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Changes in Erythrocyte Membrane Glycoproteins During Oral Cancer

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INTRODUCTION

It is well known that normal somatic cell with normal functions suddenly undergo changes either by mutation or by addition of some unknown protein to its environment and become a cancer cell. Many workers have established that new cell surface antigens appear following chemical, viral or by spontaeous transformations. It is a established and confirmed fact that antigenic determinants on the surface of erythrocytes and other cell are carried by both glycolipids and glycoproteins, but soluble blood group substances are strictly glycoproteins in nature. (Lipton et al., 1979; Harvey et al., 1981; Patel et al., 1997). These membrane glycoproteins appear to be asymmetrically distributed on the inner and outer side of the bilayer. In the erythrocyte membrane they are expressed only in the exterior surface. (Eylar et al., 1962). Fukuda and Osawa (1973) estimated that in RBC the total carbohydrate of the glycoprotein is nearly 55%. It has been widely studied that many biochemical variations such as, in cell adhesion, loss of contact inhibition, proliferative growth, antigenic properties, do take place. Hasija (1991), Lis and Sharon (1973, 1981) have shown that noticeable changes in binding qualities of blood group substances with lectins occur with the change in glycomoieties of the glycoproteins. It is also evident by crystallization test (Hasija and Saxena, 1986).

Baxi et al. (1991) and Ira et al. (1958) described the carbohydrates moieties as major erythocyte antigens and said that changes in the cell carbohydrates, during malignant development, involve the blood antigens. Rh antigens which are also in the native membrane are thought to exist as a complex Rh polypeptides and Rh related glycoproteins.

Therefore, the present studies were, undertaken to differentiate between erythrocyte membrane glycoprotein in oral cancer patient's blood as compared to normal healthy person's having same blood group and Rh typing, qualitatively and quantitatively both.

MATERIALS AND METHODS

Quantitative Estimations: The blood samples of 100 patients was collected in EDTA bottle. Blood samples of normal subjects were taken as control group. Five samples of each type of blood group and Rh typing pair were taken (A+, A-, B+, B-, AB+, AB-, O+, O-). The matching of test and control was done according to the blood group, Rh typing and to the nearest possible total leucocyte count and Haemoglobin values. Glycoprotein was extracted from erythrocyte membrane of control group and from the test group before and after radiotherapy (BT and AT).

Extraction of Glycoprotein: Glycoprotein was extracted by the method of Bolmer and Davidson (1981). The erythrocytes was washed with 0.9% sodium chloride in 5 ml Tris - HCl buffer pH 7.4; hemolyzed with 9 volumes of 10 ml and Tris 0.1 EDTA HCL buffer (pH 7.4) and centrifuged for 20 minutes. The membranes were mechanically homogenized and diluted with Tris EDTA buffer. All steps were carried out at low temperature. The above membrane suspension was stirred vigorously for 30 minutes at room temperature, centrifuged to separate aqueous layer through aspiration and again centrifuged at high speed for 1 hour at 4°C. The clear supernatent, thus obtained, contained glycopro-teins. This aspirate was dialyzed against distilled water, lyophilized and stored in refrigerator at 4°C, until analysed.

Total protein was estimated by Lowry's method (1951). Protein bound total carbohydrate was estimated by the method of Dubois et al. (1951).

Qualitative Analysis: For studying the qualitative changes in sugar specificity of the receptors on the erythrocyte membranes, binding assay was done with the help of three lectins prepared by the method of Dunsford and Bowley (1967). The Haemagglutination inhibition

reaction was performed in normal subject's blood which was taken as control.

Seed extracts of *Pisum sativum, Ricinus communis* and *Triticum vulgaris*, were used as lectin.

Red cell suspension was prepared in phosphate buffer saline (PBS) (pH 7.4). The packed cells were resuspended in PBS to prepare 10% suspension of red cells.

Sugar solution (0.2 M) of arabinose, cellobiose, fructose, fucose, galactose, glucose, lactose, maltose, mannose, melibiose, raffinose, rhamnose, ribose, xylulose, were prepared and kept frozen.

Haemagglutination inhibition reaction was tested by the method of Bhatia and Boyd (1962).

One drop of lectin (titre 1:16) was mixed with equal volume of 0.2 M sugar solution in a microtitre plate in different cavities and kept for 2 hours at room temperature. One drop of each appropriate red cell suspension was added to the respective cavity and mixed. The results were noted with naked eye after 30 minutes.

RESULTS

I. Quantitative Estimations

Total Protein: It is clear from the table that there is a reduction in the amount of total protein of the glycoprotein in oral cancer erythrocyte membrane before and after chemotherapy as compared to normal subjects. After therapy it was all the more reduced when compared with before therapy. The protein content was reduced in oral cancer by 13.0% (BT) and 36.17% (AT) as compared to control group. It is fully suggestive that this further decrease in the quantity of total protein may be due to the damage done to membrane protein by radiation.

Protein Bound Total Carbohydrates: Protein bound total carbohydrates which is generally neutral sugars were drastically reduced in all the cancer patient's erythrocyte membrane glycoproteins as compared to normal subject's, in general, before the radiotherapy but there was a little increase after the radiotherapy (Table 1). Increase in the quantity of protein bound total carbohydrates after the complete course of radiotherapy is indicative that radio therapy has certain effect to restore the glycomoieties of the erythrocyte membrane glycoprotein in maligancy, as there is an increase in the binding of sugars.

The quantitative estimations have revealed that the protein part of the glycoprotein as well as the carbohydrate bound to the protein both decrease before radiotherapy. It suggests that malignancy effects the red blood cell's surface receptors. However, after the course of treatment the protein was further decreased while the carbohydrate content increased to some extent showing that it effects the sugar binding sites on the membrane receptors.

II. Qualitative Analysis

The binding assay with haemagglutination inhibition (HI) reaction with *Ricinus communis,*

Table 1: Total protein and protein bound total carbohydrates in rbc membrane glycoprotein in oral cancer.

Blood Group and Rh typing	*Total Protein (mg/ml)*			*Protein bound total Carbohydrate (mg/ml)*		
	Control	*BT*	*AT*	*Control*	*BT*	*AT*
A+	4.28	3.66	2.93	5.72	1.37	1.75
A-	3.4	-	-	6.75	-	-
B+	4.8	3.59	2.79	5.53	1.32	1.7
B-	4.2	-	-	6.11	-	-
AB+	3.4	-	-	6.66	-	-
AB-	4.42	-	-	5.83	-	-
O+	4.6	3.92	2.43	5.65	1.46	1.91
O-	4.72	3.15	2.66	5.29	1.67	1.84

BT= Before treatment
AT= After treatment
Conclusion of 5% level of P
S.E.M. = 0.8
C.D. (5%) 1.53

Pisum sativum and *Triticum vulgaris* lectins were performed and are presented in table 2, 3, 4. With *Ricinus communis* lectin (Table 2), blood group A+, all sugars were present on the cell surface of erythrocytes but were absent in test in varying degree. In B+, Arabinose, Fructose, and Raffinose were present in control but were absent in test; in second Galatose and Mannose were absent in control but present in test before treatment, and again was absent after treatment (AT). In O- group, Fructose, Glucose and Maltose were present in control but were absent in test. With *Pisum sativum* lectin (Table 3) A+ group, Fructose, Galactose, Lactose, Maltose and Melibiose were present in control but were absent in test; in B+ Arabinose, Cellobiose, Fucose, Galactose, Melibiose, Raffinose, Ramnose and Xylose were present in control but absent in test. Secondarily Cellobiose and Melibiose were present in control but absent in test.

In O- group Galactose, Maltose, Mannose, Ribose and Xylose were present in control but absent before treatment which again appeared after treatment. Arabinose, Cellobiose, Lactose, Melibiose and Rhamnose were present in control but absent in test. With *Triticum vulgaris* lectin (Table 4) with blood group A+, Cellobiose, Lactose and Maltose were present in control but absent in test. Rest of the receptor sites were same in test and control; in blood group B+ only

Table 2: Haemagglutination-inhibition reaction with *Ricinus communis* Lectin

Blood group	*A+*			*B+*			*O+*			*O-*		
sugar	*C*	*BT*	*AT*	*C*	*BT*	*AT*	*C*	*BT*	*AT*	*C*	*BT*	*AT*
Arabinose	+	-	-	+	-	-	-	-	-	-	-	-
Callobiose	+	-	-	-	-	-	+	-	-	+	-	-
Fructose	+	-	-	+	-	-	+	-	-	+	-	-
Fucose	+	-	-	-	-	-	-	-	-	-	-	-
Galactose	+	-	-	-	+	-	-	-	-	-	-	-
Glucose	+	-	-	-	-	-	+	-	-	+	-	-
Lactose	+	-	-	-	-	--	-	-	--	-	-	-
Maltose	+	-	-	-	-	-	+	-	-	+	-	-
Mannose	+	-	-	-	+	-	+	-	-	-	-	-
Melibiose	+	-	-	-	-	-	-	-	-	-	-	-
Raffinose	+	-	-	+	-	-	-	-	-	-	-	-
Rhammose	+	-	-	-	-	-	-	-	-	-	-	-
Ribose	+	-	-	-	-	-	+	-	-	-	-	-
Xylose	+	-	-	-	-	-	+	-	-	-	-	-

C=Control BT=Before Treatment AT=After Treatment

Table 3: Haemagglutination-inhibition reaction with *Pisum sativum* Lectin

Blood group	*A+*			*B+*			*O+*			*O-*		
sugar	*C*	*BT*	*AT*	*C*	*BT*	*AT*	*C*	*BT*	*AT*	*C*	*BT*	*AT*
Arabinose	+	+	+	+	-	-	-	-	-	+	-	-
Cellobiose	+	-	-	+	-	-	+	-	+	+	-	-
Fructose	+	-	-	-	-	-	-	-	-	-	-	-
Fucose	+	+	+	+	-	-	-	-	-	-	-	-
Galactose	+	-	-	+	-	-	+	-	-	+	-	+
Glucose	+	-	-	-	-	-	-	-	-	-	-	-
Lactose	+	-	-	+	+	+	+	-	-	+	-	-
Maltose	+	-	-	-	-	-	-	-	-	+	-	+
Mannose	+	+	+	+	+	-	-	-	-	+	-	+
Melibiose	+	-	-	+	-	-	+	-	+	+	-	-
Raffinose	+	+	+	+	-	-	+	-	-	-	-	-
Rhammose	+	+	+	+	-	-	+	-	-	+	-	-
Ribose	+	+	+	+	+	-	-	-	-	-	-	-
Xylose	+	+	+	+	-	-	-	-	-	-	-	+

C=Control BT=Before Treatment AT=After Treatment

Table 4: Haemagglutination-inhibition reaction with *Tritiaum vulgaris* Lectin

Blood group	*A+*			*B+*			*O+*			*O-*		
sugar	*C*	*BT*	*AT*	*C*	*BT*	*AT*	*C*	*BT*	*AT*	*C*	*BT*	*AT*
Arabinose	-	-	-	-	-	-	-	-	-	-	-	-
Cellobiose	+	-	-	-	-	-	-	-	-	-	-	-
Fructose	-	-	-	-	-	-	-	-	-	+	-	-
Fucose	-	-	-	-	-	-	-	-	-	+	-	-
Galactose	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	-	-	-	-	+	-	-	-	-	+	-	-
Lactose	+	-	-	-	-	-	-	-	-	+	-	-
Maltose	+	-	-	-	-	-	-	-	-	-	-	-
Mannose	-	-	-	-	-	-	-	-	-	+	-	-
Melibiose	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-
Rhammose	-	-	-	-	-	-	-	-	-	-	-	-
Ribose	-	-	-	-	-	-	-	-	-	-	-	-
Xylose	-	-	-	-	-	-	-	-	-	-	-	-

C=Control BT=Before Treatment AT=After Treatment

glucose was absent in control but present in test; before treatment which again disappeared during treatment. In blood group O+ all fourteen sugars were absent in both control and test indicating same receptor site; in O- group, Fructose, Fucose, Glucose, Lactose and Mannose were present in control but absent in test. (cases in blood group and Rh typing of A-, B-, AB+ and AB-, were not available).

DISCUSSION

The names of sugars which are not mentioned above gave similar HI reaction in test and control, showing similar receptor sites on both the erythrocyte membrane glycoproteins. It is evident that the mature red cells do not synthesize any protein and therefore glycoproteins must be synthesized at an early stage of erythroid differentiation. The expression and degree of glycosylation are strictly regulated by the carbohydrate moiety which may be important for various interactions (Mali et al. 1977). Xie et al. (2000) working on P-glycoprotein expression in carcinoma of the oral region reported that P-glycoprotein was detected in 62.5% of all samples and expression was related to the chemotherapy and the differentiation of carcinoma. P-glycoprotein expression was higher in post-chemotherapy group. Ralhan et al. (1997) reported that the differential expression of P-glycoprotein may be an index of the disease prognosis in oral cancer patients in the context of the Indian populations. It can be speculated that antigen are not, direct gene product as they consist of monosacchardides rather than amino acid subunits only. The biological functions of carbohydrate moiety is not known. We can only speculate of its major involvement in the process of malignancy.

The first association between blood group A and carcinoma disease other than haemolytic disease has been demonstrated (Aird, 1995). Association of lip cancer with blood group B was also significantly demonstrated (Alexander, 1921). The blood group activities of the soluble glycoproteins were assessed by their ability to inhibit agglutination of red blood cells by specific lectins. It was found that ABO specificity of soluble glycoproteins are carried by monosaccharide units. Lectins or agglutinins are proteins containing covalently bound carbohy-drates that bind specifically to saccharide moieties in glycoproteins or glycolipids on the cell surface without modifying them chemically.

Binding is reversible and all lectins have more than one specific carbohydrate binding sites. Those lectins which can agglutinate red blood cells irrespective of any specific blood group are known as "Non specific haemagglutinins". Thus agglutination can be inhibited by the addition of relevant sugars, which competes with the cell bound residues of lectins and so displaces it from the cell. Thus inhibition of agglutination by given sugar implies the presence of that sugar on the cell surface.

De Roock et al. (2000) found that human tumor cell progression and metastatis are partially dependent on the ability of a tumor cell to adhere to the proteins of the extracellular matrix and survive at the distant location. Their results further indicate that biologically active D-amino acid containing peptides that themselves support tumor cell adhesion and can inhibit tumor cell adhesion to immobilized proteins or dermal fibroblasts.

Tewarson et al. (1993) is of the opinion that all cell surface membrane components play a prominent role in neoplastic behaviour. Any intercellular, micro environmental change may lead to alteration in the surface membrane constituents releasing certain molecules in the blood of such patients.

Present result conclusively show that quantitatively the protein part of the glycoprotein is reduced in malignancy before and after treatment. Reduction in total protein, after the treatment is significant. Protein bound total carbohydrates are also drastically reduced which shows the definite involvement of carbohydrate moieties of the glycoprotein in malignancy. However, some of the glycomoieties are restored after the treatment as there is 5-9% increase in the binding of sugars by the membrane receptors.

The protein and protein bound total carbohydrates both decrease in malignancy before and after treatment. It suggests the malignancy has its effect on the erythrocyte membrane glycoprotein as a whole. It was further observed that after the radiotherapy the protein was further decreased while the carbohydrate content was increased, showing the changes on the binding site of the membrane receptors.

Similar results have been reported by Wilma et al. (2001). Qualitative analysis shows that with the help of lectins one can even pin point the sugar which appear or disappear due to malignancy. In some cases the glycomoiety returns after the therapy as in case of normal RBC membrane. It is peritinent to record that one can speculate the susceptibility to cancer by taking the blood sample and testing it for glycoprotein. This is much easier than taking the biopsy sample in certain cases. In such cases only one drop of blood is needed and within 2 hours the results can be obtained.

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KEY WORDS RBC Membrane. Glycoproteins. Oral Cancer.

ABSTRACT Quantitative and qualitative changes in erythrocyte membrance glycoproteins during oral cancer before and after radiotherapy, were investigated in one hundred patients and compared with their normal counter parts. Quantitatively total protein and protein bound total carbohydrates (neutral sugars) in the glycoprotein extracted from the erythrocyte membrane were estimated. It showed significant reduction in total protein and protein bound total carbohydrates in cancer patients before radiotherapy. However, after the complete course of radiation there was further reduction in total protein by 23.17% and increase in total carbohydrates by 5.91%. Qualitatively, the studies reveal the appearance and disappearance of sugar before chemotherapy and after chemotherapy. The studies could be helpful in knowing the susceptibility to cancer.

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