

Erythrocyte Membrane Glycoprotein Changes During Cancer Cervix

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KEY WORDS Glycoproteins. Carcinoma Cervix. Diagnosis. Radiotherapy

ABSTRACT Erythrocyte membrane glycoproteins changes during, cancer cervix, were investigated. The total protein and protein bound total carbohydrates (neutral sugars) in the glycoprotein of cancer patients showed significant reduction before radiotherapy. There was further reduction in total protein by 11.59% whereas there was increase in total carbohydrates by 8.28% after therapy. Qualitative analysis reveals changes in membrane sugar which could be of diagnostic value for cancer cervix.

INTRODUCTION

The antigenic determinants on the surface of erythrocytes and other cell are carried by both glycolipids and glycoproteins however, soluble blood group substances are strictly glycoproteins in nature (Lipton et al., 1966; Harvey et al., 1981; Patel et al., 1997). In the erythrocyte membrane they are expressed only in the exterior surface (Elyar et al., 1962). In RBC the total carbohydrate of the glycoprotein are nearly 55% (Fuku do et al., 1973). Changes in binding qualities of blood group substances with lectins occur with the change in glyco- moieties of the glycoproteins (Hasija, 1991; Lis and Sharon, 1973, 1981). These changes in cell surface carbohydrates, during malignancy development, involve the blood antigens (Baxi et al., 1991; Ira et al., 1958).

The present studies, therefore, were undertaken to differentiate between erythrocyte membrane glycoprotein in cancer cervix patient's blood as compared to normal healthy person, qualitatively and quantitatively both.

MATERIALS AND METHODS

Quantitative Estimations: The blood samples of 100 patients was collected in EDTA bottle. Blood sample of normal subjects were taken as control : 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 chemotherapy (BT & AT) by the method of Bolmer and Davidson (1981). Total protein was estimated after Lowry et al. (1951) while 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 vulgare*, were used as lectin.

Sugar solution of 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 AND DISCUSSION

Quantitative Estimations

Table 1 shows that there is a reduction in the amount of total protein of the glycoprotein in

Table 1: Total protein and protein bound total carbohydrates in RBC membrane glycoprotein in cancer cervix.

Bt. Gr. & Rh. Typing	Total Protein (mg/ml)			Protein bound total carbohydrate (mg/ml)		
	Control	BT.	AT.	Control	BT.	AT.
A+	4.28	4.08	2.88	5.72	3.45	4.20
A-	3.4	3.14	2.87	6.75	5.72	6.72
B+	4.8	3.61	3.2	5.3	4.79	5.10
B-	4.2	4.19	3.9	6.11	5.01	5.23
AB+	3.4	3.08	2.58	6.66	6.23	6.55
AB-	4.42	-	-	5.88	-	-
O+	4.6	3.61	3.59	5.65	4.97	5.38
O-	4.72	3.89	3.26	5.29	4.95	5.00

BT = Before treatment

AT = After treatment

Conclusion at 5% level of P.

S.Em = 1.1

C.D. = 2.07

cancer patient's blood before and after chemotherapy as compared to normal subjects. Protein bound total carbohydrates were drastically reduced, in general, before the radiotherapy but there was a little increase after the radiotherapy. Increase in the quantity of protein bound total carbohydrates, after the complete course of radiotherapy, indicates restoration of glycomoieties and glycoprotein. 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 was increased to some extent showing that it effects the sugar binding sites on the membrane receptors.

Present result conclusively show that quantitatively the protein part of the glycoprotein is reduced in malignancy before and after radiotherapy. 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.

Recently, Connor et al. (2000) working on glycodelin - A reported that biological normal and neoplastic cervix have glycodelin - A which has immunosuppressive effect and has a role in cervix.

Carrilho et al. (2000) have reported mucin and simple mucin type carbohydrates are cancer associated antigens in several human tumor.

They have recently identified mucin like glycoprotein (gp 230) which is associated with malignant transformations at a preinvasive stage during carcinoma of human cervix.

Qualitative Analysis

The binding assay with haemagglutination inhibition reaction with *Ricinus communis*, *Pisum sativum* and *Triticum vulgaris* lectins are presented in table 2, 3, 4. With *Ricinus communis* lectin (Table 2), shows that with blood group A+, Cellobiose, Lactose and Maltose were present in control but absent in test, Fucose, Galactose Glucose and Maltose were present in control but absent in test, Fucose, Galactose Glucose and Mannose were absent in control but present in test (BT) while they again disappeared (AT). In A-, Galactose, Maltose, Mannose, Melibiose, Ribose and xylose were absent in control but present in test, in B-, only Xylose was present in control but absent in test (BT) while they were again present after treatment. Only Melibiose, was absent in control but was present in test. In AB+ blood group Cellobiose and Lactose were present in control but absent in test (BT) while they were present after treatment; In O+; only Fructose was the sugar which was absent in control but present in test. Only Mannose was absent in control but present in test (BT) which again appeared after treatment. In O-, Fructose, Fucose, Glucose, Lactose and Mannose were present in control but absent in test. With *Pisum sativum*, lectin, (Table 3), the result show that in blood group A+, Arabinose, Fucose, Galactose, Ribose and Xylose were absent in control but were present in test. Only Maltose was present in control but absent in test. In A-, Arabinose, Mannose, Ribbose and Xylose were absent in control but present in test; in B+ Cellobiose, Glucose and Xylose were absent in control but present in test in (BT) while they were again absent after treatment. Fucose and Raffinose were absent in control but present in test (BT) while they again disappeared after treatment. In group AB+, Arabinose, Fructose, Fucose, Galactose, Glucose, Mannose, Raffinose and Xylose were absent in control but present in test. In O+ Arabinose and Lactose were absent in control but present in test. In O- blood Only Fructose was present in control but absent in test. Lactose and Rhamnose are absent in control but present in test. With

Table 2: Haemagglutination - inhibition reaction with *Recinius communis* lectin in cancer cervix

Blood Group	A+			A-			B+			B-			AB+			O+			O-		
	C	BT	AT	C	BT	AT	C	BT	AT	C	BT	AT	C	BT	AT	C	BT	AT	C	BT	AT
Sugars																					
Arabinose	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
Cellobiose	+	-	-	-	-	-	-	+	+	-	-	-	+	-	+	-	-	-	-	+	+
Fructose	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+	+	-	-	-
Fucose	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Galactose	-	+	-	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Glucose	-	+	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	-	+	-	-
Lactose	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	-
Maltose	+	-	-	-	+	+	-	-	-	+	-	+	+	+	+	-	-	-	-	-	-
Mannose	-	+	-	-	+	+	-	+	+	+	+	+	-	-	-	+	-	+	-	-	-
Melibiose	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ribose	-	-	-	-	+	+	-	+	+	-	-	-	+	-	-	-	-	-	-	-	-
Xylose	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

C : Control
 BT : Before Treatment
 After Treatment

Table 3 : Haemagglutination - inhibition reaction with *Pisum - sativum* lectin in cancer cervix

Blood Group	A+			A-			B+			B-			AB+			O+			O-		
	C	BT	AT	C	BT	AT	C	BT	AT	C	BT	AT	C	BT	AT	C	BT	AT	C	BT	AT
Sugars																					
Arabinose	-	+	+	-	+	+	-	-	-	+	-	-	-	+	+	-	+	+	+	+	+
Cellobiose	+	+	+	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	+	+	+
Fructose	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	+	-	-
Fucose	-	+	+	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	-
Galactose	-	+	+	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	-
Glucose	-	-	-	-	-	-	-	+	-	+	-	-	-	+	+	-	-	-	+	+	+
Lactose	+	+	+	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	-	+	+
Maltose	+	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-
Mannose	-	-	-	-	+	+	-	-	-	+	-	-	-	+	+	-	-	-	+	+	+
Melibiose	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	+
Ribose	-	+	+	-	+	+	-	-	-	-	-	-	+	+	+	-	-	-	+	+	+
Xylose	-	+	+	-	+	+	-	+	-	+	-	-	-	+	+	-	-	-	+	+	+

C : Control
 BT : Before Treatment
 AT : After Treatment

Table 4: Haemagglutination - inhibition reaction with *Triticum - vulgaris* lectin in cancer cervix

Blood Group	A+			A-			B+			B-			AB+			O+			O-		
	C	BT	AT	C	BT	AT	C	BT	AT	C	BT	AT	C	BT	AT	C	BT	AT	C	BT	AT
Sugars																					
Arabinose	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-
Cellobiose	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-
Fructose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Fucose	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	+
Galactose	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	+
Glucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Lactose	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	-	-
Maltose	+	-	-	-	-	-	-	-	-	+	-	-	+	+	+	-	+	-	-	-	-
Mannose	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	+	-	+
Melibiose	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ribose	-	+	-	-	+	-	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-
Xylose	-	+	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-

C : Control
 BT : Before Treatment
 AT : After Treatment

Triticum vulgaris (Table 4) the result show that in blood group A+, Cellobiose, Lactose and Maltose were present in control but absent in test. Ribose and Xylose were absent in control but present in test (BT) while they were again absent after treatment. In blood A-, Ribose and Xylose were absent in control but present in test (BT) absent after treatment. In blood B+ Arabinose, Ribose and Xylose were absent in control but present in test (BT) and absent after treatment. In B- Arabinose, Galactose, Maltose, Mannose and Xylose were present in control but absent in test. Only Melibiose, was absent in control but present in test (BT) and was absent in after treatment. In blood AB+, Galactose and Mannose were absent in control but found present in test. Fucose and Melibiose were absent in control but present in test (BT) and absent in after treatment. In blood O+, Glucose Maltose and Melibiose were absent in control but were present in test (BT) and absent in after treatment. In blood O-, Fucose and Lactose were present in control but absent test (BT). Fucose, Galactose and Mannose were present in control but absent in test (BT) and were present after treatment again (blood group and Rh typing of AB- were not available in carcinoma cervix).

The result show that ABO specificity of soluble glycoproteins are carried by monosaccharide units. Lectins or agglutinins are proteins containing covalently bound carbohydrates 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.

Tewarson et al. (1993) also observed that change in micro environment may lead to alteration in the surface membrane constituents releasing certain molecules in the blood of such patients. Qualitative analysis reveals that with the help of lectins one can pin point the sugars which appear or disappear due to malignancy. It is pertinent to record that one can speculate the susceptibility to cancer by testing it for glycoprotein. This is a convenient method than

taking the biopsy sample in certain cases.

ACKNOWLEDGEMENT

The author is thankful to M.P. Council of Science & Technology for financial support.

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