CHANGES IN ERYTHROCYTE MEMBRANE GLYCOPROTEINS DURING LEUKAEMIA

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ABSTRACT

Changes in erythrocyte membrane glycoproteins during, leukaemia were investigated in one hundred patients. Control group contained normal ones. The total protein and protein bound total carbohydrates (neutral sugars) in the glycoproteins extracted from the erythrocyte membrane showed significant reduction in total protein and protein bound total carbohydrates before radiotherapy. However, after the radiotherapy there was further reduction in total protein by 10.41% whereas there was increase in total carbohydrates by 9.98%. Qualitative analysis reveals that with the help of lectins one can pin point the sugars which appear or disappear due to leukaemia. The test could be of a diagnostic value.

KEY WORDS

RBC membrane, Glycoproteins, Leukaemia.

INTRODUCTION

It is an established and confirmed fact that antigenic determinants on the surface of erythrocytes and other cells are carried by both glycolipids and glycoproteins. However, soluble blood group substances are strictly glycoproteins in nature (1,2,3). Glycoproteins appear to be asymmetrically distributed on the inner and outer side of the bilayer whereas in the erythrocyte membrane they are expressed only on the exterior surface (4).

It has been estimated that in RBC the total carbohydrate of the glycoprotein is nearly 55%. Many biochemical variations such as, in cell adhesion, loss of contact inhibition, proliferative growth, antigenic properties, do take place on RBC (5). Further studies have also shown that changes in binding qualities of blood group substances with lectins occur with the change in glycomoieties of the glycoproteins (6,7,8). It is also evident by crystallization test (9).

Carbohydrate moieties are described as major erythrocyte antigens and changes in the cell surface carbohydrates, during malignant development, involve the blood antigens (10, 11). Blood group structure of ABC (H) system have cell surface carbohydrates. Rh antigens which are also in the native membrane are thought to exist as complex Rh polypeptides and Rh related glycoproteins.

The present study was, therefore, undertaken to differentiate between erythrocyte membrane glycoprotein in leukaemia patient's blood as compared to normal healthy person's having same blood group and Rh typing, both qualitatively and quantitatively.

MATERIALS & METHODS

The blood samples of 100 patients were 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 chemotherapy (BT & AT).

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Glycoprotein was extracted by the method of Bolmer & Davidson (12). Total protein was estimated by Lowry's method (13). Protein bound total carbohydrate was estimated by the method of Dubois *et al* (14).

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 & Bowley (15). 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.

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 solutions (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 & Boyd (16).

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 & DISCUSSION

1. Quantitative Estimations

It is clear from Table No. 1 that there is a reduction in the amount of total protein of the glycoprotein in leukaemia patient's erythrocyte membrane before and after chemotherapy as compared to normal subjects. The protein content was also reduced in leukaemia. Protein bound total carbohydrates were drastically reduced before the treatment but there was a little increase after the treatment.

The estimations have further revealed that the protein part of the glycoprotein as well as the carbohydrate bound to the protein both decrease before treatment. It suggests that leukaemia affects 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 affects the sugar binding sites on the membrane receptors.

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. It can be speculated that antigens are not direct gene product as they consist of monosaccharides rather than amino acid subunits only. Their biological functions as carbohydrate moiety are not known. We can only speculate of its major involvement in the process of malignancy.

II. Qualitative Analysis

The binding assay with haemagglutination inhibition (HI) reaction with *Ricinus communis*, *Pisum sativum* and *Triticum vulgare* lectins were performed and are presented in Tables 2, 3, 4. The results show that in leukaemia with *Ricinus communis* lectin (Table - 2), in blood group A+, all sugars were present in control but fucose, glucose, lactose & Rhamnose were absent in test; in A-, Ribose and Xylose only were present in control and test groups, other sugars were absent; in B+, Arabinose, Fructose, Fucose and Melibiose were present in control while they were absent in test; in AB+ Fructose was the only sugar which was present in control but was absent in test. With lectin *Pisum sativum* (Table - 3), in blood group A+, all sugars were present in control but were absent in test; in A-, Arabinose, Cellobiose, Fucose, Galactose, Lactose, Mannose, Melibiose, Raffinose, Rhamnose and Xylose were present in control but were absent in test; in B+, Cellobiose, Fucose, Rhamnose, Ribose and Xylose were present in control but absent in test where as Lactose and Melibiose were present in control, which disappeared in test and again appeared after treatment. In AB+ all sugars were

present in control but absent in test; in O+ Arabinose, Fructose, Fucose, Glucose, Maltose and Mannose, Ribose and Xylose were absent in control but they appeared in test before treatment and again disappeared after treatment. Cellobiose, Galactose, Lactose, Melibiose, Raffinose and Rhamnose were present in control but absent in test. In case of Lectin, *Triticum vulgaris* (Table - 4) with blood group A+, Cellobiose, Lactose, Maltose, and Ribose were present in control but absent in test whereas Galactose, Melibiose, Raffinose and Rhamnose were absent in control but they were present before treatment and disappeared after treatment. In A-, Galactose, was absent in control but present before treatment and again disappeared after treatment, in B+, Galactose, Melibiose, Raffinose and Rhamnose were absent in control but present in test (BT) and absent after treatment. In AB+, Cellobiose, Lactose, Maltose and Ribose were present in control and absent in test; whereas Galactose, Melibiose, Raffinose and Rhamnose appeared before treatment and were absent after treatment. In O+, Galactose, Melibiose, Raffinose and Rhamnose were absent in control but present in test (BT) only. (Cases of B-, AB-, and O- were not available in leukaemia).

The first association between blood group and a disease other than haemolytic disease were demonstrated between carcinoma and blood group A (17). Same way association of cancer lip was significant with blood group - B. 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 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 compete 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 (6).

All cell surface membrane components play a prominent role in isoplastic behaviour (18). Any intracellular, micro environmental change may lead to alteration in the surface membrane constituents releasing certain molecules in the blood of such patients. Recently it has been reported that tissue factor, a transmembrane glycoprotein complexes with factor VII which initiates blood coagulation (19). It is expressed on the surface of various cells including monocytes and endothelial cells. Haemoglobin forms a specific complex with tissue factor purified from human malignant melanoma cells and enhances its precoagulant activity. The tyrosine kinase lyn also plays an important role in intracellular signalling in non transformed and leukemic erythroid cells (20).

Present results conclusively show that quantitatively the protein part of the glycoprotein is reduced in leukaemia 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 leukaemia. 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.

It has been demonstrated that maximum tolerated dose of pharmacokinetic interaction and activity compared to daunorubicin and cytarabine in patient with acute myeloid leukemia is only 49%. Patients achieved a complete remission or restored chronic phase (21). The metastasis associated proteins are expressed by normal lymphoid cells, CD44v6 serving as activator while NM 23-HI as constitutive protein. CD44v6 is considered as a marker of poor prognosis of various hematological cancers. It could be suggestive that protein expression was maintained in leukemia cells as only one of the metastasis associated proteins. The feature is similar to observations made in several adult cancers. (22).

Qualitative analysis shows that with the help of lectins one can even pin point the sugars

which appear or disappear due to leukaemia. In some cases the glycomoiety returns after the therapy as in case of normal RBC membrane.

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Table 1

Total Protein and Protein Bound Total Carbohydrates in RBC Membrane Glycoprotein (mg/ml) in Leukaemia

	Total Protein			Protein Bound Total Carbohydrate		
Bl. Gr. & Rh Typing	Control	BT	AT	Control	BT	AT
A+	4.28	3.02	2.98	5.72	1.68	2.2
A-	3.4	3.8	2.55	6.75	2.0	2.96
B+	4.8	3.92	3.05	5.3	1.61	1.98
B-	4.2	-	-	6.11	-	-
AB+	3.4	3.32	2.92	6.66	2.13	2.84
AB-	4.42	-	-	5.83	-	-
O+	4.6	3.38	3.29	5.65	1.69	2.12
O-	4.72	-	-	5.29	-	-

BT = Before treatment

AT = After treatment

Conclusion at 5% level of P:

SEM = 0.81

C.D. (5%) = 1.42

Table 2

HAEMAGGLUTINATION - INHIBITION REACTION WITH *RICINUS - COMMUNIS* LECTIN IN LEUKAEMIA

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

Control ----- C

Before treatment ----- BT

After treatment ----- AT

Haemagglutination - inhibition reaction ----- HI

+ HI reaction - Inhibition is present i.e. Sugar is present on the cell surface therefore No agglutination.

- HI reaction - No Inhibition i.e. Sugar is not present on the cell surface therefore No agglutination.

Table 3

HAEMAGGLUTINATION - INHIBITION REACTION WITH *PISUM SATIVUM* LECTIN IN LEUKAEMIA

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

Control ----- C

Before treatment ----- BT

After treatment ----- AT

Haemagglutination - inhibition reaction ----- HI reaction

+ HI reaction - Inhibition is present i.e. Sugar is present on the cell surface therefore No agglutination.

- HI reaction - No Inhibition i.e. Sugar is not present on the cell surface therefore agglutination takes place.

Table 4

HAEMAGGLUTINATION - INHIBITION REACTION WITH *TRITICUM VULGARIS* LECTIN IN LEUKAEMIA

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

Control ----- C

Before treatment ----- BT

After treatment ----- AT

Haemagglutination - inhibition reaction ----- HI reaction

+ HI reaction - Inhibition is present i.e. Sugar is present on the cell surface therefore No agglutination.

- HI reaction - No Inhibition i.e. Sugar is not present on the cell surface therefore agglutination takes place.

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