Journal of Oral Biology and Craniofacial Research 13 (2023) 740–750

Contents lists available at ScienceDirect

Journal of Oral Biology and Craniofacial Research

journal homepage: www.elsevier.com/locate/jobcr

Utility of salivary biomarkers for diagnosis and monitoring the prognosis of nicotine addiction - A systematic review

Shreya S.[a], Manoj Annamalai[b], Vasanti Lagali Jirge[b], Sneha Sethi[c,*]

[a] A B Shetty Memorial Institute of Dental Sciences, Mangalore, Karnataka, India
[b] KAHER'S KLE V.K. Institute of Dental Sciences, Belagavi, Karnataka, 590010, India
[c] Adelaide Dental School, University of Adelaide, Adelaide, 5000, Australia

A B S T R A C T

Objectives: Tobacco and smoke associated with tobacco comprises of a mixture of more than 9500 chemical compounds, most of which have been identified as harmful. Two of the most potent carcinogens found in cigarette smoke are N′-nitrosonornicotine (NNN) and polycyclic aromatic hydrocarbons (PAHs). The most commonly used method to detect and monitor nicotine addiction is via serum cotinine levels. Though considered the gold standard, there is a decline in preventive screening and diagnostic testing due to the fear of pain from invasive testing.

Data sources and study selection: A structured literature search was performed using the search engines PubMed and Google scholar following the PRISMA guidelines for systematic reviews. The titles and abstracts were retrieved and analysed, followed by full-text relevant data extraction in addition to a risk-of-bias analysis.

Data extraction and synthesis: A total of 37 studies were included in the systematic review. Salivary cotinine levels were compared between smokers and non-smokers, cigarette smokers and water pipe smokers, water pipe smokers and non-smokers. Lactate dehydrogenase salivary levels were compared between smokers and non-smokers, and salivary thiocyanate were compared between smokers and non-smokers.

Conclusions: Identifying biomarkers with high performance in terms of sensitivity and specificity will contribute to accelerating future research in this domain.

1. Introduction

Invasive diagnostic techniques have endured the gold standard testing for diagnosis and monitoring management of sickness and abnormalities, most commonly including blood tests and tissue biopsies. Whilst these tests are reliable and highly efficient with consistent diagnostic accuracy, the collection of blood samples requires venepuncture which is an invasive procedure potentially resulting in pain and phobia, and sensitivity of the technique demands the involvement of trained personnel.[1] Thus, even though the accuracy of such tests is favorable, these procedures are often viewed with apprehension and fear by the patient. As a result of the increased levels of anxiety there is a decrease in the uptake of preventive screening tests which is quintessential for early detection and prevention of disease, thereby highlighting the significance of patient acceptability towards diagnostic methodologies.

Non-invasive oral fluid testing is a popular alternative to plasma or urine for drug monitoring in treatment, workplace, criminal justice, and driving under the influence testing programs.[2] Saliva is by far the most readily available and non-objectionable fluid present in the body. Salivary diagnostics refer to the utilization of saliva as a diagnostic material. It has been reported that saliva has been previously used as a diagnostic fluid in various fields, however, there is scant literature or evidence available to support the reliability and validity of salivary biomarkers.

One of the most significant benefits of utilizing salivary biomarkers would be for monitoring the levels of nicotine in addiction programmes. Multiple reports have been published which have observed a considerable effect of cigarette smoke on the various components and constituents of saliva. Being one of the first biological fluids to face the toxicity of inhaled smoke, salivary diagnostics can be reliable and consistent.[3] Primary constituents of cigarette smoke are nicotine and cotinine. Cotinine is the principal metabolite of nicotine, and is a sensitive measure of exposure to second hand smoke (SHS). It is related to incidence of heart disease and stroke, and additionally the salivary cotinine levels are comparable to the levels observed in blood.[4] It has been recognised that there could be considerable variation regarding the extent of human exposure to carcinogens due to tobacco smoke; owing to dosage, frequency and method of smoke inhalation.[5] Apart from salivary cotinine, many other biomarkers such as salivary thiocyanate, cortisol, lactate dehydrogenase, etc., have also been explored in the diagnosis of nicotine addiction. The association between cortisol, smoking, and stress is one that is well-known.[6,7] However, the utility of salivary cortisol to

* Corresponding author.
E-mail addresses: dr.shreya.sridharan@gmail.com (S. Shreya), manojezhil1997@gmail.com (M. Annamalai), vasantijirge@live.com (V.L. Jirge), sneha.sethi@adelaide.edu.au (S. Sethi).

https://doi.org/10.1016/j.jobcr.2023.10.003
Received 12 June 2023; Received in revised form 12 September 2023; Accepted 8 October 2023

diagnose nicotine addiction is still not very popular despite ample research available for the same.[6,8,9] Lactate dehydrogenase has been advocated as a potential biomarker for diagnosing tissue damage and the extent of periodontal disease prevalent in a smoking population.[10] However, its direct relationship with nicotine addiction is an enigma that is yet to be resolved.

Tobacco and tobacco smoke comprises mixture of over 9500 chemical compounds which have been identified as hazardous to human health.[11] A total of 83 carcinogens are currently recognised – 3 in unburnt tobacco and 80 in tobacco smoke.[11] Unburnt tobacco can be consumed in the form of smokeless tobacco chewing habits which is a commonly observed habit in South Asian countries.[12] The most potent oral cancer causing carcinogens found in cigarette smoke are N′-nitrosonornicotine (NNN) and polycyclic aromatic hydrocarbons (PAHs).[5] NNN is a "tobacco-specific nitrosamines," which is a carcinogen derived from tobacco alkaloids.[5] The estimated NNN levels in mainstream cigarette smoke average to approximately 85 ± 31 ng/cigarette, based on the analysis of 50 different brands of cigarettes marketed in the United States.[13] Studies on rats have shown a direct link of oral cavity and oesophageal tumours due to the influence of NNN.[5] In addition to NNN a group of carcinogens which are formed due to the incomplete combustion of organic material called polycyclic aromatic hydrocarbons (PAH) have also been observed.[14] All of these carcinogens have been identified in tobacco smoke and have been linked to tumours of the upper respiratory tract and lungs, irrespective of route of consumption (inhalation, instillation in the trachea, or implantation in the lung).[14]

The usage of salivary diagnostics in monitoring oral diseases has been well-established, especially in cases of nicotine and alcohol consumption.[15–18] Utilizing salivary biomarkers could prove to be potentially significant due to the ease of sample collection and the technique being non-invasive.[19] Therefore, even monitoring of a patient to study the treatment outcomes could be made easier while using saliva as a testing sample as it does provide an upper hand in comparison to the self-reported smoking status[20] as at-home testing kits could be procured, thereby allowing remote monitoring as an option.[21] Thus, in the right scenario, salivary diagnostics could be a potential game-changer. However, there is a lacunae in the existing research, because while

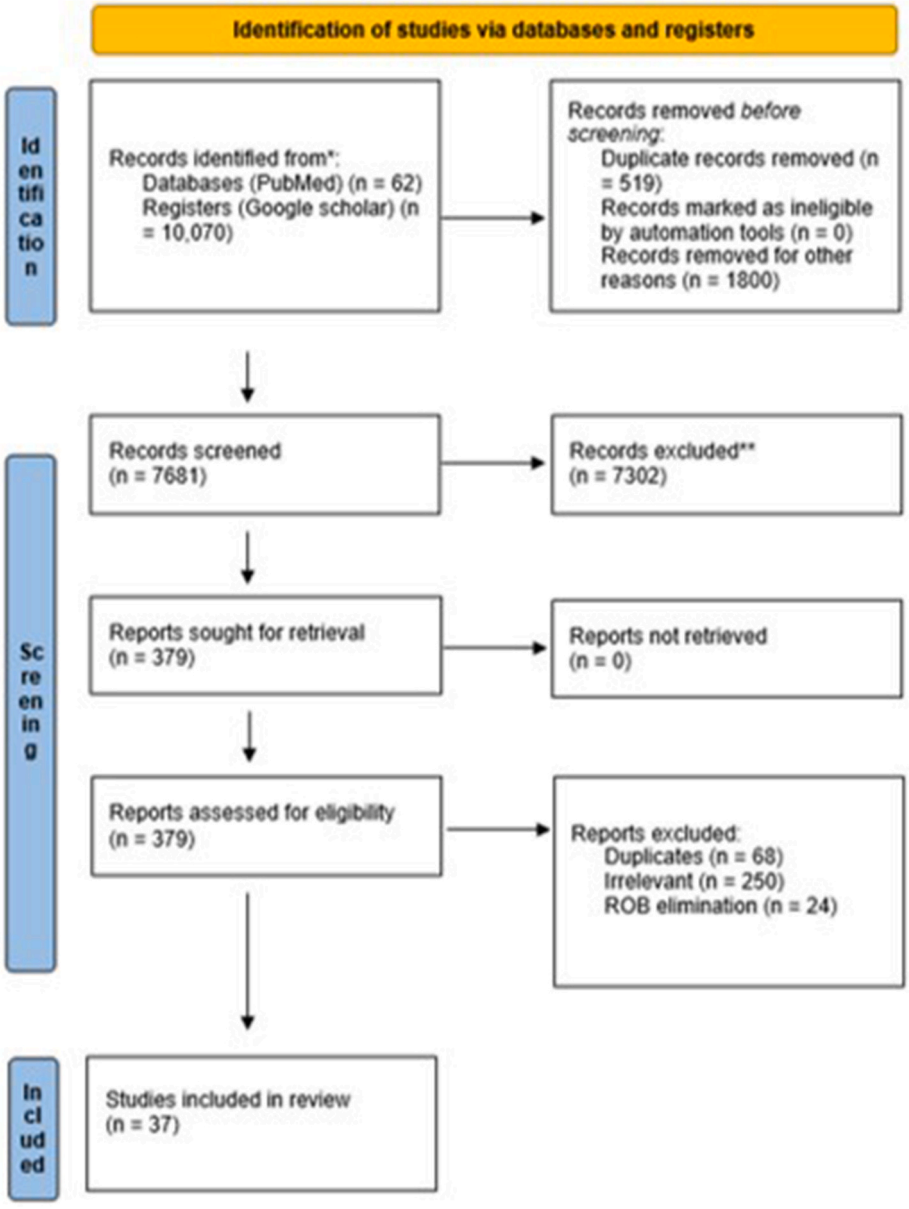

Fig. 1. PRISMA flowchart of search result.

many serum biomarkers have been established, a specific set of biomarkers which can accurately diagnose nicotine addiction and aid in monitoring the prognosis of the same is yet to be discovered. While there are systematic reviews assessing the efficacy of de-addiction programmes,[21–23] there have been no systematic reviews assessing the efficacy of salivary biomarkers in the same. Due to scarce research available evaluating the salivary cotinine levels, and its scope for utilization as a disease monitoring fluid, this review was performed to generate high quality evidence. The aim of this systematic review was to identify the levels of salivary biomarkers in individuals with nicotine consumers and non-consumers to determine the utility of saliva as a potent biomarker for the diagnosis and monitoring the nicotine levels in de-addiction programs. To the best of our knowledge, this is the first systematic review and meta-analysis to identify viable salivary biomarkers pertaining to nicotine addiction.

2. Materials and method

This systematic review has been registered in PROSPERO (CRD number: CRD42021168784) (supplementary file 1) and was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (Fig. 1).

The PICO question formulated for the purpose of this was as follows:

Participants - Adults who have experienced either passive or active exposure to nicotine.

Exposure - Any study that analysed, from an accuracy analysis perspective, a minimum of one or more objectively quantifiable biomarkers, detected in saliva.

Comparator/Control – Patient with no history of exposure or passive exposure to nicotine.

Outcomes – Levels of salivary biomarkers identified in participants and controls.

Studies that met the inclusion criteria were selected for further analysis.

A structured literature search was performed using the search engines PubMed and Google scholar from July 2020 to August 2022. The key words, “Saliva” AND “Salivary biomarkers” AND “Nicotine” were utilized to run the search engines and separate searches were performed with each individual biomarker in consideration e.g., Cotinine, Thiocyanate, Alkaline phosphatase, Lactate dehydrogenase, Carbon monoxide, NNAL, 1-OHPG and Cortisol (supplementary file 2).

Titles and abstracts were retrieved for all of the recognised studies and reviewed by two independent reviewers (SS and MA). Disagreements were resolved by discussion, consensus or involving the expertise of a third reviewer (VJ). Subsequently, full texts of all selected studies were retrieved, relevant studies were shortlisted following the predetermined inclusion and exclusion criteria;

Inclusion criteria:

1. Studies in which the combination of one or more biomarkers in saliva was evaluated, reporting results on the diagnostic accuracy for individuals presenting with active or passive exposure to tobacco products (reference standard).
2. Observational and cross-sectional test accuracy studies, longitudinal studies and interventional studies.
3. Studies published between 2005 and 2020.
4. Studies published in the English language

Exclusion criteria:

1. Studies conducted in vitro or in vivo, studies conducted on animals, and narrative reviews.
2. Articles that did not give sufficient information on group sizes or sensitivity and specificity values for the calculation of the contingency table.
3. Studies published before 2005 or after 2020.
4. Studies published in languages other than English.

Data extracted from all selected studies included; type of study (cross-sectional, longitudinal or interventional study), target and control conditions, reference standard (levels of salivary biomarkers identified), diagnostic criteria, patient characteristics including age, history of tobacco-use disorder, history of passive nicotine exposure, characteristics of the salivary sample (stimulated or unstimulated saliva; storage), index tests (number and type of host biomarkers analysed, type of technique for the detection of biomarkers; detection limit), statistical methods used and relevant results including, true positive, true negative, false positive, false negative, any equivocal results, withdrawal and classification threshold of the biomarkers.

The data was summarized using Microsoft Excel and descriptive data analyses were performed using RevMan 5.4.

The quality of the included studies was appraised by two independent reviewers (SS and MA) using the Newcastle Ottawa scale. A star (*) was awarded to the feature of the study that minimized risk of bias in each category. Studies were graded high, fair and low quality based on the number of stars; 6–9, 5–4, 3 or less, respectively.

3. Results

3.1. Study characteristics

A total of 10,070 studies were identified by searching the relevant databases, and 7681 titles and abstracts were eligible for screening after removal of duplicates (519). Based on their application, 379 studies were selected for full-text review. Out of those selected for full-text assessment, 342 were excluded, and 37 studies were included in the final pool of studies selected for this systematic review.

Among the studies included, the oldest was conducted in 2006[24] and the most recent in 2021.[25] Four studies were conducted in United States,[25–28] three in Poland,[29–31] three in Lebanon,[32–34] three in India,[35–37] and two in Canada,[38,39] four in Germany[40–42,43] two in Iran,[44,45] two in Saudi Arabia, one in South Korea,[46] one in Romania,[47] one in Nigeria,[48] one in New Zealand,[49] one in Jordan,[50] one in Israel,[3] and one in Brazil.[51] A majority of the studies had a case-control design,[3,26,30,32–34,36–38,40–43,48,49], with the rest having a cross-sectional,[25,29,31,35,45,47,50–53] or cohort study design,[27,28,39,44,13,14,25,30,32] The sample sizes ranged from 20 to 510 and the age from 18 to 68 y. All studies comprised an adult study sample (age >18 y).

The characteristics of the included studies are summarized in Table 1. The inter-reviewer reliability was 95.3 % (supplementary file 3) (see Table 2).

3.2. Study outcomes and measurements

Eleven studies compared the levels of cotinine between smokers and non-smokers[25,28,29,32,36,38,42,45,46,52,53]; two studies compared levels of lactate dehydrogenase in saliva between smokers and non-smokers[3,50]; three reported salivary levels of thiocyanate between smokers and non-smokers,[30,37,38] three studies compared salivary cotinine levels between cigarette smokers and waterpipe smokers,[32,45,53] and between non-smokers and waterpipe smokers.[32,45,53] All of the studies used the non-smoker group as the control group. Additional analysis was done on comparisons of salivary cotinine levels between passive or second-hand smokers, active smokers and non-smokers.[26–28,52]

Salivary cotinine were quantified with the High-performance liquid chromatography,[27,30,32,36,38,40,42,43,51] gas chromatography,[28,34,41,49] spectrophotometric assay,[26,37,44,48] or immunosorbent assays.[3,29,31,33,39,46,47,52]

Table 1
Characteristics of included studies.

Author, year of publication	Location of study	Mean Age	Tobacco form usage	Target conditions	Sample Size Target population	Sample Size Control	Biomarkers measured	Index texts	Characteristics of Saliva Sample used
CASE-CONTROL STUDIES									
Allwright S et al., 2005	Republic and northern Ireland	Republic of Ireland:45.5 Northern ireland:36.1	Cigarette	SSSE[a]	288	41	Cotinine	NS[a]	Non-SS[a]
Bacha ZA et al., 2007	Beirut	Waterpipe smokers - 27.5y Cigarette smokers - 36.4y Non-smokers - 37.5y	Waterpipe Cigarette	Exhaled CO[a] + saliva cotinine	Waterpipe smokers[15] Cigarette smokers[20]	20	Cotinine	HPLC[a]	Expectorated samples
Nagler RM et al., 2007	Israel	64 ± 13y	Cigarette	Saliva composition	25	25	LDH[a]	Chemistry analysis	Expectorated samples
Scherer G et al., 2007	Germany	29.1Y	Cigarette	Smoking exposure	202	100	Cotinine	LC–MS/MS[a]	Expectorated samples
Cooke F et al., 2008	Auckland	Range – 18-70y	Cigarette	NCTS[a]	50	50	Cotinine	Gas chromatography	SS[a]
Pascale S et al., 2009	Beirut	22-35y	Cigarette Waterpipe	Salivary levels	Waterpipe (103) Cigarette[42]	43	Cotinine	Colorimetric method (Saliva Smokescreen)	saliva samples collected via expectoration
Shepperd CJ et al., 2009	Germany	Non-smokers - 44y Smokers - 38y	Cigarette	Comparing methods	Smokers = 150	50	Cotinine	LC–MS/MS[a]	saliva samples collected via expectoration
Morin A et al., 2011	Canada	non smokers-41yrs, smokers-40 yrs	Cigarette	Comparing methods	142	50	Cotinine	LC–MS/MS[a]	Expectorated Saliva
Shepperd CJ et al., 2011	Germany	Non-smokers - 44y Smokers - 38y	Cigarette	Comparing methods	250	50	Cotinine	LC–MS/MS[a]	Expectorated Saliva
Lawhorn NA et al., 2013	USA[a]	Range – 18-45y	SSSE[a]	exposure to SSSE[a]	31	10	Cotinine	Centrifugation	Non-SS[a]
Parthiban et al., 2013	India	Smokers – 44y Non-smoker - 34y	Cigarette	Cotinine levels	15	15	Contine	Chromatography	Non-SS[a]
Martínez-Sánchez JM et al., 2014	Spain	Range - 18–40 y	Cigarette	SSSE[a]	25	24	Contine	LC/MS/MS[a]	SS[a]
Mueller DC et al., 2014	Germany	Smokers - 36.5y± 9.1 Non-smokers - 36.8 ± 9.7y	Cigarette	GC-TOF-MS[a]	25	25	Contine	GC-TOF-MS[a]	Non-SS[a]
Zir EE, 2016	Beirut	Range – 21 -50y	Cigarette	Nicotine dependence	100	100	Contine	Gas chromatography	Non-SS[a]
Muhammad II et al., 2017	Nigeria	Range – 18 - 68y	Cigarette	Salivary TCN[a] level	Smokers[15] Ex-smokers[18]	40	TCN[a]	Spectrophotometry	Expectorated Saliva
Neves CDC et al., 2017	Brazil	Non-smokers – 31y Light smokers – 37y Heavy smokers – 34y	Cigarette	Salivary cortisol level	14	13	Cortisol	EIA[a]	Salivettes
al'Absi M et al., 2018 2018	USA[a]	34.8 y	Cigarette	effect of early life adversity –nicotine withdrawal	112	44	Cortisol	FIA[a]- cortisol-biotin conjugation	Salivettes
Prakruthi BV et al., 2018	India	Range – 18–25y	Cigarette	effects of salivary TCN[a]	35	35	TCN[a]	Spectrophotometry	Non-SS[a]

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Table 1 (*continued*)

Author, year of publication	Location of study	Mean Age	Tobacco form usage	Target conditions	Sample Size Target population	Sample Size Control	Biomarkers measured	Index texts	Characteristics of Saliva Sample used
				levels on oral mucosa					
Flieger J et al., 2019	Poland	Range – 40–45y	Cigarette E-cigarette	salivary TCN[a] levels	Smokers[8] e cigarette[8]	8	TCN[a]	HPLC-UV –using a unique phosphatidylcholine column	Expectorated Saliva
Elbüken G et al., 2020	Turkey	Smokers - 42.6y Non-smokers - 40.8 y	Cigarette	Salivary cortisol level	25	25	Cortisol	High-sensitivity EI[a] kit	non stimulated saliva
COHORT STUDY DESIGN									
Woodward A et al., 2005	New Zealand	Range - 22–26 y	Cigarettes	SSSE[a]	11	11	Cotinine	HPLC, mass spectrometer	Un-SS[a]
Dinusha Fernando D et al., 2007	New Zealand	Range - 24–45 y	Cigarettes	SSSE[a]	5	5	Cotinine	HPLC, mass spectrometer	Un-SS[a]
Gotz NK et al., 2008	England	18->/ = 65	Cigarettes	SSSE[a]	66	48	Cotinine	gas–liquid chromatography	Un-SS[a]
Ferna'ndez E et al., 2009	Spain, Portugal, and Andorra	**Median** Spain - 39.4y Portugal & Andorra - 37.1y	Cigarettes	SSSE[a]	137	137	cotinine	Capillary gas chromatography and mass spectrometry.	Stimulated saliva samples
Hall JC et al., 2009	Greece	21-30y	Cigarettes	SSSE[a]	31	31	Cotinine	Mass spectrometry.	Un-SS[a]
Pearson J et al., 2009	USA[a]	NS[a]	Cigarettes	Tobacco smoke levels and respiratory symptom reports	46	46	Cotinine	Liquid chromatography-tandem mass spectrometry (LC-MS/MS).	Un-SS[a]
Azar R et al., 2011	Canada	18.8y	Cigarettes	Salivary CRP levels	13	Active - 10 Passive - 22	Cotinine	EIA[a]	NS[a]
St.Helen G et al., 2012	Greece	21-40y	Cigarettes	SSSE[a]	24	24	Cotinine	LC APCI MS/MS[a]	Un-SS[a]
Alfred K. Mbah AK et al., 2013	USA[a]	18-44y	Cigarettes	ETS exposure	Non-smokers[23] passive smokers (106) smokers (107)	NS[a]	Cotinine	ICA[a]	NS[a]
Rajkumar S et al., 2013	Switzerland	18-65y	Cigarettes	SSSE[a]	NS[a]	NS[a]	Cotinine	Liquid and gas chromatography	Un-SS[a]
Batty DG et al., 2014	UK[a]	51y	Cigarettes	Cotinine with mortality	2523		Cotinine	Gas-liquid chromatography	Un-SS[a]
Kim S et al., 2014	South Korea	Median – 45y	SSSE[a]	Tobacco smoke exposure	Non-smokers (77) Passive smokers (105)	107	Cotinine	GC-MS/MS[a]	Saliva collected via expectoration
Liu KH et al., 2016	South Korea	Non-smokers 34y Smokers 29.3y Non-quitters 45.3y	Cigarettes	Smoking cessation effect	Non-smokers[13] Quitters[11]	Non-quitters[9] oscillators[6]	Cotinine	HPLC[a]	Unstimulated saliva samples
Melstrom P et al., 2018	USA[a]	28-54y	E-cigarettes	SSSE[a]	6	6	Cotinine	HPLC[a]	NS[a]
CROSS-SECTIONAL STUDY DESIGN									
Bachanek T et al., 2015	Poland	Non-smokers - 29.12y Smokers = 32.08y	Cigarette	Saliva analysis	49	66	Cotinine	Cotinine test (Calbiotech)	SS[a] and Un-SS[a]
Hamad AWR et al., 2015	Jordan	19-24y	Cigarette	Effects of smoking on liver functions	50	50	ALP[a], LDH[a]	Assays	NS[a]
Suzuki N et al., 2016	Japan	Smokers: 26.8y Non-smokers: 25.0y	Cigarette	Salivary stress biomarker levels	18	31	Cortisol	ELISA[a]	Un-SS[a]

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Table 1 (*continued*)

Author, year of publication	Location of study	Mean Age	Tobacco form usage	Target conditions	Sample Size Target population	Sample Size Control	Biomarkers measured	Index texts	Characteristics of Saliva Sample used
Rudnicka MN et al., 2017	Poland	Non-smoking - 29.9y Smoking - 31.8y	Cigarette	Streptococcus mutans and Lactobacillus levels	53	63	Cotinine	Cotinine ELISA[a]test (Calbiotech)	Un-SS[a]
Fallatah AA et al., 2018	Egypt	Smokers - 22.9y Non-smokers - 24.8y Passive smokers - 25.6y	Cigarette, hookah	Salivary cotinine levels	10	10	Cotinine	EIA[a] NicAlert	Un-SS[a]
Mokeem SA et al., 2018	Saudi Arabia	Cigarette smokers - 42.4y Waterpipe smokers - 44.7y E-cig users - 28.3y Never-smokers - 40.6y	Cigarette, Waterpipe, E-cigarettes	Clinical, radiographic, periodontal inflammatory parameters	Cigarette smokers,[39], Waterpipe smokers,[40], e-cigarette[37]	38	Cotinine	ELISA[a]	Un-SS[a]
Arora KS et al., 2019	India	25-50y	Cigarette	Salivary levels	100	100	Cotinine	ELISA[a]	Un-SS[a]
Shaikh RB et al., 2019	UAE	25 to >35y	Cigarette and Midwakh	Salivary levels	Cigarette smokers[54] Midwakh smokers[52]	53	Cotinine	NicAlert strips	Un-SS[a]
Rosa MB et al., 2020	Brazil	Smokers 29.4y Non-smokers 27.6y	Cigarette	Ttactile and gustatory sensitivities	27	27	Cotinine	HPLC[a]	Un-SS[a]
Ye D et al., 2020	USA[a]	E-cigarettes - 34.9y Non-smokers - 35.6y Cigarette smokers - 40.2y Dual smokers - 39.4y	E-cigarettes, Cigarettes	Saliva and gingival crevicular fluid (GCF) profiles	Cigarette smokers[12] e-cig users[12] dual smokers[12]	12	Cotinine	EIA[a]	Un-SS[a]
Rabiei M et al., 2014	Iran	Hookah smokers - 23.5y Cigarette smokers - 24.1y Non-smokers - 21.6y	Waterpipe smoker, Cigarette	Saliva cotinine levels	Cigarette smoker[16] waterpipe smoker[16]	16	Cotinine	EIA[a]	Expectorated saliva
Jia X et al., 2022	China	18-62y	SSSE[a]	SSSE[a]	28	194	Cotinine	NS[a]	SS[a]
Ghazi A et al., 2020	Iran	35.7y	Cigarettes	Salivary levels	32	34	Cotinine	Cotinine kit (ZellBio GmbH, Germany)	Un-SS[a]
Pandarathodiyil AK et al., 2021	Malaysia	Smokers: 32.3y among smokers, and 26.00 ± 7.35 among vapers	Cigarettes, vaping	Effects on oral tissues	Smokers[30] Vapers[30]	30	LDH[a]	LDH[a] Colorimetric Assay	Un-SS[a]

[a] USA – United States of America, LDH – Lactate Dehydrogenase, TCN- Thiocyanate, EI- enzyme immunoassay, HPLC-UV - Liquid Chromatography with ultraviolet detection, FIA- Fluorescence immunoassay, GC-TOF-MS - gas chromatography coupled to time-of-flight mass spectrometry, LC/MS/MS -Liquid chromatography + tandem mass spectrometry with multiple reaction monitoring, NS – Not Specified, SSSE – Secondhand smoke exposure, CO – Carbon monoxide, SS – Stimulated Saliva, NCTS - NicAlert cotinine test strips; CRP–C Reactive Protein, ETS - environmental tobacco smoke, ICA- Immuno-chromatographic assay, UK – United Kingdom, ALP - Salivary Alkaline phosphatase, ELISA- Enzyme linked immunosorbent assay.

Table 2
Description of salivary cotinine levels among active smokers, passive smokers, and non-smokers.

Author	Mean Age	Active smokers	Passive smokers	Non-smokers	Salivary cotinine levels	
					Passive vs non-exposed	Passive vs active smokers
Lawhorn, NA (26)	Range - 18 - >45y	–	31	10	Passive - 2.6 ng/ml. Non-exposed - 0.5 ng/ml	–
Mbah AK (27)	Range - 18–44 y	107	106	23	Passive smokers- 1.1 ± .4 Non-exposed - 0 ± 0	Passive smokers- 1.1 ± .4 Active smokers 2.1 ± 1.4
Kim S (28)	Median 45 y	107	105	77	Passive smokers - 0.41 (0.035–1.08) Non-exposed - 0.27 (0.04–0.61)	Passive smokers - 0.41 (0.035–1.08); Active smokers - 135.1 (62.2–228.6)
Fallatah AA (52)	Non-smokers - 24.8 ± 6.1; Passive smokers - 25.6 ± 4.57	10	10	10	Non-exposed - 1.5 ± 0.5 Passive smokers - 2.1 ± 0.8	Passive smokers - 2.1 ± 0.8 Active Smokers - 5.2 ± 1.3;

3.3. Salivary cotinine levels

Cigarette smokers and non-smokers: The 20 studies that reported on salivary cotinine between smokers and non-smokers showed similar results.[25,28,29,32–36,38–47,52,53] Of these 20 studies, only eleven studies were eligible for meta-analysis, reasons for exclusion from meta-analysis included no control group,[40,43] reporting of cotinine as a categorical variable instead of continuous,[33] exclusive genomic analysis and inadequate information like mean values.[34,35,41,47] Meta-analysis of the included studies (Fig. 2) had a total of 506 subjects in the experimental or smokers' group and a total of 386 subjects in the control or non-smokers group revealed a mean difference of 247.66 (95 % CI 62.17–433.16; $p < 0.00001$). The heterogeneity amongst the included studies was high, indicating towards large variations in study design, analysis techniques and study setting.

Cigarette smokers and Water-pipe smokers: Four studies that reported on salivary cotinine between cigarette smokers and waterpipe smokers, three studies showed similar results,[32,33,44,45] except one.[53] Of these four studies, only three studies were eligible for meta-analysis,[32,45,53], reasons for exclusion from meta-analysis was categorical description of cotinine levels in both groups.[33] Meta-analysis of the included studies (Fig. 2) had a total of 75 subjects in the experimental or cigarette smokers' group and a total of 71 subjects in the control or waterpipe smokers group revealed a mean difference of 2.49 (95 % CI -21.21-26.20; $p = 0.11$). The heterogeneity amongst the included studies was around 55 %, indicating a more uniform study design and setting amongst the included three studies.

Water-pipe smokers and non-smokers: All four studies that reported on salivary cotinine between waterpipe smokers and non-smokers showed similar results.[32,33,45,53] Of these four studies, only three studies were eligible for meta-analysis,[32,45,53] reasons for exclusion from meta-analysis was categorical description of cotinine levels in both

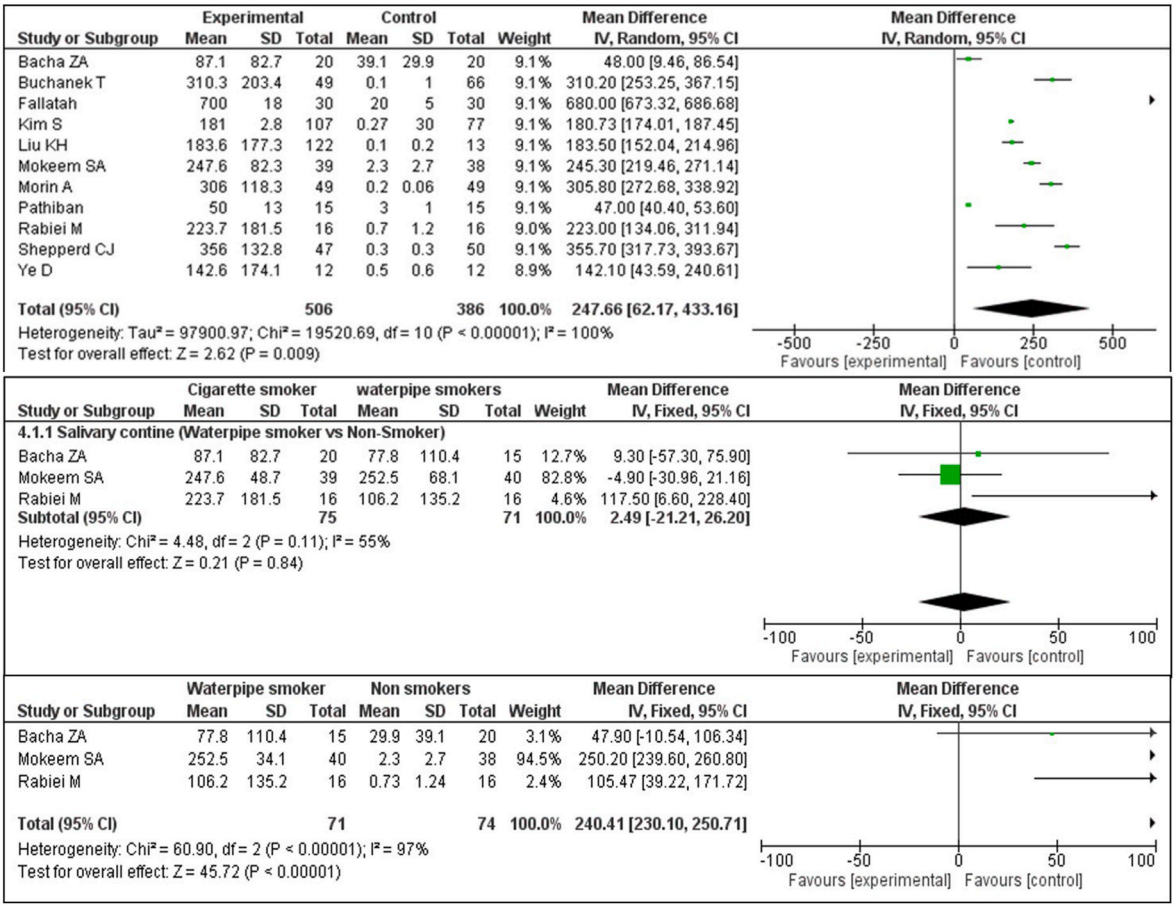

Fig. 2. Forest plots comparing salivary cotinine levels between smokers and non-smokers (upper) and smokers and water-pipe smokers (lower).

groups.[33] Meta-analysis of the included studies (Fig. 2) had a total of 71 subjects in the experimental or waterpipe smokers' group and a total of 74 subjects in the control or non-smokers group revealed a mean difference of 240.41 (95 % CI 230.10–250.71; $p < 0.00001$).

3.4. Lactate dehydrogenase levels

The two studies that reported on lactate dehydrogenase levels between smokers and non-smokers showed completely contrasting results. One study suggested an increased (86 %) detection of LDH levels in non-smokers[3] and the other study[50] suggested a lower mean level amongst non-smokers (76.92 ± 6.98) as compared to smokers (132.58 ± 11.73).

3.5. Salivary thiocyanate levels

Three studies that reported on salivary thiocyanate levels between cigarette smokers and non-smokers showed similar results.[30,37,48] Meta-analysis of the relevant studies (Fig. 3) had a total of 51 subjects in the experimental or smokers' group and a total of 83 subjects in the control or non-smokers group revealed a mean difference of 1.79 (95 % CI 1.76–1.82; $p = 0.13$). The heterogeneity amongst the included studies was around 52 %, indicating a more consistent study design and setting between the included three studies.

3.6. Passive smokers/second-hand smoke intake

The data extracted for this systematic review also enables the authors to perform an additional comparison of salivary cotinine levels between non-exposed (to smoke), active smokers and passive smokers. This was recognised as a significant insight (Table 1), which would help in investigating the actual level of carcinogenic elements (cotinine) amongst subjects who were not actively smoking but inhaled smoke due to environmental circumstances.

Passive smoker's vs non-smokers: Four studies that reported on salivary cotinine levels between passive smokers and non-exposed showed similar results.[26–28,52] A total of 252 subjects in the passive smokers' group revealed consistently higher salivary cotinine levels compared to a total of 110 subjects in the non-exposed group. Meta-analysis of the data from these four studies was not possible due to inconsistent reporting of variable information between the studies.

Passive smoker's vs active smokers: Three studies that reported on salivary cotinine levels between active smokers and passive smokers showed similar results.[27,28,52] A total of 224 subjects in the active smokers' group revealed consistently higher salivary cotinine levels compared to a total of 222 subjects in the passive smokers group. Meta-analysis of the data from these four studies was not possible due to inconsistent reporting of variable information between the studies.

4. Discussion

Consumption of tobacco and its products has had significant social, financial and health related implications on our society.[54] Nicotine, which is the primary and most dangerous component of tobacco is associated with life threatening psychological and physical impacts like habitual cravings and noticeable withdrawal effects. The majority (70–80 %) of Nicotiana is broken to cotinine by hepatosomal micro-enzymes.[55] Further break down cotinine to *trans*-3′-hydroxycotinine is due to the action of CYP2A6.[56] Nicotine is marketed in various forms such as cigarettes, e-cigarettes, hookah, nicotine gums, nicotine patches, lozenges, etc. The magnitude of the multiple effects of cigarette smoke especially on salivary components and their subsequent interactions have not been studied thoroughly despite the fact that saliva is the first biological fluid to encounter inhaled cigarettes.[3] The levels of salivary biomarkers offer a promising diagnostic adjunct owing to its simple non-invasive collection method and it could be a potential tool for large population based screening.[57] It is a well-known fact that substances present in saliva can permeate into plasma via passive diffusion or active transportation; therefore, a reliable estimation can be made by examining both saliva and blood.[29]

The present study summarizes existing evidence on principal salivary biomarkers and compares the accuracy in diagnosis and monitoring of the prognosis of nicotine de-addiction in tobacco consumers. The most important biomarkers identified included: Cotinine, Lactate dehydrogenase, Thiocyanate, NNAL 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, 1-OHPG (1-hydroxypyrene glucuronide), Alkaline phosphatase, Carbon monoxide, Cortisol.

Cotinine is the most promising biomarker and is particularly useful as it has a comparable half-lives in both plasma and saliva,[29] and also has the ability to detect tobacco components (active or due to SHS exposure use) from exposures 2–3 days old.[49] Cotinine is considered as the biomarker of choice to estimate the absorption of tobacco smoke; as it can be easily detected in various body fluids like blood, urine and saliva,[58] and because of its greater sensitivity and specificity than other biochemical tests.[59,60] The incorporation of salivary cotinine levels in the diagnostic process enhances objectivity by circumventing reliance on potentially flawed self-reported data. This objectivity is particularly valuable in assessing addiction severity and monitoring treatment efficacy. However, the meta-analysis of studies reporting an association between the levels of salivary cotinine in smokers and non-smokers had a high degree of heterogeneity amongst the included studies was high, which could be attributed to variable study designs and statistical analysis used. Whilst the studies conducted by Bacha et al., Kim et al., Liu KH et al.,[28,32,52] reported varying baseline values for salivary cotinine concentration in smokers and non-smokers, they support the fact that salivary cotinine is a reliable biomarker specific to the use of nicotine addiction.[28,32,52]

The sub-group meta-analysis of the included studies on the basis of smoking method (cigarette smokers and water pipe smokers) included a total of 75 subjects in the experimental or cigarette smokers' group and a total of 71 subjects in the control or water pipe smokers' group with a heterogeneity of around 55 %, indicating an increase in the level of methodological consistency between studies. All three studies indicated that the salivary cotinine levels in both groups were comparably close, signifying that saliva can be used to assess cotinine levels in cigarette smokers and water pipe smokers.[45,52,53]

LDH is a cytoplasmic enzyme present in essentially all major organ systems[61] which plays a crucial role in the conversion of glucose to energy which is utilized by body cells. When tissues are damaged they

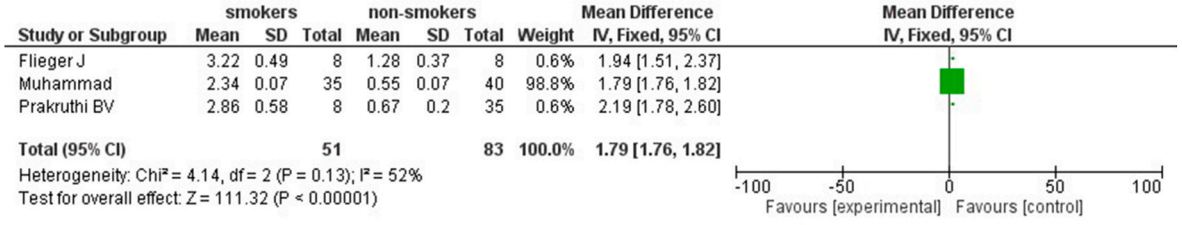

Fig. 3. Forest plots comparing salivary lactate thiocyanate levels between smokers and non-smokers.

release LDH into the blood stream or other body fluids.[62] Rise in LDH levels would be an indication of cellular damage via disease or Injury.[63] Cigarette smoking leads to an increase in serum as well as salivary LDH levels as an indicator of tissue damage in the oral cavity.[64] While the levels of salivary lactate dehydrogenase have been estimated in two studies,[3,50] both showed completely contrasting results. The issue with utilizing LDH as an exclusive biomarker for diagnosis and monitoring of nicotine addiction would be the fact that no direct causal pathway has been established between LDH and nicotine addiction. Most of the studies utilizing LDH for analysis have been conducted in conjunction with other conditions such as periodontal disease, cancer.[3,10,50,64] This indicates that further research needs to be performed specifically studying LDH levels in order to provide more concrete and reliable evidence.

Sub-groups meta-analysis of the relevant studies pertaining to salivary thiocyanate levels were with a more consistent study design and setting All three studies included concluded that the salivary thiocyanate levels in smokers was significantly higher than that of non-smokers, with Flieger et al. stating that the highest levels were observed in e-cigarette smokers.[30,37,48] The thiocyanates found in body fluids result, in part, from detoxification of hydrogen cyanide in cigarette smoke. These observations have led to utilization of serum thiocyanate levels to document adult smoking cessation. Hydrogen cyanide, a component of mainstream smoke of all cigarettes[65] is detoxified to thiocyanate ion (SCN), which can be detected in body fluids. The half-life of this ion has been demonstrated to range from 10 to 14 days in normal adults.[66] These characteristics imply that SCN could potentially be utilized as a mainstream biomarker for smoking detection and possible dose measurement. However, SCN is also found in various fruits and vegetables which are consumed as a part of day-to-day life. Therefore, baseline SCN levels in smokers and healthy individuals need to be established in order to utilize SCN as a diagnostic biomarker.

Cortisol is a stress hormone which could be influenced by cigarette smoking[67] Cigarette smoking can interfere with steroid hormone release, binding, transport, storage, metabolism, and clearance, resulting in changes in circulating hormone concentrations.[68,69] Cortisol, which is an effector of the Hypothalamus-pituitary-adrenal axis (HPA axis), increases after nicotine administration and decreases in response to acute tobacco abstinence.[70]

The relationship between salivary cortisol and smoking has been a matter of debate with conflicting reports; with some studies stating no difference between smokers and non-smokers with respect to salivary cortisol levels[67,70] and others state a lower level has been observed in non-smokers.[71] A sub group meta-analysis on the basis of cortisol levels could not be performed due to lack of sufficient data. The assessment of salivary cortisol whilst controlling for the effect of stress is another factor to be taken into consideration to avoid the confounding results.

An additional comparison of salivary cotinine levels between non-exposed (to smoke), active smokers and passive smokers was also performed. The studies reporting on salivary cotinine levels between passive smokers and non-exposed revealed consistently higher salivary cotinine levels in the smokers group.[2,26,27,52] Sub-groups meta-analysis of the data including the four relevant studies was not possible due to inconsistent reporting of variable information between the studies. The studies reporting on salivary cotinine levels between active smokers and passive smokers revealed consistently higher salivary cotinine levels in the active group.[2,27,52]

Cigarette smoke contains a large number of chemical substances with hepatotoxic potential including nicotine.[72] Liver function is assessed using mainly serum total cholesterol (TC), total protein, albumin, alkaline phosphatase (ALP), total bilirubin (TB), etc.[50] Salivary alkaline phosphatase (S-ALP) is a clinical biomarker, and its increased level indicates inflammation and destruction of healthy tissues suggesting it as a clinical biomarker.[73] A study conducted by Hamad et al.[50] showed a significant association between smoking and salivary alkaline phosphatase where S-ALP levels were increased in smokers in comparison to non-smokers. However, a meta-analysis could not be performed due to lack of sufficient data regarding the same.

While the total set of initially identified biomarkers included salivary NNAL, 1-OHPG, and carbon monoxide, the data available with respect to the same were not assessed as many of them had a high risk of bias and did not provide sufficient data.

From the literature reviewed as well as the meta-analysis, it can be concluded that salivary cotinine and salivary thiocyanate are the only biomarkers which have demonstrated an acceptable diagnostic capability and could be used for the monitoring of prognosis of nicotine addiction as well. As far as monitoring prognosis is concerned, there is a lack of research utilizing salivary biomarkers as a tool to monitor nicotine addiction. Most of the studies associated with prognosis have predominantly utilized serum and urinary biomarkers of which the most assessed is cotinine. It could be hypothesised that if a salivary biomarker does exhibit excellent diagnostic capability, then the same could be assessed as an evaluative factor in monitoring prognosis as well.

However, the same cannot be stated when it comes to salivary cortisol, lactate dehydrogenase, as well as alkaline phosphatase, the prime factors being the lack of consistent data, the association of comorbidities in the studies assessing these biomarkers, as well as the lack of good study designs with limited bias. These could be potential avenues for further research in the field of nicotine addiction as indicated by the clear deficit in the availability of established salivary biomarkers. These issues if addressed could pave the way for a new agenda in salivary diagnostics. Identifying salivary biomarkers with high sensitivity and specificity could lead to the development of non-invasive diagnostic tests for nicotine addiction that are more acceptable to patients and easier to implement in various settings. This could potentially improve early detection, intervention, and monitoring of nicotine addiction, ultimately leading to better patient outcomes and public health outcomes.

E-cigarettes, also known as electronic cigarettes or vapes, have amassed popularity in recent years as a newer alternative to tobacco products. They are often marketed as an alternative to aid in quitting smoking as they are likely to be less addictive due to the slower delivery of nicotine. The differences in cotinine levels[74] result from several factors prior level of tobacco dependence, nicotine content in e-liquids, number of puffs, e-cigarette model, electric power of the device, vapour temperature and density, nicotine concentration in the vapour (versus in liquids), volume of puffs, depth of inhalation, duration of apnoea between inhalation and exhalation and each individual's specific nicotine metabolism.[75] Therefore, when it comes to electronic nicotine delivery systems, the baseline values of biomarkers established for traditional tobacco products and these may vary. Studies have been conducted using salivary thiocyanate,[30] lactate dehydrogenase,[76] as well as salivary cotinine (Bullen 2010) in conjunction with e-cigarettes, but there is not sufficient data available to come to a consensus and establish baseline values for these biomarkers. These could be explored further as e-cigarettes and vapes are gaining more popularity of late.

The limitations of this review were that the studies included were only in the English language, did not include grey literature and full text of some potentially relevant articles could not be obtained. Another limitation is that sub-groups meta-analysis could not be performed for all of the biomarkers included, due to limited nature of data.

5. Other information

The present systematic review has been registered in the International prospective register of systematic reviews, PROSPERO [Registration ID: CRD42021168784]. The protocol is accessible with the following link: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021168784.

6. Conclusion

The present systematic review included data extracted from 37 research articles reporting salivary biomarkers in smokers, passive smokers and non-smokers as well as provided meta-analysis with a subset group. Identifying biomarkers with high performance in terms of sensitivity and specificity will accelerate future research in this domain. This could prove to be a game-changer in the field of diagnostic medicine as salivary biomarkers would be easier to obtain and would be more acceptable to the patient as well.

Data availability statement

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the corresponding author (S.S.).

Funding sources

No specific funding was procured for this research study.

Author contribution statement

Shreya S: Study design, Data extraction, Critical appraisal, drafting, and revision of manuscript. Manoj Annamalai: Data extraction, Critical appraisal, drafting, and revision of manuscript, Vasanti Lagali Jirge: conceptualization, study design, registration recruitment, drafting and manuscript review, Sneha Sethi: data analysis, writing manuscript, critical review and revision.

Open science transparency statements

(1) ***study registration:*** This systematic review has been registered in PROSPERO (CRD number: CRD42021168784)
(2) ***analytic plan registration:*** The analytic plan of this review has been described and registered with PROSPERO (CRD number: CRD42021168784)
(3) ***availability of data:*** The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the corresponding author (S.S.).
(4) ***availability of analytic code:*** NA
(5) ***availability of materials:*** NA

Declaration of competing interest

The authors have no conflicts of interest to declare.

Acknowledgement

NA.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jobcr.2023.10.003.

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