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PROGNOSTIC SIGNIFICANCE OF SERUM L-FUCOSE LEVEL IN HEAD AND NECK MALIGNANCIES

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ABSTRACT

India contributes a major pool of head and neck cancer (HNC) patients in global cancer burden. Measurement of L-fucose glycoprotein has been used as a cancer biomarker. In this study, preoperative levels of serum L-fucose glycoprotein were compared with that of postoperative levels measured at 3rd week in 50 HNC patients without distant metastasis. The mean value of serum glycoprotein L fucose was 11.33±7.39 mg % preoperatively. Well differentiated & moderately differentiated squamous cell carcinoma showed a significant drop in postoperative serum L-fucose level. Compared to stage III & IV HNC, serum glycoprotein L fucose level decreased considerably in stage I & II cancers following surgery. In conclusion, head & neck malignancies are associated with a serum glycoprotein L-fucose level exceeding the normal value. In conjunction with clinical diagnostic procedures it can be employed successfully in monitoring patients in postoperative period for complications and recurrence.

KEYWORDS: **L-fucose; serum glycoprotein; head and neck cancer; biomarker**

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INTRODUCTION

Owing to its large population, India contributes a major pool of cancer patients in global cancer burden[1,2]. Among all malignancies, Indian population is particularly predisposed to head and neck cancers (HNC) as a result of distinct demographic profile and lifestyle[3]. Intake of tobacco in various forms along with alcohol and betel nut are common in indigenous population and these are essentially associated with HNC[3-5]. More than 200,000 HNC cases are reported in India every year[1]. Although, these malignancies frequently cause significant structural deformity with functional disability, they are mostly preventable by lifestyle modification and treatable in early stage. HNC, especially oral cancers, are often preceded by a long latent period which may have noticeable precancerous changes[1,6]. The confirmative diagnosis is essentially based on histopathology[1,7]. In India, 60 to 80% of patients have advanced cancer at the first presentation itself[1]. Although histopathology is the gold standard investigation for HNC[1,7] an easy to perform, non-invasive and cost-effective technique enabling early detection is more likely to have significant impact on patient care. For several years, the focus of cancer research malignancy has been to find a unique molecular alteration associated with malignant transformation which can be utilized as a relatively inexpensive and reliable way to detect the presence of malignancy. Numerous observations indicate that glycoproteins are often elevated above the normal levels in sera of cancer patients. Measurement of protein-bound carbohydrates of glycoproteins has been used as an index to glycoprotein levels. A more recent trend has been to use the amount of given monosaccharide as a measure of glycoproteins. One of the monosaccharide is L-fucose, a methyl pentose, which is a terminal sugar in most of the plasma glycoproteins. Changes have been detected in the fucosylation pattern of these molecules in the tissue of cancer patients due to fucosyl transferase activity, which is especially high in the serum of patients suffering from highly malignant or metastatic tumors. It has been observed that the serum fucose level is raised in different groups of malignancies such as breast cancer, ovarian cancer, colorectal adenocarcinoma and leukemia as well as brain tumors[8-12]. Rise in serum fucose level is not specific for cancers, as elevated serum fucose levels have also been reported in various pathological states such as cirrhosis liver and meningitis, rickets and osteomalacia, tuberculosis, cardiovascular disorders as well as in depressive disorders [13]. However, in conjunction with clinical diagnostic procedures, serum glycoprotein L fucose levels can be used as an effective biochemical indicator in HNC and may be useful in monitoring recurrences. There is inadequate published data on, serum glycoprotein L fucose levels in HNC in Indian population. This study was done to determine the prognostic value of serum L-fucose glycoprotein level in head and neck malignancies without distant metastasis.

METHODOLOGY

1. Study Design

A prospective study was carried out after receiving Institutional ethical committee approval in a tertiary care hospital in south India in department of Otorhinolaryngology from October, 2009 to July, 2011. Informed consent was obtained from all individuals participating in the study. Preoperative levels of serum L-fucose glycoprotein were compared with that of 3rd week postoperatively in 50 HNC patients who underwent surgery. They were followed up for postoperative complications and recurrence for a period of 2 years.

2. Patients

Fifty adults aged above 18 years with histopathologically confirmed head and neck malignancies without distant metastasis, were monitored for serum L-fucose glycoprotein levels during the preoperative and postoperative period. As per the exclusion criteria, patients who are known or found to have distant metastasis, recurrent head and neck malignancy, associated malignancy other than head and neck region, cardiovascular disease, diabetes mellitus,

rheumatoid arthritis, renal diseases, hepatic diseases, cystic fibrosis, other chronic inflammatory conditions and were less than 18 years were excluded from the study group. After discharge from the hospital, patients were followed up by outpatient visits and telephonic follow up interviews.

3. Interventions

Cases were diagnosed clinically and confirmed by histopathological investigations. Specimens obtained by punch biopsy were sent for histopathology. AJCC (American Joint Committee on Cancer 2002) staging system is followed for staging of HNC *viz*. lip and oral cavity cancers, oropharyngeal cancer, hypopharyngeal cancer and laryngeal cancer. 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. Five ml of venous blood was collected, serum was separated by centrifugation and stored at $4^{O}C$ for analysis. Level of serum L-fucose was estimated in spectrophotometer (Spectronic 20, Thermo Fisher Scientific, USA) as per Winzler method [14].

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 L-fucose glycoprotein level in preoperative and postoperative period. All p values < 0.05 were considered statistically significant.

RESULTS

This study was carried out on 50 patients with clinically and histopathologically confirmed head and neck malignancies. The study group was comprised of 40 males and 10 females. Mean age of cases were 55.92±10.17 years. We found habit of smoking, alcohol consumption, betel nut chewing and tobacco chewing in 60%, 56%, 54% and 48% patients respectively. Half of the study group (50%) had habit of both alcohol consumption and smoking. Most of the patients presented with throat pain (46%, n=23) followed by difficulty in swallowing (42%, n=21). Oral lesions, oral bleeding, neck swelling and change in voice were other clinical symptoms reported in the current study. Among 50 cases of HNC, oral cavity cancer was most common (34%), whereas malignancy involving larynx, hypopharynx and oropharynx accounted for 28%, 26% & 12% cases respectively. Stage III and IV were considered as late stage or advanced stage and represented 44% & 38% cases respectively. Histopathological study of the tumour tissue taken from 50 cases showed moderately differentiated squamous cell carcinoma was the commonest type. (Table-1)

Table 1
Tumor histopathology

Tumor histopathology	Number of patients (n=50)	%
Well differentiated SCC	15	30.0
Moderately differentiated SCC	24	48.0
Poorly differentiated SCC	8	16.0
Verrucous carcinoma	2	4.0

The mean preoperative value of serum glycoprotein L fucose was 11.33±7.39 mg%. Following surgery there was a considerable drop in serum glycoprotein L fucose level in well differentiated & moderately differentiated squamous cell carcinoma (SCC) of HNC with a mean value of 7.95±4.06 mg%. (Table 2)

Table 2
Comparison of preoperative & postoperative serum glycoprotein L fucose levels in head and neck cancer patients

	Pre-operative L fucose level (mg%)	Post-operative L fucose level (mg%)	P value
Mean value	11.33±7.39	7.95±4.06	0.001
Tumour histopathology			
Well differentiated SCC	12.28±7.87	8.06±3.92	0.033
Moderately differentiated SCC	12.75±7.37	7.98±4.13	0.003
Poorly differentiated SCC	8.71±5.23	9.64±3.68	0.339
Verrucous carcinoma	2.58±0.98	2.70±1.27	0.651
Stage of malignancy			
Early stage (stage I & II)	11.53±10.16	5.55±3.50	0.053
Late stage (stage III & IV)	11.28±6.79	8.48±4.02	0.006
Site of malignancy			
Oral cavity	11.33±8.28	6.53±3.25	0.010
Oropharynx	12.20±4.79	6.32±2.81	0.039
Hypopharynx	8.80±4.59	9.86±4.87	0.508
Larynx	13.32±9.06	8.62±4.03	0.017

DISCUSSION

Head and neck malignancies constitute a diverse group of malignancies mostly arising from the aero digestive tract. It represents about 6% of all cancer cases worldwide[15]. Habits of tobacco, betel and alcohol have a strong causative association with HNC. Several cytogenetic aberrations have also been detected more commonly in betel and tobacco chewers[16,17]. Micronuclei, sister chromatid exchange and chromosomal aberrations like chromatid breaks, gaps and exchange have been observed. These chromosomal aberrations reflect DNA damage associated with betel and tobacco use which may ultimately give rise to malignant changes. HNC incidence is exceedingly high in countries where tobacco use and alcohol consumption is more common. Owing to the large population and common practice of tobacco, betel and alcohol use, head and neck malignancies are extremely high in India. More than 141,000 new cases and 104,000 deaths due to HNC are estimated to occur each year in India[18]. In a recent study conducted in Andhra Pradesh, it was found that all the three habits *viz*. smoking, alcohol, and chewing were significant risk factors in both males and females for HNC[3]. We found half of our study group had habit of alcohol consumption in combinations with smoking while chewing betel nut (54%) and tobacco were found in 54% & 48% cases respectively. As per GLOBOCAN, 2008 estimates, in India 23.3% & 7% of all cancer cases in males and females respectively are attributed exclusively to HNC[18]. The male predominance may be related to exposure to carcinogenic effects of tobacco, betel and alcohol. However, the world's highest reported incidence of HNC in women is from India[19]. In this study, the male to female ratio was 4:1 and the mean age of cases were 55.92±10.17 years. Other authors also have reported similar demographic profile of HNC in Indian population[3,4,20].

In general, oral cavity cancers are more common compared to pharyngeal and laryngeal cancers. Tongue is the most common site of malignancy, followed by cheek & lip[20]. We found oral, larynx, hypopharynx and oropharynx cancers accounted for 34%, 28%, 26% & 12% cases respectively. In a nationwide study, overall decline in age-adjusted incidence rates of oropharynx, hypopharynx, larynx and tongue cancer in both urban and rural community was documented lately[1,4]. This may be attributed to the reduction in the use of tobacco and the beneficial effect of healthcare education. However, oral cancer rate in rural women was reported to be stable[1]. In India, due to lack of diagnostic infrastructure HNC are frequently are diagnosed at advanced stage[1]. It is estimated that 60 to 80% of patients in India have advanced HNC at the first presentation[1]. We found 47.5% and 37.5% male patients in our study had stage III and stage IV cancer

respectively which may be the result of delay in seeking medical attention. Our observation is in accordance with other studies. Addala *et al*. reported that compared to female patients, a significant number of male patients were diagnosed in stage III (37.6%) and stage IV (20.6%)[3]. The importance of early detection is well established in HNC. These cancers, especially oral cancers are often preceded by precancerous conditions like leukoplakia, erythroplakia and oral submucous fibrosis for several years[1,6]. HNC at precancerous stage and early stage (stage I & II) are more amenable to treatment. However, recurrences are not uncommon even after judicial use of radiotherapy, medical therapy and surgery. Within 3-5 years of local treatment, approximately 20-36% of patients are reported to develop local and regional recurrence[21]. Serum glycoprotein-bound L-fucose levels may have valuable role in diagnosis of HNC at early stage as well as for detection of local and regional failures following surgery. In general, the mean value of normal serum is 6.84±0.13 mg% and any value exceeding 9 mg% is usually pathological. The etiology behind such abnormal raise is mostly neoplastic process rather than benign tumour and non- neoplastic conditions. We monitored serum glycoprotein L-fucose levels in preoperative and postoperative period for all 50 patients. The mean value of preoperative level of L-fucose (11.33±7.39 mg%) decreased significantly postoperatively (7.95±4.06 mg%). It was also found that preoperative and postoperative levels were significantly different in early and late stage malignancy. Early stages had a pre-operative level of 11.53±10.16 mg% and post-operative level of 5.55±3.50 mg% with a difference of 5.98 which is statistically significant (p value 0.053). Late stages had a pre-operative level of 11.28±6.79 mg% and post-operative level of 8.48±4.02 mg% with a p value of 0.006 which is strongly significant. Except hypopharyngeal cancer, all other HNC uniformly showed statistically significant reduction in L-fucose level in postoperative period from preoperative value. There was no significant difference between the pre-operative and post-operative levels of serum fucose in hypopharyngeal cancer patients, this was largely due to elevated serum fucose levels in three cases who developed pharyngocutaneous fistula in the post-operative period. One case among these three cases developed recurrence at the primary site after 4 months and displayed persistently raised L-fucose level (19.34mg%). Similar findings were reported elsewhere[6,22,23]. Several authors reported positive correlation of serum L-fucose level with cancer stage, progression, postoperative complications & recurrence. Shah *et al.* found a positive correlation of serum L-fucose & fucosidase activity with the extent of disease and response to treatment in patients with oral cancer.[6] The In another study, the fucose levels were repeated after one week of complete removal of the growth in 10 patients of head & neck malignancy and the values were found to be lowered to normal range in 7 patients[23]. Similarly in a serial postoperative follow-up study, Lambana *et al.* observed that the levels in serum fucose content was a useful parameter for judging therapeutic effectiveness and the prognosis in patients with carcinoma[13]. Contrastingly, Sawke *et al*. could not find any significant difference between preoperative and 10th postoperative day level of L-fucose in various cancer patients[12]. This may be related to the fact that L-fucose level may not readily decrease in all patients after operation owing to postoperative stress and inflammation. There are inadequate studies on L-fucose levels in different histological types of HNC to compare our finding. Parwani *et al*. conducted a study on 67 subjects, including 14 healthy individuals and 53 oral squamous cell carcinoma cases and reported lack of association of serum fucose levels with histopathological grades of oral SCC[24]. Similar results documented in another study by Shashikanth *et al*[25]. However, in this study we observed histological type of tumour has association with serum L-fucose level. Well differentiated & moderately differentiated squamous cell carcinoma (SCC) showed significant drop in postoperative serum L-fucose level compared to poorly differentiated SCC & verrucous carcinoma.

CONCLUSION

The present study highlights the importance of serum glycoprotein L-fucose levels in HNC patients. We found that most head & neck malignancies were associated with a serum glycoprotein L-fucose level exceeding the normal value. L-fucose levels were associated with tumour stage, histopathological grade and adequacy of treatment. In conjunction with clinical diagnostic procedures it can be employed successfully in monitoring patients in postoperative period for complications and recurrence. However, further studies are needed to prove this association conclusively.

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