

# Utility of serum homocysteine in oral squamous cell carcinoma patients as a potential biomarker

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## Abstract

**Background:** In India, Oral cancer is one of the most common cancers. Despite advances in treatments, prognosis for oral cancer has remained poor with a five-year survival rate of 40–50%. Therefore, it is necessary to develop effective diagnostic methods for early diagnosis and better prognosis. Homocysteine (Hcy) has been reported as a ‘tumour marker’ in various cancers such as breast cancer, colorectal cancer, lung cancer, cervical cancer.

**Aim:** To study the levels of serum Hcy in oral squamous cell carcinoma (OSCC) patients.

**Objectives:** To assess the clinical utility of serum Hcy as a potential tumour marker for OSCC cases.

**Methodology:** Serum Hcy levels were studied and compared between patients with OSCC and healthy individuals.

**Results:** Serum Hcy levels were higher in patients having OSCC.

**Conclusion:** Serum Hcy levels could be utilized as a biological marker in the diagnosis and the prognosis of OSCC patients.

**Keywords:** Homocystein, oral squamous cell carcinoma, OSCC, prognosis

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## INTRODUCTION

Oral cancer (OC) is the most common type of head and neck squamous cell carcinoma (HNSCC), with an estimated 377,713 cases in 2020.<sup>[1-4]</sup> OC accounts for 2–4% of all cancers and is most commonly diagnosed as oral squamous cell carcinoma (OSCC).<sup>[1,2]</sup>

In India, oral cavity cancer is one of the most common leading cancer sites, accounting for 19% of the total cancer cases in men and 7% of that in women. In India, out of

0.3 million deaths per year, around one-third are caused due to tobacco-related oral cancer.<sup>[5,6]</sup>

Despite advances in surgical procedures and adjuvant medicines, the prognosis for oral cancer has remained stable for decades, with a five-year survival rate of 40–50% in industrialized countries and significantly lower in developing nations.<sup>[7]</sup> Therefore, it is necessary to develop alternative diagnostic methods that are more effective in

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early diagnosis and improve the survival rate of patients and enhance the quality of their life.<sup>[6]</sup>

OSCC has a multi-factorial aetiology. The most common aetiological agents are tobacco in all forms and alcohol. However, other factors are nutritional deficiencies such as vitamin A, B, and C, human papilloma virus (HPV) infections, actinic radiation, trauma, and dental irritation.<sup>[6,8]</sup> Identifying the markers at molecular levels or biochemical markers involving the various metabolic reactions associated with the initiation and biological behaviour of individual tumours are very important in diagnosis and prognosis of cancer.<sup>[7,9]</sup>

Various authors have reported homocysteine (Hcy) as a 'tumour marker' in various cancers such as breast cancer, colorectal cancer, lung cancer, cervical cancer and many other cancers.

Hcy is an intermediate cross-linking metabolite of the methionine cycle that affects all methyl and sulphur group related metabolisms in the body, either directly or indirectly. Hcy is a sensitive indicator of folate status and an emerging biomarker of folate inadequacy.<sup>[9]</sup> Folate is essential for One-Carbon Metabolism (OCM), which encompasses amino acid metabolism, purine and pyrimidine synthesis, and formation of S-adenosylmethionine (SAM), the agent primarily responsible for DNA methylation. DNA methylation plays a critical role in gene expression, chromosomal changes, and chromosomal abnormalities. Disruption of OCM can interfere with DNA synthesis, repair and methylation, which may promote carcinogenesis.<sup>[9,10]</sup>

Folate and various vitamins and enzymes are required for OCM, where Hcy accepts a one-carbon group from folate to form methionine in a vitamin B12-requiring reaction or Hcy is degraded in a vitamin B6-requiring reaction. Impairment of either pathway may result in the accumulation of Hcy. Elevated levels of Hcy have been associated with low folate, vitamin B1, vitamin B6, and with a common polymorphism (667C--T) in the methylenetetrahydrofolate reductase gene, which reduces enzyme activity. Thus, serum Hcy can be a sensitive, integratory biomarker of disruption in OCM, resulting in cancer development.<sup>[10,11]</sup>

Various authors have found elevated levels of Hcy in lung cancer, breast cancer, cervical cancer, colorectal cancer, head and neck cancer, etc., Very few studies have been reported on Hcy levels in head and neck cancer, especially OSCC. Thus, we intend to study the role of Hcy in OSCC.

The aim of this experiment is to study the levels of serum Hcy in OSCC patients. Study is done with the objective to assess the clinical utility of serum Hcy as a potential tumour marker for OSCC.

## METHODOLOGY

Blood samples of histopathologically confirmed cases of OSCC of various grades and healthy individuals without any systemic disease and without any habit history were collected. All the study participants were advised overnight fasting to avoid any dietary influence, and blood samples were collected during the morning hours. Blood was allowed to clot for 45 min and centrifuged under 2500 rpm for 10 min. Serum separated from blood samples was transferred to an Eppendorf tube. Serum samples were stored at -20°C and sent to laboratory for further processing. Hcy was measured in the serum samples by a fully automated Snibe Maglumi 800 Immunoassay Analyzer. The Maglumi 800 Immunoassay Analyzer uses chemiluminescent immunoassay technology for the quantitative determination of serum Hcy.

Normal reference range 4–14 µmol/L of Hcy was considered.

Eighty samples were categorized into two study groups. Group 1 consisted of 40 samples of histopathologically diagnosed cases of OSCC. Group 2 consisted of 40 samples of healthy individuals.

The entire data were collected and statistically analysed using Statistical Package for Social Sciences (SPSS version 24.0, IBM Corporation, USA) for MS Windows. The inter-group statistical comparison of means of normally distributed continuous variables was carried out using independent sample t-test. The inter-group statistical comparison of medians of non-normally distributed continuous variables was done using Mann-Whitney U test.

The mean ± SD of age of cases in Group 1 and Group 2 was 50.55 ± 11.69 years and 39.00 ± 15.68 years, respectively [Table 1]. The minimum–maximum age range in Group 1 and Group 2 was 25–72 years and 22–74 years, respectively. The Inter-group comparison of type of diet among the cases and controls were studied.

**Table 1: Inter-group comparison of mean age**

	Group 1 (n=20)		Group 2 (n=20)		P
	Mean	SD	Mean	SD	
Age (years)	50.55	11.69	39.00	15.68	0.012*

Values are mean and SD, P value by independent sample t test. P<0.05 is considered to be statistically significant. \*P<0.05

Of 40 cases in Group 1, 22 (45.0%) had vegetarian diet and 18 (55.0%) had non-vegetarian diet. Of 40 controls in Group 2, 11 (55.0%) had vegetarian diet and 29 (45.0%) had non-vegetarian diet. The distribution of type of diet did not differ significantly between two study groups ( $P$  value  $>0.05$ ) [Table 2]. Inter-group comparison of tobacco use among the cases and controls was done and showed that out of 40 cases in Group 1, 15 (25.0%) had no habit, 9 (30.0%) had smokeless tobacco, 2 (5.0%) had smoking tobacco, 6 (15.0%) had smokeless plus smoking tobacco use and 8 (25.0%) used mishri. Of 40 controls in Group 2, none had any habit. The distribution of tobacco use among the cases and controls studied differs significantly between two study groups ( $P$  value  $<0.05$ ) [Table 3; Graph 1].

Inter-group comparison of average serum Hcy levels was analysed. The median serum Hcy levels in Group 1 and Group 2 was 18.55  $\mu\text{mol/L}$  and 16.85  $\mu\text{mol/L}$ , respectively. The minimum–maximum serum Hcy range in Group 1 and Group 2 was 6.0–42.7  $\mu\text{mol/L}$  and 7.2–31.1  $\mu\text{mol/L}$ , respectively. Distribution of median serum Hcy levels differs significantly between group of cases and group of controls studied ( $P$  value = 0.005) [Table 4; Graph 2]. Comparison of average serum Hcy levels between vegetarian and non-vegetarian groups was done. In cases, distribution of median serum Hcy levels is significantly higher in vegetarian group compared to non-vegetarian group ( $P$  value  $<0.05$ ). In controls, distribution of median serum Hcy levels did not differ significantly between vegetarian and non-vegetarian groups ( $P$  value  $>0.05$ ) [Table 5]. The median serum Hcy levels in group of cases with habit and group of cases without habit was 18.30  $\mu\text{mol/L}$  and 23.50  $\mu\text{mol/L}$ , respectively. The minimum–maximum serum Hcy range in habit Group and without habit Group was 6.0–42.7  $\mu\text{mol/L}$  and 13.2–29.3  $\mu\text{mol/L}$ , respectively. Distribution of median serum Hcy levels did not differ significantly between group of cases with habit and group of cases without habit ( $P$  value  $>0.05$ ) [Table 6].

## DISCUSSION

HNSCC is the most common head and neck malignancy, accounting for the sixth most common cancer worldwide. It develops from the mucosal epithelium in the oral cavity, pharynx, and larynx.<sup>[12]</sup> OC is the most common type of HNSCC, accounting for 2 to 4% of all cancers.<sup>[1,2]</sup> It is most commonly diagnosed as OSCC, with a high mortality rate.<sup>[1]</sup> OSCC accounts for 90% of all oral cancers with a five-year survival rate of 40–50%.<sup>[1,2]</sup> It is one of the most severe health problems worldwide and the primary

**Table 2: Inter-group comparison of type of diet among the cases and controls studied**

Diet type	Group 1 (n=40)		Group 2 (n=40)		P
	n	%	n	%	
Vegetarian	22	45.0	11	55.0	0.527 <sup>NS</sup>
Non-vegetarian	18	55.0	29	45.0	
Total	40	100.0	40	100.0	

Values are n (% of cases), P value by Chi-Squared test.  $P < 0.05$  is considered to be statistically significant. NS – Statistically non-significant

**Table 3: Inter-group comparison of smoking or smokeless form of tobacco use**

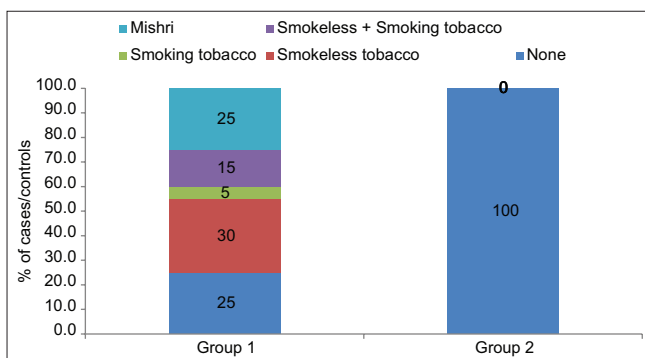
Tobacco Habit	Group 1 (n=40)		Group 2 (n=40)		P
	n	%	n	%	
None	15	25.0	40	100.0	0.001 <sup>***</sup>
Smokeless tobacco	9	30.0	0	0.0	
Smoking tobacco	2	5.0	0	0.0	
Smokeless + Smoking tobacco	6	15.0	0	0.0	
Mishri	8	25.0	0	0.0	
Total	40	100.0	40	100.0	

Values are n (% of cases), P value by Chi-Square test.  $P < 0.05$  is considered to be statistically significant. <sup>\*\*\*</sup> $P < 0.001$

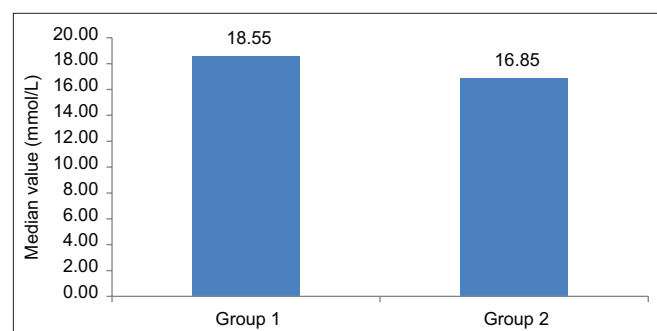
**Table 4: Inter-group comparison of average serum homocysteine levels**

	Group 1 (n=40)		Group 2 (n=40)		P
	Median	Min–Max	Median	Min–Max	
Serum homocysteine (mmol/L)	18.55	6.0–42.7	16.85	7.2–31.1	0.005*

Values are median and min–max, P value by Mann-Whitney U test.  $P < 0.05$  is considered to be statistically significant



**Graph 1: Inter-group distribution of tobacco use**



**Graph 2: Inter-group distribution of median serum homocysteine**

**Table 5: Comparison of average serum homocysteine levels according to type of diet in cases and controls**

	Group 1 (n=40)		Group 2 (n=40)		P Group 1 vs Group 2
	Median	Min-Max	Median	Min-Max	
Serum homocysteine (mmol/L)					
Vegetarian Diet (n=9/n=11)	23.60	13.8-35.7	19.70	12.1-31.1	0.125 <sup>NS</sup>
Non-Vegetarian Diet (n=11/n=9)	14.80	6.0-42.7	15.70	7.2-22.5	0.796 <sup>NS</sup>
P [Intra-group] Vegetarian vs Non-Vegetarian	0.017*		0.160 <sup>NS</sup>		

Values are median and min-max, P value by Mann-Whitney U test. P<0.05 is considered to be statistically significant. \*P<0.05, NS – Statistically non-significant

**Table 6: Comparison of average serum homocysteine levels according to habit in cases**

	Cases with habit (n=25)		Cases without habit (n=15)		P
	Median	Min-Max	Median	Min-Max	
Serum homocysteine (mmol/L)	18.30	6.0-42.7	23.50	13.2-29.3	0.631 <sup>NS</sup>

Values are median and min-max, P value by Mann-Whitney U test. P<0.05 is considered to be statistically significant. NS – Statistically non-significant

cause of death, increasing incidence in the 21<sup>st</sup> century in developing countries.<sup>[13,14]</sup> The inability to make an early diagnosis and deliver appropriate treatment appears to cause high mortality and morbidity. The main cause of OC is tobacco use in its various forms, along with excessive consumption of alcohol. Other causes are high exposure to UV light and sunlight, nutritional deficiency such as vitamin A and C, HPV infections, trauma, and dental irritation. Evidence suggests that OC can result from genetic damage associated with exposure to environmental factors. Gene mutations have been detected in OSCC in various chromosomes.<sup>[8]</sup> Molecular aberrations in oral carcinogenesis are mostly caused by genes involved in cell cycle control and regulation, resulting in a growth advantage in the mutated cell population.<sup>[9]</sup> Carcinogenesis is also linked to metabolic changes and may either promote or result from tumour progression. Thus, analysing metabolic abnormalities may be a valuable approach to understand tumour biochemistry and may provide a means to uncover new therapeutic targets.<sup>[7]</sup>

Carcinogenesis is reported to be associated with the methionine cycle, a major metabolic process. Methionine metabolism in the synthesis of various proteins influences several metabolic processes, including the synthesis of nutrients required for the optimum functioning of the cardiovascular, skeletal, and neurological systems. Methionine metabolism results in the production of Hcy which is metabolized by two pathways.

First is the re-methylation pathway, which regenerates methionine, and the second is the trans-sulphuration pathway, which first converts Hcy to cysteine and then to taurine. In essence, the intermediate metabolite Hcy is

located at a critical metabolic crossroad and, therefore, both directly and indirectly, affects all methyl and sulphur group metabolism occurring in the body.<sup>[9,15,16]</sup> Other metabolic functions of the Hcy include tissue folate recycling and serving as a precursor for cystathionine, cysteine, and other metabolites as part of choline metabolism. Increased levels of Hcy in the blood can be due to various internal and external factors.

The most important internal factor is genetic polymorphism, and the external factor is the composition of the diet. Although Hcy is not found directly in the diet, it is associated with both folate and vitamin B12.<sup>[9,17]</sup> They play an essential role in converting Hcy to methionine, where Vitamin B12 and folate act as co-enzyme and co-factor, respectively in Hcy.<sup>[18]</sup> According to literature, increased Hcy (Hhcy) is considered a risk factor in cardiovascular diseases, cerebrovascular diseases, ocular diseases, etc., Hhcy has lately been related to cancer, raising the possibility of use of Hcy as a tumour marker.

In a study conducted by Eleftheriadou *et al.*,<sup>[15]</sup> significantly higher levels of serum Hcy and low levels of serum folate in HNSCC patients were reported compared with the smoking group and healthy patients. These results are consistent with the present study results. Almadori *et al.*<sup>[7]</sup> reported significantly higher levels of serum concentrations of Hcy and lower levels of folate concentrations in HNSCC patients when compared with smokers and healthy individuals. Authors said that Vitamin B12 and folate deficiencies could cause chromosomal damage in buccal mucosa cells in tobacco users.

In our study distribution of median serum Hcy levels were significantly different between group of cases and group of controls studied (P value = 0.005) [Table 4; Graph 2]. Results of our study are consistent with the studies reported in the literature.

## CONCLUSION

This study investigated serum Hcy levels in OSCC patients and healthy individuals using the chemiluminescence immunoassay method. Increased serum Hcy levels were

observed in OSCC compared to healthy individuals. Results support the hypothesis that Hcy could be used as a tumour marker in OSCC cases. Although, further research with a larger sample size and ruling out the influence of dietary factors should be done to support this hypothesis.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

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