Serum Cotinine Level as a Tobacco Exposure Related Biomarker in Oral Cavity Malignancy

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Article History:
Received on: 20 Sep 2020
Revised on: 20 Oct 2020
Accepted on: 22 Oct 2020

Keywords:
Serum Cotinine Level,
Tobacco,
Biomarker,
Oral Cavity Malignancy

ABSTRACT

Oral cancer has posed a challenge to us with its incidence as high as around 20% of total body cancers. Aim of the study was to quantify serum cotinine level as tobacco exposure-related biomarker in controls, chronic tobacco chewers (High risk for oral cavity malignancy) and oral cavity cancer patients. This was a hospital-based case-control study of 24 months duration in controls, chronic tobacco chewers and oral cancer patients. A total of 150 subjects had enrolled after meeting the inclusion & exclusion criteria. The commonest age group of oral cavity malignancy was 41 to 50 years. Males were found to be more affected than females. Tobacco chewing was the most common habits in a male patient and mishri application in female patients. In this study, 90% of patients had a history of tobacco consumption for the last ten years or more. Frequency of tobacco chewing was more in chronic tobacco chewers (30% in more than ten times a day) compared to oral cavity malignancy group (16% in more than ten times a day). The majority (more than 50%) of patients had tumour arising from the buccal mucosa, and histopathological studies revealed that 48% of patients had well-differentiated squamous cell carcinoma. Serum cotinine level in non-tobacco exposure group is less than 1 ng/mL. Conclusion- Tobacco chewing habit is a high-risk factor for oral cancer. Chronic tobacco chewers can be included in the high-risk group for oral cavity cancer. Serum cotinine level can be used as a tumour marker for oral cancer patients.

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ISSN: 0975-7538
DOI: https://doi.org/10.26452/ijrps.v11iSPL4.4440

INTRODUCTION

Cancer is a significant cause of death in the world. The infection and contagious diseases are sufficiently controlled, but cancer is still a significant challenge to modern medicine and hence is a major health problem. Cancer is often silent, hence diagnosis is always late. Oral cavity lesions are easily accessible. In India, it is one of the most common cancers and is an important public health problem. Oral malignancy is the commonest cancer in males and 3rd common cancer in females. In India, it is one of the potentially preventable cancers; it comprises 35-40% of all Head and Neck malignancies (Daftary, 1991). Around 56000 new cases have been evaluated to happen each year, which would provoke more than 100000 individuals encountering the disease in the general population at whatever year (Kalyanpur et al., 2012). Other than 7% of all ailment passings in folks and 4% in females have been represented to be a result of oral opening danger. In the United States carcinoma of the oral cavity
comprises 1 to 2% of all carcinoma, this constitutes about 8500 patients each year, over half of who will die (Peacock et al., 2008). Oral cancers are many a time preceded a premalignant lesion. All these factors together provide an excellent opportunity for easy detection and control.

The incidence of oral cancer in our country is remarkably high, and its control is thus a tremendous national health problem. The malignant disease of the oral cavity should receive careful consideration for several reasons.

The incidence is more among the patients of a low socio-economic group, and it is usually detected late or reported to higher medical institutions in the advanced stage. The chief problem associated is the ignorance and lack of awareness of the dreadful nature of cancer results in late presentation of our patients when the growth has fungated or when the patient is not able to open the mouth because of trismus due to deep infiltration of the malignancy.

Tobacco contains thousands of chemical constituents, including major alkaloid (nicotine & cotinine) and minor alkaloids (nicotine, anabasine, anatabine, etc.). These alkaloids can react with nitrite to form nitrosamines like 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), which are called tobacco-specific nitrosamines. The sections of nicotine, cotinine, nitrate and nitrite are huge predecessors in the plan of tobacco express nitrosamines (Hoffmann and Hecht, 1985). Cotinine is a significant and notable biomarker of tobacco use. Most nicotine entering the body (70%-80%) is metabolized into cotinine. Cotinine is present in the blood, serum, saliva, urine, amniotic fluid, cervical mucus and hair of both smokers and nonsmokers exposed to tobacco smoke. It has been cited as the most useful marker for distinguishing tobacco users from non-users.

In this preliminary clinical trial, we have found that serum cotinine level can be used as a biomarker for oral cavity malignancy.

**Aim & Objective**

To quantify serum cotinine level as tobacco exposure-related biomarker in Controls, Chronic tobacco chewers (High risk for Oral cavity malignancy) and Oral cavity cancer patients.

**MATERIALS AND METHODS**

This was a hospital-based case-control study of 24 months duration in controls, chronic tobacco chewers and oral cancer patients.

**The total duration of the study**

24 months

**Sample Size**

A total of 150 subjects had enrolled in the study from the Department of General Surgery, Oncosurgery Unit, Krishna hospital, Karad, and after meeting the inclusion & exclusion criteria.

**Study group sampling for OR (Odds Ratio) analysis**

Patient demographics had collected for Controls (n=50), Chronic tobacco chewers (n=50), Oral cancer patients (n=50) by interview questionnaires.

The information ascertains had included details of age, sex, tobacco habits, duration and frequency of tobacco consumption.

**Study group for serum biomarkers**

The subjects had chosen from a single centre (Dept. of General Surgery, Oncosurgery unit), Krishna hospital for analysis of the biomarker (Cotinine).

They included 50 controls, 50 oral cancer patients and 50 chronic tobacco chewers.

All the subjects had divided into controls (Non-tobacco exposure), oral cancer patients and heavy tobacco chewers.

**Sample collection**

Blood samples had collected from all individuals included in the study before the initiation of any anticancer therapy.

**Inclusion Criteria**

1. Must have given written consent before any Investigation carried out.
2. Diagnosed Oral cancer patients.
3. Must not have taken any chemotherapy and radiotherapy for oral cancer before the inclusion in the study.
4. The person must be taking half pouch (1 pouch=70-80gm of tobacco) of tobacco per day for more than five years consecutively.

**Exclusion Criteria**

1. Patient already treated for oral cancer will not include in this study.
2. Chronic tobacco chewers but those have abstinence for more than 15 days.
Analysis

Data were statistically analyzed by using Student's unpaired t-test, which was useful to compare levels between controls, chronic tobacco chewers and orals cancer patients. Relative risks of cancer in healthy individuals was estimated by computing OR (Odds Ratio).

OBSERVATIONS/DISCUSSION

The incidence of oral cavity cancer, its distribution in the oral cavity, its age incidence and sex ratio all are very widely in different nations and races with the difference in their nutritional status, their habits, their occupation and their awareness of general health and hygiene.

In our country, the incidence of oral cavity cancer is very high as compared to western countries. Pay大师 quotes an incidence of 45% and 47% of total cancer in India. Similarly, Jussawalla quotes the incidence of 37.5% of all cancers (Jussawalla, 1973).

In this study, as shown in Table 1, the commonest age group of oral cavity malignancy was 41 to 50 years. The lowest was 37 years, and the oldest was 80 years, while in chronic tobacco chewers group also the commonest age group was 41-50 years. The lowest age was 32 years, and the oldest age was 78 years. A similar distribution was seen in the control group, also. In this study, males were found to be more affected than females. Out of 50 patients of oral cavity malignancy, 41 were male, and 9 were female, while in chronic tobacco chewer group also male preponderance visible.

In this study, as shown in Table 2 Tobacco chewing was the most common habits in male patient and mishri application in female patients. Similarly, in chronic tobacco chewer group also most common habits in male were tobacco chewing and in female was mishri application.

In this study, 90% of patients had a history of tobacco consumption since last ten years or more while in the high-risk group only 60% patients had a history of 10 years or more for tobacco consumption. Table 3 shows an increase in serum cotinine level as the duration of tobacco exposure increase in chronic tobacco chewer and oral cancer patients.

Frequency of tobacco chewing was more in chronic tobacco chewers (30% in more than ten times a day) compared to oral cavity malignancy group (16% in more than ten times a day).

The high event of an oral threat in India is associated with smokeless tobacco inclinations in a lot of the general population, which chomp tobacco in various structures. Smoking affinity for tobacco like cigarettes, stogies, pipes, etc. moreover cause oral cancer. Several reports have suggested the association of high risk of oral cancer with greater amounts of tobacco used and longer duration of use. But the reduction in risk of oral cancer was also associated with tobacco cessation. People-based case-controls ponders uncovered that cigarette smokers have been found to 2 to 5 time higher perils for oral threat than that of nonsmokers (Winn, 2001). Local effects of tobacco gnawing consolidate the unsettling influence of the oral mucosa from the dissolvable pH and coarseness, which achieve gum slomp, the introduction of the neck of the tooth, and scratched spot of the entire surface. Relentless irritation begins epithelial dysplasia of the buccal mucosa, at the end occurring in leucoplaikia that should be seen as a premalignant injury. The movement of hurtful change for these leucoplaikic districts is someplace in the scope of 6 and 30%. Nodular and spotted leucoplaikic regions will, as a rule, have the most significant movement of undermine change (Al-Ibrahim and Gross, 1990). In this examination as showed up in Table 7. majority of patients had tumour arising from the buccal mucosa (more than 50%) and Bucco-gingival sulcus as they were the commonest site among tobacco chewer for keeping tobacco in these sites. Also, the commonest type of lesion found was proliferative ulcer type, as shown in Table 4.

In this study of 50 oral cavity malignancy patients as shown in Table 5 it was found that 22 patients had ipsilateral lymphadenopathy, 4 of them had involvement of the mandible in addition to the lymphadenopathy. Five of the patients had erosion of the mandible without lymphadenopathy, and 5 of them had skin involvement. Eighteen patients did not have involvement of skin, mandible and lymph nodes.

In this study, as shown in Table 6, the histopathological studies revealed that 48% of patients had well-differentiated Squamous cell carcinoma (SCC) and 32% of patients had moderately differentiated SCC. 4% patient had well to moderately differentiated carcinoma and 16% patients with poorly differentiated SCC. In our study mean of serum cotinine level in well-differentiated SCC patients was 22.175 while SEM (Standard Error of Mean) was 5.501. The P-value for this group was 0.015, which was <0.05 suggestive of significance and meaning of serum cotinine level in moderately differentiated SCC patients was 56.05 while SEM (Standard Error of Mean) was 4.555.

In this study, Oral hygiene was poor in 60% cases.
### Table 1: Distribution of study samples according to Age and Sex

| Age group | Group-A |   | Group-B |   | Group-C |   |
|-----------|---------|---|---------|---|---------|---|
|           | Male    | Female | % | Male    | Female | % | Male    | Female | % |
| 31-40     | 07      | 00    | 14 | 11      | 00    | 22 | 10      | 02    | 24 |
| 41-50     | 13      | 02    | 30 | 13      | 02    | 30 | 18      | 04    | 44 |
| 51-60     | 09      | 03    | 24 | 07      | 03    | 20 | 06      | 03    | 18 |
| 61-70     | 09      | 04    | 26 | 07      | 04    | 22 | 04      | 01    | 10 |
| 71-80     | 03      | 00    | 06 | 03      | 00    | 06 | 02      | 00    | 04 |
| Total     | 41      | 09    | 100| 41      | 09    | 100| 40      | 10    | 100|

### Table 2: Types of tobacco consumption in Group-A

| Forms of tobacco          | Group-A |   | Group-B |   |
|---------------------------|---------|---|---------|---|
|                           | Male    | Female | % | Male    | Female | % |
| Tobacco chewing           | 22      | 00    | 44| 26      | 00    | 52 |
| Mishri application        | 00      | 09    | 18| 00      | 08    | 16 |
| Tobacco Pan + Gutakha     | 13      | 00    | 26| 10      | 00    | 20 |
| Tobacco + smoking         | 06      | 00    | 12| 06      | 00    | 12 |

### Table 3: Duration of tobacco consumption in study subjects

| Duration of tobacco consumption | Group-A |   | Group-B |   |
|---------------------------------|---------|---|---------|---|
| freq                            | %       | Serum cotinine freq | %   | Serum cotinine (ng/ml) |
| 5 to 10 years                   | 05      | 10% | 7.397 | 20 | 40% | 6.539 |
| 11 to 15 years                  | 09      | 18% | 33.050| 08 | 16% | 37.452|
| 16 to 20 years                  | 11      | 22% | 32.361| 14 | 28% | 30.731|
| >20 years                       | 25      | 50% | 28.268| 08 | 16% | 32.458|

### Table 4: Type of lesion in Group-A

| Type of lesion | No. of cases | Serum cotinine |
|----------------|--------------|----------------|
| Ulcerative     | 16           | 44.95          |
| Ulceroproliferative | 34 | 47.43         |
| Proliferative  | 00           | 00             |

### Table 5: Adjacent structures involved with a tumour in Group-A

| Structures involved                          | No. of patients |
|----------------------------------------------|-----------------|
| Lymph nodes                                  | 22              |
| Mandible                                     | 05              |
| Skin (cheek)                                 | 05              |
| Mandible + Lymph node                        | 04              |
| Mandible + Skin (Cheek)                      | 01              |
| Skin (cheek) + Lymph node                    | 04              |
| Mandible + Skin Cheek) + Lymph node          | 01              |
| None                                         | 18              |

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Table 6: Pathology in Group-A

| Histopathology      | No. of cases | Percentage (%) |
|---------------------|--------------|----------------|
| Well DSCC.          | 24           | 48             |
| Mod. D. SCC.        | 16           | 32             |
| Poorly D. SCC.      | 08           | 16             |
| Well to Mod. D. SCC.| 02           | 04             |
| Total               | 50           | 100            |

Table 7: Oral hygiene in Group-A

| Oral hygiene | No. of cases | Percentage |
|--------------|--------------|------------|
| Good         | 02           | 04         |
| Average      | 18           | 36         |
| Poor         | 30           | 60         |

Table 8: Odds Ratios of Oral Cancer For Tobacco Addiction

| Tobacco chewer: | Frequency | Ca/Co | OR   | P-value |
|-----------------|-----------|-------|------|---------|
| Duration: 5 to10yrs | 6 to 10   | 2/4   | -    |         |
|                  | 10 to 15  | 3/9   | 1.500| 1.000   |
|                  | >15       | 0/7   | 8.333| 0.1923  |
| 10 to 15yrs     | 6 to 10   | 6/1   | -    |         |
|                  | 10 to 15  | 0/3   | 30.333| 0.0333 |
|                  | >15       | 3/4   | 8.000| 0.2657  |
| 15 to 20yrs     | 6 to 10   | 4/5   | -    |         |
|                  | 10 to 15  | 4/3   | 1.667| 1.000   |
|                  | >15       | 3/6   | 1.60 | 1.000   |
| >20yrs          | 6 to 10   | 11/1  | -    |         |
|                  | 10 to 15  | 9/4   | 4.889| 0.3217  |
|                  | >15       | 5/3   | 6.600| 0.2553  |

and Average in 36% cases and good hygiene in 4% cases. Table 8 shows the odds ratio and p-value for oral cavity malignancy and high-risk group based on duration and frequency of tobacco chewing. The P-value for each group was > 0.05, which shows insignificant statistical means both groups are identical. The odds ratio for each group is more than 1.0, which also suggest a significant risk of developing oral cancer in chronic tobacco chewers.

Active tobacco chewers almost always have levels higher than 10 ng/mL and sometimes higher than 500 ng/mL (Horstmann, 1985). In this study also shown in serum cotinine level in non-tobacco exposure group is less than 1 ng/mL, in oral cancer patient group is 6.218 ng/mL. In regular tobacco, chewer group is also 5.753, which is similar to the result of Hukkanen et al., 2005 study (Hukkanen et al., 2005). The serum cotinine level in non-tobacco users is 0.245 ng/mL, which similar to result in a study (Florescu et al., 2009).

Based on the findings of this study and statistical analysis it was proved that serum cotinine level could be used as tobacco exposure-related biomarker in oral cavity malignancy and it can be a handy tool to detect early malignancy in community. Exactly when an individual is introduced to a manufactured by direct contact, taking in it or ingesting it, a substance identifying with the mixture may be found in the body. This substance is usually called a "biomarker of presentation." Biomarkers of introduction are as often as possible the consequences of assimilation and can be assessed in the pee, blood, spit and hair. The level of a specific biomarker in the body checks how much an individual may be introduced to a chemical. An ideal biomarker of the presentation should meet the going with three criteria: A biomarker of the introduction should be express to the compound being studied. The biomarker should remain long enough in the body to guarantee its assessment is reliable. The biomarker

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should be anything but difficult to accumulate and gauge - for example, be accessible in pee, blood, or saliva (Woodward et al., 1991). The usage of tobacco things and prologue to tobacco smoke may cause certified afflictions, for instance, coronary disease, respiratory contaminations and cancer. Tobacco smoke is seen as a biological toxin. It contains more than 4,000 chemicals, and more than 70 of these chemicals are known to cause, initiate, and promote cancer (Talhout et al., 2011). Smokeless tobacco products also contain many of the same toxic chemicals.

From a public health point of view, it is important to monitor the use of tobacco products and study how much of the population is exposed to tobacco. Most surveys on tobacco use and exposure to tobacco smoke are based on self-reports. However, a better indicator of exposure would be to measure the biomarkers of exposure specific to tobacco products and tobacco smoke.

The usage and treatment of tobacco things or reused introduction to tobacco smoke achieves nicotine take-up into the course framework. At the point when nicotine shows up at the liver, kidneys and lungs, it is handled into a couple of critical breakdown things known as cotinine, cotinine-N-glucuronide, nicotine-N-glucuronide, trans-3’-hydroxycotinine and trans-3-hydroxycotinine-O-glucuronide (Hukkanen et al., 2005). These breakdown things (metabolites) similarly as nicotine itself are tobacco-unequivocal biomarkers of introduction and can be assessed in pee or blood. This information can then be used to determine the extent to which a person has been exposed to tobacco products or tobacco smoke (Wall et al., 1988).

Cotinine is one of the most useful biomarkers of exposure to tobacco products and tobacco smoke (Murphy et al., 2004). It can be easily measured in urine or blood and can be detected in the body for up to four days after exposure to nicotine (Florek et al., 2003). Cotinine is specific to nicotine. It also provides a reliable measurement of exposure to tobacco products and tobacco smoke. Recently, measuring cotinine levels were used successfully to validate self-reported smoking status (Wong et al., 2012)

Serum Cotinine can be recognized using brilliant spectroscopy, feeble layer chromatography and gas chromatography after basic extraction.Among these procedures, brilliant spectroscopy won’t be discrete among nicotine and cotinine. Other methods are either time consuming, costly or less sensitive. But the major advantage of Serum Cotinine ELISA method is the rapidity, cheap and sensitive (Horstmann, 1985).

CONCLUSION
Tobacco chewing habit is a high-risk factor for oral cancer. Chronic tobacco chewers can be included in a high-risk group for oral cavity cancer. Serum cotinine level can be used as a tumour marker for oral cancer patients.

ACKNOWLEDGEMENT
Nothing to Report.

Funding Support
The authors declare that they have no funding support for this study.

Conflict of Interest
The authors declare that there is no conflict of interest for this study.

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