-Aali et al., 2018; AlQahtani et al., 2018; ArRejaie et al., 2019). The increased number of AGE-RAGE bonds has been linked to the formation of reactive oxygen species that induce the oxidative burst of periodontal tissues and cause functional changes in polymorphonuclear chemotaxis and phagocytosis and increased systemic and local inflammatory burden through elevated cytokine expression in serum and gingival fluid. In addition, Ganapathy et al. (2017) demonstrated that exposure to aerosol extracts of e-cigs may lead to significant DNA damage in oral epithelial cells expressed with high concentrations of 8-oxo-dG. Analyzing the mechanisms that modulate e-cigarette-induced DNA damage, the authors identified an increase in ROS concentration, and a decrease in TAC and the expression of proteins essential for repairing oxidative DNA damage (8-oxoguanine DNA glycosylase, OGG1). These results clearly indicate that exposure of the oral cavity to

electronic cigarette aerosol may potentially increase the risk of developing oral cancer.

Graphic presentation of the influence of smoking on oral redox homeostasis (**Figure 3**).

CONCLUSION

Alcohol abuse and smoking have been substantial public health problems for years. Numerous studies have demonstrated that those habits cause damage to almost every organ of the body, including impairing the function of the oral cavity. As a result of excessive alcohol consumption and smoking, the oxidative balance in the oral cavity might be disturbed. Smoking and alcohol abuse might be reflected in reduced oral antioxidant capacity and in an increasing oxidative damage to cellular elements. Overproduction of free radicals might leads to DNA damage which increased risk of developing oral cancer. Alcohol and smoking habits might worsen the course of the already existing periodontal disease, which could not only be the cause of tooth loss, but could be responsible for the deterioration of mental health, leads to poorer digestion of food and, most importantly, could be associated with systemic inflammation. Unfortunately, what should be emphasized, the effect of exogenous antioxidants on strengthening the endogenous antioxidant barrier of the oral cavity of smokers and alcohol abusers are inconclusive.

What should be keep in mind, alcohol abuse and smoking, not least through redox imbalances may lead to the deterioration of the oral cavity homeostasis. The other factors, such as dehydration of the mucosa, its irritation, worse oral hygiene in addicted people, may contribute to the development of the discussed pathologies in the oral cavity. Similarly, the innate/hereditary antioxidant predisposition of the organism of alcohol abusers/smokers may influence the amount of generated oxidative stress in response to the above stimulants. Unfortunately, we are not able to carry out a meta-analysis, due to the large variety of publications (experimental and clinical publications). It is necessary to inform the public about the possible adverse effects of excessive alcohol consumption and smoking to raise awareness of the possible oral health consequences.

AUTHOR CONTRIBUTIONS

SZ: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, writing—original draft. MM: conceptualization, investigation, writing—review and editing. AZ: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, writing—original draft, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphys.2021.793028/full#supplementary-material

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Streszczenie

Palenie papierosów stanowi poważny problem zdrowia publicznego i wiąże się ze zwiększoną zachorowalnością i śmiertelnością. Alternatywą dla powyższych, miały zostać nowoczesne urządzenia dostarczające do organizmu nikotynę: e-papierosy oraz „heat-not-burn products", dające użytkownikowi wrażenie podobne do zwyczajnego palenia. Obecnie pogląd mniejszej szkodliwości wyżej wspomnianych urządzeń budzi coraz więcej kontrowersji. Wykazano, że aerozol wdychany w trakcie inhalacji tych nowoczesnych urządzeń zawiera w swoim składzie szereg substancji o udowodnionym naukowo szkodliwym działaniu na zdrowie organizmu.

Jama ustna jest pierwszym miejscem kontaktu wdychanego dymu papierosowego oraz aerozoli z e-papierosów/"heat-not-burn products" z organizmem człowiek.

Dlatego celem rozprawy doktorskiej była ocena wpływu palenia papierosów tradycyjnych oraz nowoczesnych urządzeń dostarczających do organizmu nikotynę (e-papierosów oraz „heat-not-burn products") na stężenie wybranych ślinowych lipidów oraz produktów ich peroksydacji, jak również stężenie ślinowych cytokin, chemokin i czynników wzrostu.

Badaniem objęto młode osoby dorosłe (w wieku 18-30 lat), z czasem trwania nałogu nikotynowego nie krótszym niż rok i nie dłuższym niż 3 lata. Palaczy podzielono na 3 równe grupy (po 25 osób w każdej): palacze papierosów tradycyjnych, palacze e-papierosów oraz palacze „heat-not-burn products". Osoby zakwalifikowane do jednej z powyższych grup badanych mogły stosować, wyłącznie jedną z trzech powyższych metod dostarczania do organizmu nikotyny oraz charakteryzowały się brakiem stanów zapalnych w jamie ustnej, prawidłowym zakres BMI, sporadycznym spożywaniem alkoholu, brakiem w wywiadzie zażywania substancji psychoaktywnych. Grupę kontrolną stanowili niepalący z brakiem historii palenia w przeszłości,

dopasowani pod względem powyższych wymagań i płci do uczestników grup badanych. Ślina stanowiąca materiał diagnostyczny, została pobrana metodą odpluwania. Bezpośrednio po pobraniu materiału diagnostycznego uczestnicy zostali poddani badaniu stomatologicznemu, które obejmowało ocenę stanu uzębienia oraz przyzębia.

Oceniono stężenie wybranych sfingolipidów, ceramidów oraz produktów peroksydacji lipidów w ślinie niestymulowanej oraz stymulowanej, jak również stężenie ślinowych cytokin, chemokin oraz czynników wzrostu w ślinie niestymulowanej.

Wykazano istotny spadek stężenia większości badanych sfingolipidów w ślinie niestymulowanej i stymulowanej palaczy, niezależnie od sposobu dostarczania nikotyny oraz zwiększone stężenie produktów peroksydacji lipidów, wskazujące na zaburzenie równowagi redoks w gruczołach ślinowych palaczy.

Ponadto, wykazano, że stosowanie zarówno e-papierosów jak i „heat-not-burn products" wydaje się wyraźnie hamować lokalną odpowiedź immunologiczną w niestymulowanej ślinie palaczy, podczas gdy palenie tradycyjnych papierosów tylko nieznacznie nasila odpowiedź zapalną w porównaniu do osób niepalących. Hamujący wpływ na syntezę lub uwalnianie badanych cytokin z komórek może być spowodowany wpływem mentolowego aromatu do e-papierosów i „heat-not-burn products".

W pracy przeglądowej, podsumowano doniesienia na temat wpływu palenia tradycyjnych papierosów na hemostazę redox jamy ustnej.

Summary

Cigarette smoking is considered to be a serious public health problem and is associated with increased morbidity and mortality. The alternative to the above were to be modern devices that deliver nicotine to the body: e-cigarettes and "heat-not-burn products", giving the user an experience similar to regular smoking. Currently, the idea that the above-mentioned devices are less harmful is becoming more and more controversial. It has been shown that the aerosol inhaled during inhalation of these modern devices contains a number of substances with scientifically proven harmful effects on the body's health.

The oral cavity is the first place of contact of inhaled cigarette smoke and e-cigarette aerosols/heat-not-burn products with the human body.

Therefore, the aim of the doctoral thesis was to assess the effects of smoking traditional cigarettes and modern nicotine delivery devices (e-cigarettes and heat-not-burn products) on the concentration of selected salivary lipids and their peroxidation products, as well as the concentration of salivary cytokines, chemokines and growth factors.

The study included young adults (aged 18-30) with a nicotine addiction duration of not less than one year and not more than 3 years. Smokers were divided into 3 equal groups (25 people in each): smokers of traditional cigarettes, smokers of e-cigarettes and smokers of "heat-not-burn products". People classified into one of the above study groups could use only one of the three above methods of delivering nicotine to the body and were characterized by the absence of inflammation in the oral cavity, normal BMI range, occasional alcohol consumption, and no history of using psychoactive substances. The control group consisted of non-smokers with no history of smoking, matched in terms of the above requirements and gender to the participants of the study groups. Saliva, which constitutes diagnostic material, was collected by spitting method. Immediately after collecting the diagnostic

material, the participants underwent a dental examination, which included an assessment of the condition of the teeth and periodontium.

The concentration of selected sphingolipids, ceramides and lipid peroxidation products, as well as the concentration of salivary cytokines, chemokines and growth factors were assessed.

There was a significant decrease in the concentration of most of the sphingolipids in the unstimulated and stimulated saliva of smokers, regardless of nicotine delivery method, and increased concentrations of peroxidation products indicating a redox imbalance in the salivary glands of smokers.

Furthermore, it was shown that the use of both e-cigarettes and heat-not-burn products appears to significantly inhibit the local immune response in unstimulated saliva of smokers, while smoking traditional cigarettes only slightly increases the inflammatory response compared to non-smokers. The inhibitory effect on the synthesis or release of the tested cytokines from cells may be caused by the influence of menthol flavor for e-cigarettes and "heat-not-burn products".

The review summarizes reports on the impact of smoking traditional cigarettes on redox hemostasis of the oral cavity.

Informacja o charakterze udziału współautorów w publikacjach wraz z szacunkowym określeniem procentowego wkładu

Impact of Smoking on Salivary Lipid Profile and Oxidative Stress in Young Adults: A Comparative Analysis between Traditional Cigarettes, E-Cigarettes, and Heat-Not-Burn Products. Medical Science Monitor 2024: 30, 12 pp.

Imię i nazwisko współautora	Charakter Udziału	Procentowy wkład
Lek. dent. Zięba S. **doktorant**	koncepcja i planowanie badania, gromadzenie danych, analiza statystyczna, interpretacja wyników, przygotowanie manuskryptu, przegląd piśmiennictwa.	72 %
Prof. dr hab. n. med. Błachnio-Zabielska A.	współudział w planowaniu badania, gromadzeniu danych, analizie statystycznej, interpretacji danych.	5 %
Dr hab. n. med. Maciejczyk M.	współudział planowaniu badania, zbieraniu danych, analizie statystycznej, interpretacji danych, przygotowaniu manuskryptu.	5 %
Mgr Pogodzińska K.	współudział w zbieraniu danych, analizie statystycznej, interpretacji danych.	3 %
Prof. dr hab. n. med. Szuta M.	współudział w planowaniu badania, zbieraniu danych, interpretacji danych.	3 %
Prof. Lo Giudice G.	współudział w planowaniu badania, analizie statystycznej, interpretacji danych.	1 %
Dr Lo Giudice R.	współudział w zbieraniu danych, analizie statystycznej, interpretacji danych,	1 %
Prof. dr hab. n. med. Zalewska A.	współudział planowaniu badania, zbieraniu danych, analizie statystycznej, interpretacji danych, przygotowaniu manuskryptu, przeglądzie piśmiennictwa.	10 %

Oświadczam, że procentowy wkład oraz charakter udziału doktorantki lek. dent. Sary Zięba w powyższej publikacji jest zgodny ze stanem faktycznym.

Oświadczam, że wszyscy współautorzy wyrazili zgodę na wykorzystanie powyższej publikacji w rozprawie doktorskiej lek. dent. Sary Zięby.

Podpis

Sara Zięba

Comparison of smoking traditional, heat not burn and electronic cigarettes on salivary cytokine, chemokine and growth factor profile in healthy young adults–pilot study. Frontiers in Physiology. 2024: 15, 10 pp.

Imię i nazwisko współautora	Charakter Udziału	Procentowy wkład
Lek. dent. Zięba S. **doktorant**	koncepcja i planowanie badania, gromadzenie danych, analiza statystyczna, interpretacja wyników, przygotowanie manuskryptu, przegląd piśmiennictwa.	73 %
Dr hab. n. med. Maciejczyk M.	współudział w projektowaniu badania, zbieraniu danych, analizie statystycznej, interpretacji danych oraz przygotowaniu manuskryptu.	5%
Dr n. med. Antonowicz B.	współudział w zbieraniu danych, redagowaniu manuskryptu.	3 %
Porydzaj A.	współudział w zbieraniu danych, redagowaniu manuskryptu.	3 %
Prof. dr hab. n. med. Szuta M.	współudział w zbieraniu danych, redagowaniu manuskryptu.	3 %
Prof. Lo Giudice G.	współudział w redagowaniu i wizualizacji manuskryptu.	1 %
Dr Lo Giudice R.	współudział w analizie formalnej, redagowaniu i wizualizacji manuskryptu.	1 %
Krokosz S.	współudział w zbieraniu danych, redagowaniu manuskryptu, wizualizacji danych.	1 %
Prof. dr hab. n. med. Zalewska A.	współudział w koncepcji i planowaniu badania, zbieraniu danych, analizie statystycznej, gromadzeniu danych, interpretacji wyników, przygotowaniu manuskryptu.	10 %

Oświadczam, że procentowy wkład oraz charakter udziału doktorantki lek. dent. Sary Zięba w powyższej publikacji jest zgodny ze stanem faktycznym.

Oświadczam, że wszyscy współautorzy wyrazili zgodę na wykorzystanie powyższej publikacji w rozprawie doktorskiej lek. dent. Sary Zięby.

Podpis

Sara Zięba

Ethanol- and Cigarette Smoke-Related Alternations in Oral Redox Homeostasis. Frontiers in Physiology. 2022: 12, 19 pp.

Imię i nazwisko współautora	Charakter Udziału	Procentowy wkład
Lek. dent. Zięba S. **doktorant**	wybór tematu i koncepcji pracy przeglądowej, zbieranie danych, analiza formalna, przegląd piśmiennictwa, napisanie artykułu.	85 %
Dr hab. n. med. Maciejczyk M.	współudział w koncepcji pracy przeglądowej, przeglądzie piśmiennictwa, edycji manuskryptu.	5 %
Prof. dr hab. n. med. Zalewska A.	współudział w koncepcji pracy przeglądowej, zbieraniu danych, analizie formalnej, napisaniu artykułu.	10 %

Oświadczam, że procentowy wkład oraz charakter udziału doktorantki lek. dent. Sary Zięba w powyższej publikacji jest zgodny ze stanem faktycznym.

Oświadczam, że wszyscy współautorzy wyrazili zgodę na wykorzystanie powyższej publikacji w rozprawie doktorskiej lek. dent. Sary Zięby.

Podpis

Sara Zięba

Oświadczenia współautorów

Białystok, 17.06.2024 r.

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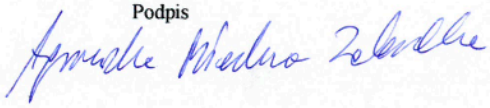
Oświadczenie

Oświadczam, iż mój udział w przygotowaniu publikacji:

"**Impact of Smoking on Salivary Lipid Profile and Oxidative Stress in Young Adults: A Comparative Analysis between Traditional Cigarettes, E-Cigarettes, and Heat-Not-Burn Products**" autorów: Zięba S., Błachnio-Zabielska A., Maciejczyk M., Pogodzińska K., Szuta M., Lo Giudice G., Lo Giudice R., Zalewska A., opublikowanej w Medical Science Monitor 2024; 13:30:e942507, wchodzącej w skład rozprawy doktorskiej lek. dent. Sary Zięby wynoszący 5%, polegał na: współudziale w projektowaniu badania, zbieraniu danych, analizie statystycznej oraz interpretacji danych.

Jednocześnie wyrażam zgodę na wykorzystanie przez lek. dent. Sarę Zięba, publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Podpis



Białystok, 17.06.2024 r.

dr hab. n. med. Mateusz Maciejczyk
Zakład Higieny, Epidemiologii i Ergonomii
Uniwersytet Medyczny w Białymstoku
ul. Mickiewicza 2c
15-089 Białystok

Oświadczenie

Oświadczam, iż mój udział w przygotowaniu publikacji:

"**Impact of Smoking on Salivary Lipid Profile and Oxidative Stress in Young Adults: A Comparative Analysis between Traditional Cigarettes, E-Cigarettes, and Heat-Not-Burn Products**" autorów: Zięba S., Błachnio-Zabielska A., Maciejczyk M., Pogodzińska K., Szuta M., Lo Giudice G., Lo Giudice R., Zalewska A., opublikowanej w Medical Science Monitor 2024; 13:30:e942507, wchodzącej w skład rozprawy doktorskiej lek. dent. Sary Zięby wynoszący 5%, polegał na: współudziale w projektowaniu badania, zbieraniu danych, analizie statystycznej, interpretacji danych oraz przygotowaniu manuskryptu

Jednocześnie wyrażam zgodę na wykorzystanie przez lek. dent. Sarę Zięba, publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Podpis

dr hab. n. med.
Mateusz Maciejczyk
ADIUNKT

Białystok, 17.06.2024 r.

mgr Karolina Pogodzińska
Zakład Higieny, Epidemiologii i Zaburzeń Metabolicznych
Uniwersytet Medyczny w Białymstoku
ul. Mickiewicza 2c
15-089 Białystok

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. dent. Sarę Zięba, publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Podpis

Karolina Pogodzińska

Kraków, 18.06.2024 r.

Prof. dr hab. n. med. Mariusz Szuta
Zakład Chirurgii Stomatologicznej
Collegium Medicum Uniwersytetu Jagiellońskiego
ul. Montelupich 4
31-155 Kraków

Oświadczenie

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Podpis

Prof. dr hab. n. med. Mariusz Szuta
specjalista chirurgii szczękowo-twarzowej
otolaryngolog
1477700

Messina, 17/06/2024

Prof. Giuseppe Lo Giudice
Department of Biomedical and Dental Sciences
and Morphofunctional Imaging
Messina University
98100 Messina, Italy.

Declaration

I declare that my participation in the preparation of the publication:
"**Impact of Smoking on Salivary Lipid Profile and Oxidative Stress in Young Adults: A Comparative Analysis between Traditional Cigarettes, E-Cigarettes, and Heat-Not-Burn Products**" by the authors: Zięba S., Błachnio-Zabielska A., Maciejczyk M., Pogodzinska K., Szuta M., Lo Giudice G., Lo Giudice R., Zalewska A., published in Medical Science Monitor 2024; 13:30:e942507, included in the doctoral dissertation of PhD Candidate Sara Zięba amounting to 1%, consisted of: statistical analysis and data interpretation.

I give Sara Zieba permission to use the above publication in her PhD thesis

Signature

Messina, 17/06/2024

Dr Roberto Lo Giudice
Department of Human Pathology of the Adult
and Evolutive Age. G. Barresi
Messina University
98100 Messina, Italy.

Declaration

I declare that my participation in the preparation of the publication:
"**Impact of Smoking on Salivary Lipid Profile and Oxidative Stress in Young Adults: A Comparative Analysis between Traditional Cigarettes, E-Cigarettes, and Heat-Not-Burn Products**" by the authors: Zięba S., Błachnio-Zabielska A., Maciejczyk M., Pogodzinska K., Szuta M., Lo Giudice G., Lo Giudice R., Zalewska A., published in Medical Science Monitor 2024; 13:30:e942507, included in the doctoral dissertation of PhD Candidate Sara Zięba amounting to 1%, consisted of: statistical analysis and data interpretation

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Signature

Białystok, 17.06.2024 r.

Prof. zw. dr hab. n. med. Anna Zalewska
Zakład Stomatologii Zachowawczej
Uniwersytet Medyczny w Białymstoku
ul. Marii Skłodowskiej-Curie 24A
15-276 Białystok

Oświadczenie

Oświadczam, iż mój udział w przygotowaniu publikacji:

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. dent. Sarę Zięba, publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Podpis

KIEROWNIK
Zakładu Stomatologii Zachowawczej
prof. dr hab. Anna Zalewska

Białystok, 17.06.2024 r.

dr hab. n. med. Mateusz Maciejczyk
Zakład Higieny, Epidemiologii i Ergonomii
Uniwersytet Medyczny w Białymstoku
ul. Mickiewicza 2c
15-089 Białystok

Oświadczenie

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. dent. Sarę Zięba, publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Podpis

dr hab. n. med.
Mateusz Maciejczyk
ADIUNKT

Białystok, 17.06.2024 r.

dr n. med. Bożena Antonowicz
Zakład Chirurgii Stomatologicznej
Uniwersytet Medyczny w Białymstoku
ul. Marii Skłodowskiej Curie 24A
15-276 Białystok

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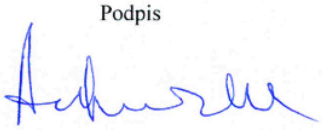
Podpis



Białystok, 17.06.2024 r.

Aleksandra Porydzaj
Studenckie Koło Naukowe przy Zakładzie Stomatologii Zachowawczej
Uniwersytet Medyczny w Białymstoku
ul. Marii Skłodowskiej Curie 24A
15-276 Białystok

Oświadczenie

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Podpis

Aleksandra Porydzaj

Kraków, 18.06.2024 r.

Prof. dr hab. n. med. Mariusz Szuta
Zakład Chirurgii Stomatologicznej
Collegium Medicum Uniwersytetu Jagiellońskiego
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Podpis

Prof. dr hab. n. med. Mariusz Szuta
specjalista chirurgii szczękowo-twarzowej
otolaryngolog
1477700

Messina, 17/06/2024

Prof. Giuseppe Lo Giudice
Department of Biomedical and Dental Sciences
and Morphofunctional Imaging
Messina University
98100 Messina, Italy.

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I give Sara Zieba permission to use the above publication in her PhD thesis.

Signature

Messina, 17/06/2024

Dr Roberto Lo Giudice
Department of Human Pathology of the Adult
and Evolutive Age. G. Barresi
Messina University,
98100 Messina, Italy.

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I give Sara Zieba permission to use the above publication in her PhD thesis.

Signature

Białystok, 17.06.2024 r.

Stanisław Krokosz
Studenckie Koło Naukowe przy Zakładzie Stomatologii Zachowawczej
Uniwersytet Medyczny w Białymstoku
ul. Marii Skłodowskiej Curie 24A
15-276 Białystok

Oświadczenie

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. dent. Sarę Zięba, publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Podpis

Stanisław Krokosz

Białystok, 17.06.2024 r.

Prof. zw. dr hab. n. med. Anna Zalewska
Zakład Stomatologii Zachowawczej
Uniwersytet Medyczny w Białymstoku
ul. Marii Skłodowskiej-Curie 24A
15-276 Białystok

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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. dent. Sarę Zięba, publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Podpis

KIEROWNIK
Zakładu Stomatologii Zachowawczej
prof. dr hab. Anna Zalewska

Białystok, 17.06.2024 r.

dr hab. n. med. Mateusz Maciejczyk
Zakład Higieny, Epidemiologii i Ergonomii
Uniwersytet Medyczny w Białymstoku
ul. Mickiewicza 2c
15-089 Białystok

Oświadczenie

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"**Ethanol- and cigarette smoke-related alternations in oral redox homeostasis**" autorów: Zięba S., Maciejczyk M., Zalewska A., opublikowanej w Frontiers in Physiology 2021; 12:793028, wchodzącej w skład rozprawy doktorskiej lek. dent. Sary Zięby wynoszący 5%, polegał na: współudziale w koncepcji pracy poglądowej, przeglądzie piśmiennictwa oraz napisaniu artykułu.

Jednocześnie wyrażam zgodę na wykorzystanie przez lek. dent. Sarę Zięba, publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Podpis

dr hab. n. med.
Mateusz Maciejczyk
ADIUNKT

Białystok, 17.06.2024 r.

Prof. zw. dr hab. n. med. Anna Zalewska
Zakład Stomatologii Zachowawczej
Uniwersytet Medyczny w Białymstoku
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Jednocześnie wyrażam zgodę na wykorzystanie przez lek. dent. Sarę Zięba, publikacji w postępowaniu o nadanie stopnia doktora nauk medycznych.

Podpis

KIEROWNIK
Zakładu Stomatologii Zachowawc
prof. dr hab. Anna Zalewsk

Uchwały Komisji Bioetycznej

UNIWERSYTET MEDYCZNY
w Białymstoku
KOMISJA BIOETYCZNA
15-089 Białystok, ul. Jana Kilińskiego

Białystok, 26.11.2020 r.

Uchwała nr: APK.002.343.2020

Komisja Bioetyczna przy Uniwersytecie Medycznym w Białymstoku, po zapoznaniu się z projektem badania zgodnie z zasadami GCP/ Guidelines for Good Clinical Practice /- **w y r a ż a z g o d ę** na prowadzenie tematu badawczego: „Ocena parametrów stresu oksydacyjnego, obrony antyoksydacyjnej oraz białek odpowiedzi swoistej i nieswoistej w ślinie palaczy papierosów tradycyjnych, elektronicznych oraz systemów do podgrzewania tytoniu" przez lek. dent. Sarę Ziębę wraz z zespołem badawczym z UMB.

Przewodnicząca Komisji Bioetycznej przy UMB

prof. dr hab. Otylia Kowal-Bielecka

Białystok, 30.03.2023 r.

Uchwała nr: APK.002.175.2023

Na podstawie art. 29 ust. 2 i 14 ustawy dnia 5 grudnia 1996 r. o zawodach lekarza i lekarza dentysty (t.j. Dz. U. z 2022 r. poz. 1731 z późn. zm.), Komisja Bioetyczna przy Uniwersytecie Medycznym w Białymstoku, po zapoznaniu się z projektem badania zgodnie z zasadami GCP/ Guidelines for Good Clinical Practice /- **w y r a ż a z g o d ę** na prowadzenie tematu badawczego: „Ocena parametrów stresu oksydacyjnego, obrony antyoksydacyjnej oraz białek odpowiedzi swoistej i nieswoistej w ślinie palaczy papierosów tradycyjnych, elektronicznych oraz systemów do podgrzewania tytoniu" przez lek. dent. Sarę Ziębę wraz z zespołem badawczym z UMB.

Planowany okres realizacji od 30.03.2023 r. do I.2025 r.

Przewodnicząca Komisji Bioetycznej przy UMB

prof. dr hab. Otylia Kowal-Bielecka