

RESEARCH ARTICLE

Comparison of Serum Fucose Levels in Leukoplakia and Oral Cancer Patients

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Abstract

Background: Tumor markers, designated as a broad group of substances produced by malignancies, could be in the form of biochemical substances, immunological substances, cell surface changes and genetic alterations. Cancer, a disorder of cellular behavior is characterized by alteration of serum glycoproteins. L-fucose, a hexose, which is the terminal sugar in most of the plasma glycoproteins, may be useful as a tumor marker for the detection, monitoring and prognostic assessment of malignancies. The aim of the study was to ascertain the role of serum fucose as a biomarker for early detection of oral cancer and to compare serum fucose levels in healthy controls, leukoplakia and oral cancer patients. **Materials and Methods:** The study included 60 (100.0%) subjects, who were grouped as 20 (33.3%) control subjects, 20 (33.3%) squamous cell carcinoma patients and 20 (33.3%) leukoplakia patients. Fucose estimation was done using UV-visible spectrophotometry based on the method as adopted by Winzler using cysteine reagent. The results were analyzed statistically using ANOVA with Bonferroni post hoc tests. **Results:** Results showed a high significance in serum fucose in oral squamous cell carcinoma (OSCC) and leukoplakia subjects compared to normal controls. There was a gradual increase in the values noted from control to leukoplakia and to squamous cell carcinoma. **Conclusions:** Estimation of serum fucose may be a reliable marker and can be used as an effective diagnostic biomarker in oral squamous cell carcinoma patients.

Keywords: Cancer - leukoplakia - oral squamous cell carcinoma (OSCC) - serum fucose

Asian Pac J Cancer Prev, 16 (17), 7497-7500

Introduction

Oral squamous cell carcinoma is the most common malignancy known in the head and neck region. It remains one of the major causes of deaths worldwide and it is one of the major health hazards in India as well (Pindborg, 1981).

Approximately 80,000 new cases are diagnosed each year (Krishna et al., 2014), mainly due to consumption of different forms of available tobacco products (Khovichunkit et al., 2008; Kumar and Muniyandi, 2010). Smoking and other forms of tobacco use can lead to development of potentially malignant disorders (Raval et al., 2001; Moore et al., 2010). Potentially malignant disorders (PMD-WHO, 2005) such as leukoplakia, erythroplakia, OSMF are at high risk of malignant transformation (Gurudath et al., 2012) so; these patients can be good candidates for studying molecular changes in

them. It has been seen that PMD's have an increased risk of malignant transformation rate of 0.6% to 36% (Shah et al., 2008). PMD's can be identified clinically so oral physicians can play a major role in the diagnosis at the prior level (Tsuda and Moore, 2001). Despite the recent advances in cancer treatment, the outcome and prognosis of oral squamous cell carcinoma is still relatively poor (Lotfi et al., 2015). This may be attributed to delayed and late diagnosis of neoplasm when the tumor is in advanced stage (Oliver et al., 2000). Advancement in the tumor growth may lead to changes in the certain substances in serum which are known as "tumor markers" (Kadam et al., 2011). These markers can be used for early diagnosis and can help in early treatment of oral squamous cell carcinoma in high risk patients (Oliver et al., 2000; Hassona et al., 2014).

Classically, a marker is synthesized by the tumor and released into circulation at the cell surface in large

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quantities by malignant cells (Kimura et al., 2000; Silvia et al., 2001). Diagnosis of early cancer is difficult, it remains asymptomatic for long time and by the time patient seek advice it would have invaded deeper structures which makes prognosis poor. For these biochemical substances to be clinically significant, it must be present in appreciable amount in tissues. Glycoproteins can be detected in the body fluids which are synthesized by malignant cells as biochemical products (Tormey et al., 1982). Altered properties of cancerous cells are expressed at the cell surface and these surface glycoconjugates are important because these molecules are associated with tumor progression, metastasis and cell to cell adhesion (Kanagi, 1997). Glycoproteins are composed of different types of monosaccharides, one of them is L-fucose, a hexose, six carbon sugar. This sugar is required for optimum cell function (Parwani and Parwani, 2011).

Normally, fucose is present in low concentration in body fluids but it is increased in malignant diseases (Kanagi, 1997; Elkins et al., 2003). It is also important for proper immune regulation and is widely present in macrophages. Tumor cells escape recognition by modulating their surface by increasing fucosylation and leads to abnormal tumor growth (Youakim and Herscovics, 1985; MacDougall et al., 1987; Shah et al., 2008).

Fucosylation of glycoproteins is one of the important features that mediate several specific biologic functions. Monitoring serum fucose levels could be a novel approach for the early detection and diagnosis of the malignant diseases that may lead to better prognosis for oral cancer patients (Parwani and Parwani, 2011).

The objective of the present study was to ascertain the role of serum fucose as a biomarker for early detection of oral cancer and compare serum fucose levels in healthy controls, leukoplakia and oral cancer patients.

Materials and Methods

Subjects were selected randomly who reported to Department of Oral Medicine & Radiology, serum analysis was done in Department of Biochemistry. The clinical diagnosis of leukoplakia and oral cancer was confirmed by histopathological examination. An informed consent was obtained from all the participants. The study included 3 groups: 20 (33.3%) healthy individuals with (mean age range of 37.15±7.08), 20 (33.3%) patients with leukoplakia with (mean age range 46.80±10.35) and

20 (33.3%) oral cancer patients with (mean age range 57.85±8.99), who had the habit of tobacco and alcohol consumption. The total sample size was 60 (100.0%), out of which males were n=35 (58.3%) i.e. Control n=12 (60.0%), Leukoplakia n=13 (65.0%), Carcinoma (OSCC) n=13 (65.0%) and female were n=25 (41.7%) i.e. Control n=8 (40.0%), Leukoplakia n=7 (35.0%), Carcinoma (OSCC) n=10 (50.0%).

Exclusion criteria for study group was past history of any major illness such as liver disease, tuberculosis, diabetes and hypertension, patients undergoing radiotherapy or chemotherapy for cancer or any history of malignancy other than oral cancer. Controls were excluded on the basis of tobacco or alcohol consumption.

Blood was drawn from the healthy subjects for the purpose of the study, whereas in patients' with oral cancer and leukoplakia it was drawn as part of routine hematological investigation prior to biopsy. Blood was collected and transferred to sterilized plain tubes and allowed to clot at room temperature. Then the clot was separated and serum centrifuged at 3000 rpm for 15 min. The sample was stored at 4°C and assays were performed within 48 hours. Fucose estimation was done according to the method of Winzler (Winzler RJ, 1955) absorbance was read at 400 nm and 430 nm using UV - visible spectrophotometer (Systronics 117). The color

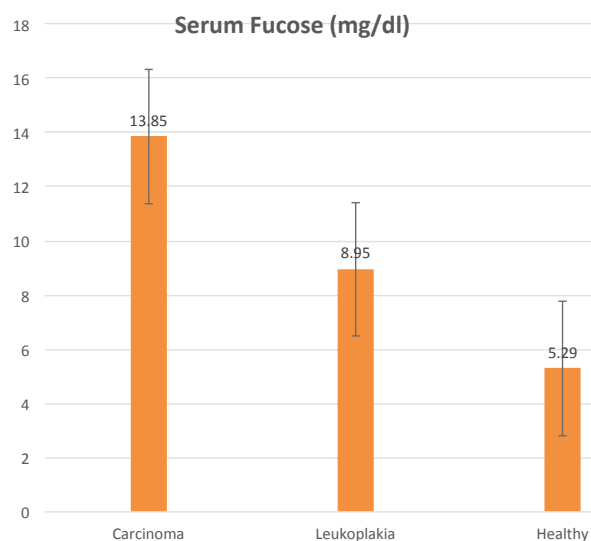


Figure 1. Comparison of Mean Serum Fucose Level in Carcinoma, Leukoplakia and Healthy Control Groups

Table 1. Bonferroni Post Hoc Test for Pairwise Comparisons of the Groups

Comparison between Groups	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
1.Control	2	-8.47799*	0.97038	0	-10.8716 -6.0844
	3	-3.39955*	0.95756	0.002	-5.7616 -1.0375
2.Leukoplakia	1	8.47799*	0.97038	0	6.0844 10.8716
	3	5.07845*	0.98184	0	2.6566 7.5003
3.OSCC	1	3.39955*	0.95756	0.002	1.0375 5.7616
	2	-5.07845*	0.98184	0	-7.5003 -2.6566

*The mean difference is significant at the 0.05 level

produced by hexoses under these conditions is corrected by determining absorbance at two wavelengths.

Statistical analysis

Data are parametric and, followed, normal variation. The biochemical values of this study were subjected to statistical analysis using SPSS software IBM version 16. ANOVA with Bonferroni post hoc test was used to compare and correlate different parameters in study.

Results

The mean serum fucose levels among Carcinoma, Leukoplakia and healthy Control groups were assessed. The mean serum L-Fucose level in OSCC group was 13.85 ± 4.34 mg/dl, whereas it was 8.95 ± 1.92 mg/dl in leukoplakia group as compared to control group it was 5.29 ± 2.18 mg/dl (Figure 1). Table 1 shows correlation between the groups. Bonferroni post hoc test was done which shows significant correlation between all the groups, the carcinoma and leukoplakia group, and carcinoma and control group as well as between leukoplakia and control group.

Discussion

Oral cancer is the sixth most common cause for cancer related death (Abbasi et al., 2014; Hassona et al., 2014) and it accounts for 30-40% of all cancers in India. Malignant cells are responsible for the increase or decrease of biochemical substances in the blood, which can be detected in body fluids even in minute quantities using sensitive techniques to identify early malignant changes in the tissues. These substances are known as tumor markers (Bathi et al., 1991). The markers are the substances produced in a broad category by malignant cells which can be used for detection and monitoring the therapy in malignancies. Tumor markers help in diagnosis as well as in prognosis of the malignancies (Taneja et al., 2009).

Glycoproteins are primarily composed of protein and carbohydrate in which the sugars are firmly linked to the peptide chain. Increase in glycoproteins, serum fucose levels has been associated with different types of malignancies like cancer of cervix, breast, oral cavity and lymphomas.

L-Fucose is found in many glycoproteins, along with families of blood group antigens. Changes are detected in fucosylation pattern in these molecules in cancer patients due to their fucosyltransferase activity, and which will be having high levels in patients with malignancies (Wang et al., 1995; Fernandez-Rodriguez et al., 1997).

In the current study an increased level of serum L-fucose in the study group was noted when compared to patients with leukoplakia and normal individuals.

In this study the normal fucose level in the control group is found to be 5.29 ± 2.18 mg/dl with levels ranging between 4.259 and 7.124 mg%. In contrast, the established normal fucose level by (Arya and Bhatnagar, 1974) found to be 4.92 ± 0.87 mg/dl and by (Sharma and Sur, 1967) was 4.6 ± 0.13 mg/dl. The difference could be because of employment of different techniques, the different

sample size and different age groups of individuals who participated in the study.

Serum fucose level in the present study was significantly elevated among cancer patients (13.85 ± 4.34 mg/dl) when compared to leukoplakia (8.95 ± 1.92 mg/dl) and control group (5.29 ± 2.18 mg/dl). (Figure 1). It was observed that there was no relationship of serum fucose levels with age and sex of the patients included in the study.

The reason for the elevation of fucose levels in serum is not clearly understood but according to various authors, they suggested that rise in serum fucose levels could be due to increased destruction and proliferation of diseased tissues.

(Seibert et al., 1947; Shetlar et al., 1947) suggested that elevation of fucose levels in serum is due to tissue destruction and release of preformed fucose at the site or it is because of increased tissue proliferation.

In cases of carcinoma of oral cavity, the possibility exists that may be a cancer products getting filtered in the blood stream or it may be due to manifestation of generalized effect of oral carcinoma on the body metabolism. It is important to exclude other degenerative and proliferative disorders while evaluating serum fucose levels in oral carcinoma because elevated levels of serum fucose also has been reported in various pathological conditions like liver cirrhosis, osteomalacia, TB, CVS disorders, meningitis and in depressive disorders. Elevated levels of serum fucose is also been noted in other malignancies like in cases of breast cancer, ovarian cancer, colorectal cancer and leukemias. An alteration in levels of serum fucose may be due to tumor burden and inflammatory reactions. Various authors found decrease in levels of serum fucose after successful treatment of the malignancies after successive long periodic follow-ups.

In conclusion, Identification of reliable biological tumor markers or substance associated with neoplasia that can be used for the detection, staging and evaluation has been the goal of many investigators. Analysis of the markers can be an additional tool for diagnosis, prognosis and treatment monitoring of cancer patients. There was progressive elevation in serum L-fucose level in oral cancer, showing positive correlation of fucose level and malignancy.

The biomarker showed good sensitivity, specificity and efficiency for oral cancer. Therefore, the evaluation of serum L-fucose would be of good help in assessing early malignant change in increasing the accuracy of clinical diagnosis and also in assessing the spread and invasiveness of oral cancer.

Acknowledgements

We extend sincere appreciation to the Deanship of Scientific Research, King Saud University, Riyadh, Saudi Arabia for funding/supporting this research through Research Group No-IRG 14-31.

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