RESEARCH ARTICLE

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Molecular and Lifestyle Determinants of Oral Squamous Cell Carcinoma in Bangladesh: Dietary Patterns Independent of HPV and *TERT* Promoter Mutations

Rokhshana Akter Liza[1], Enayetur Raheem[2], Sonia Akter[1], Md. Nadimul Hasan[3], Ami Hasan Jamil[1], Abdullah Al Thufayal[1], Md. Morshedul Alam[4], Ashekul Islam[5], M. Rezaul Karim[1]*

Abstract

Background: Oral squamous cell carcinoma (OSCC) is the sixth most prevalent cancer globally and constitutes a major public health burden in Bangladesh, with approximately 7.5% of all cancer-related deaths. Although tobacco and betel-quid use are recognized risk factors, dietary patterns may also contribute to OSCC development. Objective: This study investigated the association of lifestyle and dietary factors with OSCC in Bangladeshi patients and explored the potential involvement of *TERT* promoter mutations and human papillomavirus (HPV) infection. **Methods:** A hospital-based, case–control study was conducted involving 47 histopathologically confirmed OSCC patients and 100 age- and sex-matched healthy controls without cancer or chronic illness. Sociodemographic, lifestyle, and dietary data were collected through a structured questionnaire. Tumor DNA was analyzed for *TERT* promoter mutations and HPV DNA using PCR and Sanger sequencing. Statistical analyses were performed using multivariable logistic regression to identify significant associations. **Results:** Excessive betel-quid chewing was strongly associated with OSCC ($p < 0.01$). Among 39 sequenced OSCC samples, 25 single-nucleotide polymorphisms (SNPs) at position –245 (A>G) and one hotspot mutation at position –124 (G>A) of the *TERT* promoter were identified substantially lower than frequencies reported in other populations. No HPV DNA was detected in any sample. Certain dietary patterns such as low fruit and vegetable intake and high consumption of betel quid were linked to increased OSCC risk. **Conclusion:** Our findings suggest that, in Bangladeshi patients, specific dietary and lifestyle factors, rather than HPV infection or *TERT* promoter mutations, contribute significantly to OSCC development. Targeted public health strategies emphasizing nutritional awareness and cessation of betel-quid use are recommended.

Keywords: Oral squamous cell carcinoma- Dietary patterns- Betel quid- Tobacco use

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Introduction

Oral squamous cell carcinoma (OSCC) arises from the mucosal epithelium of the lips, oral tongue, buccal mucosa, gingiva, hard palate, and floor of the mouth, representing a serious health burden in developing countries [1–3]. Approximately 84–97% of oral malignancies are squamous cell carcinomas [3, 4]. Globally, OSCC ranks as the sixth most common cancer, with Southeast Asia exhibiting the highest prevalence due to region-specific cultural and lifestyle practices [5, 6].

In South and Southeast Asia including Bangladesh, India, Pakistan, Sri Lanka, and Taiwan—the high incidence of OSCC is linked primarily to habitual betel-quid chewing, bidi smoking, and alcohol consumption [7–9]. Betel quid (locally known as paan) consists of betel leaf, areca nut, tobacco flakes, and lime. Regular use induces chronic mucosal irritation, predisposing users to oral precancerous and cancerous lesions. Recently some studies in the USA have shown an increasing incidence of OSCC due to human papillomavirus (HPV) infection [10, 11]. Additional risk factors include periodontal disease, nutritional deficiency, poor oral hygiene, and limited access to cancer screening and healthcare services [12].

*[1]Laboratory of Cancer Biology, Department of Biotechnology and Genetic Engineering, Jahangirnagar University, Savar, Dhaka 1342, Bangladesh. [2]Biomedical Research Foundation, Dhaka 1212, Bangladesh. [3]National Institute of Cancer Research and Hospital, Mohakhali, Dhaka, Bangladesh. [4]Department of Biochemistry and Microbiology, North South University, Dhaka 1229, Bangladesh. [5]Department of Biochemistry and Molecular Biology, Mawlana Bhashani Science and Technology University, Tangail 1902, Bangladesh. *For Correspondence: mrkarimcu@gmail.com. Rokhshana Akter Liza and Enayetur Raheem have equal contribution in this study.*

Bangladesh has one of the densest populations in South Asia. Every year almost 200,000 people are diagnosed with cancer [13]. More than 13,985 of them have cancer of the lip, oral cavity, or pharynx, with a 7.5% mortality rate (Globocan 2020) [14, 15]. Despite a high national prevalence of HPV infection (7.7% among women) [15, 16], its association with OSCC in Bangladesh remains unconfirmed. Recent global evidence suggests that HPV-related OSCC is increasing in Western countries; however, such trends are not evident in South Asia [16–18].

Apart from the most common and well-established etiological factors, little is known about the relationship between nutrition and the development of OSCC. A few studies have examined the global impact of eating habits on OSCC [19–21]. Dietary patterns may play a significant role in OSCC etiology. Studies have shown an inverse relationship between the consumption of fruits and vegetables and oral cancer risk, whereas other research found that an unbalanced diet and inadequate nutrition can increase pharyngeal and oral cancer [19, 22]. According to some research, high intake of red meat, refined carbohydrates, and alcohol correlates with increased incidence, of OSCC, while others found no link [23, 24]. Although several studies showed an inverse relationship, one study found a link between milk and cheese consumption and oral cancer [5, 19]. Evidence regarding dairy products remains inconsistent [5]. Genetic factors are also considered important risk factors in the development of OSCC, such as mutations in the *TERT* promoter, have also been implicated in oral carcinogenesis [25, 26]. A study carried out by Copper et al. gathered information regarding first-degree relatives. They discovered that out of 105 individuals, 31 had upper aero-digestive tract and respiratory cancers [27]. However, no comprehensive study to date has examined the combined influence of dietary factors and *TERT* promoter mutations on OSCC in Bangladesh.

The present study aimed to assess epidemiological, clinical, dietary, and molecular determinants of OSCC among Bangladeshi patients. Understanding these factors is crucial for designing targeted prevention and awareness programs tailored to regional lifestyles.

Materials and Methods

Study Population

This hospital-based case–control study included 47 patients with histopathologically confirmed oral squamous cell carcinoma (OSCC) who underwent surgery and whose tissue samples were available for analysis. All patients were diagnosed between April 26, 2019, and November 14, 2020, at the Department of Dental and Maxillofacial Surgical Oncology of the National Institute of Cancer Research and Hospital (NICRH), Dhaka, Bangladesh. Patients who declined to participate were excluded. NICRH is one of the largest public cancer hospitals in the country, primarily serving lower-income populations due to government-subsidized healthcare. Consequently, the present study may not fully represent patients from higher socioeconomic backgrounds. Furthermore, patient recruitment coincided with the onset of the COVID-19 pandemic, during which access to patients and all recruitment activities were fully suspended.

Tumor tissues were collected from different oral cavity sites of the 47 OSCC patients. Of these, 39 samples were selected for reliable polymerase chain reaction (PCR) amplification and sequencing analyses. A total of 100 healthy individuals were recruited as controls, matched to cases by age, sex, income, and sociodemographic characteristics. Control participants had no previous history of cancer or chronic illness. Most participants in both groups were rural residents, married, uneducated, and economically disadvantaged, with a higher prevalence of female participants. The study was approved by the Ethics Committee of the National Institute of Cancer Research and Hospital (Ref. No. NICRH/Ethics/2019/431), and written informed consent was obtained from all participants. All experimental procedures were performed in accordance with relevant ethical guidelines and regulations.

Clinical and Biochemical Evaluation

All male and female participants aged 18 years and older, regardless of religion, ethnicity, or socioeconomic status, were eligible for inclusion. Exclusion criteria comprised patients with a history of any malignancy other than OSCC and those older than 80 years. Clinical assessment was performed for all OSCC patients upon presentation at NICRH. Baseline investigations included complete blood count (CBC), serum creatinine, random blood sugar (RBS), serum glutamic pyruvic transaminase (SGPT), hepatitis B surface antigen (HbsAg), electrocardiogram (ECG), chest X-ray (CxR), thyroid-stimulating hormone (TSH), serum electrolytes, and serum albumin. An oral biopsy was subsequently performed to confirm the diagnosis of OSCC.

Data Collection for Epidemiological Analysis

A structured questionnaire was used to conduct face-to-face interviews with both patients and controls. The questionnaire collected information on demographics, lifestyle behaviors, and risk factors such as smoking, alcohol consumption, and betel-quid chewing. Demographic variables included age, sex, marital status, education, income, and place of residence. Data regarding the daily number of cigarettes smoked and betel quids consumed were recorded, along with information on exposure to secondhand smoke. Details on oral hygiene practices, first clinical symptoms, family history of cancer, and lifetime sexual behavior were obtained through interviews and medical records. All interviews were conducted by trained personnel who were blinded to participant group status to minimize interviewer bias.

Dietary Assessment

Detailed information on daily dietary patterns was collected using the structured questionnaire to evaluate associations between food intake and OSCC. The dietary patterns of 47 OSCC cases and 100 controls were analyzed. Standard serving sizes for each food item were defined according to the dietary guidelines of the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine, and Metabolic Disorders (BIRDEM).

The reported food consumption rates were converted into grams or milliliters per day and compared with the BIRDEM recommendations. Tea consumption was recorded as cups per day, and seasonal variation in fruit and vegetable intake was noted. Food intake was categorized as "high" or "low" depending on whether it exceeded or fell below the recommended level [28].

The dietary components were grouped into ten major categories comprising a total of 82 food items: rice as the primary course; bread and ruti as secondary staples; red meat and meat-based foods such as kebabs and burgers; white meat and poultry-based dishes; fish (river and sea) and eggs; leafy vegetables including red amaranth, cauliflower, cabbage, water spinach, and pumpkin leaves; non-leafy vegetables such as potatoes, okra, eggplant, carrots, tomatoes, and pumpkins; beverages including tea, coffee, milk, and tea with condensed milk; fruits such as mangoes, guavas, bananas, apples, oranges, and tropical varieties; and sweets, rice-based desserts, and soft drinks. These food groups were selected to represent typical Bangladeshi dietary habits. Case–control statistical analysis was then performed to examine the relationship between dietary patterns and OSCC risk.

OSCC Sample Collection for Molecular Analysis

Tumor specimens were obtained from the 47 OSCC patients at the Department of Dental and Maxillofacial Surgical Oncology, NICRH. Approximately 10 g or less of resected tumor tissue was collected in 1X phosphate-buffered saline (PBS) and immediately stored in a −20°C mini cooler (Tarson 526030) placed within an icebox containing frozen gel packs. Samples were transported to the laboratory within two hours and stored at −20°C until further processing.

DNA Extraction from Tumor Tissues

A portion of approximately 25 mg from each resected tumor specimen was washed in 1X PBS and finely minced using a sterile surgical blade. DNA extraction was performed using the PureLink Genomic DNA Mini Kit (K1820-01) according to the manufacturer's protocol. In brief, the homogenized tissue was incubated in 180 µL of digestion buffer with 20 µL of proteinase K at 55°C for approximately four hours until complete digestion was achieved. Subsequently, 20 µL of RNase A was added and incubated at room temperature for two minutes. After adding 200 µL of lysis buffer and vortexing, DNA was precipitated using 200 µL of absolute ethanol, washed twice with wash buffer, and finally eluted using elution buffer. DNA concentration and purity were assessed using a Nanodrop spectrophotometer.

Detection of Human Papillomavirus (HPV) by Polymerase Chain Reaction

Nested PCR was performed to detect HPV DNA targeting the L1 gene of HPV-16. Two sets of primers, MY09/MY11 and GP5+/GP6+, were used to amplify 450 bp and 140 bp fragments of the HPV L1 open reading frame, respectively, as previously described [28]. PCR reactions were performed in a final volume of 25 µL containing 5 µL of template DNA (200–400 ng), 2.5 µL of 10X ExTaq buffer, 2 µL of dNTPs, 1 µL of each primer (10 pM), and 0.12 µL of ExTaq DNA polymerase. The PCR cycling conditions were as follows: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 53°C for 30 seconds, and extension at 72°C for 30 seconds, with a final extension at 72°C for 3 minutes. The nested PCR used identical cycling conditions except for an annealing temperature of 43°C. Amplified products were visualized on agarose gel using ethidium bromide staining. Samples were considered HPV-positive when a 140 bp product specific to the L1 antigen was observed.

Detection of TERT Promoter Mutations

Mutations in the *TERT* promoter region were analyzed by PCR amplification and Sanger sequencing. The 260 bp *TERT* promoter fragment encompassing the −124 C>T and −146 C>T mutation sites were amplified using the previously described primer pairs: forward 5′-CCC ACG TGC GCA GCA GGA C-3′ and reverse 5′-CTC CCA GTG GAT TCG CGG GC-3′ [25]. PCR reactions were performed in a 25 µL mixture containing 200–400 ng of genomic DNA, 12.5 µL of GoTaq G2 Green Master Mix (Cat# M7822), and 10 pmol of each primer. The amplification conditions included an initial denaturation at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 1 minute, annealing at 60°C for 30 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes. PCR products were purified using the FavorPrep Gel/PCR Purification Mini Kit (Cat. No: FAGCK001) and sent to Macrogen Korea for sequencing. Both forward and reverse reads were analyzed for each sample.

Statistical Analysis

All statistical analyses were performed using R software (version 4.2.0) on a macOS platform. Descriptive statistics were used to summarize participant characteristics. Bivariate analyses were followed by multivariable logistic regression to identify independent risk factors associated with OSCC. Both crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) were calculated after adjusting for demographic and behavioral covariates. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. The chi-square (χ^2) test with Yates' continuity correction was applied to determine significant group differences. A p-value less than 0.05 was considered statistically significant.

Results

Demographic and Clinical Characteristics

In this study, a total of 47 patients diagnosed with oral squamous cell carcinoma (OSCC) and 100 healthy control subjects were included (Table 1). The median age of the OSCC group was 56 years (inter-quartile range [IQR] 45–60), compared to 50 years (IQR 45–57) in the control group. Among the OSCC patients, 27 (57%) were female, and among controls, 60 (60%) were female. When stratified by age category, 15 patients (32%) and 45

Table 1. General Demographic Data of OSCC Patients and Controls

Characteristic	Treatment, N = 47	Control, N = 100
Age, yr (Median, IQR)	56 (45, 60)	50 (45, 57)
Age group		
< 50	15 (32%)	45 (45%)
50 - 64	21 (45%)	52 (52%)
>= 65	11 (23%)	3 (3.0%)
Gender		
Female	27 (57%)	60 (60%)
Male	20 (43%)	40 (40%)
Education		
Uneducated	20 (43%)	39 (39%)
Primary	16 (34%)	37 (37%)
SSC	6 (13%)	16 (16%)
HSC	2 (4.3%)	5 (5.0%)
Graduate	3 (6.4%)	3 (3.0%)
Occupation		
Farmer	9 (19%)	1 (1.0%)
Housewife	21 (45%)	43 (43%)
Business	2 (4.3%)	11 (11%)
Service Holder	9 (19%)	22 (22%)
Others	6 (13%)	23 (23%)
Income		
<5,000	3 (6.4%)	7 (7.0%)
5,000-10,000	5 (11%)	28 (28%)
10,000-20,000	23 (49%)	25 (25%)
>20,000	16 (34%)	40 (40%)
Marital status		
Married	46 (98%)	99 (99%)
Unmarried	1 (2.1%)	1 (1.0%)
Residence		
Rural	44 (94%)	90 (90%)
Urban	3 (6.4%)	10 (10%)

controls (45%) were under 50 years; 21 patients (45%) and 52 controls (52%) were aged 50–64 years; and 11 patients (23%) and 3 controls (3%) were aged 65 years or older.

Educational attainment differed between groups: among cases, 20 (43%) had never attended formal school and 16 (34%) had primary education only, whereas among controls, 39 (39%) were uneducated and 37 (37%) had primary education. Occupation data indicated that 21 (45%) of female cases were housewives. Income levels showed that 23 patients (49%) were in the Bangladesh Taka 10 000–20 000 monthly bracket, the largest income subgroup among cases; among controls, 40 (40%) belonged to the >20 000 Taka group. Regarding place of residence, most subjects lived in rural areas: 44 cases (94%) and 90 controls (90%); only 3 cases (6%) and 10 controls (10%) were urban dwellers.

First clinical symptoms of OSCC patients

Our study collected information on the initial clinical symptoms seen when patients were admitted to hospitals. Every patient had various clinical symptoms, which were typical signs of OSCC. These symptoms included swollen or grainy vesicles, red or white lesions, abnormal bleeding, pain and discomfort, numbness, etc., inside the mouth. It is evident that Bangladeshi patients' behavior of avoiding clinical examinations and regular disease screening has significantly impacted the emergence of deadly diseases such as oral cancer. The leading causes of this, as we have already mentioned, are poverty, illiteracy, and lack of awareness. Nearly all Bangladeshi patients are admitted to the hospital with clinical symptoms due to the delayed diagnosis of diseases. According to our research, practically all OSCC patients have red or white lesions with accompanying pain. However, these symptoms do not necessarily meet the diagnostic standards. Patients with the symptoms mentioned above are thought to have OSCC. Other diagnostic procedures, such as a biopsy, are required for confirmation.

Figure 1 shows the first clinical symptoms of patients.

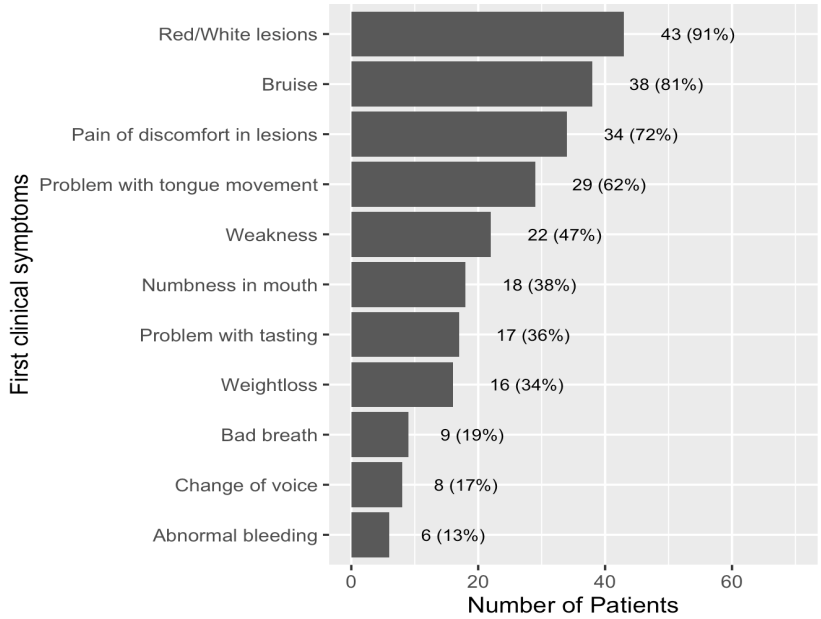

Figure 1. Percentage distribution of first clinical symptoms of Bangladeshi OSCC Patients

Table 2. Association between Risk of Oral Cancer and Risk Factors

Characteristic	Treatment, N = 47	Control, N = 100	p-value
Smoking	15 (37%)	27 (27%)	0.3
(Missing)	6	0	
Exposure to secondhand smoke	31 (76%)	79 (79%)	0.7
(Missing)	6	0	
Betel quid	33 (80%)	53 (53%)	0.002
(Missing)	6	0	
Gul	9 (22%)	4 (4.0%)	0.002
(Missing)	6	0	
Smoking and Betel quid	9 (19%)	15 (15%)	0.5
Betel quid and Gul	8 (17%)	1 (1.0%)	<0.001
Smoke/day			0.018
< 5	31 (66%)	79 (79%)	
>= 5	11 (23%)	20 (20%)	
(Missing)	5 (11%)	1 (1.0%)	
Betel quid/day			<0.001
< 5	12 (26%)	75 (75%)	
>= 5	30 (64%)	24 (24%)	
(Missing)	5 (11%)	1 (1.0%)	
Brushing per day			<0.001
1	37 (79%)	33 (33%)	
2	9 (19%)	58 (58%)	
3	1 (2.1%)	9 (9.0%)	
Have sharp teeth	6 (13%)	11 (11%)	0.8
Denture teeth	0 (0%)	5 (5.0%)	0.2
Any lesions inside the mouth before cancer detection			<0.001
N/A	0 (0%)	100 (100%)	
No	29 (62%)	0 (0%)	
Yes	18 (38%)	0 (0%)	
Tooth powder use	13 (28%)	47 (47%)	0.026
Uses tree branch for brushing	9 (19%)	13 (13%)	0.3
First degree family member affected by oral cancer	2 (4.3%)	2 (2.0%)	0.6
(Missing)	0	1	
Second degree family member affected by oral cancer	2 (4.3%)	4 (4.0%)	>0.9
(Missing)	0	1	
Family members affected by other cancer	5 (11%)	18 (18%)	0.2
(Missing)	0	1	

In our investigation, almost every patient had symptoms. Multiple symptoms were discovered in one patient. A maximum of 91% of patients had lesions inside their mouths that were red or white. For 72% of patients, lesions cause burning pain and discomfort. Speaking, drinking, and eating was problematic for most oral cancer patients – roughly 62%. A numbness in the mouth affected over 38% of the patients. Other issues were weight loss, weakness, bleeding from the mouth, taste problems, bad breath, and voice changes, reported by 34%, 47%, 13%, 36%, 19%, and 17% of patients, respectively.

Etiological and Lifestyle Risk Factors

Figure 2 illustrates the distribution of main risk exposures in the OSCC group. Among the 47 cases, 33 (72%) reported habitual betel-quid chewing, and 31 (66%) reported exposure to second-hand tobacco smoke. Nine cases (19%) used the smokeless tobacco product "gul," and nine (19%) both smoked and chewed betel-quid; only two cases (4%) reported the combined use of betel-quid, gul, and smoking. None of the participants with OSCC reported alcohol consumption.

In the bivariate analysis (Table 2), habitual betel-quid chewing (33/47 vs 53/100; p = 0.002) and gul (tobacco powder) use (9/47 vs 4/100; p = 0.002) were significantly associated with OSCC. Stratification by consumption frequency showed that participants consuming five or more betel-quids per day had markedly increased

Table 3. Multivariable Relationship between Predictive Risk Factors and the Disease Outcome

Characteristic	OR	95% CI	p-value
Age (yr)	1.09	1.04, 1.16	0.002
Female	2.61	0.51, 16.2	0.3
Education			
Uneducated			
Primary	0.71	0.21, 2.25	0.6
SSC	0.41	0.07, 2.06	0.3
HSC	0.53	0.05, 4.33	0.6
Graduate	2.02	0.05, 44.3	0.7
Income			
<5000			
5000-10000	0.28	0.03, 2.34	0.2
10000-20000	3.74	0.63, 27.1	0.2
>20000	1.09	0.16, 8.41	>0.9
Residence			
Rural			
Urban	0.24	0.02, 1.97	0.2
Smoking	3.07	0.58, 19.6	0.2
Exposure to secondhand smoke	0.49	0.13, 1.80	0.3
Gul	8.51	1.67, 54.6	0.014
Betel quid	4.62	1.64, 14.9	0.006
Doesn't use toothpowder	7.33	2.42, 26.6	<0.001

proportions of OSCC (30/47) compared to those with fewer than five per day (12/47), $p < 0.001$.

In multivariable logistic regression adjusted for age, sex, education, income, and place of residence (Table 3), habitual betel-quid chewing was associated with an odds ratio (OR) of 4.62 (95% CI: 1.64–14.9; $p = 0.006$) compared to non-users. Gul use yielded OR = 8.51 (95% CI: 1.67–54.6; $p = 0.014$). Moreover, not using tooth-powder (a proxy for inadequate oral hygiene practice) was associated with OR = 7.33 (95% CI: 2.42–26.6; $p < 0.001$). The overall model's discriminative capacity was good (AUC = 0.855).

Dietary Pattern Associations

Dietary pattern data (Table 4) showed, for example, that among cases, only 6 (13%) reported high intake of roti (flatbread), compared to 56 (57%) controls ($p < 0.001$). Using multivariable modelling adjusted for age, sex and income (Table 5), low roti consumption versus high consumption was associated with OR = 15.8 (95% CI: 4.97–61.4; $p < 0.001$), and moderate consumption was associated with OR = 6.69 (95% CI: 1.43–34.3; $p = 0.017$). Other food groups (red meat, rice, non-leafy vegetables) did not show statistically significant associations after adjustment, although confidence intervals were wide and estimates imprecise.

HPV and TERT Promoter Mutation Analysis

Among 41 tumor DNA samples tested by nested PCR for HPV-16 L1 gene, no HPV DNA was detected in the OSCC cases (Supp Table 2). In *TERT* promoter sequencing (39 samples), one hotspot mutation at –124 bp upstream

Table 4. Association between the Risk of Oral Cancer and Food Habit

Characteristic	Treatment, N = 47	Control, N = 100	p-value
Red meat			>0.9
High	3 (6.4%)	6 (6.1%)	
Low	44 (94%)	93 (94%)	
(Missing)	0	1	
Egg			0.003
High	0 (0%)	15 (15%)	
Low	47 (100%)	84 (85%)	
(Missing)	0	1	
Rice			>0.9
High	44 (94%)	91 (92%)	
Low	3 (6.4%)	8 (8.1%)	
(Missing)	0	1	
Leafy vegetables			0.039
High	2 (4.3%)	0 (0%)	
Low	21 (45%)	34 (34%)	
Moderate	24 (51%)	65 (66%)	
(Missing)	0	1	
Non-leafy vegetables			0.017
Low	39 (83%)	63 (64%)	
Moderate	8 (17%)	36 (36%)	
(Missing)	0	1	
Fish			<0.001
High	0 (0%)	34 (34%)	
Low	30 (64%)	40 (40%)	
Moderate	17 (36%)	25 (25%)	
(Missing)	0	1	
White meat			0.065
High	24 (51%)	54 (55%)	
Low	20 (43%)	45 (45%)	
Moderate	3 (6.4%)	0 (0%)	
(Missing)	0	1	
Roti			<0.001
High	6 (13%)	56 (57%)	
Low	34 (72%)	36 (36%)	
Moderate	7 (15%)	7 (7.1%)	
(Missing)	0	1	
Milk			0.005
High	1 (2.1%)	19 (19%)	
Low	46 (98%)	80 (81%)	
(Missing)	0	1	
Fruits			0.3
High	0 (0%)	5 (5.1%)	
Low	46 (98%)	90 (91%)	
Moderate	1 (2.1%)	4 (4.0%)	
(Missing)	0	1	

Table 5. Association between Food Intake and the Disease Outcome

Characteristic	OR	95% CI	p-value
Age (years)	1.08	1.03, 1.14	0.002
Sex			
F			
M	0.87	0.31, 2.34	0.8
Income (Tk)			
<5,000			
>20,000	1.27	0.24, 7.82	0.8
10,000-20,000	5.3	1.04, 33.5	0.055
5,000-10,000	0.33	0.05, 2.30	0.2
Red meat			
High			
Low	0.91	0.13, 7.65	>0.9
Rice			
High			
Low	3.69	0.38, 30.5	0.2
Non-leafy vegetables			
Low			
Moderate	0.8	0.26, 2.31	0.7
Roti			
High			
Low	15.8	4.97, 61.4	<0.001
Moderate	6.69	1.43, 34.3	0.017

of the ATG start site was identified (Supp Table 1). In contrast, 25 samples (64%) exhibited the single nucleotide polymorphism (SNP) rs2853669 at position –245 (A > G). One of the 25 samples included two mutations, one each at positions -245 and -124. No mutations at –146 bp (C>T) were detected.

Discussion

In this study of Bangladeshi OSCC cases and matched controls, we observed that lifestyle and dietary patterns exerted a stronger association with OSCC than did HPV infection or canonical *TERT* promoter hotspot mutations. Our data indicate that habitual betel-quid chewing and use of the smokeless tobacco product gul are major risk factors in this population, while low intake of roti (a staple flatbread) emerged as a notable dietary correlate. The absence of HPV DNA and the low frequency of classical *TERT* promoter mutations (1/39) suggest that the pathogenesis of OSCC in this cohort may diverge from patterns seen in Western populations [10, 12, 29–32]. This study was conceived as an exploratory investigation into the unique etiological patterns of OSCC in Bangladesh—an area where data on the interplay between *TERT*, HPV, and local dietary exposures remain scarce. The significant findings for key risk factors such as betel-quid use (OR = 4.62) and smokeless tobacco (OR = 8.51) serve as strong preliminary signals rather than conclusive population estimates.

The predominance of betel-quid chewing among OSCC patients (72%) aligns with reports from other parts of South Asia, where areca nut and betel derivatives are widely used [5, 7, 33–35]. The OR estimate of 4.62, after adjustment, reinforces the strong etiologic role of betel-quid use. Smokeless tobacco in the form of gul also

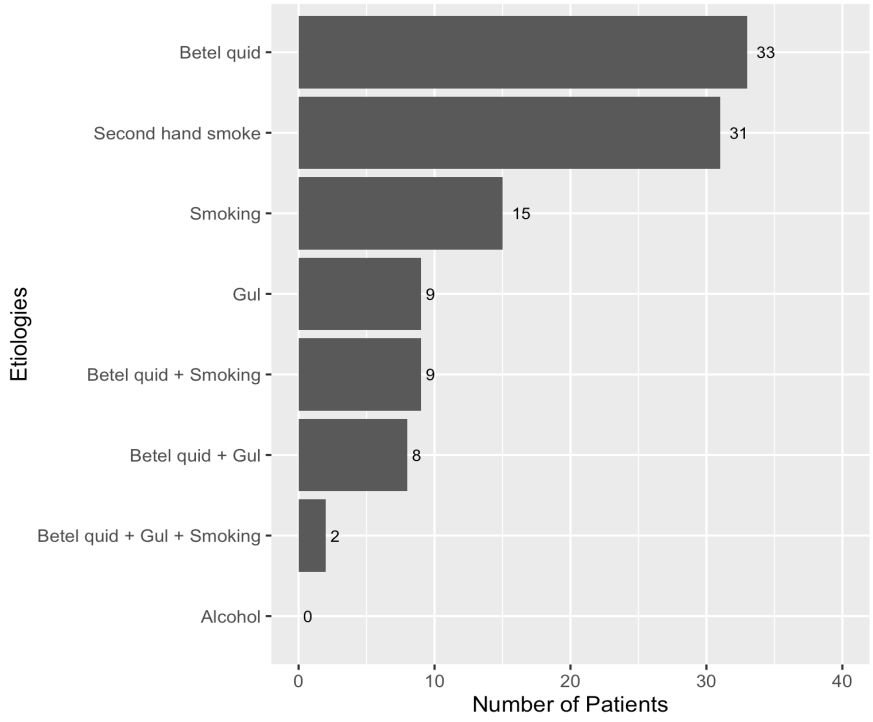

Figure 2. Underlying Etiologies of Bangladeshi OSCC Patients. In Bangladesh chewing betel quid is the most common cause of OSCC (70%), followed by second hand smoke (66%). Since no patients in this study were drinking alcohol, alcohol has no effect.

showed a very high OR (8.51). These findings underscore the behavioral specificity of oral carcinogenesis in this region and support public health efforts to target such exposures.

Oral hygiene, indirectly assessed through non-use of tooth powder, showed a strong association with OSCC (OR = 7.33). However, this measure should be interpreted cautiously, as non-use of tooth powder does not necessarily equate to poor oral hygiene and may reflect other unmeasured hygiene practices. It may instead serve as a proxy indicator of broader oral care behaviors that could contribute to chronic mucosal irritation or infection. Nevertheless, this finding is consistent with previous reports linking poor oral sanitation and periodontal disease with an increased risk of oral cancer [34, 36, 37]. Although associations for betel-quid use and oral hygiene remained robust after adjustment, results should be interpreted in light of the variables assessed and the exploratory design of this study.

Our dietary analysis particularly the finding that low roti consumption relates to higher OSCC risk is intriguing though requires cautious interpretation. The concept that lower consumption of roti would increase risk seems counterintuitive unless roti intake serves as a surrogate for other protective dietary or socioeconomic patterns. It is possible that individuals with higher roti consumption may indicate a more diverse diet or the inclusion of complementary foods such as lentils (dal) and legumes, which are commonly consumed together in Bangladeshi households and may contribute protective nutritional benefits. Conversely, low roti intake could reflect reduced dietary variety, lower caloric sufficiency, or unmeasured socioeconomic constraints. We have also added an explicit caution to readers against interpreting roti consumption per se as a direct protective factor against OSCC. The wide confidence intervals warrant replication in larger cohorts.

The complete absence of HPV-16 DNA in the 41 tested OSCC cases contrasts markedly with reports from Western populations, where HPV-associated oropharyngeal cancers have increased and HPV positivity may reach up to 70%. This finding suggests that HPV may play a minimal or negligible role in OSCC in this Bangladeshi cohort, consistent with earlier study [18]. Begum H. et al. (2017) used the HPV-16 L1 gene to detect HPV infection in cervical cancer patients in Bangladesh, identifying HPV-16 as the most prevalent high-risk genotype in this population [38]. The L1 region is highly conserved and widely employed for reliable HPV detection and genotyping. However, infection with other high-risk HPV types, such as HPV-18, HPV-31, or HPV-33, cannot be ruled out. Similarly, the low prevalence of *TERT* promoter hotspot mutations (~2.5%) suggests that the molecular oncogenesis pathways may diverge from those in other geographic regions where *TERT* promoter alterations are more common [25, 26, 39, 40-42]. However, the high frequency of the rs2853669 SNP at –245 observed in our cohort is noteworthy. Giunco S. et al. [42] reported that the co-occurrence of the rs2853669 SNP with *TERT* promoter hotspot mutations (-124 C>T and -146 C>T) was associated with an increased risk of OSCC progression. A more detailed consideration of its potential functional role in this population particularly in the context of chronic exposure to betel quid may help to better explain its contribution to disease susceptibility and progression. Several limitations must be acknowledged. First, as a hospital-based case–control study, selection bias cannot be excluded, and participants recruited from a public hospital likely represent lower- and lower-middle-income groups.Consequently, the findings may not fully reflect the broader Bangladeshi population, particularly higher-income groups with different lifestyle patterns, healthcare access, and environmental exposures. Because socioeconomic status is closely linked to risk factors such as tobacco and betel-quid use, nutrition, and health-seeking behavior, the observed associations may be more characteristic of lower socioeconomic groups. This limits the generalizability of the results to more affluent or urban populations. Future multicenter studies involving diverse socioeconomic settings are needed for a more nationally representative understanding.

Second, though controls were matched by age, sex, income, and education, the matching did not perfectly balance the groups (e.g., income categories), which may introduce residual confounding. Third, although the questionnaire was piloted and reliability tested, self-reported dietary and lifestyle data remain subject to recall bias. Fourth, the sample size, particularly for molecular analyses, was modest, leading to wide confidence intervals and limited power to detect modest associations. Finally, the cross-sectional design precludes inference of causality; longitudinal studies would strengthen inference.

Despite these limitations, this study offers novel insight into OSCC epidemiology in Bangladesh, highlighting the importance of region-specific risk factors (betel-quid and smokeless tobacco use, dietary patterns) and the relative absence of HPV and classical *TERT* promoter mutations. These data underscore the need for tailored public health strategies and further molecular research.

In conclusion, the development of oral squamous cell carcinoma in this Bangladeshi cohort appears to be strongly associated with habitual betel-quid chewing, use of smokeless tobacco products, and suboptimal oral hygiene practices, whereas infection with HPV and canonical *TERT* promoter hotspot mutations appear to play minimal roles. Additionally, a low intake of roti emerged as a dietary correlate of OSCC risk, though this finding requires confirmation in larger, prospective studies. Efforts to reduce betel-quid and smokeless tobacco use, promote oral hygiene, and further explore dietary influences are recommended. Future research should also examine functional effects of the rs2853669 *TERT* promoter polymorphism and trace other molecular pathways pertinent to OSCC in South Asia.

Author Contribution Statement

All authors contributed significantly to preparing the manuscript. MRK led the initial design, experimental planning, writing, editing, and preparation of the manuscript. Statistical analysis was performed by ER. Data were collected by RAA, SA, and AAT. RAA and AHJ have written the different parts of the manuscript.

Molecular data were prepared by AHJ and AAT. MNH helped with patient recruitment and data collection. MAA and AI revised the article critically and contributed to the overall preparation of the manuscript.

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Data Availability

The manuscript contains all the necessary data.

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Ethical Declaration

National Institute of Cancer Research and Hospital (NICRH) has approved the research (Ref no. NICRH/Ethics/2019/431)

Conflict of Interest

The authors claim that there are no conflicting interests.

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