RESEARCH ARTICLE

Editorial Process: Submission:11/30/2024 Acceptance:12/22/2025 Published:12/26/2025

Risk factors of Esophageal Cancer in Punjab- A Case Control Study

Harmanjeet Kaur[1], Usha Dutta[2], Savita Attri[3], Nalini Gupta[4], Mini P Singh[5], Akhilesh K Yadav[6], Kim Vaiphei[7], Rajesh Dixit[8], JS Thakur[1]*

Abstract

Background: Esophageal cancer is a significant health concern globally. In Punjab, where pesticides use and heavy metal exposure are widespread esophageal squamous cell carcinoma is among the leading cancers. This study aims to investigate the association between these environmental factors and esophageal carcinoma, for instituting preventive strategies. **Methods:** We conducted a case-control study in Punjab, with 380 carcinoma cases and 760 age, gender, and district-matched controls from hospital and community settings. The participants completed a case report form with validated questions on risk factors. The urine, water, oral cytology, blood, and esophageal biopsies samples were collected from a subset of the population to evaluate pesticide metabolites, heavy metal exposure, cytological changes and infections like H. pylori and HPV. **Findings:** The mean age of cases was 57.17 ± 9.54 (SD) years, similar to that of controls, 56.96 ± 8.93 (SD)years (p>0.05).The key risk factors for esophageal carcinoma were, Dimethylphosphate presence in urine (5.41 (95% CI: 1.42-20.67, p<0.05), tobacco use (OR 1.60, 95% CI 1.24-2.06, p < 0.001), alcohol use (OR 1.65, 95% CI 1.31-2.08, p < 0.001) and hot beverages (OR 1.81, 95% CI 1.44-2.28, p < 0.001), with population attributable fraction of 86.7%, 10.8%, 16.5%, and 16.7%, respectively.. The daily intake of fruits (OR 0.74, 95% CI 0.59-0.92, p = 0.008) and vegetables (OR 0.81, 95% CI 0.65-0.99, p = 0.045) had protective association, with inadequate intake contributing to 27% and 26.8% of risk, respectively. The exposure to heavy metals from drinking water was higher in both groups but not statistically significant. **Interpretation:** This study confirms established risk factors like tobacco, alcohol, and diet contribute to esophageal squamous cell carcinoma in Punjab, while identifying pesticide exposure as a new risk factor. It calls for stricter regulations, public health interventions, and further research into environmental risks.

Keywords: Carcinoma esophagus- risk factors- prevention- pesticides- case-control study

Asian Pac J Cancer Prev, **26 (12)**, 4323-4330

Introduction

Esophageal carcinoma is the eighth most common cancer globally. Esophageal squamous cell carcinoma (ESCC), which accounts for approximately 80% of all esophageal cancer cases, has a complex and multifactorial aetiology. Its incidence varies significantly across geographic regions and has changed over time. Numerous studies, including those from India, have explored the roles of environmental exposures, dietary habits, cultural practices, genetic mutations, and polymorphisms in the development of ESCC [1, 2]. Given the extremely poor prognosis of ESCC and the limited prospects for early detection or effective treatment, a deeper understanding of its risk factors is essential. Such insights could offer valuable opportunities for primary prevention.

In the Punjab state of India, esophageal carcinoma ranks among the top five cancers in the Malwa region. Among males, the age-adjusted incidence rates (AAR) per 100,000 population were reported as 6.2 in SAS Nagar, 5.6 in Sangrur, 4.9 in Chandigarh, and 3.8 in Mansa. For females, the AARs were 7.0 in Mansa, 6.2 in Sangrur, and 3.3 in SAS Nagar [3]. Punjab is also known for its intensive agricultural practices and widespread pesticide use. Exploring the potential link between pesticide exposure and ESCC is especially relevant in regions where agriculture plays a central role in the economy. While a few studies conducted in the state suggest an association between pesticide toxicity and the occurrence of various cancers [4]. specific evidence linking pesticide exposure

*[1]Department of Community Medicine and School of Public Health, Post Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, Punjab, India. [2]Department of Gastroenterology, PGIMER, Chandigarh, Punjab, India. [3]Department of Paediatrics, PGIMER, Chandigarh, Punjab, India. [4]Department of Cytology and Gyna ecological Pathology, PGIMER, Punjab, India. [5]Department of Virology, PGIMER, Punjab, India. [6]CSIR-Indian Institute of Toxicology Research, Lucknow, India. [7]Department of Histopathology, PGIMER, Punjab, India. [8]Centre for Cancer Epidemiology, Tata Memorial Centre, Mumbai, Maharashtra, India. *For Correspondence: jsthakur64@gmail.com*

to ESCC remains limited [4]. Similarly, although reports have highlighted heavy metal toxicity in the region, [5] its role in the development of ESCC has not yet been investigated. This gap in knowledge highlights the need for focused research to generate relevant data and evidence.

The objective of this study is to investigate the potential association between pesticide and heavy metal exposure, along with other established risk factors, and the occurrence of esophageal squamous cell carcinoma (ESCC) in Punjab.

Materials and Methods

Study design: A matched case-control study was conducted to assess the prevalence of risk factors and their association with the occurrence of esophageal squamous cell carcinoma in Punjab, India.

Study setting: The study was carried out at a tertiary care referral hospital in Northern India between 2018 and 2023 [6]. The data collection took place from 2019 to 2022, with slower and intermittent recruitment during the COVID-19 pandemic period (2020–2021).

Study participants

Cases: The patients aged 30–74 years with histologically confirmed primary invasive ESCC, who had resided in Punjab for at least the past five years, were included as cases. They were recruited from the gastroenterology and radiotherapy departments at PGIMER, Chandigarh, and through population-based cancer registries in Sahibzada Ajit Singh Nagar and Chandigarh.

Controls

For each case, two age- (±5 years), sex-, and district-matched controls were recruited from both hospital and community settings. The hospital-based controls were selected from the gastroenterology and hematology departments and had no mucosal abnormalities in the esophagus. The community-based controls were drawn from the same geographic population as the cases. The matching consistency was monitored periodically throughout the data collection period. The participants with a personal or family history of upper gastrointestinal malignancies, gastroesophageal reflux disease (GERD), Barrett's esophagus, esophageal inflammation, or ulcers were excluded. A written informed consent was obtained from all participants.

Data collection and study variables

The diagnostic confirmation of Esophageal Squamous Cell Carcinoma cases was done through biopsy, while control participants were confirmed to be free of abnormal esophageal mucosal changes and symptoms. The major exposures included ever use of tobacco, ever consumption of alcohol, consumption of burning hot tea, and dietary habits such as daily intake of fruits and vegetables. The environmental and occupational exposures included use of underground water, involvement in pesticide mixing/loading/application, proximity (<3 km) to farmland where pesticides are applied, exposure to fumigants, and animal contact. The potential confounders included living area, education, socioeconomic status, and other lifestyle factors, while age, gender, and district of residence were accounted for through individual matching.

The data on lifestyle, occupational, dietary, and environmental risk factors were collected using a pre-designed, pilot-tested, interviewer-administered questionnaire. The information on all the variables was self-reported by participants. The oral health was assessed by clinical examination, including the identification of edentulism and calculation of the DMFT score. All assessments were conducted similarly for cases and controls using standardized tools and procedures by the trained personnel. The biological sampling and laboratory analyses (e.g., for pesticide metabolites and heavy metals) followed the same collection and testing protocols across groups, ensuring comparability.

The selection bias was minimized through individual matching of controls to cases based on age (±5 years), gender, and district of residence. The information bias was addressed by using a structured questionnaire and standardized clinical examination procedures. The measurement bias was reduced by applying uniform protocols across cases and controls and blinding laboratory staff to case/control status.

Laboratory analysis

The urine samples were collected from 300 participants (100 cases, 200 controls), with every 3rd enrolled participant providing a sample; 100 (50 cases, 50 controls) were randomly selected for pesticide metabolite analysis using LC-MS.

Similarly, the drinking water samples from 300 participants (100 cases, 200 controls) were analyzed for heavy metals via ICP-MS (Agilent 7500). The oral brush cytology samples (n=300) were collected in SurePath media and processed using liquid-based cytology. H. pylori IgG antibodies were measured using an ELISA kit (Calbiotech, USA). DNA from esophageal biopsies was extracted (Qiagen) for HPV detection via PCR and gel electrophoresis, followed by L1 gene sequencing for genotyping.

Sample size

The sample size for the case and control groups was determined with two controls per case, assuming a 5% significance level, two-sided alpha risk of 5%, 90% power, a 10% non-response rate to detect the smallest risk (OR of 2.00 for exposure to pesticides on ESCC and 8.35% prevalence of the risk factor in community [4] and an adjustment of about 15% for confounders.

Statistical analysis

The descriptive statistics used to analyze the quantitative data included frequencies, percentages, means, and standard deviations. The multivariate analysis was performed with using disease diagnosis as dependent variable and independent variables included known and putative risk factors. For questionnaire-based data, conditional logistic regression was used to calculate adjusted odds ratios (OR) and 95% CIs. The SPSS version 26, (with $p<0.05$ considered as significant) was

used for performing statistical analysis. The unadjusted OR, using unconditional logistic regression, assessed the association of pesticide metabolites in urine and heavy metals in water with ESCC using subgroup analysis. The population attributable fraction for each risk factor was determined using, Pe × (OR - 1) / OR, where Pe refers to the proportion of cases that had been exposed to the risk factor [7].

For pesticide metabolite analysis, the concentrations above the limit of detection were used as an indicative for presence of pesticide and the geometric mean concentration for each metabolite in urine samples was calculated. For heavy metals analysis, data was presented as median and interquartile range due to non-uniform data distribution. The sensitivity analysis was performed using a complete-case approach, excluding participants with missing data to minimize bias. The non-parametric tests were also used for skewed laboratory variables to reduce the influence of extreme values and assess the robustness of results.

Results

A total of 446 patients with histologically confirmed ESCC were deemed eligible for the study. Of these, 66 patients were excluded due to their inability to complete both the questionnaire and biological sample collection, primarily owing to poor health status. Thus, 380 ESCC cases were included in the final analysis. Among controls, 816 hospital-based individuals were initially eligible. However, 56 were excluded due to individual matching issues, resulting in 760 matched controls for the final analysis (Figure 1).

The mean age of ESCC cases was 57.17 ± 9.54 (SD) years, comparable to that of controls 56.96 ± 8.93 (SD) years (p>0.05) However, significant differences were observed in educational attainment ($p < 0.05$), with 41.6% of cases being illiterate compared to 24.3% of controls. A greater proportion of cases were engaged in elementary occupations (15.8%) and skilled agricultural work (18.2%) compared to controls. Additionally, a higher percentage of cases belonged to the lower (13.7%) and upper-lower (49.7%) socioeconomic classes compared to controls (8.0% and 45.1%, respectively) (Table 1).

The unadjusted and adjusted odds ratios (ORs) with 95% CIs for the risk factors were calculated to assess associations with ESCC. The adjusted ORs were derived from multivariable logistic regression, controlling for age, sex, education, occupation, socioeconomic status, and relevant environmental factors, selected based on prior evidence and their relevance as potential confounders.

On multivariate analysis, ever use of tobacco was associated with a higher risk compared to never use (aOR 1.60, 95% CI 1.24-2.06, $p < 0.001$), as was ever use of alcohol compared to never use (aOR 1.65, 95% CI 1.31-2.08, $p < 0.001$). The consumption of burning hot tea was also identified as a significant risk factor, with adjusted ORs of 1.81with 95% CI (1.44-2.28, $p < 0.001$). The consumption of fruits at least once per day was found to be associated with a lower risk of ESCC compared to those who consumed fruits less than once per day (aOR 0.74, 95% CI 0.59-0.92, $p = 0.008$). Similarly, consuming vegetables at least once per day was also found to be associated with a lower risk of ESCC compared to consumers of vegetables in frequencies of less than once per day (aOR 0.81, 95% CI 0.65-0.99, $p = 0.045$). The use of underground water was associated with a higher risk compared to no use (aOR 1.49, 95% CI 1.21-1.83, $p < 0.001$). Similarly, being edentulous (having no teeth) was found to be associated with a higher risk compared to having more than 20 teeth present (aOR 1.48, 95% CI 1.09-2.05, $p = 0.014$). The involvement in pesticides mixing, loading & application is also found to increase the risk. (aOR 1.38, 95% CI 1.01-1.89, p=0.04) The vegetables consumption less than once per day, fruits consumption less than once per day, consumption of very hot tea, alcohol consumption, tobacco use, underground water use, being edentulous and being involved in pesticide application were found to have 27%, 26.78%, 16.72%, 16.48%, 10.75%, 10.21%, 5.38% and 5.36%

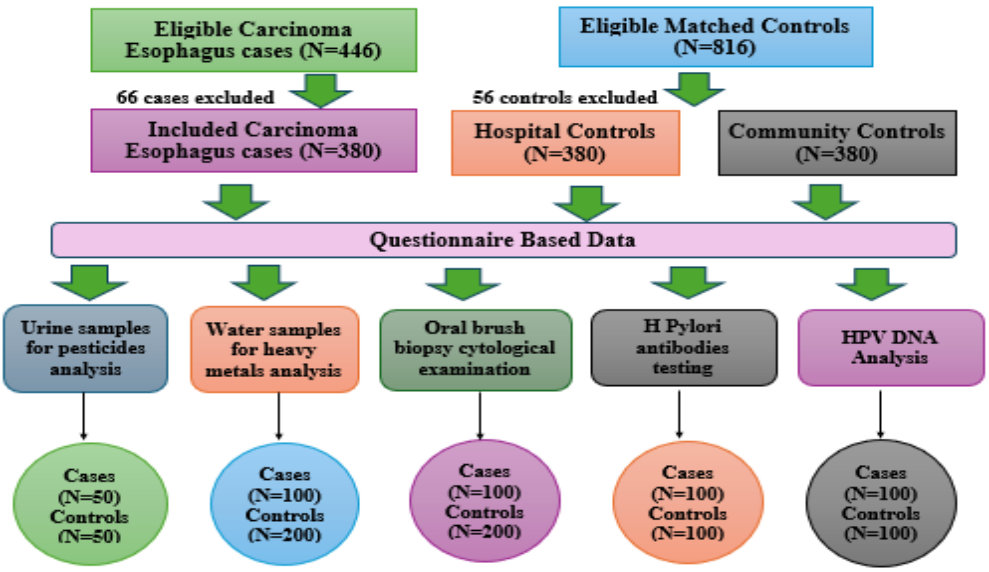

Figure1. Flow Diagram of Sampling Strategy

Table 1. Sociodemographic Characteristics of Carcinoma Esophagus Cases and Controls in Punjab

Sociodemographic variable	Carcinoma esophagus cases (N=380)	Controls (N=760)	p-value
Mean age (SD)	57.17 (9.54) years	56.96 (8.93) years	0.714
Age group			
31-40	30 (7.9)	60 (7.9)	0.996
41-50	65 (17.1)	127 (16.7)	
51-60	134 (35.3)	273 (35.9)	
61-70	151 (39.7)	300 (39.5)	
Gender			
Males	224 (58.9)	448 (58.9)	1
Females	156 (41.1)	312 (41.1)	
Education			
Illiterate	158 (41.6)	185 (24.3)	<0.001
Primary	54 (14.2)	128 (16.8)	
Middle school	44 (11.6)	67 (8.8)	
High school	79 (20.8)	157 (20.7)	
Intermediate	10 (2.6)	87 (11.4)	
Graduation or professionals	35 (9.2)	136 (17.9)	
Religion			
Hindu	225 (59.21)	326 (42.89)	<0.001
Sikh	152 (40)	425 (55.92)	
Muslims	3 (0.78)	3(0.39)	
Christians	0 (00)	6 (0.78)	
Area of living			
Rural	287 (75.5)	497 (65.4)	<0.001
Urban	93 (24.5)	263 (34.6)	
Occupation (As per International Standard Classification of Occupation,)			
Armed forces occupations	2 (0.5)	0 (00)	0.005
Clerical support workers	2 (0.5)	6 (0.8)	
Craft and related trade workers * *	172 (45.3)	339 (44.6)	
Elementary occupation	60 (15.8)	86 (11.3)	
Managers	21 (5.5)	33 (4.3)	
Plant and machine operators,	7 (1.8)	16 (2.1)	
Professionals	27 (7.1)	74 (9.7)	
Service and sales workers	11 (2.9)	36 (4.7)	
Skilled agriculture ,and forestry workers	69 (18.2)	118(15.5)	
Technicians & Associate professors	9 (2.4)	52 (6.8)	
Body mass index			
<18.5 kg/m^2	61 (16.1)	15 (2.0)	<0.001
18.5-22.9 kg/m^2	130 (34.3)	200 (26.4)	
23.0-24.9 kg/m^2	56 (14.8)	157 (20.7)	
>25 kg/m^2	132 (34.8)	387 (51.0)	
Socio economic status (BG Prasad scale)			
Lower	52 (13.7)	61 (8.0)	0.001
Upper lower	189 (49.7)	343 (45.1)	
Lower middle	108 (28.4)	263 (34.6)	
Upper middle	31 (8.2)	93 (12.2)	

Footnote: Figures in the parenthesis represent percentages **Craft and related trade workers (include homemakers)

Table 2. Association of Conventional Factors with Occurrence of Carcinoma Esophagus in Punjab

Risk factors	Cases (N=380)	Controls (N=760)	p-value	Unadjusted odds ratio 95% CI,	Adjusted odds ratio 95% CI *	Population attributable risk
Ever tobacco use	109 (28.7)	87 (11.4)	<0.001	1.84 (1.46-2.33), <0.001	1.60 (1.24-2.06), <0.001	10.75%
Ever alcohol consumption	159 (41.8)	177 (23.3)	<0.001	1.66 (1.35-2.04),<0.001	1.65 (1.31-2.08), <0.001	16.48%
Consumption of burning hot tea	142 (37.4)	108 (14.2)	<0.001	1.98 (1.60-2.45), <0.001	1.81 (1.44-2.28), <0.001	16.72%
Fruits intake (≥1 time per day)	30 (7.9)	160 (21.1)	<0.001	0.71 (0.58-0.87) <0.001	0.74 (0.59-0.92), 0.008	26.70%
Vegetables intake (≥1 time per day)	33 (8.7)	178 (23.4)	<0.001	0.70 (0.58-0.85) <0.001	0.81 (0.65-0.99), 0.045	27%
Use of underground water	118 (31.1)	165 (21.7)	<0.001	1.24 (1.03-1.49), 0.025	1.49 (1.21-1.83), <0.001	10.21%
Edentulous	63 (16.6)	73 (9.6)	<0.001	1.53 (1.13-2.08), 0.006	1.48 (1.09-2.05), 0.014	5.38%
Involved in pesticides mixing, loading & application	74 (19.5)	81 (10.7)	<0.001	1.39 (1.09-1.75), 0.007	1.38 (1.01-1.89), 0.0391	5.36%
Distance of <3 km from the farmland where pesticide application occurs	280 (73.7)	514 (67.6)	<0.036	1.12 (0.95-1.33), 0.182	1.01 (0.83-1.23), 0.922	
Exposure to fumigants	179 (47.1)	258 (33.9)	<0.001	1.27 (1.08-1.51), 0.005	1.14 (0.95-1.38), 0.152	
Contact with animals (Ruminants, canines, poultry etc.)	237 (62.4)	370 (48.7)	<0.001	1.26 (1.07-1.47), 0.005	1.15 (0.95-1.39), 0.156	

Footnotes: Figures in the parenthesis represent percentages; Abbreviation- OR, odds ratio; CI, confidence interval; *Adjusted for living area, education, socioeconomic status, tobacco use, alcohol use, hot beverages consumption, fresh fruits and green vegetables intake, animal contact, fumigants use, drinking water source, working on fields, involvement in pesticides application. Age, gender and districts were matched at time of data collection

population attributable fraction respectively [Table 2].

In urine sample analysis on subset of the study participants, among ESCC cases (N=50), 96% (48/50) had at least one detectable pesticide metabolite (equal to or above the limit of detection) in their urine samples , while among controls (N=50), 70% (35/50) had detectable pesticide metabolites. (p<0.05). The unadjusted OR for the presence of any pesticide metabolite was found to be 10.28 (95% CI: 2.21-47.90, p<0.05). Dimethylphosphate (DMP), a common metabolite of organophosphates, was detected in 92% of ESCC cases and 68% of controls. (p<0.05).The unadjusted OR for the presence of DMP is 5.41 (95% CI: 1.42-20.67, p<0.05), suggesting a significantly higher likelihood of its presence in ESCC cases compared to controls. Similarly, Methamidophos (Acephate), was detected in 14% cases and 8% controls. (p>0.05).(Table 3) Among ESCC cases, the geometric mean level of DMP was 25.51 µg/L, while in controls, the geometric mean level was significantly lower at 6.86 µg/L (p<0.05) (Table 3). The mean levels of Aminomethyl Phosphonic Acid were higher in cases (133.40µg/L) as compared to controls (55.68 µg/L).(p>0.05).No significant differences were observed for other pesticides, including Methamidophos and Chlorpyriphos.

The uranium was present in 286 out of 300 water samples. In cases, 7.44% exceeded the permissible limit (30µg/L), compared to 18.23% in controls. For selenium, detected in 255 water samples, 8.33% of cases exceeded the 10µg/L limit, while 12.28% of controls did. The arsenic was detected in 299 samples, with 3% of cases and 8.04% of controls surpassing the 10µg/L limit. Mercury was detected in 226 samples, with 5.33% of cases and 8.60% of controls exceeding the 1µg/L limit. For the levels of heavy metals, median values (IQR) were calculated and the concentrations were as follows: Uranium (67.23 µg/L vs 114.17 µg/L), Selenium (25.51 µg/L vs 28.59 µg/L), Arsenic (11.34 µg/L vs 36.69 µg/L), and Mercury (8.28 µg/L vs 1.92 µg/L) for cases and controls, respectively. Nickel's median concentration was 15.83 µg/L, while iron was detected at 429.48 µg/L in controls (Table 4).

For liquid-based oral cytology, 81% of ESCC cases and 78.5% controls showed squamous epithelial cells along with bacterial colonies. None of the cases well as controls showed evidence of intraepithelial lesions or malignancy. About 29.20% serum samples of ESCC cases were found to be positive for H pylori infection, while among controls it was 26.28% serum. (p>0.05). For Human Papilloma Virus (HPV), 3.57% ESCC cases were found to be positive, while among controls none of the case was positive for HPV. (p>0.05). On genotyping,

Table 3. Various Pesticide Metabolites in the Urine Samples of Carcinoma Esophagus Cases and Controls from Punjab

Type of pesticide metabolite***	Limit of detection (µg/L)	Carcinoma esophagus cases (N=50)	Controls (N=50))	Total (N=100)	p-value
Any pesticide metabolite (at least 1)	-	48(96)	35(70)	83 (83)	<0.001
Dimethylphosphate (Common metabolite of organophosphates)	7.46	46 (92)	34 (68)	80(80)	0.003
Methamidophos (Acephate)	2.71	7(14)	4 (8)	11(11)	0.338

Footnote: Figures in the parenthesis represent percentages; Abbreviation; Carcinoma esophagus cases, Esophageal Squamous Cell Carcinoma cases; CI, Confidence Interval

Table 4. Concentrations of the Heavy Metals (Detected in Quantities above the Permissible Limits) in Drinking Water Samples Taken from Carcinoma Esophagus Cases and Controls in Punjab

Heavy metal	Permissible limit (µg/L)	ESCC cases	Cases Median (IQR)	Controls	Controls (Median (IQR)	P-value (Median test)
Uranium (N=286)	30	7/94 (7.44)	67.23 (53.23)**	35/192 (18.23)	114.17 (293.96)	0.408
Selenium (N=255)	10	7/84 (8.33)	25.51 (25.82)	21/171 (12.28)	28.59 (65.39)	1
Arsenic (N=299)	10	3/100 (3)	11.34	16/199 (8.04)	36.69 (85.97)	1
Mercury (N=226)	1	4/75 (5.33)	8.28 (11)	13/151 (8.60)	1.92(1)	0.294
Nickel (N=127)	10	0/39 (00)	-	7/88 (7.95)	15.83 (40.89)	
Iron (N=83)	300	0/9 (00)	-	2/74 (2.70)	429.48	

Footnote: Figures in the parenthesis represent percentages; The denominator in ESCC and Controls section represent the number of positive samples for presence of heavy metal, however numerator represents the number of samples having heavy metals content above the permissible limits. ** the unit of measurement for levels of heavy metals was µg/L

HPV type 6 was detected in positive samples.

Discussion

The study in Punjab identified major ESCC risk factors, including tobacco and alcohol use, hot beverages, low fruit and vegetable intake, edentulism, and underground water use. Notably, urinary dimethylphosphate a marker of pesticide exposure was significantly associated with ESCC, highlighting an emerging risk in agricultural regions. These findings align with the study's objective to assess both established and novel risk factors.

Several socioeconomic factors, such as lower education and income levels, were associated with increased ESCC risk. Although low socioeconomic status has been identified as a risk factor in previous studies, our findings suggest it may influence cancer risk indirectly through its impact on health behaviors, environmental exposures, and health literacy [8, 9]. These associations are consistent with global evidence but highlight region-specific challenges in Punjab, where widespread agricultural practices and pesticide use may exacerbate risk.

The tobacco smokers in the study had approximately twice the odds of developing ESCC compared to controls, consistent with previous epidemiological findings [10, 11]. In Northeast India, the use of betel quid and chewing tobacco has been identified as a significant ESCC risk factor [12, 13]. In the present study, although higher odds of ESCC were observed among tobacco chewers in Punjab, the association was not statistically significant ($p > 0.05$). The population attributable fraction for tobacco use was also lower than that reported in other studies [14, 15].

Similarly, alcohol consumption was significantly associated with an increased risk of ESCC, in line with findings from several studies across different Indian states [16, 17]. The population attributable fraction for alcohol use in the current study was higher than previously reported [15]. A regular consumption of very hot tea was also associated with a significantly elevated risk of ESCC, consistent with earlier research [18].

Our findings also support the protective role of fruit and vegetable intake, aligning with earlier research highlighting their importance in lowering cancer risk [19, 20]. Previous studies have reported low consumption of fruits and green vegetables among the population in Punjab [21]. Additionally, edentulism was associated with higher odds of developing ESCC in this study, consistent with findings from previous research [22].

In contrast to some previous studies, no significant association was observed between red meat consumption, pickle intake, or contact with animals and ESCC risk in this study [23, 24]. Additionally, although fumigants have been implicated in cancer risk elsewhere, their link to ESCC remained inconclusive, indicating the need for further investigation [25].

Despite extensive research, the association between pesticide exposure and ESCC remains inconclusive. In this study, questionnaire-based data showed no significant risk associated with farm work exposure, consistent with findings from a study in Tanzania [26]. However, individuals involved in pesticide mixing, loading, and application had higher odds of developing ESCC (OR: 1.38) compared to those not involved, aligning with findings from previous studies [4, 16].

Rather than relying on proxy measures to assess agrochemical exposure, precise biomonitoring is essential for a clearer understanding of associated health risks. In this study, dimethylphosphate was detected five times more frequently in ESCC patients than controls, with significantly higher mean concentrations among cases. While few studies have explored the link between DMP and ESCC risk in the general population [27], the absence of reference values for urinary pesticide metabolites limits meaningful interpretation. Additionally, the presence of aminomethylphosphonic acid, a breakdown product of glyphosate, suggests ongoing exposure likely from illegal pesticide sales highlighting the urgent need for stronger market regulation and enforcement to ensure public health safety.

Heavy metal exposure, particularly to uranium, selenium, and arsenic, was notable in this study. Previous research has reported elevated levels of uranium and selenium in Punjab's drinking water, and our findings further support these concerns [28, 29]. Arsenic, a Group 1 carcinogen classified by IARC, and mercury, a Group 2B possible carcinogen, were detected above permissible limits. Additionally, nickel and iron levels exceeded safe thresholds; however, current evidence linking these metals

to ESCC risk remains limited. These findings highlight the need for ongoing monitoring and assessment of heavy metals in drinking water sources in the region.

In current study the percentage of study subjects found to be positive for HPV infection were found to be low in comparison to previous studies and is not found to be risk factor for ESCC [30]. Similar to previous literature, no significant association between, H Pylori and ESCC is found in current study [31].

This is the first case-control study in Punjab to investigate the association between pesticide metabolites and ESCC. The strengths of this study include age, gender, and region matching; adjustment for key confounders; a large sample size; diverse exposure assessments; and detailed lifestyle data. However, limitations include potential recall and interviewer bias, residual confounding despite adjustments, and limited generalizability due to recruitment from a single tertiary hospital and the study being confined to Punjab, which has a distinct agrarian population.

This study underscores the urgent need for stricter pesticide regulation, particularly organophosphates, in agricultural regions like Punjab. The public health efforts should target modifiable risks, such as tobacco and alcohol use, while promoting oral hygiene and healthy diets. The future research should focus on precise pesticide exposure assessments, cancer mechanisms, and the role of heavy metals using robust designs. The long-term studies and meta-analyses are essential to confirm findings and guide policies on environmental and occupational safety, integrating environmental health into cancer prevention strategies in rural areas.

Author Contribution Statement

HK: Conceptualization, Methodology, Data curation, Investigation Writing – original draft, Writing – review & editing. JST: Conceptualization, Funding acquisition, Resources, Critical review, Analysis UD: Conceptualization, Resources, Critical review, Analysis, Review. SA: Resources, Review. NG: Resources, Review. MPS: Resources, Review, KV: Review, AKY: Investigation, Review. RD: Review, All the authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

Acknowledgements

This study was supported by the Indian Council of Medical Research (ICMR) through a fellowship to Dr. Harmanjeet Kaur and by the Department of Health & Family Welfare, Punjab for funding laboratory investigations. We thank the Indian Institute of Toxicological Research, Lucknow, for sample analysis and are grateful to all participants and their families for their contributions.

Funding

This research was funded by Indian Council of Medical Research, New Delhi and Department of Health & Family Welfare, Punjab, India

Study Approval

The study was conducted as part of a doctoral research project and was approved by the competent authority of the Postgraduate Institute of Medical Education and Research, Chandigarh.

Ethical approval

An ethical clearance was obtained from the institutional ethical review committee of Postgraduate Institute of Medical Education and Research (PGIMER, Chandigarh) (Ref no. NK/4352/PhD/5319, Dated 21.09.2018).

Data availability statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest statement

The authors disclose that there are no competing financial or non-financial interests or conflicts of interest that may have influenced the outcome or interpretation of this study.

References

1. Kinner HK, Carminati A. New Research on Esophageal Cancer. Nova Publishers; 2007.
2. Dar NA, Bhat GA, Shah IA, Iqbal B, Makhdoomi MA, Nisar I, et al. Hookah smoking, nass chewing, and oesophageal squamous cell carcinoma in kashmir, india. Br J Cancer. 2012;107(9):1618-23. https://doi.org/10.1038/bjc.2012.449.
3. Tata Memorial Centre (TMC)-Centre for Cancer Epidemiology (CCE), Postgraduate Institute of Medical Education and Research (PGIMER). Cancer Burden in Chandigarh and Punjab State Report: 2017 – 2018. [internet] 2020 [cited on 2024 September 23]. Available from: https://pgimer.edu.in/PGIMER_PORTAL/PGIMERPORTAL/home.jsp#
4. Thakur JS, Rao BT, Rajwanshi A, Parwana HK, Kumar R. Epidemiological study of high cancer among rural agricultural community of punjab in northern india. Int J Environ Res Public Health. 2008;5(5):399-407. https://doi.org/10.3390/ijerph5050399.
5. Sharma R, Dutta D. A study of heavy metal pollution in groundwater of malwa region of punjab, india: Current status, pollution and its potential health risk. Int J Eng Res. 2017;07:81-91. https://doi.org/10.9790/9622-0703038191.
6. Kaur H, Thakur JS, Dutta U, Attri S, Gupta N, Singh M, et al. Study of modifiable risk factors for instituting evidence-based preventive strategy for carcinoma esophagus in punjab: A study protocol. Int J Noncommun Dis. 2020;5:149. https://doi.org/10.4103/jncd.jncd_33_19.
7. Benichou J. Methods of adjustment for estimating the attributable risk in case-control studies: A review. Stat Med. 1991;10(11):1753-73. https://doi.org/10.1002/sim.4780101113.
8. Islami F, Kamangar F, Nasrollahzadeh D, Aghcheli K, Sotoudeh M, Abedi-Ardekani B, et al. Socio-economic status and oesophageal cancer: Results from a population-based case-control study in a high-risk area. Int J Epidemiol. 2009;38(4):978-88. https://doi.org/10.1093/ije/dyp195.
9. Dar NA, Shah IA, Bhat GA, Makhdoomi MA, Iqbal B, Rafiq R, et al. Socioeconomic status and esophageal squamous cell carcinoma risk in kashmir, india. Cancer Sci. 2013;104(9):1231-6. https://doi.org/10.1111/cas.12210.
10. Ganesh B, Talole SD, Dikshit R. Tobacco, alcohol and tea drinking as risk factors for esophageal cancer:

A case-control study from mumbai, india. Cancer Epidemiol. 2009;33(6):431-4. https://doi.org/10.1016/j.canep.2009.09.002.
11. Pandeya N, Williams GM, Sadhegi S, Green AC, Webb PM, Whiteman DC. Associations of duration, intensity, and quantity of smoking with adenocarcinoma and squamous cell carcinoma of the esophagus. Am J Epidemiol. 2008;168(1):105-14. https://doi.org/10.1093/aje/kwn091.
12. Phukan RK, Ali MS, Chetia CK, Mahanta J. Betel nut and tobacco chewing; potential risk factors of cancer of oesophagus in assam, india. Br J Cancer. 2001;85(5):661-7. https://doi.org/10.1054/bjoc.2001.1920.
13. Talukdar FR, Ghosh SK, Laskar RS, Mondal R. Epigenetic, genetic and environmental interactions in esophageal squamous cell carcinoma from northeast india. PLoS One. 2013;8(4):e60996. https://doi.org/10.1371/journal.pone.0060996.
14. Okello S, Churchill C, Owori R, Nasasira B, Tumuhimbise C, Abonga CL, et al. Population attributable fraction of esophageal squamous cell carcinoma due to smoking and alcohol in uganda. BMC Cancer. 2016;16:446. https://doi.org/10.1186/s12885-016-2492-x.
15. Negri E, La Vecchia C, Franceschi S, Decarli A, Bruzzi P. Attributable risks for oesophageal cancer in northern italy. Eur J Cancer. 1992;28a(6-7):1167-71. https://doi.org/10.1016/0959-8049(92)90479-l.
16. Notani PN. Role of alcohol in cancers of the upper alimentary tract: Use of models in risk assessment. J Epidemiol Community Health. 1988;42(2):187-92. https://doi.org/10.1136/jech.42.2.187.
17. Talagala IA, Nawarathne M, Arambepola C. Novel risk factors for primary prevention of oesophageal carcinoma: A case-control study from sri lanka. BMC Cancer. 2018;18(1):1135. https://doi.org/10.1186/s12885-018-4975-4.
18. Zhao L, Liu CL, Song QK, Deng YM, Qu CX, Li J. Association between dietary behavior and esophageal squamous cell carcinoma in yanting. Asian Pac J Cancer Prev. 2014;15(20):8657-60. https://doi.org/10.7314/apjcp.2014.15.20.8657.
19. Joshi SC, Saxena SR, Satyawali VN, Joshi A, Nigam P, Singh VK, et al. Oesophageal carcinoma--a study of risk factors (emphasis on nutrition) in a teaching hospital of kumaon region of uttarakhand. J Assoc Physicians India. 2009;57:631-5.
20. Niu C, Liu Y, Wang J, Liu Y, Zhang S, Zhang Y, et al. Risk factors for esophageal squamous cell carcinoma and its histological precursor lesions in china: A multicenter cross-sectional study. BMC Cancer. 2021;21(1):1034. https://doi.org/10.1186/s12885-021-08764-x.
21. Thakur JS, Jeet G, Pal A, Singh S, Singh A, Deepti SS, et al. Profile of risk factors for non-communicable diseases in punjab, northern india: Results of a state-wide steps survey. PLoS One. 2016;11(7):e0157705. https://doi.org/10.1371/journal.pone.0157705.
22. Abnet C, Kamangar F, Islami F, Nasrollahzadeh D, Brennan P, Aghcheli K, et al. Tooth loss and lack of regular oral hygiene are associated with higher risk of esophageal squamous cell carcinoma. Cancer Epidemiol Biomark Prev. 2008;17:3062-8. https://doi.org/10.1158/1055-9965.EPI-08-0558.
23. Yang CX, Wang HY, Wang ZM, Du HZ, Tao DM, Mu XY, et al. Risk factors for esophageal cancer: A case-control study in south-western china. Asian Pac J Cancer Prev. 2005;6(1):48-53.
24. Dar NA, Islami F, Bhat GA, Shah IA, Makhdoomi MA, Iqbal B, et al. Contact with animals and risk of oesophageal squamous cell carcinoma: Outcome of a case-control study from kashmir, a high-risk region. Occup Environ Med. 2014;71(3):208-14. https://doi.org/10.1136/oemed-2013-101802.
25. Alavanja MC, Hofmann JN, Lynch CF, Hines CJ, Barry KH, Barker J, et al. Non-hodgkin lymphoma risk and insecticide, fungicide and fumigant use in the agricultural health study. PLoS One. 2014;9(10):e109332. https://doi.org/10.1371/journal.pone.0109332.
26. Mmbaga EJ, Mushi BP, Deardorff K, Mgisha W, Akoko LO, Paciorek A, et al. A case-control study to evaluate environmental and lifestyle risk factors for esophageal cancer in tanzania. Cancer Epidemiol Biomarkers Prev. 2021;30(2):305-16. https://doi.org/10.1158/1055-9965.Epi-20-0660.
27. Lari S, Pandiyan A, Vanka J, Jee B, Yamagani P, Kumar SB, et al. Residual dialkyl phosphate metabolite concentrations of organophosphate pesticides among indian farmworkers: Implication of exposure and hazard assessment. J Occup Environ Med. 2023;65(11):e722-e31. https://doi.org/10.1097/jom.0000000000002960.
28. Thakur J, Prinja S, D S, Rajwanshi A, Arora S, Prasad R, et al. Genotoxicity and adverse human outcomes among people living along major waste water drains in punjab, india. World J Pharm Res. 2014;4:895-908.
29. Virk HS. High levels of heavy metals contamination in ground water of Punjab State, India. 2017. https://doi.org/10.13140/RG.2.2.20273.79204
30. Ludmir EB, Stephens SJ, Palta M, Willett CG, Czito BG. Human papillomavirus tumor infection in esophageal squamous cell carcinoma. J Gastrointest Oncol. 2015;6(3):287-95. https://doi.org/10.3978/j.issn.2078-6891.2015.001.
31. Malik A, Nadeem M, Naseem K, Amjad W, Zahid R, Haider M, et al. S464 helicobacter pylori infection and risk of esophageal carcinoma: A systematic review and meta-analysis. Official journal of the American College of Gastroenterology | ACG. 2021;116:S205. https://doi.org/10.14309/01.ajg.0000774328.61075.a0.