Original Article

Fucose: A biomarker in grading of oral cancer

Satish Kumar, Mona Saxena[1], Kandakurtis Srinivas, Vinod Kumar Singh[1]

Department of Oral Medicine, Career Post Graduate Institute of Dental Sciences and Hospital, [1]Department of Biochemistry, Career Institute of Medical Sciences and Hospital, Lucknow, Uttar Pradesh, India

Address for correspondence:
Dr. Mona Saxena,
Ram Lodge, 417/214,
Newazganj, Lucknow - 226 003,
Uttar Pradesh, India.
E-mail: drmonasaxena@gmail.com

ABSTRACT

Introduction: Early diagnosis of cancer helps a great deal in the management of oral cancer patients. Number of proteinous markers have been employed for this purpose. Majority of them are not specific. Recently conjugated oligosaccharide with proteins and lipids have gained considerable importance in the present postgenomics and postproteomic period in the diagnostic and prognostics of cancer cases. **Materials and Methods:** In this study, serum fucose levels were estimated in 50 control cases and 75 cases of oral cancer by the method of Dische and Shettles as adopted by Winzler. **Results:** Serum fucose levels were found to be significantly higher in oral cancer cases (46.63 ± 5.29 mg/dl) as compared to the control cases (7.22 ± 0.26 mg/dl). The stepwise elevated serum fucose levels were found to be correlated with the histopathological grading of oral cancer. **Conclusions:** Estimation of such fucose conjugated proteins is suggestive to be good biomarkers in the diagnosis of oral cancer cases as well as in assessing the prognosis of such cases.

Key words: Fucosylation, oral cancer patients, prognosis, serum 6-deoxy-L-galactose

Introduction

The prevalence of oral cancer is particularly high among men and is the eight most common cancer worldwide.[1] It has been estimated that more than 43% of cancer deaths worldwide are due to tobacco (90% in the oral cavity),[2] unhealthy diet, physical inactivity, and infections.[3] India accounts for 86% of the world's oral cancer cases. Several molecular markers have been used for the identification of oral cancer.[4,5] Currently, estimation of conjugated oligosaccharide with proteins and lipids has gained considerable importance that is associated with cell division or oncogenesis.[6]

It has been reported that tumor cells modulate their surface by increasing fucosylation levels to escape recognition which contribute to several abnormal characteristics of tumor cells.[2,7] Hence, monitoring serum/tissue fucose levels could be a promising approach for the early detection, diagnosis, and prognosis of various cancer types.

Materials and Methods

The intended study included 125 subjects divided into two groups. Group-I included 50 age and sex matched healthy subjects from patients attendants to serve as controls. Group-II included 75 oral cancer patients. Healthy subjects with no history of systemic or localized illness such as allergies, renal problems, hypertension, and diabetes are included as controls. Patients with known systemic conditions such as diabetes, hypertension, pregnancy, allergies, infections, and liver diseases are excluded. Oral cancer subjects

Access this article online

Quick Response Code:

Website: www.njms.in

DOI: 10.4103/0975-5950.183869

How to cite this article: Kumar S, Saxena M, Srinivas K, Singh VK. Fucose: A biomarker in grading of oral cancer. Natl J Maxillofac Surg 2015;6:176-9.

were further classified into subgroups by histological gradings. A written informed consent was taken from the subject, and Institutional Ethical Clearance is also obtained.

About 4–5 ml blood sample from each subject is collected by venous arm puncture from an antecubital vein into a sterile centrifuge tube, and allowed to clot at room temperature followed by centrifugation at 3000 rpm for 15 min to get clear sample of serum. The serum was transferred into eppendorf tubes and stored at 4°C and was assessed within 48 h. The serum fucose level estimation was done based on the method of Dische and Shettles[8] as adopted by Winzler.[9]

Fucose is a 6-carbon methyl pentose in glycoproteins. It is assayed by dissolving ethanol precipitated proteins of serum in alkali, and reacting them with ice-cold sulfuric acid H_2O mixture, forming a colored furfural derivative. Further cysteine reagent is added which couples with the yellow chromophore. Thus, the final colored product formed is measured at 396 nm and 430 nm using ultraviolet–visible spectrophotometer. Absorbance at two wavelength is noted to correct interference caused by chromophore from other sugars, so the difference in these two wavelength is the absorption of fucose alone.

For each serum sample two 15 mm × 150 mm test tubes were prepared. One was marked "serum blank (S-1)" and the other "serum test (S-2)." 0.1 ml of serum and 1 ml of 70% ethyl alcohol were added to each tube and mixed on a vortex mixer. The tubes were centrifuged at 3000 rpm for 15 min, the supernatant was decanted, and the precipitate was again suspended in 1 ml of 70% ethyl alcohol. It was recentrifuged for 15 min, and the supernatant was completely decanted. The precipitate was dissolved in 3 ml of 0.2N NaOH. Bound fucose is released from complex carbohydrate. The reagent blank and standard tubes were prepared by adding 1 ml of distilled water and 1 ml of working fucose standard (20 µg/ml) to appropriately marked 15 mm × 150 mm tubes respectively, serum blank (S-1) and serum test (S-2) contain 1 ml of prepared sample. All the tubes were placed in a rack in an ice water bath. 4.5 ml ice-cold sulfuric acid-water mixture was added to each tube. Content in all the tubes were mixed on a vortex mixture and tubes were kept in a boiling water bath for exactly 3 min. Then they were cooled under tap water. 0.1 ml of cysteine reagent was added to the "reagent blank," "standard" and "serum test (S-2)." 0.1 ml of water was added to the "serum blank (S-1)." They were immediately mixed on a vortex mixer and kept at room temperature for 60–90 min. After that, the solutions were transferred to appropriate cuvette, and the absorbance was read at 396 nm and 430 nm using spectrophotometer (Systronics 117) set at zero with the reagent blank. ANOVA and Turkey's *post hoc* test were used to analyze the data, and analysis was performed on IBM SPSS statistics software.

Results

Subjects of groups were age and gender matched. The mean age of normal and oral cancer groups ranged from 32.60 ± 2.34 years to 33.80 ± 2.56 years, respectively. ANOVA revealed similar age among groups, i.e., not differed statistically.

The mean fucose level of normal and oral cancer groups were 7.22 ± 0.26 mg/dl and 46.63 ± 5.29 mg/dl, respectively. The mean fucose level of oral cancer subjects was significantly higher ($P < 0.001$) than the control group [Table 1].

In all the three histopathological grades of oral cancer, the mean fucose level increases with severity, i.e., from mild (15.11 ± 1.01) to moderate (31.43 ± 2.83) to severe (71.27 ± 3.41) condition as shown in Table 2.

It is evident that fucose levels were highest in the severe grade of oral cancer subjects. Thus, the present study shows that fucose levels are increasing significantly ($P < 0.001$) from mild to severe grade of oral cancer. Hence, it is beneficial in modern medicine for grading of oral cancer patients.

Discussion

Cancer is the second most common cause of morbidity as well as the mortality in the community.[1] However, due to lack of signs and symptoms majority of the patients come in the late stages of the disease. Therefore, the cancer detection is one of the most useful tools in the preclinical stages as well as in different stages of malignancy. The gold standard for detection of cancer is the biopsy which is often not possible in certain tumors like glioma's of the brain. Thus, there has been a need to detect cancer by estimating certain biomolecules called as markers which are directly produced by malignant cells

Table 1: Serum fucose levels (mean ± standard error)

Normal (mg/dl)	Oral cancer (mg/dl)
7.22 ± 0.26	46.63 ± 5.29

Table 2: Serum fucose levels (mean ± standard error) of three groups according to grades in mg/dl

Groups	Histopathological/clinical grades					
	n	Mild	*n*	Moderate	*n*	Severe
Oral cancer	18	15.11 ± 1.01	21	31.43 ± 2.83	36	71.27 ± 3.41

or by nontumor cells.[10] A large number of proteinous tumor markers are available in the market, but none is very specific.[4,5,10] Hence, an attempt has been made to find out a marker based on oligosaccharides which result due to alteration of the carbohydrate structure in the cancer cells. The first successful report was based on n-glycosylation at a specific Asn residue of a glycoprotein in cases of pancreatic carcinoma, one of the most difficult cancers to diagnose.[6] Therefore, in the present study fucosylated glycobiomarkers have been used to detect the malignancy in oral cancer cases. Our present study indicates a direct relationship of fucose with the stage of oral cancers.

Glycosylation is involved in a variety of biological phenomenon including birth, differentiation, growth, inflammation, etc.[10,11] Among different types of oligosaccharides, fucose is one of the important carbohydrate in oligosaccharide chain. This fucosylation is mainly found in glycoprotein and glycolipids of living beings. Hence, altered fucosylation of glycoproteins is the most representative types of glycan-related cancer biomarker.[10,11] Physiologically during normal growth and development, the fucose level increases but the rise is within normal limits, i.e., 7.22 ± 0.26 mg/dl that is important for normal biological functions but a rise more than normal limits is an indicative of oral disease. We have found an increase in the level of fucose in sera reflecting enhanced fucosylation. In the present study related to oral cancer, the levels of fucose are found to be significantly higher (46.63 ± 5.29 mg/dl) than normal subjects (7.22 ± 0.26 mg/dl).

Thus, our study indicates a clear relationship between degree of the fucosylation and the stage of oral cancer. It is likely that enhanced fucosylation seen in our study is due to the effect of nicotine and other hydrocarbons present in tobacco taken in the form of smoking or chewing and is due to formation of nitrosamine like carcinogenic metabolite.[11]

GDP-fucose formed either by *de novo* synthesis from D-glucose or mannose in the presence of GDP synthesizing enzymes called as GDP-mannose-4,6 dehydrates and FX protein. By salvage pathway, GDP-fucose is synthesized from free fucose obtained either by catabolism of fucosylated glycans in the lysosome or by diet utilizing fucose kinase and GDP-pyrophosphorylase enzymes. Subsequently, GDP-fucose is transported by carrier protein from cytosol to lumen of golgi apparatus where fucosyltransferase enzyme transfers the fucose moiety to oligosaccharide glycoprotein for N-glycosylation of protein at Asn amino acid site.[10,12] Smoking and other hydrocarbons are very well known to be enzyme inducers.[13] It is likely that either one of the above three proteins or one of them is induced at the genetic loci enhancing the transcription of this protein and thus there is enhanced glucosylation and which in turn is responsible for the proliferation of the cells in oral cancer. This increase in the enzyme perhaps depends on the duration of exposure to such carcinogens which in turn decides the stage of oral cancer. The grade dependent increase of fucose in our study clearly supports the above-stated mechanism of carcinogenesis.

In this study, the normal fucose level in the control group is 7.22 ± 0.26 mg/dl. In contrast, to the established normal fucose level by Parwani RN and Parwani SR[14] was found 5.32 ± 0.67 mg/dl.

Serum fucose level in the present study was significantly found to be elevated among cancer patients (46.63 ± 5.29 mg/dl) when compared to control group (7.22 ± 0.26 mg/dl) as observed by Parwani and Parwani,[14] Bose *et al*.[15] in their study. According to Shah *et al*.[16] high fucosylation one of the characteristic features of malignancies is mainly due to increased activity of fucosyltransferase activity in malignant tissue.

According to Parwani and Parwani, 2011[14] there is no relationship of serum fucose level with histopathological grading but our study shows a significant change in histopathological gradings of oral cancer (mild is 15.11 ± 1.01, moderate is 31.43 ± 2.83 and in severe is 71.27 ± 3.41) showing that level of serum fucose is significantly increased in a stepwise fashion depending on the severity of carcinogenesis as reported earlier by Baxi *et al*.[17]

Conclusions

It is concluded that estimation of serum fucose levels may be used as a biomarker in the diagnosis as well as prognosis of different histopathological grades of oral cancer.

Acknowledgments
Grateful acknowledgments to Prof. R.C. Saxena, Retired Director and Head, KGMU, Chairman, IEC, CPGIDSH; Dr. K. Arvinda, Dr. Jyoti Gupta, and Dr. Vasu S. Saxena of CPGIDSH for their timely help.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1. Stewart BW, Kleihues P, editors. World Cancer Report. Lyon: WHO, International Agency for Research on Cancer; 2003.

2. Rao VR, Krishnamoorthy L, Kumaraswamy SV, Ramaswamy G. Circulating levels in serum of total sialic acid, lipid-associated sialic acid, and fucose in precancerous lesion and cancer of the oral cavity. Cancer Detect Prev 1998;22:237-40.
3. Reibel J. Tobacco and oral diseases. Update on the evidence, with recommendations. Med Princ Pract 2003;12 Suppl 1:22-32.
4. Gokul S. Salivary diagnostics in oral cancer. In: Ogbureke KUE, editor. Oral Cancer. China: InTech; 2012. p. 227-48.
5. Sidransky D. Emerging molecular markers of cancer. Nat Rev Cancer 2002;2:210-9.
6. Nakata D. Increased N-glycosylation of Asn^{88} in serum pancreatic ribonuclease 1 is a novel diagnostic marker for pancreatic cancer. Sci Rep 2014;4:6715.
7. Malati T. Tumour markers: An overview. Indian J Clin Biochem 2007:22:17-31.
8. Dische Z, Shettles LB. A specific color reaction of methylpentoses and a spectrophotometric micromethod for their determination. J Biol Chem 1948;175:595-603.
9. Winzler RJ. Determination of serum glycoproteins. Methods Biochem Anal 1955;2:279-311.
10. Miyoshi E, Moriwaki K, Terao N, Tan CC, Terao M, Nakagawa T, *et al.* Fucosylation is a promising target for cancer dagnosis and therapy. Biomolecules 2012 30;2:34-45.
11. Chhavi S. Studies on involvement of tumor suppressorgene and cell cycle regulation in gynecological malignancies. Thesis. Lucknow: K.G's Medical University; 2011.
12. Becker DJ, Lowe JB Fucose: Biosynthesis and biological function in mammals. Glycobiology 2003;13:41R-53R.
13. Saxena RC, Saxena M. Compendium of drugs. Biochemical Basis. 2nd ed. Lucknow: NMTC Productions; 2010.
14. Parwani RN, Parwani SR. Quantitative evaluation of serum fucose in oral squamous cell carcinoma patients. J Cancer Res Ther 2011;7:143-7.
15. Bose KS, Gokhale PV, Dwivedi S, Singh M. Quantitative evaluation and correlation of serum glycoconjugates: Protein bound hexoses, sialic acid and fucose in leukoplakia, oral sub mucous fibrosis and oral cancer. J Nat Sci Biol Med 2013;4:122-5.
16. Shah M, Telang S, Raval G, Shah P, Patel PS. Serum fucosylation changes in oral cancer and oral prescancerous conditions: Alpha-L-fucosidase as a marker. Cancer 2008;113:336-46.
17. Baxi BR, Patel PS, Adhvaryu SG, Dayal PK. Usefulness of serum glycoconjugates in precancerous and cancerous diseases of the oral cavity. Cancer 1991;67:135-40.