Original Article

Indian Journal of Pathology: Research and Practice Volume 4
Number1January - June 2015
DOI : https://dx.doi.org/10.21088/ijprp.2278.148X.4115.2

Sialic Acid: Indicator of Alcohol Abuse in Male Prior to Liver Disease

Dhiraj J. Trivedi*, Akshata Shinde, Chhaya D. Trivedi***

*Professor and head, Department of Biochemistry, SDM College of Medical Sciences and Hospital, Sattur, Dharwad, Karnataka, India. **M.Sc. Student (Organic Chemistry) Christ University, Bangalore Karnataka, India

Abstract

Change in glycosylation is quick and dramatic process which leads to various pathological conditions. Over expression of sialylation is noted in various diseases. Though sialylation is one of the prominent features associated with acute phase protein, Desialylation results in clearance of protein or tinted cell. Impact of alcohol on Sialic acid of cell membrane needs to be investigated. In present study we estimated effect of alcohol on Sialic acid of erythrocyte membrane in male alcoholics without liver disease and correlated its value with GGT, a biomarker of alcohol abuse. We found significant increase in Sialic acid level in plasma with noteworthy decrease in membrane Sialic acid levels. Plasma GGT revealed Positive correlation with plasma Sialic acid where as negative correlation with membrane Sialic acid. As alcohol has definite effect on Sialic acid. Sialic acid can be used as marker to differentiate non-alcoholic from alcoholic before any clinical symptoms appear.

Keywords: Alcoholics; Sialic Acid; Glycoprotein's; Erythrocyte; Cell Membrane.

Introduction

Glycosylation is a common post-translational modification associated with normal physiological functions of biomolecule in the cell. It is subjected to regulation in response to cellular location and metabolic state of the cell.[1] Glycosylation is robust as it allows masking of variation but in the appropriate context. Glycan molecule when attached to free protein or lipid it offers stability by manipulating their turnover. When they are integrated in membrane, function as receptor – ligand interaction, cell-cell signaling and cell recognition. However, a precise molecular mechanism involved is still unknown[2] Sialic acids, a family of 9-carbon containing acidic monosaccharide, forms terminal sugar in glycan unit of glycoconjugates which is present either on the cell surface or in circulation [3]. All vertebrates make some form of Sialic acid because it is needed for proper development and growth[4]. Sialic acid offers negative charge and plays vital role in cell surface interaction [5] protection of cell from membrane proteolysis[6], determine the half-life of protein[7] and has some innate immunity function[8] Sialylation is the most prominent feature associated with serum acute phase glycoprotein[9] Over expression of Sialic acid has been noted in cancer,[10] Rhematoid arthritis, [11] Cardio vascular and Ischemic heart disease, Diabetes mellitus[12], and congenital glycation disorders[13]. Desialylation results in early clearance of protein from circulation or senescence of red blood cells. Loss of Sialic acid from the red blood cell membrane surface is blamed for loss of negative charge and its altered osmotic fragility or red blood cell indices. Changed in Sialic acid levels are observed during pathogenesis which recovers back to normal on recovery of disease condition [14,15]. Anemia, altered erythrocyte indices

Corresponding Author: Dhiraj J. Trivedi, Professor, Department of Biochemistry, SDM College of Medical Sciences and Hospital, Manjushree Nagar, Sattur, Dharwad, Karnataka- 580009

E-mail: dhiraj99trivedi@gmail.com

with distorted cell morphology, increased osmotic fragility and reduced life span of red blood cell are common findings among alcoholic patients[17]. Carbohydrate deficient Transferrin, loss of membrane bound glycoprotein enzyme Gama Glutamyl transpeptidase (GGT) and Na, K ATPase indicate impact of ethanol on glycoconjugates. Even change in microenvironment of cell has demonstrated change in membrane composition[18,19] Current trend in cell biology is to study the mechanism of regulatory cellular function through specific structural and functional molecules rather than classical enzymes. Abnormal protein glycation is highly involved in disease development [20] Though change in glycosylation occurs quickly and dramatically in various pathological conditions very less is known about its cause, mechanism and significance behind such aberrance. Oligosaccharide chain of glycoprotein is extremely heterogeneous and exact chemical nature of glycoprotein epitomes is basically unknown. Ability of alcohol to fluidize and swell plasma membrane is well documented, which is likely to be the result of altered orientation of glycoconjugates embedded in cell membrane. Both, glycoprotein and glycolipid have Sialic acid as terminal sugar unit. Thus study of Sialic acid of glycoconjugates on cell membrane perhaps has importance in diagnosis and healing among alcohol abuse. Hence present study was undertaken to estimate Sialic acid level in serum and in erythrocyte membrane to explore its prognostic value in alcohol abuse before any clinical symptoms of liver disease appear. Also levels of Sialic acid were correlated with serum GGT, an existing biomarker in alcohol abuse.

Materials and Methods

Present observational case control study was approved by Institutional ethical committee. One hundred male persons in the age group 25 to 48 years were enrolled as study population. Fifty Male subjects visiting psychiatric or Medicine outpatient department of SDM medical Hospital and habituated to alcohol intake, minimum three times a week, in the age group of 25 to 48years and not having any reported hepatic complications were considered under Alcoholic group. Fifty ages matched healthy male individuals from blood donors list of our hospital blood bank having absolutely no history of alcohol intake or any other habits or any reported systemic disease were selected and enrolled to form control group.

After obtaining informed written consent from each individual, 4.0 ml venous blood sample was collected in 0.4ml of 3.8% Na-citrate as anticoagulant by taking all aseptic precaution. Blood sample was centrifuged at 3000RPM for 5minutes. Plasma and packed erythrocyte were separated. Plasma sample was subjected for estimation of Gama Glutamyl-transpeptidase (GGT), Alanine amino transferase (ALT) and Aspartate amino-transferase (AST) enzymes activity by spectrophotometric assay kit obtained from Sigma – Aldrich. Total Plasma Sialic acid was estimated using diphenylamine method as described by WinzlerBJ [21] Erythrocyte membrane was isolated using the modified method of Dodge JT [22]. Erythrocyte suspension was hemolysed using 20 mMol/L of phosphate buffer of pH 7.4 and centrifuged. The procedure was repeated using the same solution but 10 mMol/L and 5 mMol/L concentration in order to wash off the hemoglobin from the erythrocyte ghosts. Isolated erythrocyte membrane was subjected for Protein estimation by Lowery's method using BSA as standard and Total membrane bound Sialic acid estimation by diphenylamine method using N-Acetyl neuraminic acid as standard. All data were expressed as mean standard deviation (SD). Comparison of the levels of SA and GGT in serum of alcohol-dependent individuals and control subjects were performed using Student's t -test. For correlation analysis, the Spearman correlation coefficients (r) were calculated

Result and Discussion

The main observations emerged out from present study on alcohol abuse male without any liver disease is, significant increase of total Sialic acid levels in plasma (813.38 ± 86.24; $P<0.001$) with noteworthy corresponding decrease of erythrocyte membrane bound Sialic acid (79.24 ± 49.02; $P<0.001$) when compared to control. Though decreased level of membrane protein was observed, it remained non-significant. On comparing the activity of liver enzymes, ALT and AST, which remained unchanged but activity of GGT has shown threefold increase (72.4 ± 9.2) in alcohol abuse as compared to control group(21.8 ± 11.6). Also plasma Sialic acid and GGT have shown positive correlation ($r = + 0.423$; $p< 0.001$). Whereas membrane bound Sialic acid and plasma GGT have shown negative correlation ($r = -0.284$; $P<0.001$).

GGT is a known biomarker of current alcohol abuse [23]. Thus observed elevated levels of GGT among alcoholic group suggest cells are presently under the influence of alcohol. Simultaneously normal levels of Liver enzymes ALT and AST validates unaffected liver function. Our observation is in concurrence with

finding of Daniel SP (2000) [24] Our result of Sialic acid are in agreement with the findings of Cylwik B (2009) [25] Alcohol metabolism is known to induce oxygen deficit (hypoxia), it alters NADH / NAD ratio resulting in the formation of ROS which leads to imbalance in cell redox state. Both, acute and chronic alcohol consumption can increase ROS production and leads to oxidative stress [26]. A positive correlation between lipid peroxidation marker Malondialdehyde and Sialic acid [27] and decomposition of Sialic acid from the oxidatively stress aged erythrocyte have been reported [28]. The impact of alcohol on various tissues depends on alcohol concentration in blood, individual difference in clearance and duration of exposure. During the course of alcohol exposure, erythrocytes by compulsion get exposed to oxidative stress resulting in membrane protein and lipid oxidation [29]. Membrane bound Sialic acid is related to glycophorin A, oxidation of protein may shed off negatively charged Sialic acid causing altered rheological properties and increased membrane fluidity observed frequently in alcohol abuse [30,31] Combined effect of induction of plasma sialidase and inhibition of sialyltransferase observed in alcohol abuse [32] may result in loss of Sialic acid from erythrocyte membrane and increased level in plasma. Desialylation per se is responsible for clearance of tinted biomolecule or cell whereas, heavily sialylated plasma protein is responsible for immunological function. Thus does this selective sialylation and desialylation indicates an adaptive process to protect the organism from excessive damage and cell death remains to be explored [33]. Our findings of increased serum Sialic acid and decreased concentration of membrane Sialic acid correlates with scientific reports [34,35]. Similar results have been reported in many other oxidative stress related diseases [36] Alcohol, an organic solvent, can diffuse across membrane and metabolized by enzyme Alcohol dehydrogenase resulting in generation of free radicals and acetaldehyde. Acetaldehyde is highly reactive and toxic byproduct having strong affinity for protein and forms adduct [37]. Adducts contribute to cell damage because it is recognized as foreign and generates immune molecules against them [38,39]. In turn body senses it as unwanted and clears them off but, this leads to loss of membrane bound glycoconjugates also. Positive correlation of GGT with serum Sialic acid and negative correlation with membrane Sialic acid may be used as marker in prognosis of damage due to alcohol abuse.

Thus, altered levels of Sialic acid observed in plasma and erythrocyte membrane may be the result of an individual or cumulative effect of Oxidative stress, Acetaldehyde or Sialo enzymes on glycoconjugates. Again there is a reason to assume increased serum sialic acid may have been derived from various organ cells which are also exposed to similar microenvironment change. Thus glycoconjugate of cell membrane is the important target in biological action of ethanol exposure. Thus SA determination helps differentiate between alcoholic from non-alcoholic before any symptoms of liver disease appear and can be used as marker of alcohol abuse diseases.

Table 1 : Biochemical characteristics of the male Alcoholic and Control group subjects.

	Control group n=50 All Male Mean ± SD	Alcoholic group n=50 All Male Mean ± SD
Age group (years)	37± 09	42 ± 07
Total membrane protein (microgram/mg membrane)	5.6 ± 0.01	5.2 ± 0.8^{NS}
Total membrane bound sialic acid (microgram/gm protein)	134.82 ± 42.38	79.24 ± 49.02*
Total serum sialic acid mg/L	563.56 ± 138. 21	813.38 ± 86.24**
Gama glutaryltranspeptidase U/L	21.8 ± 11.6	72.4 ± 9.2**
Plasma AST U/L	39.4 ± 12.8	42.6 ± 23.1^{NS}
Plasma ALT U/L	22.7 ± 8.2	28.2 ± 10.4^{NS}

*P<0.01, **p<0.001 NS= Non Significant

References

1. Lau KS, Partridge EA, Grigorian A.. Dennis JW; Complex N-glycan number and degree of branching cooperate to regulate cell proliferation and differentiation, Cell; 2007; 129; 123-134.
2. Varki A. Biological roles of oligosaccharides: all of the theories are correct. Glycobiology. 1993;

3:97–130. [PMD: 8490246]

3. Chen X, Varki A. Advances in the biology and chemistry of sialic acids. ACS Chem Biol. 2010; 5:163–176. [PMD: 20020717]
4. *Schwarzkopf M*, *Knobeloch KP*, *Rohde E*, Hinderlich S, Wiechens N, Lucka L, Horak I, et al. Sialylation is essential for early development of mice, Proc. Natl. Acad. Sci, USA, 2002, 99,5267-70 [PMD: 11929971]
5. Paulson JC, Glycoproteins What are the sugar chains for; trends in Biochemsci, 1989, 14, 272-276 [PMID 2672447]
6. Gorog P Pearson JD. Sialic acid moieties on surface GP protect endothelial cells from proteolytic damage; J Pathol, 1985; 146;205-212 [PMID-2993570]
7. Bork K, Horstkorte R, Weidermam W. Increasing the sialylation of therapeutic GPs the potential of the Sialic acid biosynthetic pathway; J Pharma Sci, 2009; 98; 3499-3508 [PMID – 19199295]
8. Barbel S Blaum, Jonathan P Hannan, Andrew P Herbert, David Kavanagh, Dusan Uhrín, Thilo Stehle. Structural basis for Sialic acid–mediated self-recognition by complement factor H. Nature Chemical Biology, 2014; [DOI: 10.1038/nchembio.1696]
9. Lin SY, Chen YY, Precise mapping of increased sialylation pattern and the expression of acute phase protein accompanying murine tumor progression , J proteome Res.2008,73293-3303 [PMID -18549263]
10. Kokoglu E, Sonmez H, Uslu E, Uslu I; Sialic acid levels in various types of cancer. Cancer Biochemistry Biophysics; 1992, 13(1):57-64 [PMID-1343847]
11. Alturfan AA, Uslu E, Alturfan EE, Hatemi G, Fresko I, Kokoglu E; Increased serum Sialic acid levels in primary osteoarthritis and inactive rheumatoid arthritis. Tohoku J Exp Med. 2007 Nov; 213(3): 241-8 [PMID-17984621]
12. Pickup JC, Crook MA. Serum sialic acid and heart disease. BMJ. 1993 Aug 21; 307(6902): 503 [PMC-1678804]
13. Freeze HH, Aebi M. Altered glycan structures: the molecular basis of congenital disorders of glycosylation. Curr. Opin. Struct. Biol, 2005;15: 490–498. [PMD: 16154350]
14. Dhiraj J. Trivedi, Chhaya D. Trivedi, Kaveri Hallikeri, Ravindra Udupa; Salivary Sialic acid as marker of oral cancer; Int. J. Int sci. Inn. Tech. Sec. B, Apr. 2012, 1(1), 48-50.[ijiit.webs.com/documents/120101-09.pdf]
15. Stibler H, Borg S, Reduction of the Sialic acid and galactose concentration in erythrocyte membrane in alcoholics, Drug Alcohol Depend, 1982,10(1), 85-98 [PMD- 7173044]
17. S Maruyama, C Hirayama, S Yamamoto, M Koda, A Udagawa, Y Kadowaki, et al. Red blood cell status in alcoholic and non-alcoholic liver disease. J Lab Clin Med 2001; 138: 332-337
18. Resmi H, Akhunlar H, Temiz Artmann A, Guner G. In vitro effects of high glucose concentrations on membrane protein oxidation, G-actin and deformability of human erythrocytes. Cell Biochem Funct. 2005; 23(3):163-8 [PMID-15386536]
19. S Mazumdar, U sarkari, D sengupta; Biochemical profile of erythrocyte membrane of jaundiced neonates; Indian Journal of expremental Biology, 2000; 38(01), 91-94
20. Dube DH, Bertozzi CR. Glycans in cancer and inflammation - potential for therapeutics and diagnostics. Nat Rev Drug Discovery. 2005; 4(6):477–488 [PMID-15931257]
21. Winzler BJ Determination of serum glycoprotein In:Glick D eds Methods of biochemical analysis, VolII,Newyork ,Interschenco Publishers Inc. 1955, 279-312
22. Dodge, J. T., Mitchell C. and Hanahan, D. J. The preparation and chemical characteristics of hemoglobin free ghosts of human erythrocytes. Archives of Biochemistry and Biophysics; 1963 ; 100, 119–130
23. Niemela O; Biomarkers in alcoholism; Clin Chim Acta. 2007 Feb; 377(1-2):39-49 [PMID-17045579]
24. Daniel SP, Kaplan MM; Evaluation of Abnormal Liver-Enzyme Results in Asymptomatic Patients; N Engl J Med; 2000; 342:1266-1271 (Review Article)
25. Cylwik B, Krawiec A, Chrostek L, Supronowicz Z, Szmitkowski M; The effect of chronic alcohol drinking on the total concentration of Sialic acid and lipid bound Sialic acid; Pol. Merkur Lekarski; 2009, 27(158), 101-104 (PMD-19856873]
26. Wu D; Cederbaum AI; Alcohol oxidative stress and free radical damage; Alcohol research & health; 2003; 27; 277-84 [PMID –15540798]
27. Iijima R, Ichikawa T, Yamazaki M Sialic acid attenuates the cytotoxicity of the lipid hydroperoxides HpODE and HpETE. Carbohydr Res. 2009 May 12; 344 (7): 933-35 [doi: 10.1016/j.carres.2009.02.025]
28. MM Mehdi, Prabhakrsingh, SI Rizvi; erythrocyte sialic acid content during aging in humans:

correlation with markers of oxidative stress; disease Markers; 2012; 32,179-86.

29. Grattagliano I, Vendemiale G, Didonna D, Errico F, Bolognino A, Pistone A, Cofano M, Signorile A, Ciannamea F, Altomare E;Oxidative modification of protein in chronic alcoholics; Boll Soc Ital Biol Sper; 1995; 71(7-8),189-95 [PMD-85199495]
30. Mori I, Hiramatsu M, Toda N, Koide Y, Miyagawa ; Effects of alcohol on membrane fluidity of human erythrocyte; Acta Med Okayama; 1994; 48: 117-22.
31. Hadengue A, Razavuan SM, Del Pino M, Simon A, Levenson J; Influence of sialic acid on erythrocyte aggregation in hypercholesterolemia; Thromb Haemost; 1996; 76; 944-949.
32. Waszkiewicz N, Szajda SD, Zalewska A, Szulc A, Kêpka A, Minarowska A, et al; Alcohol abuse and glycoconjugate metabolism; Folia Histochem Cytobiol; 2012; 50(1): 1-11 [doi: 10.2478/18690]
33. Grewal PK, The Ashwell receptor mitigates the lethal coagulopathy of sepsis; Nat. Med 2008; 14: 648–655 [PubMed-18488037]
34. Stibler H, Borgs, Reduction of Sialic acid and galactose concentration in erythrocyte membrane in alcoholics; Drug Alcohol Depend ; 1982, 10(1); 85-98 [PMD 7173044]
35. F. Schellenberg, F. Beauge, C. Bourdin, J.M. Bourre, J. Weill ; Alcohol intoxication and sialic acid in erythrocyte membrane and in serum transferrin; Pharmacology Biochemistry and Behavior; 1991; 39(2),443–447
36. Hadengue AL, Del-Pino M, Simon A, Levenson J; Erythrocyte disaggregation shear stress, sialic acid, and cell aging in humans; Hypertension; 1998; 32(2): 324-30 [PMID-9719062]
37. Tuma DJ, Casey CA; Dangerous byproduct of alcohol breakdown: focus on adducts; Alcohol Research and health; 2003; 27,285-290 [PMD-15540799]
38. Lin RC, Lumeng L, Shahidi S; Protein-acealdehyde adducts in serum of alcoholic patients, Alcoholism: Clinical and experimental research; 1990; 14,438-443 [PMD-2378429]
39. Warrall S, De JerseyJ, Shanley BC, Wilce PA; Antibodies against acetaldehyde modified epitopes: presence in alcoholic non alcoholic liver disease and control subjects; Alcohol and Alcoholism; 1990; 25, 509-517 [PMD-1708257]