



## ESTIMATION OF SERUM LIPID PROFILE, SERUM CALCIUM AND ALKALINE PHOSPHATASE AND SERUM PROTEIN IN PATIENTS OF HEAD AND NECK CANCER UNDERGOING RADIOTHERAPY.

**Dr. Wajid Ali Shah**

Assistant Professor, Department of Pathology, Vardhmaan Mahaveer Medical College & Safdarjung Hospital, New Delhi.

**Dr. Abhishek Shrivastava**

Assistant Professor, Department Of Radiation Oncology, Atal Bihari Vajpayee Government Medical College, Vidisha-464001

**Dr. Varsha Mandloi\***

Senior Resident, Department Of Radiation Oncology, Gandhi Medical College Bhopal- 462001 \*Corresponding Author

### ABSTRACT

**BACKGROUND:** Carcinogenesis leads to various biochemical changes in the body. Alteration in the metabolism of lipids may be associated as they have a key role in the maintenance of integrity of cell. Elevated levels of calcium and alkaline phosphatase are frequently observed in advanced cancer. The aim of the study is to evaluate the correlation of serum lipid profile, calcium, alkaline phosphatase and proteins in head and neck cancer patients with histopathological grading and staging.

**MATERIAL & METHODS:** Sixty cases of histopathologically proven carcinoma of oral cavity and oropharynx were analysed. Data were collected and reviewed from the patient's case files. Patients were analysed for age and sex distribution, tumour staging, metastasis and overall survival. Serum lipid profile and serum levels of calcium, alkaline phosphatase and proteins were evaluated. Statistical analysis was done using chi square test.

**RESULTS:** Decreased LDL-C, HDL-C and VLDL-C levels were observed in head and neck cancer patients. Serum calcium and alkaline phosphatase were not found to be significantly elevated. Serum total protein and albumin levels were decreased.

**CONCLUSION:** Cancer adversely affects the cellular structure and metabolism. We didn't any significant correlation in the parameters with grading and staging, further studies should be carried out in a large number of patients to conform the role of these parameters with special attention to modifiable parameters in malignancies and their relation with grading and staging of cancer, which could be used as prognostic markers.

**KEYWORDS :** Head and neck cancer, lipid profile, serum calcium, alkaline phosphatase, proteins.

### INTRODUCTION

Head and neck cancer [HNC] is the sixth most common type of cancer in the World, representing about 6% of all cancer cases.<sup>(1,2)</sup> In the HNC, surgery and radiotherapy [RT] are curative treatments; while chemotherapy [CT] enhances the effects of irradiation, and routinely used as part of combined modality treatment.<sup>(3,4)</sup> Several biochemical changes occur in the body during carcinogenesis. Association of lipids and their metabolism needs detailed studies as lipids are the part of cellular integrity. Also antineoplastic therapies also influence lipid profile.<sup>(5)</sup> Elevated levels of calcium and alkaline phosphatase are frequently observed in advanced cancer.<sup>(6, 7)</sup> Tobacco carcinogens generates free radicals and reactive oxygen species, which causes high rate of oxidation/peroxidation of polyunsaturated fatty acids, further releasing peroxide radicals, leading to carcinogenesis.<sup>(8,9)</sup> Oxygen free radicals have a role in the initiation, promotion and progression of carcinogenesis which alters the serum protein and albumin level.<sup>(10,11)</sup>

This study was undertaken to evaluate the correlation of serum Lipid Profile, Calcium, Alkaline Phosphatase and proteins in patients of head and neck cancer undergoing radiotherapy and their correlation with histopathological grading and staging and to find out modifiable parameters.

### MATERIAL AND METHOD

Sixty cases of histopathologically proven carcinoma of oral cavity and oropharynx were analysed. Data were collected and reviewed from the patient's case files. Patients with age <70 years, both sexes, with histologically proven locally advanced oral cavity/oropharyngeal squamous cell cancer, with Karnofsky performance score/scale  $\geq 70$ , having normal haematological investigations, renal and liver function test were studied. Patients with age >70 years, having prior head and neck irradiation in the past and with histology other than squamous cell carcinoma were excluded from the study. Tests were performed from fasting blood sample. The measurements were done by Biosystems' kits using A25 and A15 analyzers based on the spectrophotometric principle.

Lipid profile estimation includes measurement of serum total cholesterol, HDL, triglycerides, LDL and VLDL by cholesterol oxidase mediated enzymatic accelerated non-color forming reaction. Friedewald formula was used to calculate LDL-C and VLDL-C.

Calcium and Alkaline Phosphatase kits worked on Arsenazo III and rate of 4-nitrophenol formation respectively which is measured spectrophotometrically.

Protein in the sample reacts with copper (III) ion in alkaline medium forming a colored complex that can be measured by spectro photometry.

Albumin in the sample reacts with bromocresol green in acid medium forming a colored complex that can be measured by spectrophotometry.

### STATISTICAL ANALYSIS:

Data was expressed as mean(x) and standard deviation (SD), Using Mann-Whitney/Wilcoxon Two-Sample Test (Kruskal-Wallis test for two groups) which was equivalent to Chi square. Value of P < 0.05 was considered significant, and P>0.05 was considered statistically insignificant.

### RESULTS

#### Table 1 shows Classification of SCC

Grading (Broader's Classification)	Cases	Percent
Well differentiated squamous cell carcinoma	34	56.66%
Moderately differentiated squamous cell carcinoma	14	23.33%
Poorly differentiated squamous cell carcinoma	12	20.00 %
Total	60	100.00%

#### Table 2 shows the details of laboratory variables among cases.

Variables	Cases	
	Mean $\pm$ SD	Range

TC(mg/dl)	140.7±18.76	107-200
LDLC (mg/dl)	123±21.4	88-202
HDLC (mg/dl)	45.15±8.04	26-70
VLDLC(mg/dl)	24.77±4.11	15-45
TGL(mg/dl)	141.5±21.86	75-225
Calcium(mg/dl)	10.41±1.29	7.5-14
Alkaline Phosphatase. (IU/L)	91.53±17.38	44-145
Protein (gm. %)	5.11±0.67	4.6-8
Albumin (gm. %)	4.5±0.52	3-5.5
Globulin (gm. %)	1.68±0.233	1.3-2.6
A/G ratio	2.08±0.52	1.25-4.4

**Table 3 shows Correlation of Studied Parameters with TNM Staging of Head and Neck Cancer**

Variables	Stage I	Stage II	Stage III	Stage IV	P Value
TC (mg/dl)	102.5±11.89	110.4±19.13	98.48±12.72	105.45±14.8	0.362
LDLC (mg/dl)	100.8±22.07	101±24.23	105.5±13.7	104.9±17.4	0.945
HDLC (mg/dl)	33.9±8.19	31±8.74	30.9±7.42	29.0±3.46	0.755
VLDLC (mg/dl)	21.5±5.28	24.4±2.89	21.3±5.43	21.8±4.23	0.4307
TGL (mg/dl)	112.8±31.57	113.9±34.25	122.32±31.42	114±23.32	0.4451
Calcium (mg/dl)	8.23±1.49	9.19±0.87	9.18±1.75	9.19±0.60	<0.001
Alk.phosp (iu/l)	98.75±14.48	111.37±15.29	111.92±25.6	97.1±12.32	0.0026
Protein (gm. %)	5.05±0.71	5.35±0.58	5.62±0.067	5.88±0.55	0.1197
Albumin (gm. %)	3.13±0.64	3.95±0.74	3.67±0.53	3.35±0.55	0.094
Globulin (gm. %)	1.95±0.29	1.99±0.34	1.94±0.114	1.95±0.27	0.9371
A/g Ratio	1.5±0.51	1.41±0.50	1.45±0.50	1.58±0.51	0.793

**Table 4 shows correlation of histopathological grading with studied parameters.**

Parameter Assesed	Well Differentiated	Moderately Differentiated	Poory Differentiated	P Value
TC (mg/dl)	113±14.35	117±19.74	122±12.74	0.5108
LDLC (mg/dl)	107.31±20.86	101.18±21.35	108.78±18.19	0.1801
HDLC (mg/dl)	27.89±8.64	30.95±10.36	30.5±5.85	0.9192
VLDLC (mg/dl)	21.54±6.32	24.5±7.05	25.14±7.12	0.8101
TGL (mg/dl)	115±31.7	121±32.33	121±32.63	0.830
Calcium(mg/dl)	9.17±1.46	9.30±2.15	9.95±1.24	0.1034
Alk.phosp(IU/L)	95.83±22.97	102.09±13.45	101.57±12.68	0.588
Protein(gm. %)	5.52±0.21	5.85±0.24	5.20±0.43	0.285
Albumin (gm. %)	3.06±0.53	3.52±0.45	3.74±0.44	0.876
Globulin (gm. %)	1.56±0.63	1.72±0.64	1.48±0.58	0.876
A/g Ratio	2.5±0.571	2.15±0.573	2.067±0.325	0.926

No significant correlation of any of the parameters found with histopathological grading.

**DISCUSSION**

Carcinoma of oral cavity and oropharynx is the commonest malignancy seen among the Indian population. <sup>[12]</sup>Most of the patients in the present study were suffering from carcinoma of tongue and buccal mucosa. This may be due to excessive tobacco use, poor oral hygiene and negligence. <sup>[13]</sup>Tobacco carcinogens are known to induce generation of free radicals and reactive oxygen species, which are responsible for high rate of peroxidation of polyunsaturated fatty acids. This peroxidation further releases peroxide radicals. These peroxide radicals affect essential constituents of cell membrane and might be one of the leading causative factors for carcinogenesis. <sup>[14, 15]</sup>In cancerous conditions,

the increase in the lipid peroxidation may be a reason for the greater utilization of lipids including total cholesterol, lipoproteins and triglycerides for membrane biogenesis. Cells derive these lipids from the circulating lipoproteins and degradation of these major lipoprotein fractions can result in lowering of blood lipid levels. <sup>[15,16]</sup>

In present study age range of patients was 31 – 70 years, with maximum no. of cases (69%) in age group 41-60 years. Male: female ratio was 3:1(45:15). Although other studies have shown male predominance, our study had slightly higher male: female ratio. <sup>[17,18, 19]</sup>

Our study showed a significant decreased LDL-C, HDL-C and VLDL-C levels, which was in agreement with other studies. <sup>[17,19,20]</sup>

Hypercalcemia is one of the established complications and most common life threatening metabolic disorder associated with advanced neoplastic condition. Raised Calcium was found by all others in 2.6-17.4% of cases, which could be due to majority of patients were of advanced states and hypercalcemia is well known in patients with advanced stages of the disease, metastatic growth and bone involvement, but this finding was not found in our study. This is in agreement with similar study mentioned in the literature. <sup>[20]</sup>

Although majority of our cases being advanced, none of the patients had distant metastasis to the viscera or long bones as per known radio graphical & clinical findings. Similar results were obtained for serum alkaline phosphatase levels which were found in normal range and were not support the results of other studies. <sup>[21,22]</sup>

There are many quantitative changes of serum protein and immunoglobulin fractions in patients with cancer of various sites, excluding those with leukemic and lymph proliferative disorders. The commonest change in serum proteins of patients with neoplastic disease is a reduction in albumin concentration and elevations of alpha globulins, especially alpha2 fraction. Malnutrition is a common problem in cancer patients that results in devastating quality of life, economic and survival issues.

Serum protein and albumin in oral cancers were reported decreased in our study which was similar to the study conducted by Abhishek Singh et al (2012) <sup>[23]</sup>.

Studied parameters showed no correlation with grading. There is no evidence to support the present finding, but it needs a detailed evaluation with more meticulous efforts having more number of cases in the studies.

**CONCLUSION**

Present study shows decreased LDL-C, HDL-C and VLDL-C level in head & neck cancer patients. Hypercalcemia and raised serum alkaline phosphatase were not significantly found in our study. Further studies are needed to know the alkaline phosphatase isoenzyme patterns in malignant diseases. Serum total protein and albumin were decreased. However, serum electrophoretic analysis of serum proteins is required, which will throw light in further analyzing the patients.

**REFERENCES**

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002.CA Cancer J Clin 2005;55:74-108.
2. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127:2893-917.
3. Bonner JA, Harari PM, Giralt J, Azarnia N, Shin DM, Cohen RB, et al.Radiotherapy plus cetuximab for squamous cell carcinoma of the head and neck. N Engl J Med 2006;354:567-78.
4. Chaukar DA, Das AK, Deshpande MS, Pai PS, Pathak KA, Chaturvedi P et al. Quality of life of head and neck cancer patient: Validation of the European organization for research and treatment of cancer QLQ C30 and European organization for research and treatment of cancer QLQ H and N 35 in Indian patients. Indian J Cancer 2005;42:178-84.
5. Simo CE, Orti LA, Sena FF, Contreras BE. "Blood cholesterol in patients with cancer". An Med Interna 1998; 15:363-6.
6. Wysolmerski JJ, Broadus AE. "Hypercalcemia of Malignancy: the central role of parathyroid hormone-related protein". Annu Rev Med. 1994; 45:189-200.
7. Stewart AF. Clinical practice. "Hypercalcemia associated with cancer". N Engl J Med 2005;352:373-9.
8. Reibel J. "Tobacco and oral diseases: an update on the evidence, with recommendations". Med Princ Pract 2003; 12:22-32.
9. Cogliano V, Straif K, Baab R, Grosse Y, Secretan B, Ghissassi FEI. "Smokeless tobacco and tobacco-related nitrosamines". Lancet Oncol 2004; 5:708.

10. Boonpipattanapong T, Chewatanakornkul S. "Preoperative carcinoembryonic antigen and albumin in predicting survival in patients with colon and rectal carcinoma". *J Clin Gastroenterol*. 2006; 40(7):592-5.
11. Onate-Ocana LF, Aiello-Crocifoglio V, Gallardo-Rincon D, Herrera-Goepfert R, Brom-Valladares R, Carrillo JF, et al. "Serum albumin as a significant prognostic factor for patients with gastric carcinoma". *Ann Surg Oncol*. 2007; 14(2):381-9.
12. Sandeep Samant, M D; K. Thomas Robbins, M D; Parvesh Kumar, M D; Jennie Z. Ma, Phd; Francisco Vieira, M D; Catherine Hanchett, B A. *Arch Otolaryngol Head Neck Sugery*. 2001; 127(12):1451-1456. Doi:10.1001/Archtol.127.12.1451.
13. Carbo N, Costelli P, Tessitore L, et al. Anti-tumour necrosis factor treatment interferes with changes in lipid metabolism in a tumour cachexia model. *Clin Sci* 1994; 87:34-55.
14. Petersen PE. The World Oral Health Report 2003: Continuous improvement of oral health in the 21st century – the approach of the WHO Global Oral Health Programme. *Community Dent Oral Epidemiol* 2003; 31 (Suppl. 1).
15. Strengthening the prevention of oral cancer: the WHO perspective. *Community Dent Oral Epidemiol* 2005; 33:397-9.
16. Schatzkin A, Hoover RN, Taylor PR, Ziegler RG, Carter CL, Albanes D, et al. Site-specific analysis of total serum cholesterol and incident cancers in the National Health and Nutrition Examination Survey. *Cancer Res* 1988; 48:452-8.
17. Patel PS, Shah MH, Jha FP, et al. "Alterations in plasma lipid profile patterns in head and neck cancer and oral precancerous conditions". *Indian J Cancer* 2004; 41:25-31.
18. Nimonkar PV, Borle RM. "Hypercalcemia in patients of oral squamous cell carcinoma". *J Maxillofac Oral Surg* 8(3):230-232.
19. Simranjit Singh, Venkatapathy et al. "Alterations in serum lipid profile patterns in oral cancer". *J natural science, Biology and Medicine* / July 2013 / vol 4 / issue 2; 374-378.
20. A. Anuradha, L.K. Veerathu et al. "Lipid profile in Oropharyngeal Cancers in Southern India". *J cancer research* 2013, 2, 99-104.
21. Vivane, Van Hoof et al. "Alkaline phosphatase isoenzyme patterns in malignant disease". *Clinical chemistry* 38/12, 2546-2551 (1992).
22. Sharon L. Ehrmeyer, Brian L. Joiner. "A Cancer-associated, Fast, Homocysteine-sensitive Electrophoretic Form of Serum Alkaline Phosphatase Cancer". *Res* 1978; 38:599-601.
23. Abhishek Singh Nayyar, Mubeen Khan, "Serum total protein, albumin and advanced oxidation protein products (AOPP) - implications in oral squamous cell carcinoma". *Malaysian J Pathol* 2012; 34(1):47-52.