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Available online at [www.ijit.net](http://www.ijit.net)**Research Article****ALCOHOL IMPACT ON MEMBRANE GLYCOPROTEIN: A LECTIN STUDY****DR. DHIRAJ J. TRIVEDI<sup>1\*</sup>, CHANDRU MC<sup>2</sup>, AKSHATA SHINDE<sup>3</sup>, CHHAYA D. TRIVEDI<sup>1</sup>***1 \*Professor and head, Department of Biochemistry, SDM College of Medical Sciences and Hospital, Sattur, Dharwad, Karnataka, India**2. Professor, Department of Biochemistry, KIMS, Hubli, Karnataka, India**3. M.Sc.Students( Organic chemistry) Christ University, Bangalore Karnataka, India***ABSTRACT**

Alcohol intoxication has damaging effect on cell membrane components. Increased fluidity, altered biophysical properties, altered osmotic fragility and loss of membrane bound enzyme activity support this fact. Carbohydrate moieties of glyco-conjugates are no exception. Lectin, carbohydrate binding protein detects such aberrations and provide important tool to Glycobiologist. In present study we found increased exposure of M. charantia agglutinin recognizable galactose epitopes on the erythrocyte surface in alcoholics without any liver disorders. Decreased levels of membrane bound sialic acid suggest desialylation possible cause for exposed penultimate galactose epitopes. Though, mechanism needs to be evaluated present study points over exposure of galactose epitopes on erythrocyte membrane of alcoholics.

**KEY WORDS:** Alcohol, Cell membrane, Lectin, Erythrocytes.**INTRODUCTION**

Alcohol intoxication in humans and certain animals clearly indicate that ethanol has damaging effect on cell membrane components which affects the physicochemical properties. <sup>(1,2)</sup> Alcohol intake affects liver, Brain, stomach cells and is associated with alteration in erythrocyte cell morphology. <sup>(3)</sup> Erythrocytes from alcoholics have reduced cell count, increased cell size (macrocytosis) and varied cell shape which make them more susceptible for lysis as compared to normal ones. Increased osmotic fragility <sup>(4)</sup> shortens RBC life span in alcoholics. <sup>(5)</sup>

Persistently increase level of an organic solvent, ethanol, in blood and increase ROS induced oxidative stress causes decomposition of erythrocyte membrane components. <sup>(6)</sup> Increase fluidity and altered biophysical property loses many membrane transporters and membrane bound enzymes. <sup>(7,8)</sup> Increase activity of Gama glutaryltranspeptidase (GGT) a membrane bound glycoprotein enzyme in serum is an indicator of alcohol abuse.

Carbohydrate moieties of glycoproteins and glycolipids are distributed on the outer surface of the cell membrane. They interact with antibodies, enzymes, hormones, lectin, viruses and other molecules. <sup>(9)</sup> They have an enormous potential for

encoding biological information but precise role played by these glyco molecules are yet to be understood.

Lectin, carbohydrate binding protein, specifically recognizes sugar units from cell surface glycoconjugates and provides important tool to Glycobiologist. <sup>(9)</sup> Lectin based experiments have revealed that cell surface glycoconjugates are transformed during disease state <sup>(10,11,12,13)</sup> and hence it is expected that each such alterations should have a characteristic pattern of lectin binding <sup>(14)</sup> As there is a considerable reason to presume characteristic glycoprotein changes on the surface of erythrocyte owing to Oxidative impact of ethanol on cell components or acetaldehyde adduct formation and change in membrane rheological properties in alcoholics. Present lectin based investigation was undertaken to assess any such alteration in glyco-conjugates on erythrocyte membrane of alcohol abuse without liver disease by using Galactose >N Acetyl galactosamine binding specific Momordica charantia agglutinin (MCA).

**MATERIALS AND METHODS**

Present case control study was approved by Institutional ethical committee. One hundred males in the age group 25 to 48 years were enrolled as study population. Fifty Male subjects forming

alcoholic group were from outpatient clinics of psychiatry or Medicine department of SDM College of medical Sciences and Hospital, Dharwad (Karnataka). Persons in 25 to 48 years age group, habituated to alcohol intake for minimum 3 years and maximum of 5 years, having frequency of at least two to three times a week, without any reported hepatic complications were considered under Alcoholic group. Fifty, age matched healthy male individuals from blood donors list of our hospital blood bank having absolutely no history of alcohol intake or any other habit or any reported systemic disease were selected and enrolled as control group.

After obtaining informed written consent form each individual, 1.8 ml venous blood sample was collected in 0.2ml of 3.8% Na-citrate by taking all aseptic precaution. Blood sample was centrifuged at 3000 rpm for 5 minutes.

Plasma was separated from packed erythrocyte. Plasma sample was subjected for Gamaglutamyl transferase (GGT) enzyme by colorimetric assay kit obtained from Sigma – Aldrich and Total Plasma sialic acid was performed using diphenylamine method described by Winzler BJ.<sup>(15)</sup>

Packed erythrocytes fraction was used for hemagglutination assay.

#### 1. Preparation of 2% Erythrocyte suspension in PBS and Hemagglutination test:

The packed erythrocyte fraction obtained by centrifugation was washed thrice in 0.05M Phosphate Buffered Saline (PBS) pH 7.2. From this washed erythrocyte 2% v/v suspension was prepared for hemagglutination titer assay.

Hemagglutination assay was carried out in U type, 96 well microtiter plate using purified MCA as per the procedure described by Rudiger H.<sup>(16)</sup>

Hemagglutination test was performed thrice on each sample of alcoholics and control subjects. Hemagglutination titer values obtained for MCA in alcoholics were compared with control group.

#### 2. Extraction and purification of the lectin from seeds of Momordica charantia: All operations were done at 4 °C as described by Rudiger.<sup>(16)</sup> About 10 gm of seeds from ripe fruit of Momordica charantia (Bitter melon, Karela) were dehulled and ground into a fine powder. The flour was defatted using acetone and air dried at room temperature. The powder obtained was subjected to extraction procedure involving Phosphate buffered saline,

followed by acetone fractionation and Sephadex purification.

- Protein Estimation of purified lectin M. charantia Agglutinin:** Protein content of purified Momordica charantia Agglutinin (MCA) was estimated by Lowery's method<sup>(17)</sup> using BSA as standard
- Sugar inhibition studies:**<sup>(18)</sup> The Hemagglutination inhibition assay for purified lectin was performed as follow: 100 µL of different sugar solutions (0.4 M) were placed in first well of the U type 96 well microtiter plate, followed by two fold serial dilution in PBS was made. 100 µL of the purified lectin (70 µg) was added to each well. To this 100 µL of 2% erythrocytes suspension, prepared in PBS, was added. The reaction mixture was incubated for 60 min at 37°C. After 60 minutes the hemagglutination was visualized and titers was noted. Also MCA was tested for its blood group specificity and found to be nonspecific for any particular blood group.

## RESULT

Protein content of Purified M. charantia agglutinin was estimated as 700microgram/ml.

Results of sugar inhibition test performed on our purified lectin MCA indicated specificity towards Galactose >N-acetyl Galactosamine > Lactose sugars. The results were in concordance with other workers.<sup>(19)</sup>

Table 1: Hemagglutination Titer and overall biochemical characteristics of study subjects

Variable	Control group Mean ± SD	Alcoholic group Mean ± SD	P value
Number of subjects (n)	50	50	
Age group (years)	34 ± 3.2	38 ± 1.5	
Duration of Alcohol abuse (Years)	00.00	3.67 ± 0.45	HS
Gama glutamyl transferase (GGT) U/L	21.8 ± 11.6	72.4 ± 9.2	P<0.005
Total membrane protein(microgram/mg membrane)	5.5 ± 0.01	5.4 ± 0.04	NS
Total membrane bound sialic acid (micromole/gm protein)	140.84 ± 32.38	87.54 ± 51.92	P<0.01
Lectin required ( miligram)	0.0025 ± 0.001	0.0116 ± 0.010	P<0.001
Hemagglutination Titer Units/mg protein	86.68 ± 53.70	38.09 ± 20.28	P<0.000

HS – highly significant, NS – Non significant

Among one hundred study subjects fifty males belong to Alcoholic group had mean age 38 ± 1.5 years whereas, males from control group had 34 ± 3.2 years mean age. Mean duration of alcohol abuse in alcoholics was 3.67 ± 0.45 years.

Even in absence of any reported hepatic disease, GGT which is a marker enzyme for alcohol abuse have shown significant (P<0.005) more than threefold increase when compared with nonalcoholic control group.

There was no difference in erythrocyte membrane protein, but significant (P<0.01) decrease was

observed in total membrane bound sialic acid between two groups. Amid alcoholic group, Plasma GGT and Membrane bound total sialic acid have presented a negative correlation.

Though large variation in titre range was observed among the group due to serial dilution method, still alcoholics ( $38.09 \pm 20.28$ ) had significantly ( $P < 0.000$ ) decreased hemagglutination titre value as compared to control ( $86.68 \pm 53.70$ ) group.

## DISCUSSION

Detrimental impact of ethanol on cell membrane is well documented. The effect is uniformly observed on all the membrane biomolecules viz. lipid, protein and carbohydrates. Most reports stands for oxidative damage, whereas few have shown protein adduct with acetaldehyde as a damaging cause<sup>(20)</sup> Membrane bound glycoconjugates which are exposed on the outer surface of erythrocyte became obvious target. Significant decrease in hemagglutination titre observed in present study approves change in glycol-conjugates on erythrocyte cell membrane from alcoholics. As MCA have specificity towards galactose sugar, suggests increased number of galactose epitopes on erythrocyte membrane of alcoholic. Normally sequence of oligosaccharide has galactose as penultimate sugar, which is terminated by sialic acid. Whenever there is attack on sialic acid galactose epitopes are exposed. Increased number of M. charantia agglutinin recognizable epitopes detected on erythrocyte from alcoholic group in present study may be the result of desialylation. As documented desialylation could be a natural process for clearance of tainted cell or biomolecules<sup>(21,22,23,24)</sup> Decreased level of membrane bound sialic acid observed in present study suggests flaking of this molecule from membrane oligosaccharide due to adduct formation with acetaldehyde. Loss of membrane glycoprotein A, Increased activity of membrane bound glycoprotein GGT in plasma and decreased activity of Na-K ATPase<sup>(25)</sup> reported in alcoholics' supports changes in glycol-conjugates on exposure to ethanol. These alterations seems to be the result of an adaptive process of tolerance towards ethanol in alcoholics or natural process for clearance of tainted molecules or may be the result of ethanol induced oxidative damage.

Though mechanism involved needs to be evaluated, present data clearly points towards over exposure of M. charantia agglutinin recognizable galactose or N-Ac-galactosamine epitopes on the surface of erythrocytes in alcohol dependence.

Further it will be interesting to evaluate pattern of glycol-conjugates in alcohol abstinence.

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