Acta Medica Okayama

Volume 37, *Issue* 6 1983 *Article* 1

DECEMBER 1983

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Abstract

Glycoproteins play a significant role in neoplastic transformations. Both the levels of fucose and the activity of fucosyl transferase, which mediates the assembly of the oligosaccharide moieties of the glycoprotein chains, have been found to be elevated in neoplastic conditions. Since these elevations are common features of a variety of neoplastic cells, these two have been designated as non-specific markers of malignancy. In the present study, the fucose level and fucosyl transferase activity were determined in the sera of cancer patients and an attempt was made to establish a relationship between the two. It was found that both the fucose levels and fucosyl transferase activities showed considerable elevation in the five cancer groups studied, establishing them as useful diagnostic parameters. However, it was also observed that the rate of increased fucosyl transferase activity was not fully reflected in the resulting serum fucose levels in a few cases.

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*PMID: 6666675 [PubMed - indexed for MEDLINE]

Acta Med. Okayama 37, (6), 457-462 (1983)

SERUM FUCOSYL TRANSFERASE ACTIVITY AND SERUM FUCOSE LEVELS AS DIAGNOSTIC TOOLS IN MALIGNANCY

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Received Febrary 23, 1983

Abstract. Glycoproteins play a significant role in neoplastic transformations. Both the levels of fucose and the activity of fucosyl transferase, which mediates the assembly of the oligosaccharide moieties of the glycoprotein chains, have been found to be elevated in neoplastic conditions. Since these elevations are common features of a variety of neoplastic cells, these two have been designated as non-specific markers of malignancy. In the present study, the fucose level and fucosyl transferase activity were determined in the sera of cancer patients and an attempt was made to establish a relationship between the two. It was found that both the fucose levels and fucosyl transferase activities showed considerable elevation in the five cancer groups studied, establishing them as useful diagnostic parameters. However, it was also observed that the rate of increased fucosyl transferase activity was not fully reflected in the resulting serum fucose levels in a few cases.

Key words : glycoprotein-fucose-fucosyl transferase, non specific marker.

Glycoproteins play a significant role in growth regulation and in neoplastic transformation (1, 2). L-fucose, a methyl pentose, is usually found in the terminal nonreducing end and the chitobiose structure of the reducing end of the oligosaccharide chain of glycoproteins. The assembly of the oligosaccharide moieties takes place by the stepwise addition of monosaccharides from their respective nucleotide sugar by the action of glycosyl transferases, which are specific for both the donor and acceptor molecules (3).

Changes in the cell surface occurring during malignant transformation are believed to effect the surface glycoproteins and glycolipids (4). In animal models it has been shown that virally transformed cells contain relatively large amounts of fucose containing glycoproteins (5). It has also been observed that there is an elevation of serum fucose levels in patients with malignant tumors (6). Similar observations have been made in the case of fucosyl transferase which is responsible for linking fucose to the polysaccharide chains (7-8).

It has also been found that the addition of a specific sugar residue to the terminal end of the oligosaccharide chain may stop further glycosylation of the glycoprotein and cause its secretion. Fucose has been demonstrated to be one

such residue (9-11).

Unlike other tumor antigen markers associated with a limited spectrum of tumors, increased fucosyl transferase activity, and hence fucose production, could be a common feature of a variety of neoplastic cells. Therefore the relatively non-specific markers of serum fucose and fucosyl transferase may have wider clinical application. The present study furnishes information with respect to the possible relationship between the two markers in the sera of patients with different forms of malignancies.

MATERIALS AND METHODS

The patients of this study were selected from the outpatient department of Chittaranjan Cancer Hospital among those who have not received any previous treatment. Matched controls included ten healthy females aged twenty-five to fifty and nine healthy males aged thirty to fifty-five.

The patients were divided into five groups depending on the tumor type : cervical carcinoma, laryngeal carcinoma, bronchogenic carcinoma, oral carcinoma, and lymphoma. There were twelve cases of cervival carcinoma, nine of which were histologically classified as squamous cell carcinoma grade II-III and the other three as adenocarcinoma grade I = II ; 14 cases of oral carcinoma of squamous cell type I-III, and twelve cases of laryngeal carcinoma of squamous cell carcinoma grade II-III. Bronchoscopy was performed on seven bronchogenic carcinoma patients of whom three were diagnosed as adenocarcinoma grade I-II and two as squamous cell variety grade II-III. There were eight cases of lymphoma, most of them being non-Hodgkin and, histologically, predominantly lymphocytic. Blood was collected intravenously and allowed to stand for 4 hours for collection of serum. A portion of the serum was stored at −80 ℃ until use for the fucosyl transferase assay.

Changes in the glycoprotein content of the serum were determined by measuring the quantitative changes in glycosyl moieties at the terminal end of the glycoprotein chains. The protein bound serum fucose content of the serum was measured by the method of Dische and Shettles (12). The protein in 0.1 ml of serum was precipitated with 95 % ethanol. The precipitate was dissolved in 1 ml of 0.1 N NaOH, and heated with 4.5 ml conc. H_2SO_4 (6 : 1) for exactly 3 min. After cooling 0.1 ml of 3 % cysteine reagent was added, and the preparation was kept at room temperature for 60-90 min. Methyl pentose (20 μg/ml) was used as a standard. The optical density was measured at 396 mμ and 430 mμ, in order to correct for non-specific colour development.

The activity of serum fucosyl transferase was determined by measuring the rate of transfer of free fucose moieties to the glycoprotein chains. The determination was carried out after the method of Chatterjee *et al.* (13). The standard incubation medium contained 50 mM Tris HCl (pH 7.0), 2.5 mM EDTA (pH adjusted to 7.0), 0.1 % Triton X, about 0.5 mg ovomucoid as acceptor (Trypsin inhibitor Type 11-0, Sigma), 3 μM of GDP −C^{14} fucose (specific activity 192.0 mCi/m mol, New England Nuclear, Boston, Mass), and serum which contained approximately 50 μg of homogenate protein in a total volume of 100 μl. After incubation at 37 ℃ for 60 min. the reaction was terminated with 1 % PTA in 0.5 N HCl. The precipitate was collected on 0.22 μ Millipore Filters (Millipore Corporation, Bedford, Mass) and washed three times with the same medium. The filters were further washed with a 2 : 1 mixture of chloroform-methanol and dried in the scintillation vials ; radioactivity was determined after addition

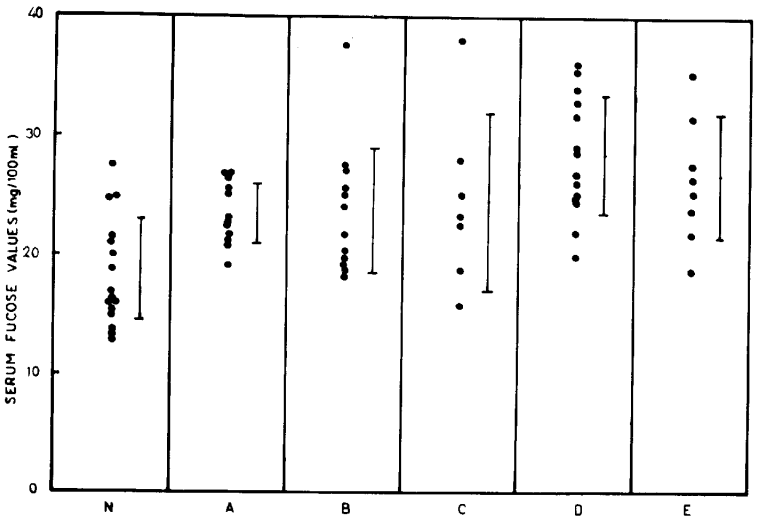

Fig. 1. Serum fucose values expressed as mg/100 ml serum in normal subjects and in patients with different types of malignancies : N=Normal, A=Cervical carcinoma, B=Laryngeal carcinoma, C=Bronchal carcinoma, D=Oral carcinoma and E=Lymphoma. P values are <0.05 in A, B, D, E, but <0.1 in C, compared to the normal subjects.

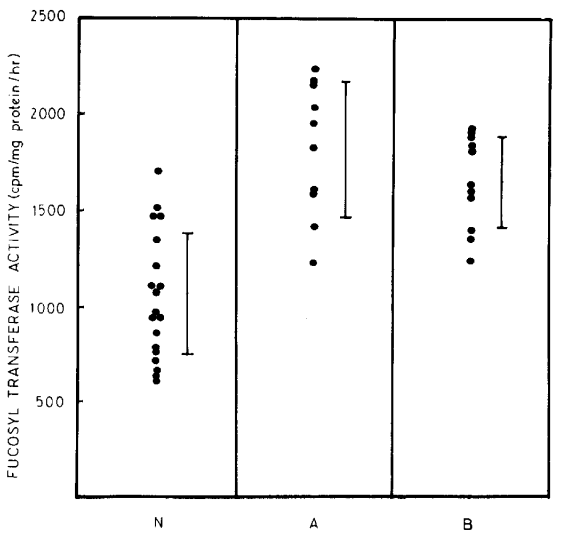

Fig. 2. Serum fucosyl transferase activity expressed as cpm/mg protein/h. N=Normal, A=Cervical carcinoma and B=Oral carcinoma. P values are <0.05 in A, B compared to the normal subjects.

of 10 ml of toluene based fluor. Serum protein was estimated according to the method of Lowry *et al.* (14). The results were statistically analysed by Student's *t* test.

The mean serum fucose values in normal males and females was 18.8 ± 4.2 ; in cervical carcinoma, 23.7 ± 5.3 ; in oral carcinoma, 27.7 ± 5.4 ; in bronchogenic carcinoma, 24.6 ± 7.5, and in lymphoma, 26.3 ± 5.2 mg/100 ml serum, serum fucose values were significantly elevated in all cases of malignancies ($p < 0.05$) except the bronchogenic carcinoma cases ($p < 0.1$) as shown in Fig. 1.

Fucosyl transferase activity in normal persons was 1059 ± 325 ; in cervical carcinoma patients, $1822 + 345$, and in oral carcinoma patients, 1647 ± 242 cpm/mg protein/h, the latter two being the only groups tested of the five groups of patients with malignancies. These results show that the fucosyl transferase activity was significantly high in both malignancy groups ($p < 0.05$) compared to the normal group (Fig. 2).

DISCUSSION

A great number of studies have been devoted to tumor-associated serum glycoproteins, the biological specificity of which has been found to reside in the carbohydrate portion of the macromolecules (1-2). Alterations in the specificity of the level of glycosyl transferase during malignant transformations are reflected in alterations in the levels of the carbohydrate moieties (15-18). Higher activity of fucosyl transferase in the tumor may result in faster completion and secretion of some tumor-associated glycoprotein antigens (13).

In the fucosyl transferase assay done in this study, ovomucoid was used as the glycoprotein acceptor as was done by Ip *et al.* (19) and Reddy *et al.* (20). The rate of fucose transfer to the substrate is proportional to the time of incubation and quantity of serum added. Since there were more than one type of *fucocyl* transferase in the serum, each being substrate specific, discrepancies are to be found in the results of different groups of workers. Glycosyl transferase enzymes are primarily located in the Golgi apparatus (21), but recent biochemical (22, 23) and electron microscopic evidence (24) shows that these enzymes are also present on the cell surface. It has been suggested that abnormal levels of glycosyl transferase in the circulation are due to the shedding of plasma membrane material from the neoplastic cells (25, 26).

In the present experiment it was observed that both the serum fucosyl transferase activity as well as serum fucose levels were considerably elevated in cases of malignancies. The results indicate that in combination, these two parameters could act as useful parameters for diagnostic purposes. It was noticed, however, that rate of increase in fucosyl transferase activity, paticularly in individual patients of the cervical carcinoma group was not proportionally reflected in the serum fucose levels in all cases. This could be due to the fact that of the total fucosyl transferase released from the tumor cell into the circulation of the host, a substantial portion may be defective or converted to an inactive from after synthesis, a supposition which needs further investigation.

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