J. Maxillofac. Oral Surg. (2024) 23:808–815
https://doi.org/10.1007/s12663-024-02234-0

ORIGINAL ARTICLE

Investigating Tobacco's Impact on DNA Repair Genes and Risks in Oral Precancer and Cancer: A Comprehensive Research Study

Shadab Mohammad[1] · Sana Farooqui[1] · Saurabh Srivastava[2,6] · Tan Ching Siang[2] · Sathvik Belagodu Sridhar[3] · Irfan Ahmad[4] · Saad Alamri[5]

Received: 5 February 2024 / Accepted: 6 June 2024 / Published online: 15 June 2024
© The Association of Oral and Maxillofacial Surgeons of India 2024

Abstract

Objective This study aimed to explore genetic variations associated with DNA repair mechanisms to enhance the management of both oral cancer (OC) and oral precancer (OPC).

Methods A cohort of 380 patients diagnosed with OC and OPC, comprising 220 males and 160 females, was analyzed. Participants were categorized based on their tobacco-chewing habits, with corresponding control groups established. Key genetic markers investigated for polymorphisms included OGG1, APE1, and XRCC1.

Results The XRCC1 Arg280H variant demonstrated significant associations with the susceptibility to both OC and OPC across various models. Further analyses, incorporating factors such as tobacco and alcohol consumption, unveiled a correlation between the XRCC1 Arg194Trp variant and an elevated risk of developing head and neck cancer. Stratified analyses also revealed an increased risk of OC or OPC based on the specific site of the cancer.

Conclusion The study underscores the importance of XRCC1 polymorphisms, particularly XRCC1 Arg280H and XRCC1 Arg194Trp, within the genetic framework of OC and OPC. Understanding these genetic associations provides valuable insights for the potential development of targeted interventions aimed at individuals predisposed to these conditions.

Keywords Genetic polymorphism · Oral cancer · OGG1 · APE1 · XRCC1

Introduction

Cancer, a complex outcome of dysregulated cell division and genetic mutations, remains a formidable global health challenge. The World Health Organization (WHO) projected over 20 million new cancer cases and more than 10 million deaths worldwide in 2021 [1], underscoring the urgent need

✉ Shadab Mohammad
shadab31aug@yahoo.com

✉ Saurabh Srivastava
saurabhsrivastava.kgmu@gmail.com

Sana Farooqui
sana17feb@gmail.com

Tan Ching Siang
tcsiang@kpjuc.edu.my

Sathvik Belagodu Sridhar
sathvik@rakmhsu.ac.ae

Irfan Ahmad
irfancsmmu@gmail.com

Saad Alamri
saralomari@kku.edu.sa

[1] Department of Oral & Maxillofacial Surgery, King George's Medical University, Lucknow, UP, India

[2] School of Pharmacy, KPJ Healthcare University College, Nilai, Malaysia

[3] RAK College of Pharmacy, RAK Medical & Health Sciences University, Ras Al Khaimah, UAE

[4] Department of Clinical Laboratory Sciences, College of Applied Medical Science, King Khalid University, Abha, Saudi Arabia

[5] Department of Biology, College of Science, King Khalid University, Abha, Saudi Arabia

[6] Era College of Pharmacy, Era University, Lucknow, India

for advanced approaches to early detection and intervention [2].

This study focuses on oral cancer (OC), ranking as the sixth most prevalent cancer globally, with particular attention to its impact in Asia. Late-stage diagnoses, observed in approximately 70% of cases, result in a dismal five-year survival rate of about 20% [3]. Recognized risk factors include tobacco usage, betel-quid chewing, alcohol consumption, inadequate oral hygiene, poor dietary habits, and viral infections such as human papillomavirus (HPV) [4, 5]. Genetic variations within DNA repair genes, crucial for maintaining genomic stability, play a pivotal role in the complex origins of oral cancer.

The DNA repair mechanism, pivotal for correcting genetic damage and preventing mutations, encompasses a intricate network of genes. Notably, the OGG1 gene located on chromosome 3p25, responsible for encoding the 8-oxoG repair enzyme, harbors a polymorphism (1245C > G) associated with an increased cellular mutation rate, contributing to various cancers, including oral cancer [6]. Playing a crucial role in DNA repair mechanisms and combating oxidative stresses within cells, apurinic/apyrimidinic endonuclease 1 (APE1) serves as a versatile enzyme essential for preserving genome stability. Its involvement in DNA repair processes correlates with resistance to anticancer drugs, mitigating the effects of radiotherapy, promoting tumor aggressiveness, and predicting unfavorable prognoses. [7].

Situated on chromosome 19q13.2, XRCC1 encodes a crucial protein involved in rectifying single-stranded DNA. Alterations in XRCC1, such as (C > T) in exon 6, (G > A) in exon 10, and (G > A) in exon 9, impact DNA repair efficiency and are associated with cancer development. Understanding these genetic nuances is pivotal for unraveling the intricacies of oral cancer etiology [8, 9].

Advancements in science and technology have led to the emergence of innovative diagnostic methodologies, surpassing conventional approaches. In this regard, the identification and comprehension of genetic polymorphisms within DNA repair genes hold promise for early detection of oral cancer. These genetic markers can serve as valuable indicators, enabling targeted screening and personalized intervention strategies, addressing the broader spectrum of cancer detection [10, 11].

The emphasis on early detection as a cornerstone for mitigating oral cancer-related fatalities underscores the urgent need for noninvasive, rapid, and accurate detection methods. Healthcare professionals, equipped with knowledge of both clinical and genetic markers, play a pivotal role in early identification, promptly referring patients for further evaluation [12]. Furthermore, comprehensive cost-effectiveness analyses are imperative to assess the feasibility of screening programs and evaluate the economic and societal implications of these genetics-based diagnostic approaches [13].

Integrating the understanding of DNA repair genes into the broader context of oral cancer not only enhances our understanding of its etiology but also lays the groundwork for innovative, personalized strategies in early detection and intervention. The convergence of genetic insights and advanced diagnostics holds the key to transforming the trajectory of oral cancer outcomes on a pan-Asian scale and beyond [4, 14].

Materials and Methods

Subjects

Before becoming part of the research study, all individuals willingly provided informed, written consent. The scope of this case–control study included 150 patients exhibiting OPC lesions, 110 individuals diagnosed with OC, and 120 healthy volunteers who actively participated. A comprehensive questionnaire was administered to collect personal information, including demographics (age, gender, and employment), family and personal history, habits, and specific details about tobacco use (smoking or smokeless). General and systemic examinations, along with clinical investigations, were meticulously documented.

The participants were categorized into three primary groups based on their characteristics. The first group comprised OC cases with tobacco use (60 out of 110), further divided into males (40 out of 60) and females (20 out of 60), as well as OC cases without tobacco use (50 out of 110), with males (30 out of 50) and females (20 out of 50). The second group included OPC cases with two subgroups—those with tobacco use (80 out of 150), males (45 out of 80) and females (35 out of 80), and those without tobacco use (70 out of 150), with males (40 out of 70) and females (30 out of 70). The third group constituted healthy controls, again with tobacco use (60 out of 120), males (35 out of 60) and females (25 out of 60), and without tobacco use (60 out of 120), with males (30 out of 60) and females (30 out of 60).

Ethical guidelines set by the institutional ethics committee were strictly adhered to throughout the experiment, with all research activities conducted post-approval from the ethical committee. Individuals diagnosed with OC, and pre-oral cancer, encompassing conditions like leukoplakia and submucous fibrosis, were enlisted as participants from the Department of Oral and Maxillofacial Surgery at King George's Medical University, Lucknow, India, with collaboration and ethical clearance for the study. The confirmation of participants' inclusion in the research was based on histopathological analysis. Specifically, OC patients with tumors in buccal mucosa, alveolus, retromolar trigone ($n = 50$), and tongue ($n = 30$) underwent treatment and surgery at

the mentioned facilities, with histopathology verifying the presence of oral squamous cell carcinoma. Control patients, selected based on their genetic history and after providing consent, were recruited from hospital blood banks and dentistry clinics. The matching criteria comprised factors such as age, gender, cigarette usage, and specific genetic polymorphisms, including XRCC1 Arg280His, XRCC1 Arg399Gln, XRCC1 Arg194Trp, OGG1 Ser326Cys, and APE1 Asp148Glu.

Additionally, subjects were stratified based on their tobacco habits, distinguishing between exclusive chewers, exclusive smokers, individuals with mixed tobacco habits, and those free of such habits. It is noteworthy that genetic polymorphism played a pivotal role in the extensive categorization of subjects within this study.

Isolation of DNA

Extraction of DNA from Buccal Swab

Genomic DNA was carefully extracted from buccal swab samples. Initially, the swabs were placed in a lysis buffer with specific components, and after incubation, extraction was carried out using a solution of phenol, chloroform, and isoamyl alcohol. The resulting mixture underwent several steps, including centrifugation and treatment with RNase A, followed by precipitation with cooled isopropanol. The final DNA pellet was re-suspended and stored at − 20 °C or − 80 °C [15].

Extraction of DNA from Blood Sample

To extract genomic DNA from whole blood, red blood cells (RBCs) were broken down using a hypotonic buffer. The resulting pellet was then mixed with a solution containing PBS, cell lysis buffer, and proteinase K. After an incubation period, the DNA was extracted with phenol, followed by washing with chloroform, isoamyl alcohol, and absolute alcohol. The clean pellet, dried under a laminar airflow chamber, was stored at − 20 °C to − 80 °C after re-suspension in nuclease-free water or 1 × TE buffer [16, 17]. Refer to Fig. 1 for a detailed overview of the DNA extraction process.

Genotyping

The PCR–RFLP method was used to find XRCC1 genotypes. In a 25 µl solution with genomic DNA, MgCl2, dNTPs, Taq, and primers, thermal cycling occurred. The resulting mixtures were digested with MspI or RsaI, followed by electrophoresis on 6% polyacrylamide gels. Stained DNA (ethidium bromide) was viewed under UV light for analysis. For the codon 280 polymorphism, the mixture was digested with

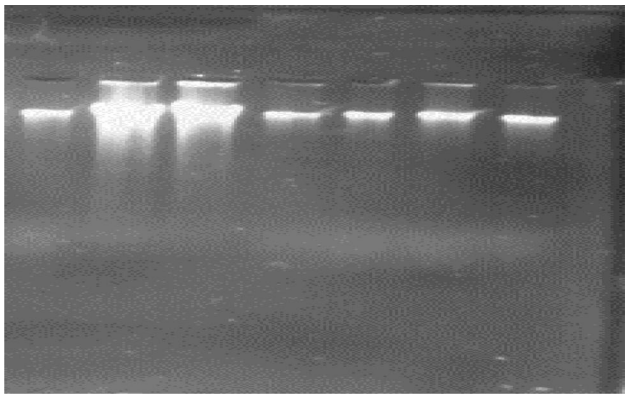

Fig. 1 Isolation of DNA from buccal swab and blood sample

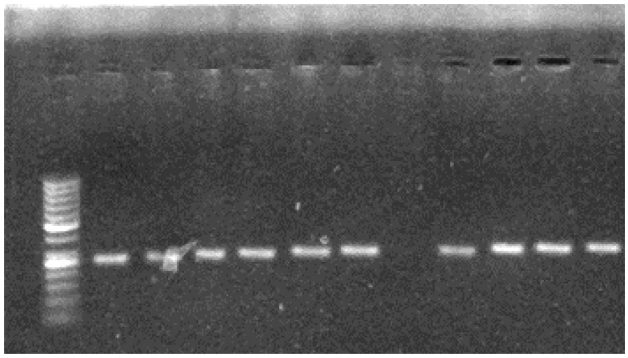

Fig. 2 Genotype analysis of DNA

RsaI, following the same steps as for codon 194 [18, 19]. See Fig. 2 for a visual overview of the DNA genotypic analysis.

Statistical Analysis

We investigated the connection between a particular genetic variation, namely the XRCC1 Arg194Trp polymorphism, and its association with the risk of oral cancer (OC). By employing various genetic models and taking ethnicity into account, we examined pooled odds ratios (ORs) alongside 95% confidence intervals (CI). Statistical analyses were conducted using Stata 11.0, and we performed sensitivity analyses. Additionally, potential publication bias was assessed using Egger's linear regression test and scrutinizing funnel plots (Table 1).

Results

In this research, we scrutinized a group of 380 individuals diagnosed with diverse forms of cancer, comprising 220 males and 160 females, with ages spanning from 28 to

Table 1 Concise overview of the study groups, including the number of cases in each category, tobacco use status, and gender distribution

Groups	Cases	Tobacco user	Gender (M/F)
OC with tobacco	60/110	Yes	40/20
OC without Tobacco	50/110	No	30/20
OPC with Tobacco	80/150	Yes	45/35
OPC without Tobacco	70/150	No	40/30
Healthy controls with Tobacco	60/120	Yes	35/25
Healthy controls without Tobacco	60/120	No	30/30

Table 2 Calculated mean age of individuals in different groups

Age	Oral cancer (110)	Oral precancer (150)	Healthy volunteers (120)
Cumulative mean age	40 ± 20.2	28.10 ± 7.80	29 ± 10.50

62 years. The average age of 150 patients with oral precancer (OPC) was calculated to be 28.10 ± 7.80, comprising 85 males and 65 females. For the 110 cases of oral cancer (OC), which included subtypes such as Oral Submucous Fibrosis and Leukoplakia, the calculated mean age was 40 ± 20.2, with 70 males and 40 females. The healthy control group of 120 individuals showed a calculated mean age of 29 ± 10.50, with 65 males and 55 females. The calculated mean age for each group is detailed in Table 2. PCR–RFLP successfully genotyped all subjects with OC, OPC, and the control group.

The most common sites of infection were found in the buccal cavity and tongue. According to the study findings, among 380 patients with oral cancer (OC), 28.94% (110 patients), among 380 patients with oropharyngeal cancer (OPC), 39.47% (150 patients), and among 380 healthy controls, 31.57% (120 individuals) had tobacco use (either smoking or smokeless) for a duration of up to 10 years. In the OC group, 72.72% (80 of 110) exhibited tobacco-chewing habits, while in the OPC group, 60% (90 of 150) had a tobacco habit. Notably, half of the healthy controls (60 of 120) were regular cigarette users but did not suffer from OC, OPC, or related conditions like leukoplakia or submucous fibrosis, yet remained susceptible to OPC or OC. Conversely, the remaining half did not use tobacco and had no prior history of OC or OPC (Table 3).

Table 3 Statistical data on individuals and their habits

Habits	Oral cancer (110)	Oral precancer (150)	Healthy volunteers (120)
Tobacco consumption	72.72%	60%	50%
Non tobacco use	27.27%	40%	50%

Table 4 Frequencies of genetic polymorphism among individuals

Genotype polymorphism	Frequencies
Arg194Trp	
Trp/Arg	9.82
Trp/Arg	42.09
Arg/Arg	51.20
Arg280His	
His/His	1.56
His/Arg	20.13
Arg/Arg	80.57
Arg390Gln	
Gln/Gln	8.20
Gln/Arg	40.66
Arg/Arg	50.32
Asp148Glu	
Asp/Asp	0.15
Glu/Glu	0.55
Asp/Glu	0.53
Ser326Cys	
Cys/Cys	38.8
Ser/Ser	37.8
Ser/Cys	20.4

Genotype and Frequency Allele

We explored the intricate relationship between genetic susceptibility and tobacco consumption, a crucial environmental risk factor for oral cancer (OC). Subjects with the XRCC1 gene demonstrated an increased susceptibility to developing leukoplakia when exposed to tobacco. The genotype frequencies for codon 194 (Trp/Trp, Trp/Arg, Arg/Arg) and codon 280 (His/His, His/Arg, Arg/Arg) were thoroughly examined, and the frequencies for codon 399 (Gln/Gln, Gln/Arg, Arg/Arg) are succinctly outlined in Table 4.

Maintaining Hardy–Weinberg equilibrium in all distributions added robustness to our findings. The XRCC1 Arg194Trp polymorphism demonstrated a significant association with an increased risk of OC or OPC, a link that persisted even after excluding studies involving individuals with oral leukoplakia.

Statistical Data of Variants of Genetic Polymorphism

We extensively investigated the association strength by calculating odds ratios (ORs) with precise confidence intervals. In a comprehensive meta-analysis involving 380 cases, encompassing 150 oral precancer (OPC), 110 oral cancer (OC), and 120 healthy controls, the XRCC1 Arg194Trp

polymorphism demonstrated a clear link to an elevated risk of OC or OPC. This was evidenced by significant OR values (OR = 1.51, 95% CI 1.10–1.93, $P = 0.01$; OR = 1.60, 95% CI 1.10–2.40, $P = 0.05$; OR = 1.60, 95% CI 1.24–2.14, $P = 0.003$). Importantly, even after excluding studies involving oral leukoplakia, a notable association persisted between the XRCC1 Arg194Trp polymorphism (TrpTrp/ArgTrp versus ArgArg) and the risk of OC (OR = 1.50, 95% CI 1.24–1.91, $P = 0.001$).

Delving deeper into subgroups based on ethnicity, genotyping method, tumor location, and publication year, a nuanced evaluation was performed. Notably, the relationship between the XRCC1 Arg280His genotype and OC risk exhibited a diminished significance in these diverse subgroups. Interestingly, in alternative genetic models and stratified analyses, no substantial heterogeneity or significant relationships were observed. While only one study examined the impact of smoking and the Arg280His polymorphism on OC risk, the findings underscored the need for further investigations.

The study found differences in the OGG1 Ser326Cys allele occurrence in control groups based on age, sex, smoking, and alcohol habits. Patients with the Cys + allele faced a 2.8 times higher risk of OC ($P < 0.001$; 95% CI 2.00:4.98 and $\chi 2 = 20.10$; OR 3.40). Conversely, the Ser + allele in the control group showed a significant tenfold protective effect against OC or OPC ($P < 0.001$; 95% CI 0.05–0.60 and $\chi 2 = 12.51$; OR 0.32). Additional analysis revealed more Ser-326Cys allele carriers among patients (Table 5).

Similarly, APE-Asp148Glu Asp + carriers had a higher occurrence in OC patients than controls ($P = 0.42$; 95% CI 0.86–3.55 and $\chi 2 = 0.10$; OR 1.66). Furthermore, the control group had a higher frequency of APE-Asp148Glu Glu/Glu compared to patients ($P = 0.341$; 95% CI 0.50–1.67 and $\chi 2 = 0.10$; OR 0.87), suggesting a lower risk of OC or OPC associated with smaller tumor size.

The allele frequencies of XRCC1 Arg194Trp, XRCC1 Arg280His, XRCC1 Arg399Gln, OGG1 Ser326Cys, and APE1 Asp148Glu were determined to be 0.45, 0.30, 0.43, 0.55, and 0.30, respectively. Survival analysis indicated a higher hazard ratio for APE1 Asp148Glu polymorphisms (AHR, adjusted hazard ratio = 2.49; 95% CI = 1.65–2.65), suggesting a susceptibility to OC or OPC. However, logistic regression analysis did not firmly establish an association between APE1 polymorphisms and OC development.

Examining the SNP rs1130409 (Asp148Glu) of the APE1 gene in 380 individuals revealed distinct genotypic distributions. Notably, APE1 codon 148 variations displayed a significant association with OC or OPC patients (> 48 years) having the Glu/Glu genotype, indicating an increased risk.

Notably, individuals carrying the OGG1 Ser326Cys Ser allele (Ser +) exhibited a remarkable 12-fold protective effect against OSCC in the control group ($P < 0.001$; 95% CI 0.02–0.47 and $\chi 2 = 12.22$; OR 0.10). However, these findings were not statistically significant in the patient group (P 0.001; 95% CI 0.02–0.47 and $\chi 2 = 12.22$; OR 0.10), and the control group had significantly higher OGG1 Ser326Cys Ser/Ser and OGG1 Ser326Cys Cys/Cys genotype frequencies than the patient group. Table 4 provides detailed insight into the genetic polymorphism data.

Table 5 Different data of variants of genetic polymorphism

Genotype	OR	CI	*P*-value
Arg194Trp			
Trp/Arg	1.51	1.10–1.93	0.01
ArgArg/TrpTrp	1.62	1.10–2.40	0.05
TrpTrp/ArgTrp/ ArgArg	1.71	1.24–2.36	0.03
Ser326Cys			
Ser/Ser	0.427	0.314–0.663	0.001
Cys/Cys	9.57	2.10–43.03	0.001
Ser/Cys	3.00	1.44–3.46	0.005
Arg280His			
Arg/Arg	0.97	0.86–1.20	0.755
Arg/His	1.10	0.85–1.35	0.410
His/His	0.85	0.65–1.08	0.240
Arg399Glu			
Arg/Arg	1.00	0.92–1.08	0.840
Arg/Glu	1.09	0.95–1.10	0.966
Glu/Glu	0.90	0.83–1.20	0.930
Asp148Glu			
Asp/Asp	1.47	0.66–3.22	0.310
Asp/Glu	0.65	0.29–1.46	0.320
Glu/Glu	0.54	0.20–1.26	0.400

This thorough analysis highlights the intricate interplay between genetic variations and the varied risks associated with oral cancer and precancerous conditions, offering valuable insights for further research and clinical applications.

Discussion

In the quest for enhanced DNA extraction protocols, our study employed a meticulous approach involving buccal swabs and blood samples. Compared to conventional methods, our protocol exhibited a superior predicted yield, boasting 70–95 ng/l/swab and 30–50 ng/l/20 ml collection, a twofold improvement. Adherence to rigorous research handling practices is pivotal, as they intricately influence DNA yield and purity. Immediate immersion in a cell lysis solution was pivotal to avert potential degradation of DNA in buccal swabs and blood samples.

The stability of freshly collected DNA samples from buccal cells, even after PCR amplification, proved comparable to those stored at − 20 °C for three days [20]. Furthermore,

our findings underscored the resilience of extracted DNA yield and PCR amplification, remaining unscathed by a week-long storage period at either 4 °C or − 20 °C [21]. This robust stability emphasizes the utility of buccal and blood samples for securing high-quality DNA, with blood samples exhibiting resilience for storage durations exceeding 4 months at − 20 °C in an EDTA-coated container [22].

Unraveling the oral cancer landscape revealed a correlation with tobacco habits among patients, either through smoking or smokeless consumption. Interestingly, even those without habitual tobacco use displayed a prevalence of oral precancerous conditions [23]. A future study involving 120 healthy volunteers will scrutinize the impact of tobacco consumption or abstinence on oral cancer development.

Transitioning to the molecular level, the significance of DNA repair genes in maintaining genomic integrity emerged as a focal point. The intricate dance between genetic variations in XRCC1 polymorphisms (Arg399Gln, Arg194Trp, Arg280His), OGG1 Ser326Cys, and APE1 Asp148Glu, extended its reach beyond oral cancers, connecting to diverse malignancies like bladder, breast, colorectal, lung, and endocrine cancers [24].

Meta-analyses emerged as a powerful tool to reconcile conflicting results from diverse studies, mitigating random variations and bolstering statistical robustness. The polymorphism landscape was dissected further, spotlighting the impact of OGG1 Ser326Cys on OC and OPC risk. Our meta-analysis delineated a substantial risk increase associated with the mutant G allele, particularly in the GG genotype [5, 25].

The intricate relationship between smoking habits and OGG1 Ser326Cys polymorphism was explored, with unexpected results indicating no significant correlation. A meticulous assessment of other genetic polymorphisms, including XRCC1 Arg399Gln, Arg280His, and Arg194Trp, unveiled nuanced findings, with Arg399Gln displaying no significant association with OC or OPC risk.

Additionally, XRCC1 polymorphisms, specifically Arg280His in the APE-binding domain, did not definitively link to the risk of OC or OPC in our meta-analysis. Speculation surrounds structural changes that may influence interactions with APE, but validation requires further studies with larger sample sizes. The discussion then shifted to XRCC1 Arg194Trp, positioned between DNA polymerase-b and poly (ADP ribose) polymerase-interacting domains.

The study found a higher risk with the Arg194Trp genetic model, challenging previous research. Importantly, significance was noted in the heterozygote model when combining Trp/Trp genotypes, emphasizing the need for further exploration in future studies with larger samples [24, 26].

In the realm of APE1 Asp148Glu, an exploration into the BER process uncovered significant associations, affirming its potential role in oral carcinogenesis. The study positioned APE1 Asp148Glu as a novel risk factor for breast cancer and OC or OPC. As the complex interplay of APE1 polymorphisms continues to be unraveled, larger sample sizes and functional studies will be crucial to validate our findings.

The significance of XRCC1 variant carriers in oral leukoplakia risk underscored the intricate relationship between DNA repair capabilities and disease susceptibility. The study illuminated how genetic polymorphisms, such as XRCC1 399Gln, might influence DNA repair effectiveness and modulate cancer risk, particularly in conjunction with tobacco exposure [4]. This multifaceted relationship warrants further exploration and underscores the need for longitudinal studies to validate these associations.

Conclusion

In conclusion, our exhaustive analysis delved comprehensively into the corpus of published data, revealing a robust and salient association between the XRCC1 Arg399Gln polymorphism and an elevated predisposition to both oral cancer (OC) and oral precancer (OPC), spanning across both homozygote and recessive models. Moreover, a pivotal interconnection surfaced concerning the XRCC1 Arg280His polymorphism and its implication in the susceptibility to OC or OPC, explicated under both heterozygote and dominant models. Despite these pivotal revelations, the inherent constraints of our meta-analysis underscore the compelling necessity for more expansive population cohorts and meticulously designed case–control studies to definitively elucidate the pivotal roles played by XRCC1 Arg399Gln and Arg280His polymorphisms in the ontogenesis of OC or OPC.

Additionally, our extensive comparative scrutiny unveiled a discerning revelation, denoting an absence of any discernible association between XRCC1 Arg194Trp, Arg399Gln, and Arg280His polymorphisms, OGGI Ser326Cys, and APE1 Asp148Glu polymorphisms with the risk of OC or OPC. Noteworthy is the meta-analysis's inability to establish any linkage between XRCC1 polymorphisms Arg399Gln and Arg280His with OC or OPC risk. Intriguingly, the XRCC1 Arg194Trp polymorphism, while lacking a conclusive association with OC or OPC risk, manifested an affiliation with an increased susceptibility to head and neck cancer (HNC) in subgroup analyses when adjusted for tobacco (smoked or smokeless) and alcohol consumption. Furthermore, its role in OC or OPC risk manifested conditionally on specific cancer sites, emerging as a palpable risk factor in stratified analyses.

Acknowledgements The authors are thankful to the Deanship of Scientific Research, King Khalid University, Abha, Saudi Arabia, for

financially supporting this work through the Large Research Group Project under Grant No. R.G.P.2/44/45.

Author Contributions S.S.; S.M.; and S.F. contributed to conceptualization. S.S. and S.F. were involved in methodology and writing—original draft preparation. S.S.; S.F.; and I.A. provided software and contributed to validation; E.S. and K.A. were involved in formal analysis; S.S and E.S. contributed to investigation and visualization; S.B.S; S.S.; I.A.; and S.F were involved in resources; S.S. contributed to data curation and project administration; S.S.; S.F.; T.C.S; S.B.S; I.A., S.A; and S.M. were involved in writing—review and editing; S.M., S.S., S.F., and S.A. contributed to supervision; and I.A. and S.F. were involved in funding acquisition. All authors have read and agreed to the published version of the manuscript. Authorship in the manuscript was limited to those authors only who have contributed substantially to the work reported.

Data Availability Data are contained within the article.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical Approval The study was conducted in accordance with the declaration, and approval by the Institutional Ethics Committee of King George's Medical University, Lucknow, UP, India (protocol code 97 ECM II A/P7), for studies involving humans' samples.

Informed Consent Informed consent was obtained from all subjects involved in the study.

References

1. Siegel RL, Miller KD, Fuchs HE, Jemal A (2021) Cancer Statistics, 2021. CA Cancer J Clin 71:7–33. https://doi.org/10.3322/caac.21654
2. Sung H, Ferlay J, Siegel RL et al (2021) Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71:209–249. https://doi.org/10.3322/caac.21660
3. Yuwanati M, Gondivkar S, Sarode SC et al (2021) Oral health-related quality of life in oral cancer patients: systematic review and meta-analysis. Future Oncol 17:979–990. https://doi.org/10.2217/fon-2020-0881
4. Ford PJ, Rich AM (2021) Tobacco use and oral health. Addiction 116:3531–3540. https://doi.org/10.1111/add.15513
5. Law Z-J, Khoo XH, Lim PT et al (2021) Extracellular vesicle-mediated chemoresistance in oral squamous cell carcinoma. Front Mol Biosci 8:629888. https://doi.org/10.3389/fmolb.2021.629888
6. Rapado-González Ó, López-Cedrún JL, López-López R et al (2021) Saliva gene promoter hypermethylation as a biomarker in oral cancer. J Clin Med 10:1931. https://doi.org/10.3390/jcm10091931
7. Donís SP, González AP, Alves MGO et al (2021) MLH1, MSH2, MRE11, and XRCC1 in oral leukoplakia and oral squamous cell carcinoma. Appl Immunohistochem Mol Morphol 29:613–618. https://doi.org/10.1097/PAI.0000000000000929
8. Mozaffari HR, Rostamnia M, Sharifi R et al (2021) A PRISMA-compliant meta-analysis on association between X-ray repair cross complementing (XRCC1, XRCC2, and XRCC3) polymorphisms and oral cancer susceptibility. Gene 781:145524. https://doi.org/10.1016/j.gene.2021.145524
9. Wang Y-Y, Fang P-T, Su C-W et al (2021) Excision repair cross-complementing group 2 upregulation is a potential predictive biomarker for oral squamous cell carcinoma recurrence. Oncol Lett 21:450. https://doi.org/10.3892/ol.2021.12711
10. Lu K-W, Chen J-C, Lai T-Y et al (2010) Gypenosides causes DNA damage and inhibits expression of DNA repair genes of human oral cancer SAS cells. In Vivo 24:287–291
11. Anantharaman D, Samant TA, Sen S, Mahimkar MB (2011) Polymorphisms in tobacco metabolism and DNA repair genes modulate oral precancer and cancer risk. Oral Oncol 47:866–872. https://doi.org/10.1016/j.oraloncology.2011.06.015
12. Galíndez MF, Carrica A, Zarate AM et al (2021) DNA repair, NFKβ, and TP53 polymorphisms associated with potentially malignant disorders and oral squamous cell carcinoma in argentine patients. Oral Surg Oral Med Oral Pathol Oral Radiol 131:339–346. https://doi.org/10.1016/j.oooo.2020.11.004
13. Ahmed F, Bolooki H, Ariyaratnam S, Pemberton MN (2021) Oral cancer: raising awareness of the importance of suspicion in early detection. Prim Dent J 10:10–12. https://doi.org/10.1177/2050168420980959
14. Wu C-N, Chang W-S, Shih L-C et al (2021) Interaction of DNA repair gene XPC With smoking and betel quid chewing behaviors of oral cancer. Cancer Genom Proteom 18:441–449. https://doi.org/10.21873/cgp.20270
15. Pißarreck M, Moreira F, Foley M (2021) Evaluation of maximum DNA yield from a new noninvasive buccal collection device following various extraction protocols. Genet Test Mol Biomarkers 25:376–380. https://doi.org/10.1089/gtmb.2020.0341
16. Yoshizaki K, Hirata A, Matsushita H et al (2021) PCR-based genotyping assays to detect germline APC variant associated with hereditary gastrointestinal polyposis in Jack Russell terriers. BMC Vet Res 17:32. https://doi.org/10.1186/s12917-020-02731-7
17. Goel H, Mathur R, Syeda S et al (2021) Promoter hypermethylation of LATS1 gene in oral squamous cell carcinoma (OSCC) among North Indian population. Asian Pac J Cancer Prev 22:977–982. https://doi.org/10.31557/APJCP.2021.22.3.977
18. Nigam K, Samadi FM, Srivastava S et al (2021) Smoking and XPC gene polymorphism interact to modulate the risk of oral cancer. J Maxillofac Oral Surg 20:607–611. https://doi.org/10.1007/s12663-020-01340-z
19. Shanmugam A, Hariharan AK, Hasina R et al (2021) Ultrasensitive detection of tumor-specific mutations in saliva of patients with oral cavity squamous cell carcinoma. Cancer 127:1576–1589. https://doi.org/10.1002/cncr.33393
20. do Nascimento GJF, da Silva LP, de Matos FR et al (2020) Polymorphisms of matrix metalloproteinase-7 and -9 are associated with oral tongue squamous cell carcinoma. Braz Oral Res 35:e019. https://doi.org/10.1590/1807-3107bor-2021.vol35.0019
21. Montanez-Gonzalez R, Vallera AC, Calzetta M et al (2021) A PCR-RFLP method for genotyping of inversion 2Rc in *Anopheles* coluzzii. Parasit Vectors 14:174. https://doi.org/10.1186/s13071-021-04657-x
22. Zhang L, Liu Y, Zheng HJ, Zhang CP (2019) The oral microbiota may have influence on oral cancer. Front Cell Infect Microbiol 9:476. https://doi.org/10.3389/fcimb.2019.00476
23. de Melo BAC, Vilar LG, de Oliveira NR et al (2021) Human papillomavirus infection and oral squamous cell carcinoma—a systematic review. Braz J Otorhinolaryngol 87:346–352. https://doi.org/10.1016/j.bjorl.2020.10.017
24. Cheung LC, Ramadas K, Muwonge R et al (2021) Risk-based selection of individuals for oral cancer screening. J Clin Oncol 39:663–674. https://doi.org/10.1200/JCO.20.02855
25. Li H, Shi L, Wei J et al (2016) Cellular uptake and anticancer activity of salvianolic acid B phospholipid complex loaded

nanoparticles in head and neck cancer and precancer cells. Colloids Surf B Biointerfaces 147:65–72. https://doi.org/10.1016/j.colsurfb.2016.07.053

26. Farooqui S, Mohammad S, Srivastava S et al (2020) A study on metabolic, nutritional and biochemical profile of tobacco users with and without oral precancer lesions. J Maxillofac Oral Surg 19:269–272. https://doi.org/10.1007/s12663-019-01200-5

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.