ABSTRACT: Lipids have a key role in the maintenance of cell integrity. Hypolipidemia is related with increased risk of cancer and mortality. Elevated calcium and alkaline phosphatase has also been seen in cancer patients having metastasis. Alteration in serum proteins also has been reported. Hence present study was undertaken in 100 histopathologically confirmed head and neck cancer patients, in Department of Pathology Gandhi Medical College Bhopal, after taking informed written consent and ethical clearance. Lipid Profile, Serum Calcium, Alkaline Phosphatase, Serum Protein with Albumin were estimated and compared with age and sex matched healthy controls. Statistical analysis was done using chi square test. Total cholesterol (TC), high density lipoprotein-cholesterol (HDLC), low density lipoprotein-cholesterol (LDLC), Very low density lipoprotein-cholesterol (VLDLC) all decreased in head & neck cancer patients. Hypercalcemia was seen in 35% of patients. Raised Alkaline phosphatase (ALP) was seen in 8 % cancer patients. Serum calcium was found to be increasing with advancing stage. Further studies should be carried out in large number of patients to confirm the role of these parameters with special attention to modifiable ones and their relation with staging and grading of cancer, which can be used as prognostic markers.

KEYWORDS: Cancer (Head &Neck), Lipids, Calcium, Alkaline Phosphatase, Proteins.

INTRODUCTION: Carcinogenesis leads to various biochemical changes in the body. Lipids might be associated with cancers because they play a key role in the maintenance of cell integrity. Elevated levels of calcium and Alkaline Phosphatase are frequently observed in advanced cancer. Oxygen free radicals have a role in the initiation, promotion and progression of carcinogenesis which alters the serum protein and albumin level. Hypolipidemia predisposes or effect of cancer is still unclear. Furthermore, antineoplastic therapies also influence lipid profile. Increased risk of cancer and associated mortality has been reported with low serum cholesterol.

So this study was undertaken to evaluate the correlation of serum Lipid Profile, Calcium, Alkaline Phosphatase and proteins in cancer patients and their correlation with histopathological grading and staging and to find out modifiable parameters. 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. But the findings of alterations in plasma lipid profile in oral cancer patients strongly warrant an in depth study.

Hypercalcemia is reported to be associated with advanced neoplastic diseases - hematological malignancies, solid tumors like carcinoma of the breast, lung and pancreas with varied incidence,
however in the head and neck malignancies the relative incidence is comparatively low.\textsuperscript{11} Hypercalcemia is caused by localized or generalized resorption of bone, due to synthesis of substance/substances having parathormone like activity, osteoclast activating factor, a group of cytokines, growth factors,1. 25-dihydroxy vitamin D - secreted by tumor cells which stimulate the production of cyclic adenosine monophosphate and causes increased resorption of bone, probably by reacting with receptors for parathormone.\textsuperscript{12}

Elevated Alkaline Phosphatase in cancer patients usually signifies metastasis to bones or livers. Malignancies of the prostate, colon, breast, lung, thyroid and other organs can metastasize to bone or liver. However, primary cancers in various organs can generate Alkaline Phosphatase elevations in the absence of metastasis.\textsuperscript{13-15}

The commonest change in serum proteins of patients with neoplastic disease is a reduction in albumin concentration and elevation of alpha globulins and is also associated with weight loss with highest frequency and severity in gastrointestinal malignancies.\textsuperscript{6} Low serum albumin has also been shown to be an independent indicator for prognosis in cancer patients with unknown primaries.

MATERIALS AND METHODS: The present case control study comprises of newly diagnosed histologically confirmed- 100 head and neck cancer cases received in the Department of Pathology, Gandhi Medical College, Bhopal between 1\textsuperscript{st} Oct. 2012 to 31\textsuperscript{st} Oct. 2013 after taking written informed consent. Ethical clearance was taken from institutional ethical committee.

Controls: Age and sex matched subjects who do not have any renal, hepatic or cardiac dysfunction. Patients having any cardiac, renal and hepatic dysfunction & those on chemotherapy and Radiotherapy were excluded.

Tests were performed from fasting blood sample. The measurements were done by Biosytems’ 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 VLDLC.

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 spectrophotometry.

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:

| AGE (YEARS) | NUMBER OF CASES | NUMBER OF CONTROLS |
|-------------|-----------------|--------------------|
|             | Total | Male | Female | Total | Male | Female |
| 31-40       | 8     | 7    | 1      | 6     | 3    | 3      |
| 41-50       | 36    | 29   | 7      | 32    | 30   | 2      |
| 51-60       | 36    | 20   | 16     | 44    | 24   | 20     |
| 61-70       | 17    | 10   | 7      | 15    | 9    | 6      |
| >70         | 3     | 2    | 1      | 3     | 2    | 1      |
| TOTAL       | 100   | 68   | 32     | 100   | 68   | 32     |

### TABLE 1: AGE AND SEX WISE DISTRIBUTION OF PATIENTS OF HISTOPATHOLOGICALLY DIAGNOSED CASES OF HEAD OF NECK CANCER PATIENTS

In present study age range of patients was 32 – 80 years, with maximum no. of cases (72%) in age group 41-60 years. Male: female ratio was 2.12:1(68:32).

- Out of 100 cases- 66 were of normal BMI, 24 were overweight & 10 % were obese. The BMI of cases and controls were almost same. (BMI range: underweight-16-18.5, normal-18.5-25, overweight-25-30, obese->30).
- Out of 100 cases 46 %, 22 %, 12 % and 5 % had malignancy of buccal mucosa, tongue, retromolar trigone and hard palate respectively. Rest 15% of the cases had malignancies of alveolus, tonsillolingual sulcus, soft palate, lip, pyriform fossa and epiglottis.
- Out of 100 cases 76% were tobacco chewers, 11% were alcoholic, 9% were smokers and 4% were not having any addiction.

| Grading (Broader’s Classification) | Cases | Percent | Lymph nodes positive |
|------------------------------------|-------|---------|----------------------|
| Well differentiated squamous cell carcinoma | 54    | 54.00%  | 16(29.63%)           |
| Moderately differentiated squamous cell carcinoma | 32    | 32.00%  | 11(34.37%)           |
| Poorly differentiated squamous cell carcinoma | 14    | 14.00%  | 11 (78.5%)           |
| Total                              | 100   | 100.00% | 38                   |

### TABLE 2: SHOWING DISTRIBUTION ACCORDING TO GRADING

Maximum cases (54%) were of well differentiated squamous cell carcinoma and lymph node metastasis was found maximum in poorly differentiated squamous cell carcinoma.
Stage (TNM) | No. of cases | Percentage
---|---|---
Stage I | 24 | 24.00%
Stage II | 24 | 24.00%
Stage III | 40 | 40.00%
stage IV | 12 | 12.00%
Total | 100 | 100.00%

**TABLE 3: SHOWING DISTRIBUTION OF CASES ACCORDING TO TNM STAGING**

Maximum number of cases was stage III (40 %)

| Variables | CASES | CONTROLS | P value |
| --- | --- | --- | --- |
| Mean ±SD | Range | Mean ±SD | Range |
| TC (mg/dl) | 111.27±16.95 | 68-176 | 150.7±19.68 | 107-200 | 0.364 |
| LDL (mg/dl) | 105.56±20.70 | 90-220 | 130±24.4 | 88-202 | <0.042* |
| HDLC (mg/dl) | 31.35±9.07 | 20-58 | 51.15±9.04 | 26-70 | 0.0005* |
| VLDLC (mg/dl) | 23.27±6.31 | 12-36 | 27.87±6.11 | 15-45 | 0.005* |
| TGL (mg/dl) | 118.45±33.88 | 60-190 | 139.5±30.76 | 75-225 | 0.629 |
| Calcium (mg/dl) | 11.24±1.818 | 7.5-15.5 | 10.41±1.29 | 7.5-14 | 0.560 |
| Alkaline Phosphatase. (IU/L) | 104.96±21.72 | 78-245 | 94.53±18.3 | 44-145 | <0.001* |
| Protein (gm. %) | 5.99±0.724 | 4.5-7.7 | 6.51±0.67 | 4.6-8 | <0.001* |
| Albumin (gm. %) | 4.06±0.627 | 3.5-5.5 | 4.5±0.52 | 3.5-5.5 | 0.0001* |
| Globulin (gm. %) | 1.91±0.316 | 1.3-2.4 | 1.9±0.303 | 1.3-2.6 | 0.0695 |
| A/G ratio | 2.13±0.541 | 1.25-4.1 | 2.08±0.52 | 1.25-4.4 | 0.0822 |

**TABLE 4: SHOWING BASELINE CHARACTERISTICS OF VARIOUS PARAMETERS STUDIED IN HEAD AND NECK CANCER**

1. Serum TC in 87%, LDL in 86%, HDLC in 68% cases was lowered.
2. Serum calcium and alkaline phosphatase levels were raised in 35% cases.
3. Total Protein in 38% and albumin in 52% were lowered whereas globulin level rose in 15% cases.

| PARAMETERS ASSESED | WELL DIFFERENTIATED | MODERATELY DIFFERENTIATED | POORY DIFFERENTIATED | P VALUE |
| --- | --- | --- | --- | --- |
| TC (mg/dl) | 112±16.35 | 110±19.74 | 110±12.74 | 0.5108 |
| LDL (mg/dl) | 107.31±20.86 | 101.18±21.35 | 108.78±18.19 | 0.1801 |
| HDLC (mg/dl) | 30.79±8.64 | 32.65±10.86 | 30.5±5.85 | 0.9192 |
| VLDLC (mg/dl) | 22.9±6.32 | 23.5±6.15 | 26.14±6.81 | 0.8101 |
### Table 5: Shows Correlation of Histopathological Grading with Studied Parameters

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

| VARIABLES               | STAGE I (Mean ± SD) | STAGE II (Mean ± SD) | STAGE III (Mean ± SD) | STAGE IV (Mean ± SD) | P VALUE |
|-------------------------|---------------------|----------------------|-----------------------|----------------------|---------|
| TC (mg/dl)              | 106.5±14.89         | 110.9±17.13          | 112.95±17.37          | 115.83±18.8          | 0.362   |
| LDLC (mg/dl)            | 104.8±24.07         | 105±21.33            | 105.5±19.7            | 107.9±17.4           | 0.945   |
| HDLC (mg/dl)            | 29.9±9.19           | 32±8.74              | 31.9±10.42            | 31.0±3.46            | 0.755   |
| VLDLC (mg/dl)           | 22.5±6.18           | 22.4±6.89            | 24.3±6.13             | 22.8±6.23            | 0.4307  |
| TGL (mg/dl)             | 115.8±36.57         | 112.9±36.25          | 124.37±33.42          | 115±32.33            | 0.4451  |
| CALCIUM (mg/dl)         | 9.99±1.49           | 10.19±0.87           | 12.03±1.75            | 13.19±0.60           | <0.001* |
| PROTEIN (gm. %)         | 97.5±14.48          | 101.37±17.29         | 113.92±26.6           | 97.1±12.32           | 0.0026* |
| ALK.PHOSP (IU/L)        | 6.095±0.781         | 5.93±0.78            | 5.86±0.067            | 6.38±0.55            | 0.197   |
| ALBUMIN (gm. %)         | 4.183±0.64          | 3.95±0.74            | 3.97±0.53             | 4.35±0.55            | 0.094   |
| GLOBULIN (gm. %)        | 1.95±0.292          | 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 6: Shows Correlation of Studied Parameters with TNM Staging of Head and Neck Cancer

Serum level of calcium and alkaline phosphatase are increasing with stage of cancer.

| PARAMETERS | CASES | CONROLS | P value | CASES | CONROLS | P value |
|------------|-------|---------|---------|-------|---------|---------|
| TC         | 158±24.89 | 164.69±18.8 | 0.35    | 147.81±17.6 | 152.59±20.7 | 0.157   |
| LDLC       | 144.97±29.17 | 147.97±21.9 | 0.553   | 127.02±21.6 | 131±25.96  | 0.337   |
| HDLC       | 48.98±10.662 | 49.28±9.92  | 0.9047  | 50.77±9.93  | 51.76±9.90 | 0.5718  |
| VLDLC      | 32.7±8.7    | 34.19±7.3  | 0.1825  | 27.30±6.8   | 27.87±5.65 | 0.990   |
| TGL        | 178.33±38.92 | 169.21±47.4 | 0.2786  | 139.5±28.26 | 139.86±34.86 | 0.8271  |

### Table 7: Serum Lipid Profile Comparision Between No Habit of Tobacco (NHT) and With Habit of Tobacco (WHT) Subjects in The Oral Cancer and Control Group

No significant alteration of lipid profile seen with addiction of tobacco.
DISCUSSION:

| Study (year)                      | Age-group(years) | Male: female |
|----------------------------------|------------------|-------------|
| Singh et al(2013)16              | 30-75            | 1.14:1      |
| Nimonkar et al(2008)11           | 28-72            | 1.3:1       |
| A. Anuradha et al(2013)17        | 25-70            | 1.5:1       |
| Present study2014                | 41-70            | 2.125:1     |

AGE AND SEX DISTRIBUTION

All studies show male predominance although our study had much higher male: female ratio.

| Study (year)       | Type of cancer                                | Results                                                                                                                                                                                                 | p- value       |
|--------------------|-----------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| Patel et al(2004)16| Head and Neck cancer and oral precancerous conditions (opc) | Lowered plasma cholesterol, HDL level, VLDL level and triglycerides in head and neck cancer patients than opc                                                                                     | TC- 0.008      |
|                    |                                               |                                                                                                                                                                                                     | HDLC-0.000     |
| Simranjit singh et al(2013)17 | Oral cancer                  | Significant decreased total cholesterol, HDL-C and Triglycerides                                                                                                                                 | TC-<0.001      |
|                    |                                               |                                                                                                                                                                                                     | HDLC-<0.001    |
|                    |                                               |                                                                                                                                                                                                     | TGL-<0.001     |
| A. Anuradha et al(2013)18 | Oropharyngeal cancer | Significant decreased total cholesterol, HDL-C and VLDL-C                                                                                                                                              | TC-0.006       |
|                    |                                               |                                                                                                                                                                                                     | HDL-0.005      |
|                    |                                               |                                                                                                                                                                                                     | LDL-0.005      |
| Present study(2014)| Head and Neck cancer                         | Significant decreased LDL-C, HDL-C and VLDL-C Level                                                                                                                                                   | LDL-<0.042     |
|                    |                                               |                                                                                                                                                                                                     | HDL-0.0005     |
|                    |                                               |                                                                                                                                                                                                     | VLDL-0.005     |

SERUM LIPID PROFILE IN HEAD AND NECK CANCER

| Study (year)                      | Result                                      |
|----------------------------------|---------------------------------------------|
| Mundy & Martin(1982)3            | Serum calcium raised in 6.6% cases          |
| Won C and Decker(1983)19         | Serum calcium raised in 2.6% cases          |
| Yoneda and Nishimura(1991)20     | Serum calcium raised in 4.4% cases          |
| Lang-Kummer(1997)21              | Serum calcium raised in 6.00% cases         |
| Nimonkar PV(2009)11              | Serum calcium raised in 17.4% cases         |
| Present study(2014)              | Serum calcium raised in 35% cases           |

SERUM CALCIUM IN HEAD AND NECK CANCER
Raised Calcium was found by all others in 2.6-17.4% of cases, whereas our study found rise in much higher- 35% cases, which could be due to majority of patients- 52% being stage III and IV and hypercalcemia is well known in patients with advanced stages of the disease, metastatic growth and bone involvement. Although majority of our cases being advanced, none of the patients had distant metastasis to the viscera or long bones as per known radiographical & clinical findings, although radiographical and clinical findings were not known in few cases.

| Study                           | Result                                      |
|---------------------------------|---------------------------------------------|
| Sharon L. Ehrmeyer et al(1978)  | Raised (p value <0.005)                     |
| Vivian O et al(1992)            | Raised (p value <0.05)                      |
| Present study(2014)             | Raised (p value <0.001)                     |

**SERUM ALKALINE PHOSPHATASE IN HEAD AND NECK CANCER**

Serum protein and albumin in oral cancers were reported decreased by Abhishek Singh et al (2012)\(^22\) with P value <0.001 similar to present study of head and neck cancers.

**CORRELATION OF STUDIED PARAMETERS WITH GRADING AND STAGING OF HEAD AND NECK CANCER:**

- Studied parameters showed no correlation with grading.
- Serum calcium values increases with stage of cancer and this rise in mean serum calcium was found to be statistically significant (p value<0.05). These results were comparable with studies done by Nimonkar PV(2009)\(^11\)
- The mean serum alkaline phosphatase values raised from stage I to stage IV cancer, and this was found to be statistically significant (p-value <0.05). This is similar to the results obtained by Leo L. Stolbach (1969)\(^23\) and Bassalyk LS,(1992)\(^24\) which explains the ectopic production of alkaline phosphates at tumor site.

**SUMMARY AND CONCLUSION:** Present study of Head & Neck cancer shows

- Decreased LDL-C, HDL-C and VLDL-C Level in head & neck cancer patients.
- Hypercalcemia in 35% patients which could be due to advanced stages of the disease, metastatic growth and bone involvement.
- Raised serum alkaline phosphatase but not significantly. ALP is valuable in advanced disease for indicating survival and progress. 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.
- Rising serum calcium with advancing stage, while other parameter i.e. serum Lipid profile, alkaline phosphatase, protein and Albumin showed no significant correlation with grading and staging in head & neck cancer patients.

Further studies should be carried out in large number of patients to confirm the role of these parameters with special attention to modifiable parameters in malignancies and their relation with staging and grading of cancer, which could be used as prognostic markers.
Abbreviations used:
- Total cholesterol (TC)
- High density lipoprotein-cholesterol (HDLC)
- Low density lipoprotein cholesterol (LDLC)
- Very low density lipoprotein- cholesterol (VLDLC)
- Triglycerides (TGL)
- Alkaline Phosphatase (ALP)

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Date of Submission: 19/01/2014.
Date of Peer Review: 20/01/2014.
Date of Acceptance: 05/02/2014.
Date of Publishing: 21/02/2014.