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Trace elements as potential biomarkers for oral squamous cell carcinoma

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Oral cancer encompasses a range of tumors affecting the oral cavity, throat, and salivary glands. Oral Squamous Cell Carcinoma (OSCC) is the most common type, particularly in Southeast Asia, where it constitutes one-third of all body cancers. Patients with advanced OSCC generally experience much poorer outcomes compared to those with early-stage disease. This highlights the need for reliable biomarkers to better understand cancer development. Trace elements, crucial for various physiological processes, have been proposed as potential biomarkers for cancer due to their roles in formation and progression. This study investigates changes in serum levels of trace elements—copper, iron, zinc, and magnesium—in patients with OSCC compared to healthy age-matched controls. Conducted in Telangana, the study involved 100 OSCC patients and 100 controls. Methods used for trace element estimation include Ferrozine, DBDC, colorimeter, and calmagite. The study aims to evaluate how individual and combined risk factors, such as smoking, alcohol use, and tobacco chewing, affect trace element concentrations. Results revealed that OSCC patients had significantly higher serum copper levels, while their serum iron, zinc, and magnesium levels were lower. The study also found that combined risk factors had a greater impact on trace element levels than individual risk factors.

Keywords: Copper, Iron, Magnesium, Oral squamous cell carcinoma, Serum levels, Zinc

Oral cancer, a common form of oral cancer, affects the oral cavity, throat, and salivary glands. Oral squamous cell carcinoma (OSCC) is the most prevalent form of oral cancer, originating from the squamous epithelial cells in the mouth. It primarily impacts areas such as the floor of the mouth and the tongue. Oral squamous cell carcinoma (OSCC) is the most common form, with it being the 6th most frequent malignancy and 3rd most common cancer worldwide. In India, it is the 2nd most prevalent malignancy, with 119,992 new cases and 72,616 fatalities annually. In Taiwan, it ranks as the fourth most common cancer among men, largely due to the prevalence of alcohol and tobacco use. Risk factors for OSCC include alcohol consumption, smoking, and betel quid chewing. Conventional treatment methods include surgery, chemoradiotherapy, and medications like EGFR and COX-2 inhibitors.Oral cavity cancers linked with HPV infection increase mortality rates by about 2% per year in men and 1% per year in women. Treatment causes significant alterations in the oral cavity, potentially affecting essential functions such as swallowing, speech, chewing, and salivation, which can significantly diminish the patient's quality of life[1-4].

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OSCC is often undetected before reaching advanced stages due to inadequate early diagnosis and less awareness. This aggressive epithelial cancer is a major public health concern, leading to poor clinical outcomes and high morbidity rates. However, reduction in smoking and advanced early detection strategies led to a gradual drop in cancer-related deaths. Trace elements, such as magnesium, copper, zinc, and iron, are crucial for genome stability and integrity in human cells. Their involvement in various malignancies, including pancreatic, lung, stomach, breast, and oral cancers, and liver cirrhosis, has been thoroughly studied[5-7].

Research on cancer should focus on discovering novel biochemical indicators, such as trace element variations, to identify potential risk factors and reliable biomarkers. Trace elements like copper and zinc contribute to the body's defense against carcinogens[8]. Studies have shown that serum zinc levels are slightly elevated in patients with oral squamous cell carcinoma (OSCC) and oral submucous fibrosis (OSMF). Oral submucous fibrosis incidence increases with clinical stages, and serum copper levels are also elevated in these patients. Conversely, zinc levels have been associated with a reduced risk of esophageal squamous cell carcinoma (ESCC)[9]. Research consistently shows that serum levels of essential metals like zinc and copper are linked to the risk of oral cancer, with an inverse correlation between copper levels and oral cancer risk and a positive association with oral cancer risk[10].

Head and neck cancer risk factors are consistent across locations, but the degree of risk associated with certain factors varies. Tobacco use is a significant risk factor for laryngeal cancer, while alcohol intake is significantlylinked to oral cavity and oropharyngeal malignancies[11]. Studies have found that certain components are higher in tumor tissues, while iron, zinc, and copper levels are lower in patients with lip and oral cavity malignancies than in healthy controls. Lee *et al*. found substantial changes in plasma levels at different phases of oral squamous cell carcinoma (OSCC)[12]. Another study found out that copper levels gradually increased from pre-cancerous to cancer phases, but blood levels of magnesium, iron, and zinc were lower in both patient groups[13]. Shishir *et al*. found a significant decrease in serum zinc levels in control groups, while copper levels were elevated. Further research indicated that copper and zinc levels are significantly higher in the plasma of cancer patients compared to controls[14]. Tobacco smoking is reported as the greatest risk factor for laryngeal cancer, while alcohol consumption is associated with an increased risk of oropharyngeal and oral cavity cancers.

Effect of iron (Fe) in OSCC

Iron plays a vital role in the growth and development of oral squamous cell carcinoma (OSCC), influencing its progression and prognosis. Cancer cells alter their iron metabolism to enhance iron intake by increasing the expression of proteins involved in iron absorption. This process activates tumor necrosis factor-alpha and nuclear factor-kappa B, resulting in oxidative stress, the production of reactive oxygen species, and the activation of survival signaling pathways in cancer cells. Iron deficiency anemia in OSCC is more responsive to iron depletion than in normal cells, leading to ferroptosis, a form of iron-dependent cell death. While iron supplements are used to treat iron deficiency, researchers are also developing drugs that target the ferroptosis pathway as a potential treatment for OSCC[15].

Effect of Copper (Cu) in OSCC

Elevated serum copper levels are linked to various cancer types, including OSCC, due to its role in oxidative stress. Copper is an essential component of enzymes involved in oxidation processes, such as tyrosinase, ceruloplasmin, amine oxidase, and cytochrome oxidase, which are crucial for maintaining cellular homeostasis. In cases of Potentially Malignant Disorders (PMDs) like OSCC, increased oxidative stress is often observed, leading to increased copper levels[16]. This can create a microenvironment conducive to cancer development by promoting oxidative damage to cellular components and disrupting cellular homeostasis. Copper's role in OSCC is also linked to its effects on tissue remodelling and collagen production. The consumption of areca nut, a known carcinogen, has been shown to influence copper levels in the oral cavity. Copper's involvement in enzymatic functions and tissue repair is essential, but its dysregulation can contribute to the development and progression of oral cancer[17]. Further research is needed to fully understand these mechanisms and their implications for prevention and treatment strategies.

Effect of Zinc (Zn) in OSCC

Zinc is a crucial trace element in the development and progression of oral squamous cell carcinoma (OSCC). It is essential for DNA repair, immune function, and apoptosis. Zinc deficiency can significantly influence OSCC pathogenesis by increasing cellular proliferation and tumorigenesis, primarily through impaired DNA synthesis and repair. Zinc also regulates apoptosis, disrupting pathways that control cell death, leading to enhanced survival of malignant cells. Zinc acts as an antioxidant, counteracting oxidative stress. Deficiency in zinc is associated with increased invasiveness and angiogenesis, which is crucial for tumor growth. Maintaining optimal zinc levels is essential for effective therapeutic strategies for managing OSCC[18].

Effect of Magnesium (Mg) in OSCC

Magnesium (Mg) is an essential mineral in living cells, vital for numerous physiological processes.Magnesium is the fourth most abundant mineral in the human body and is crucial for the development of soft tissues and bones. For a healthy adult, the advised daily intake of magnesium ranges from 250 to 350 mg. It significantly impacts the cell cycle by influencing transphosphorylation and DNA synthesis. Mg regulates the timing of spindle and chromosome cycles through its effects on intracellular concentrations. As cells grow, Mg levels decrease until they reach a threshold that permits spindle formation, which then leads to spindle breakdown and cell division. Mg is crucial for key rate-limiting steps in the cell cycle, including the initiation of DNA disrupted in cancerous cells. Additionally, processes governed by calcium (Ca) and calmodulin rely on adequate Mg levels, as low Mg can disrupt these processes. Ca's metabolic effects are mediated indirectly through its competition with Mg for binding sites on membranes. Research indicates that patients with oral squamous cell carcinoma (OSCC) have lower Mg concentrations in blood serum and saliva compared to those with potentially malignant disorders (PMDs) and healthy individuals, suggesting Mg could be a potential marker for tumor development[19,20].

Materials and Methods

The study utilizes both primary and secondary data to examine the levels of trace elements in sera of oral squamous cell carcinoma. The OSC cancer study is a hospital-based case-control study performed in Telangana with a total of 100 patients and 100 controls. The samples with gross tumor appearance (T) oral tissues were imminently collected after surgical excision or after punch biopsy. OSCC samples were analyzed based on histopathological findings and clinical examinations, tumor characteristics and staging was done by a senior pathologist, based on WHO classification. A well-structured questionnaire was prepared and designed to collect the information from the respondents. Patients were interviewed and data concerning the patient's demographic factors and family history of cancer was noted. Information regarding age, gender, smoking, alcohol intake, chewing (Paan, Jaradha, betel, and Gutka), and previous cancer diagnosis were also comprehended. The clinical information for these cases was acquired from medical reports relating to tumor size, Grade, and axillary nodes with their prior consent (Fig. 1).

Estimation of Iron, Copper, Zinc and Magnesium

The methods of Ferrozine DBDC, colorimeter and calmagite were used for the estimation of trace elements of Fe, Cu, Zn and Mg.

Estimation of Iron by Ferrozine method using ERBA kit

Pipette into clean dry test tubes labeled as blank (B), standard (S), sample blank (SB) and test (T). All the contents were mixed well and incubated at room temperature for 5 min. The absorbance was measured for the Blank (Abs B), Standard (Abs S), Sample blank (Abs SB) and Test (Abs T). against distilled water.

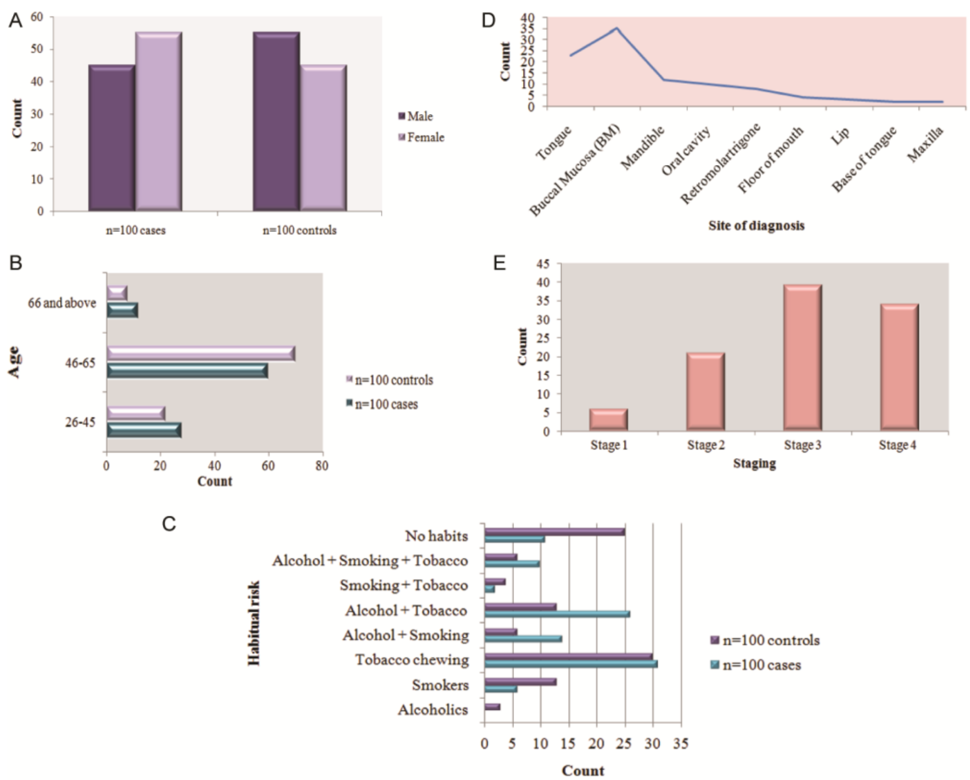

Fig. 1 — Clinical characteristics of OSCC patients and healthy controls as per (A) Gender; (B) Age; (C) Habitual risk; (D) Site of diagnosis; and (E) Staging

Estimation of Copper by the method of DBDC

1 ml of serum, 1 ml of 1N HCl, and 1 ml of 12.5% trichloroacetic acid were mixed and allowed to stand for 10 minutes at room temperature, and then centrifuged at 2000 rpm for 5 minutes. Three test tubes were set up and labeled as S, T, and C. Mix well and stand for 15 minutes, then remove the upper layer (1.0 ml layer) and read the carbon tetrachloride layers in a colorimeter at 470 nm. Read control (C) against carbon tetrachloride and test and standard against DBDC.

Estimation of Zinc by the colorimetric method by ERBA kit

Zinc in an alkaline medium reacts with Nitro-PAPS to form a purple-colored complex. The intensity of the complex formed is directly proportional to the amount of zinc present in the sample. Reagents used include buffer reagent, color reagent, distilled water, and zinc standard (S).

Estimation of Magnesium by the calmagite method by ERBA kit

Magnesium combines with calmagite in an alkaline medium to form a red colored complex. Interference of calcium and proteins is eliminated by the addition of specific chelating agents and detergents; intensity of the color formed is directly proportional to the amount of magnesium present in the sample. buffer reagent, Color reagent, distilled water Magnesium standard (S) Sample. Mix well and incubate at room temperature for 5 min. Measure the absorbance of the standard and Test sample against Blank, within 30 min.

Statistical analysis

In this section, the collected data is analyzed, and the study results are tabulated to show the distribution of trace elements in OSCC patients and control groups. All parameters in the study were statistically analyzed for mean values, standard deviation, range, and P-values. ANOVA was conducted using SPSS version 15 (SPSS, Chicago, IL) for Windows to compare the mean, standard deviation, and P-values of the parameters.

Results and Discussion

The age range for OSCC cases varied from 9 to 87 years in males and from 27 to 75 years in females. In the control group, the age range was from 21 to 80 years in males and from 22 to 87 years in females. However, many of the ages mentioned in the case sheets were subjective. The exact age of 100 OSCC cases (45 males and 55 females) and 100 controls (55 males and 45 females) was available for analysis. The mean age at which OSCC was identified was 49.30±15.55 years in males and 84.20±11.26 years in females. To understand the role of gene mutations/polymorphisms in the onset of the disease, the cases and controls were divided into four categories: <25 years (1% cases and 3% controls), 26 to 45 years (27% cases and 19% controls), 46 to 65 years (60% cases and 70% controls), and above 66 years (12% cases and 8% controls). The highest percentage of OSCC cases was identified between 46 and 65 years in both the case and control groups. Regarding the primary tumor site, there was a clear predominance on the buccal mucosa, accounting for 35 cases (35.0%), followed by the tongue with 23 cases (23.0%). The mandible, oral cavity, and retromolar trigone accounted for 12, 10, and 8 cases respectively (12%, 10%, and 8%). In the present study, Stage III showed the highest frequency (39%) compared to Stage IV (34%) and Stage II (21%). Stage I showed a very low frequency (6%) compared to other stages. Histopathologically, OSCC was graded as well-differentiated squamous cell carcinoma (WD SCC), moderately differentiated (MD SCC), and poorly differentiated (PD SCC) based on the degree of differentiation.

Trace elements distribution and degree of differentiation

Iron

The mean serum levels of iron were significantly lowered in OSCC patients (113.2±27.4) in comparison to controls (118.7±28.2) (Table 1). The serum Iron levels were found to be higher in poorly differentiated as compared to the well differentiated squamous cell carcinoma (Table 2).

Copper

The mean serum levels of copper were significantly (p=0.0001) raised in OSCC patients (132.5±17.0) in comparison to controls (103.7±17.3) (Table 1). The levels of serum copper were found to be lower in poorly differentiated squamous cell

Table 1 — Trace elements distribution in patients and control group

Trace element	MEAN±SD (Patients) N=100	MEAN±SD (Controls) N=100	P value
Iron	113.2±27.4	118.7±28.2	0.16
Copper	132.5±17.0	103.8±17.3	0.0001
Zinc	93.1±17.7	106.9±18.4	0.0001
Magnesium	1.71±0.2	1.9±0.3	0.0001

Table 2 — Trace elements according to Degree of differentiation

Trace element	WD SCC (N=64)	MD SCC (N=31)	PD SCC (N=5)	F-Statistics	P-value
Iron	129.4±12.7	129.9±20.9	134.0±15.1	.671	.000
Copper	119.5±28.8	117.7±28.4	114.5±22.9	.101	.000
Zinc	91.5±16.9	95.5±18.9	99±22.5	.800	.000
Magnesium	1.72±0.29	1.6±0.2	1.7±0.2	.248	.000

carcinoma (Table 2) as compared to moderate and well differentiated squamous cell carcinoma.

Zinc

The mean serum levels of Zinc were significantly (p=0.0001) lowered in OSCC patients (93.1±17.7) in comparison to controls (106.9±98.59) (Table 1). Zinc levels were found lower in WD SCC (Table 2).

Magnesium

The mean serum levels of magnesium were also significantly (p=0.0001) lowered in OSCC patients (1.71±0.2) in comparison to controls (1.92±0.3) (Table 1). The levels of magnesium were found unaltered with the degree of differentiation (Table 2).

Trace Elements values in correlation to risk factors

Alcohol alone

No cases were recorded with this lone risk factor for the trace elements Iron, Copper, Zinc and Magnesium (Table 3).

Smoking

Smoking risk factor when correlated to Iron levels, the mean values were observed as 111.06±5.37 and 113.99±7.85 for cases and controls respectively and smoking alone risk factor is found to be insignificant with iron levels. Copper mean values were observed as 133.22±5.03 and 101.61±4.84 for cases and controls respectively, and the correlation observed as significant. Zinc mean values were observed as 89.58±4.49 and 107.16±4.81 for cases and controls respectively, and the correlation observed as significant. Magnesium mean values were observed as 1.72±0.08 and 1.86±0.10 for cases and controls respectively, and the correlation observed as significant.

Chewing

Chewing risk factor when correlated to Iron levels, the mean values were observed as 114.62±7.96 and 118.02±10.36 for cases and controls respectively and chewing alone risk factor is found to be insignificant with iron levels. Copper mean values were observed as 133.65±5.87 and 102.98±5.37 and for cases and controls respectively, and the correlation observed as significant. Zinc mean values were observed as 93.82±5.48 and 105.80±6.26 for cases and controls respectively, and the correlation observed as significant. Magnesium mean values were observed as 1.70±0.06 and 1.91±0.10 for cases and controls respectively, and the correlation observed as significant.

Alcohol + Smoking

Alcohol and smoking combination risk factor when correlated to Iron levels, the mean values were observed as 117.21±6.53 and 123.06 ±6.30 for cases and controls respectively and Alcohol+smoking risk factor is found to be significant with iron levels. Copper mean values were observed as 133.22±5.03 and 101.61±4.84 for cases and controls respectively, and the correlation observed as significant. Zinc mean values were observed as 89.58±4.49 and 107.16±4.81 for cases and controls respectively, and the correlation observed as significant. Magnesium mean values were observed as 1.72±0.0 and 81.86±0.10 for cases and controls respectively, and the correlation observed as significant.

Alcohol + Chewing

Alcohol + Chewing risk factor when correlated to Iron levels, the mean values were observed as 112.85±8.26 and 118.39±9.26 for cases and controls respectively and risk factor is found to be significant with iron levels. Copper mean values were observed as 131.96±5.35 and 105.86±4.89 for cases and controls respectively, and the correlation observed as significant. Zinc mean values were observed as 96.42±4.95 and 106.25±5.99 and for cases and controls respectively, and the correlation observed as significant. Magnesium mean values were observed as 1.73±0.061 and 1.90±0.07 for cases and controls respectively, and the correlation observed as significant.

Smoking + Chewing

Smoking + Chewing risk factor correlated to Iron levels, the mean values were observed as 106.26±3.97 and 118.26±11.46 for cases and controls respectively and is found to be significant with iron levels. Copper mean values were observed as 137.21±5.06 and 107.59±6.51 for cases and controls respectively, and the correlation observed as significant. Zinc mean

Table 3 — Trace elements in correlation to risk factors

Risk Factors		Iron	Copper	Zinc	Magnesium
Alcohol	Cases (Mean ± SD)	Nocases	Nocases	Nocases	Nocases
	Controls(Mean±SD)	121.97±7.49	98.52±2.98	106.28±7.69	1.91±0.13
	95%CI	--	--	--	--
	t-stat	--	--	--	--
	p-value	--	--	--	--
Smoking	Cases (Mean±SD)	111.06±5.37	133.22±5.03	89.58±4.49	1.72±0.08
	Controls (Mean±SD)	113.99±7.85	101.61±4.84	107.16±4.81	1.86±0.10
	95%CI	1.05-4.81	30.23-32.99	16.28-18.88	0.11-0.17
	t-stat	3.08	45.28	26.72	10.9
	p-value	0.0024	<0.0001	<0.0001	<0.0001
Chewing	Cases (Mean±SD)	114.62±7.96	133.65±5.87	93.82±5.48	1.70±0.06
	Controls (Mean±SD)	118.02±10.36	102.98±5.37	105.80±6.26	1.91±0.10
	95%CI	0.82-5.98	29.01-32.20	10.34-13.62	0.19-0.23
	t-stat	2.6	38.55	14.4	18.01
	p-value	0.01	<0.0001	<0.0001	<0.0001
Alcohol+ Smoking	Cases (Mean±SD)	117.21±6.53	129.35±4.32	89.05±4.98	1.72±0.07
	Controls (Mean±SD)	123.06±6.30	105.30±5.76	110.25±4.83	1.96±0.09
	95%CI	4.06-7.63	22.63-25.46	19.83-22.57	0.22-0.26
	t-stat	6.45	33.4	30.56	21.05
	p-value	<0.0001	<0.0001	<0.0001	<0.0001
Alcohol+ Chewing	Cases (Mean±SD)	112.85±8.26	131.96±5.35	96.42±4.95	1.73±0.06
	Controls (Mean±SD)	118.39±9.26	105.86±4.89	106.25±5.99	1.90±0.07
	95%CI	0.39-7.99	24.67-27.52	8.29-11.36	0.15-0.19
	t-stat	4.47	36.01	12.65	18.44
	p-value	<0.0001	<0.0001	<0.0001	<0.0001
Smoking+ Chewing	Cases (Mean±SD)	106.26±3.97	137.21±5.06	93.88±2.06	1.74±0.04
	Controls (Mean±SD)	118.26±11.46	107.59±6.51	106.89±1.45	1.88±0.09
	95%CI	9.61-14.39	27.99-31.25	12.51-13.50	0.12-0.16
	t-stat	9.89	35.29	15.64	14.22
	p-value	<0.0001	<0.0001	<0.0001	<0.0001
Alcohol+ Smoking+ Chewing	Cases (Mean±SD)	108.96±9.28	131.49±5.44	92.70±5.46	1.70±0.07
	Controls (Mean±SD)	114.38±5.35	103.47±7.68	104.07±5.12	1.85±0.09
	95%CI	3.31-7.53	26.16-29.88	9.89-12.85	0.13-0.17
	t-stat	5.06	29.77	15.19	13.16
	p-value	<0.0001	<0.0001	<0.0001	<0.0001
Nohabits	Cases (Mean±SD)	118.08±9.18	131.11±2.67	99.07±5.55	1.74±0.17
	Controls (Mean±SD)	119.50±10.53	133.92±6.93	100.01±5.81	1.84±0.23
	95%CI	1.33-4.17	1.35-4.27	0.64-2.52	0.04-0.15
	t-stat	1.02	3.78	1.17	3.49
	p-value	0.3106	0.0002	0.2434	0.0006

values were observed as 93.88±2.06 and 106.89±1.45 for cases and controls respectively, and the correlation observed as significant. Magnesium mean values were observed as 1.74±0.04 and 1.88±0.09 for cases and controls respectively, and the correlation observed as significant.

Alcohol + Smoking + Chewing

All the combination risk factors together correlated to Iron levels, the mean values were observed as 108.96±9.28 and 114.38±5.35 for cases and controls respectively and is found to be significant with iron levels. Copper mean values were observed as 131.49±5.44 and 103.47±7.68 for cases and controls respectively, and the correlation observed as significant. Zinc mean values were observed as 92.70±5.46 and 104.07±5.12 for cases and controls respectively, and the correlation observed as significant. Magnesium mean values were observed as 1.70±0.07 and 1.93±0.10 for cases and controls respectively, and the correlation observed as significant.

No habits

Iron levels, the mean values were observed as 118.08±9.18 and 119.50±10.53 for cases and controls respectively and no habits status is found to be insignificant with iron levels. Copper mean values were observed as 131.11±2.67 and 133.92±6.93 for cases and controls respectively, and the correlation observed as insignificant. Zinc mean values were observed as 99.07±5.55 and 100.01±5.81 for cases and controls respectively, and the correlation observed as insignificant. Magnesium mean values were observed as 1.74±0.17 and 1.84±0.23 for cases and controls respectively, and the correlation observed as insignificant.

Discussion

Investigating the elemental makeup of tumor tissue in head and neck cancer in correlation to risk factors elucidates their impact concerning the prognosis and survival of cancer patients. To the extent of our knowledge, our study marks the inaugural attempt to conduct a qualitative analysis of elemental presence within tumor tissue samples from OSCC patients and controls and correlates these elements with alcohol, smoking, chewing, and combination risk habits. Minor alterations in trace element concentrations can profoundly impact cellular metabolism and broader physiological functions and offer valuable diagnostic insights, aiding in the detection of potential deficiencies, toxicities, or underlying health issues[21]. Regardless of the intricacy in presenting precise and steadfast evidence for the role of trace elements in cancer from small observational studies, and challenges in expounding in-depth insights from the epidemiological literature, they have great possibilities for use as biomarkers. We focused our study on the composite relationships of trace metals in the risk for the development of OSCC.

In our study, trace element iron mean serum levels were significantly lowered in OSCC patients in comparison to controls, which is in accord with a study by Anees *et al*.,[22] but contrarily, considering the degree of differentiation in our study, iron values increased slightly higher with increasing tumor size and histopathological grading in the study. Both in animals and humans, primary neoplasms tend to emerge at sites in the body where iron accumulates markedly[23] and decrease in iron levels probably due to the requirement of iron for growth of tumor. In our study correlating to risk factors, iron levels did not reveal any significance when lone risk factors were considered, but all the combination risk factors showed statistical significance. Serum iron levels in patients and controls with no habits showed statistical insignificance owing to the fact that the risk factors might alter trace elements composition.

Essential dietary micronutrients such as iron and copper have amassed recent attention in studies investigating their roles in the onset and advancement of these lesions. In the present study, copper levels elevated in patients with OSCC in accordance with a study wherein the patients who habitually chewed areca nut exhibited soaring serum copper levels, which is analogous with findings from a study that accounts for augmented tissue and salivary copper levels in OSMF[24]. Serum copper levels in patients and controls with no habits showed statistical insignificance owing to the fact that the risk factors might alter trace elements composition. Considering the degree of differentiation, the serum copper levels in leukoplakia and OSMF patients showed a progressive increase correlating with the severity of dysplasia and concurrent with the phase of the ailment and its development in cancers of colorectal and breast[25]. Our study observed that poorly differentiated Squamous cell carcinoma had lower copper serum levels than well differentiated Squamous cell carcinoma, due to utilization of copper for the growth of tumor and poorly differentiated being less vascularized than well differentiated tumors and carcinomas[24,26]. In our study, copper in correlation to all risk factors showed statistical significance, areca nut propelling elevated copper levels in habitual chewers, is consistent with a study by Trivedy *et al*., who reported increased copper levels in patients with gutka intake[27]. Copper levels increase in serum of cancer patients as well as in tumor tissue, due to the need of copper to facilitate augmentation, metastasis, and angiogenesis. These interpretations suggest that the serum copper levels may serve as a biomarker for cancer decline and can be calculated to scrutinize the efficiency of therapy.

The most profuse trace element, Zinc, plays crucial roles in various bodily functions including proliferation, immune response, DNA repair, and antioxidant activity. In accordance with our study, a marked decrease in Zn levels in saliva was recorded in oral carcinoma patients contrary to healthy volunteers and Oral Leukoplakia patients[28]. A few studies have uncovered lower salivary Zn levels in OL patients, accredited to the diminishing immune status of these individuals[29], while some studies contrastingly show amplified serum Zn levels in cancer of the oral cavity and testis[30]. In our study, correlating Zn with habitual risk factors, all the risk factors show statistical significance, and studies report that smoking and alcohol abuse may attribute to a greater risk of OSCC when low levels of zinc were observed in nails[31]. Serum zinc levels in patients and controls with no habits showed statistical insignificance owing to the fact that the risk factors might alter trace elements composition. Zinc supplementation may provide advantages in the initial phases of cancer progression compared to throughout therapy; however, zinc assists in protection from inflammatory response due to the effect of radiotherapy in oropharyngeal cancers[32].

Magnesium plays a crucial role in the cell cycle, and its insufficiency serves as a significant factor in the transformation of precancerous cells, debatably serving as a screening marker to track the advancement of potentially neoplastic ailments. The serum concentration of magnesium remains notably stable in healthy individuals, serving as a protective factor against a range of diseases. In our study, we observed reduced magnesium levels in the serum of patients compared to controls, which is in accordance with a study wherein notable minor magnesium levels were observed in sera and saliva of OSCC patients against those with PMDs and healthy controls[33]. When correlated with habitual risk factors, magnesium levels in our study showed a clear statistical significance. Some studies highlight that smoking has an effect on the salivary trace element constitution, raising the saliva's magnesium levels[34], while a study verified that the presence of magnesium was associated with smoking, prognosis, and survival. In potentially malignant disorders, patients with tobacco practices exhibited elevated serum magnesium levels compared to the control group. Conversely, in patients with tobacco habits, magnesium levels in saliva and sera were diminished compared to those without malignancy[35], maybe due to utilization of magnesium for cell processes. Magnesium levels in the serum of patients and controls with no habits showed statistical insignificance owing to the fact that the risk factors might alter trace elements composition.

Though some studies did not find any indication of trace element pointers in oral cancer[36], assessment of serum trace elements can be an affordable and non-intrusive choice for screening, diagnosis, and monitoring oral Squamous cell carcinoma. Changes in immunological status and dysfunction of the immune system have also been linked to the initiation and advancement of malignancies. Hence, these parameters can be employed as biomarkers that provide important tools in prognosis for oral squamous cell carcinoma.

Conclusion

This study provides insights into the distribution of trace elements in OSCC and their correlation with risk factors. Elevated copper and decreased zinc levels in OSCC patients suggest their potential role in OSCC development and progression. Magnesium levels were lower in OSCC patients but did not correlate with tumor differentiation. The findings emphasize the need for further research into trace elements as biomarkers and potential therapeutic targets for OSCC.

Conflict of interest

All authors declare no conflicts of interest.

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