

**EVALUATION OF SERUM SIALIC ACID LEVELS
IN HEAD AND NECK MALIGNANCIES****RATHAN SHETTY K. S¹ AND ARUNAVA KALI*²***¹Department of Otorhinolaryngology, Mahatma Gandhi Medical College & Research Institute, Pondicherry, India.**²Department of Microbiology, Mahatma Gandhi Medical College & Research Institute, Pondicherry.***ABSTRACT**

Head and neck cancers (HNC) have emerged as a leading cause of cancer related mortality and morbidity worldwide. Especially in developing countries, due to a lack of awareness and healthcare facilities, most HNC cases are diagnosed at advanced stages. Currently, there are no reliable non-invasive diagnostic modalities to detect HNC and follow up treated cases. Serum sialic acid is a promising cancer biomarker which could be crucial in HNC diagnosis. The aim of this study was to analyse pre and post-operative levels of serum sialic acid in HNC patients in comparison with healthy controls. We have estimated the pre- and post-operative serum sialic acid levels in 65 patients with histopathologically confirmed HNC who underwent surgery and compared them with those of 65 healthy controls. Compared to the control group, HNC patients had statistically significant elevation in serum sialic acid levels over normal mean value. The post-operative serum sialic acid levels showed statistically significant reduction and correlated well with postoperative events. Serum sialic acid is a non-invasive and cost-effective technique which may be employed as an adjunct to histopathology for early detection and monitoring of HNC patients.

KEYWORDS: Serum sialic acid; head and neck cancer; oral cancer; biomarker**ARUNAVA KALI**Department of Microbiology, Mahatma Gandhi Medical College
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INTRODUCTION

Over the past ten years, head and neck cancers (HNC) have emerged as a leading cause of cancer related mortality and morbidity globally. It is the 6th most common cancer worldwide and attributes to over 400,000 new cases every year.¹ HNC deaths are strongly associated with tobacco and alcohol use and are essentially preventable if diagnosed at an early stage. Due to lack of awareness and healthcare facilities in India, most patients have locally advanced diseases at the first presentation itself.² HNC, especially oral cancers, are often preceded by long latent period which may have noticeable precancerous changes.^{2, 3} These precancerous or early stage lesions are more amenable to therapy than advanced cancers. Incidence of second primary cancers in patients with HNC is not uncommon.⁴ The importance of HNC surveillance is well documented in literature.⁴ Currently, histopathology is the gold standard investigation for HNC. However, a cost-effective, reliable noninvasive diagnostic modality to detect new HNC cases and follow up treated cases remains to be established. Serum glycoproteins are promising cancer biomarker since these are often expressed by cancer cells in an altered way and are associated with tumour progression and metastasis.⁵ Alterations in cell surface glycosylation are frequently documented in neoplastic transformations. Sialic acid is widely distributed in mammalian tissue as a major constituent of cell membrane glycoproteins. It determines surface properties of cells *viz.* invasiveness, adhesiveness and immunogenicity. Serum sialic acid levels are found elevated in diverse groups of malignancies⁵⁻⁷ and inflammatory conditions.^{8, 9} However, it has not been evaluated effectively in HNC. Therefore, this study was done to determine the significance of serum sialic acid levels in head and neck malignancies without distant metastasis.

METHODOLOGY

1. Study Design

The current study was carried out in department of Otorhinolaryngology in a tertiary care hospital in south India from October, 2009 to November, 2011 after obtaining Institutional ethical committee clearance. All individuals who participated in this study were informed about the study and consent was obtained. It was a single blinded study. The pathologists were unaware of the serum sialic levels and laboratory personnel involved were unaware of the identification of cases and controls.

2. Patients

Sixty five adults aged with histopathologically confirmed head and neck malignancies without distant metastasis, were compared with 65 healthy controls for serum sialic acid levels. All cases were diagnosed clinically and confirmed by histopathology. Staging of HNC was done as per American Joint Committee on Cancer 2002 (AJCC) staging system. The study group included patients who were older than 18 years and were not suffering from metastatic or recurrent HNC, other malignancies and chronic inflammatory diseases. Individuals with habits of tobacco chewing, smoking, alcohol consumption, betel nut chewing and age less than 18 years were excluded from the control group.

3. Interventions

The treatment was administered depending on the stage of the tumour. Distant metastasis to bone, lung and liver were excluded using plain chest x-rays, abdominal ultrasound. Contrast enhanced CT scan was taken in those patients who showed suspicious lesions on chest x-ray or abdominal ultrasound. Preoperative and postoperative (3 weeks after surgery) serum sialic acid levels were estimated. Five ml of venous blood was collected, centrifuged; serum was separated and stored at 4°C for analysis. Serum sialic acid was estimated by diphenylamine method.⁵ Serum was treated with 5% TCA followed by boiling to release protein bound sialic acid which was filtered and treated with diphenylamine reagent to get pinkish red color. The intensity was read at 530 nm.

4. Statistical Analysis

All clinical & laboratory data entered in Microsoft Excel 2007 spreadsheet and statistical analysis was done in SPSS statistical software version 17.0. Student t test was used to compare serum sialic acid level in preoperative and postoperative period. All p values < 0.05 were considered statistically significant.

RESULTS

The study group of 65 HNC patients included 49 males and 16 females. The mean age in male and female patients was 56.53 ± 11.91 and 54.37 ± 10.1 years respectively. Control group was comprised of

50 males and 15 females. The mean age of cases & controls were 56 ± 11.46 and 53.32 ± 10.05 years respectively. Among 65 HNC cases, oral cavity cancer was most common (n=27, 41.53%), followed by hypopharyngeal (n=16, 24.61%), oropharyngeal (n=12, 18.46%) and laryngeal cancers (n=10, 15.38%). Oral lesion (35.38%), oral bleeding (12.30%), difficulty in swallowing (43.07%), difficulty in breathing (9.23%), change in voice (32.30%), throat pain (41.53%) and neck swelling (29.23%) were most common clinical feature. The mean preoperative serum sialic acid levels in all 4 types of HNC were significantly high compared to that of control group (table 1).

Table 1
Comparison of serum sialic acid levels of HNC patients and healthy controls

Site of cancer	Preoperative serum sialic acid (mg%)	Serum sialic acid in controls (mg%)	Degree of freedom	Two tailed p value
Oral cavity (n=27)	97.66 \pm 34.21	42.60 \pm 10.89	26	< 0.0001
Oropharyngeal (n=12)	117.38 \pm 29.08	42.60 \pm 10.89	11	< 0.0001
Hypopharyngeal (n=16)	113.18 \pm 36.87	42.60 \pm 10.89	15	< 0.0001
Laryngeal (n=10)	101.39 \pm 22.07	42.60 \pm 10.89	9	< 0.0001
Total HNC (n=65)	105.69 \pm 32.83	42.60 \pm 10.89	64	< 0.0001

Staging of HNC cases were done according to American Joint Committee on Cancer staging system. We followed up all patients after surgery and postoperative serum sialic acid levels were compared with preoperative values. Patients with oral cavity and laryngeal cancer mainly had early stage (Stage I and II) cancer (table 2).

Table 2
AJCC staging of different head and neck cancers

Stages	Oral cavity cancer (n=27)	Oropharyngeal cancer (n=12)	Hypopharyngeal cancer (n=16)	Laryngeal cancer (n=10)
Stage I	9	3	2	2
Stage II	7	2	4	4
Stage III	7	4	6	2
Stage IV	4	3	4	2

The distribution of serum sialic acid preoperative and postoperative levels varies with stages (table 3). However, there was statistically significant reduction of serum sialic acid levels in postoperative period in all stages of HNC.

Table 3
Comparison of preoperative and postoperative serum sialic acid levels of HNC patients.

Stage of cancer	Preoperative serum sialic acid (mg%)	Postoperative serum sialic acid (mg%)	Degree of freedom	Two tailed p value
Stage I (n=16)	72.4 ±19.7	40.6 ±8.8	15	< 0.0001
Stage II (n=17)	105.6 ±27.4	43.7 ±10.2	16	< 0.0001
Stage III (n=19)	118.6 ±29.4	51.2 ±16.3	18	< 0.0001
Stage IV (n=13)	127.6 ±26.2	64.8 ±26.7	12	< 0.0001

In postoperative period, 3 hypopharyngeal cancer patients developed pharyngocutaneous fistula and the 3rd week post-operative serum sialic acid levels remained elevated. Among these 3, one patient had recurrence at the primary site after 4 months with serum sialic acid level of 112.36 mg%.

DISCUSSION

Head and neck malignancies are a diverse group of cancers originating mainly from aero digestive tract. HNC incidence trends have shown a worldwide increase in cancer burden. It accounts for 400,000 new cases all over the world every year. Owing to large population and common practices like intake of tobacco in various forms along with alcohol and betel nut, Indian population is particularly predisposed to HNC, which accounts for over 104,205 cancer related deaths per year in both sexes. Cancers of the tongue and buccal mucosa are especially common in Indian men, as a sequelae of the local custom of chewing betel leaf and tobacco. As per Globocan 2008 statistics, HNC is the 2nd most common malignancy in Indian men after lung cancer.¹⁰ Several authors have documented a male preponderance with a common male to female ratio of 3:1 in head and neck malignancies.^{11, 12} Findings of this study are in accordance with these results. We found a similar sex ratio of 3.1:1. Although there are reports of increasing incidence of HNC among young,¹³ we found most cases in middle age & older population. The mean age of HNC patients were 56±11.46 years. HNC are known to have distinct site predilection which differs widely across the globe pertaining to the difference in demography, lifestyle and local customs. While pharynx cancer is most predominant in France, oral cavity is the commonest site of malignancy in India.¹¹ This is mainly due to

the carcinogenic effects of tobacco, betel nut chewing. In this study, out of 65 HNC cases, 27 patients had oral cavity cancer estimating about 41.53% of total HNC cases in our hospital. This was followed by hypopharyngeal (24.61%), oropharyngeal (18.46%) and laryngeal cancers (15.38%). Most patients with oral cavity cancer presented with oral lesion, oral bleeding and difficulty in swallowing. Sialic acids comprise a diverse family of acierated neuraminic acid derivatives and are widely distributed as major components of human cell surface glycoprotein.⁵ Overexpression of cell surface sialic acid glycoproteins in various cancers reflects primarily proliferative rather than destructive processes.¹⁴ However, increase in serum sialic acid has also been reported in non-neoplastic conditions where tissue destructive and inflammatory component are mainly responsible. Van Beek *et al.* reported increased sialic acid density in surface glycoprotein of transformed and malignant cells which may be related to spread of cancer cells as a result of lack of adhesion with neighboring cells.¹⁵ Increased negative charge imparted by sialic acid of surface glycoprotein creates repulsive force between cells. Being a major constituent of surface glycoproteins, serum level of sialic acid is raised above normal in a wide range of malignancies where it can be utilized for early diagnosis and follow up of treated cases. We have compared the serum sialic acid mean values of different types of HNC with mean values of normal controls (table 1). Oral

cavity, oropharyngeal, hypopharyngeal, laryngeal cancers as well as HNC on the whole had significantly raised serum sialic acid levels compared to that of normal controls with two tailed p value < 0.0001. The usefulness of serum sialic acid in monitoring treated cases were assessed by comparing preoperative and postoperative levels in different types of HNC (table 3). A significant postoperative drop in serum sialic acid levels were found in all stages of HNC, irrespective of their primary tumour. Reduced postoperative values indicate adequacy of treatment. Out of 16 hypopharyngeal cancer patients, 3 developed pharyngocutaneous fistula and showed no reduction in serum sialic acid levels on 3rd week of post-operative period. One case among these 3 cases developed recurrence at the primary site after 4 months and displayed persistently raised serum sialic acid level (112.36 mg%). Our findings are similar to other studies. Joshi *et al.* evaluated sialic acid levels in patients with oral cancer & precancerous lesions and found mean serum level in oral precancer and oral cancer group was statistically significant in comparison with healthy controls.¹⁶ However, there were no significant difference in sialic acid levels between oral cancer & precancerous lesions. Contrastingly, Taqi *et al.* have recommended use of serum sialic acid in diagnosis based on their finding that it can differentiate between patients with oral precancer and oral cancer.¹⁷ Both of these studies found higher levels of serum sialic acid in advanced cancers. Similar findings reported by

others.¹⁸ We found a steady increase in the preoperative mean level in HNC with higher T stages. The drop of serum sialic acid levels in postoperative period correlated well with postoperative events. It was persistently elevated only in case of postoperative complications like pharyngocutaneous fistula and recurrence. In addition to serum, sialic acid levels in saliva have also been evaluated by different authors in oral carcinoma.^{19, 20} Vajaria *et al.* reported elevation of salivary sialic acid were significantly higher than serum levels.²⁰ However, salivary sialic acid assay might have potential limitation. Oral inflammatory conditions are common and may result in erroneous results.

CONCLUSION

Oral cancer was most common among accounting for 41.53% HNC cases in our hospital. In this present study, serum sialic acid levels displayed statistically significant elevation over normal mean value in oral cavity, oropharyngeal, hypopharyngeal and laryngeal malignancies. The reduction in its value in postoperative period was statistically significant in most HNC, irrespective of their Tumour stage. Persistent elevation of serum sialic acid levels was associated with postoperative complications & recurrence. Hence, serum sialic acid could be employed as a non-invasive simple method for both early detection of new case as well as monitoring of treated patients with HNC.

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