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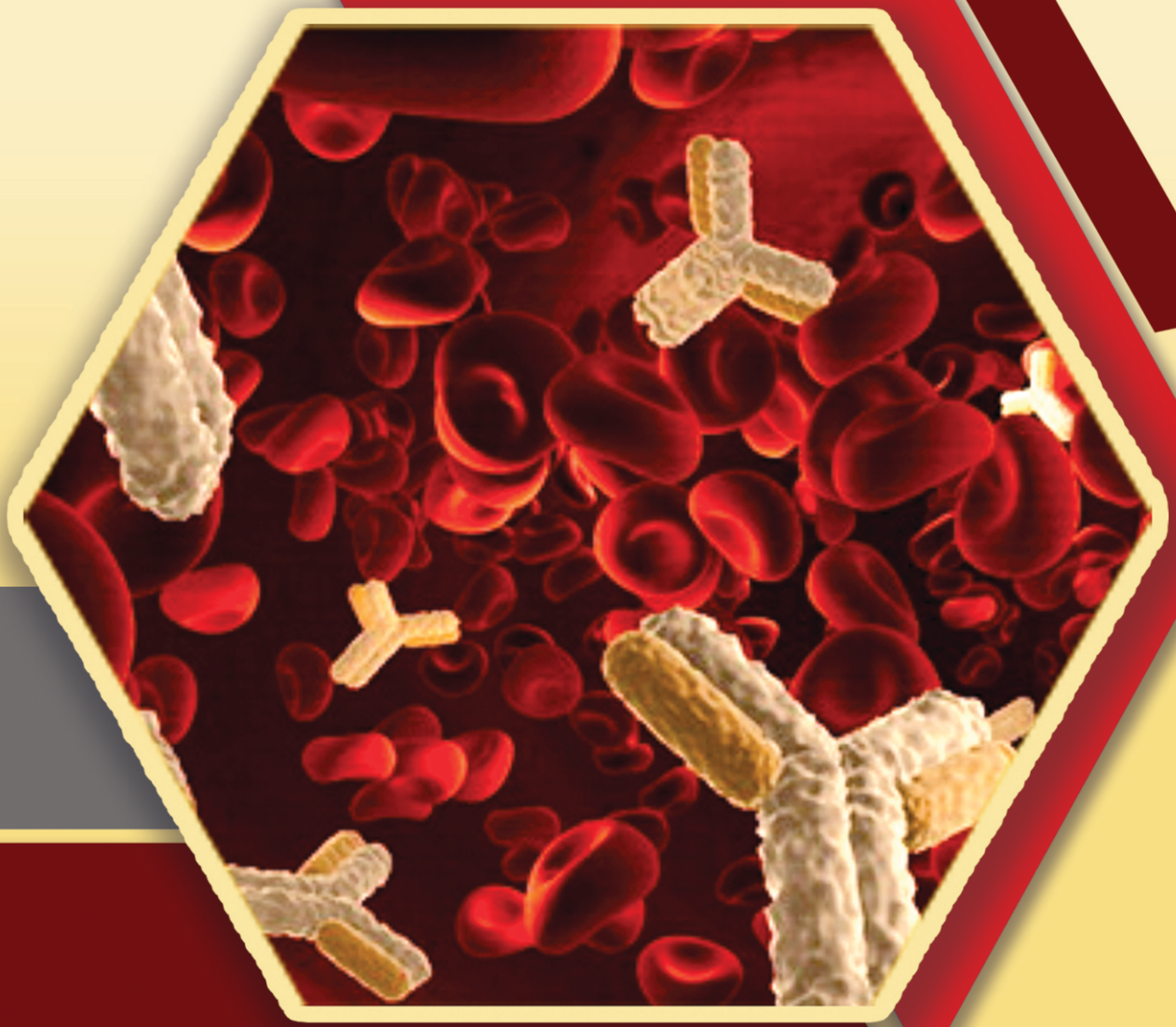
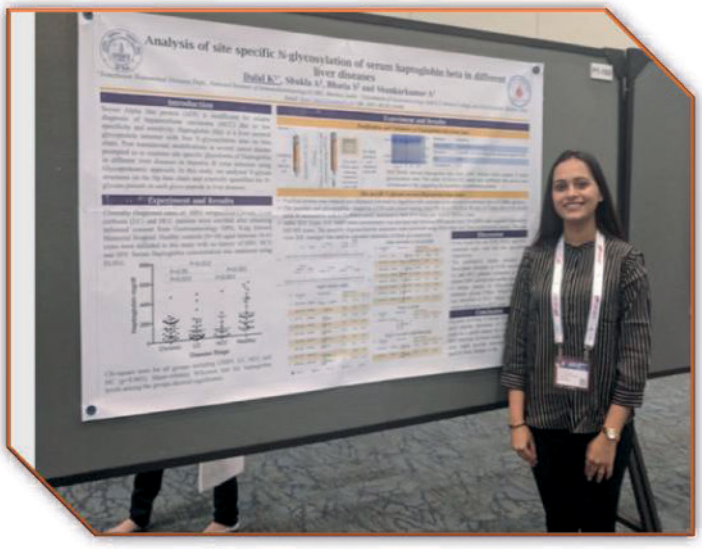


ICMR - NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY

INDIAN COUNCIL OF MEDICAL RESEARCH
NATIONAL INSTITUTE OF IMMUNOHAEMATOLOGY

The National conference on "Upcoming avenues for elimination of Viral Hepatitis in India" was organised to celebrate World hepatitis day by ICMR-National Institute of Immunohaematology on 29th July, 2018 at Durbar Hall, Haffkine Institute of Testing, Research and Training. The event coincided with the National Program for Prevention and Control of Hepatitis by Government of India.

National conference was inaugurated by Dr. Avinash Supe, Dean, Seth G S Medical College and KEM hospital. Dr. Arora, Dr. Ghosh, Dr. Philip, Dr. Aggawal and Dr. Kale delivered lectures on Viral hepatitis and its elimination.



Ms. Kruti Dalal presented a poster at Global Hepatitis Summit 2018, the 16th International Symposium on Viral Hepatitis and Liver Disease, Toronto, Canada held from 14th – 17th June, 2018.

Protein glycosylation in Hepatitis B related liver diseases

Kruti Bhavik Dalal

Protein glycosylation is a ubiquitous post translational modification, playing important role in pathophysiological processes. Glycomic and glycoproteomic analyses involve the characterization of oligosaccharides (glycans) conjugated to proteins. Glycans are produced through non-template driven series of competitive enzymes that extend the nascent chain. As most serum glycoproteins are from hepatic origin, characterization of glycans in the liver diseases would lead to better understanding of molecular pathogenesis of liver diseases including cancer. Only FDA approved marker for liver cancer, alpha-fetoprotein is not sensitive and specific enough for diagnosis of hepatocellular carcinoma. Hence, there is urgent need to explore specific marker for diagnosis, prognosis and therapeutic monitoring of the liver diseases in HBV infection. Technical advancement in the glycomics and glycoproteomics may allow a more comprehensive understanding of liver disease related glycosylation in biological fluid and tissue samples in HBV related liver diseases.

Introduction:

Glycosylation is a common co/post translational modification of proteins. Approximately 70% of human proteins are glycosylated. [1] Glycosylation is catalyzed by enzymatic additions of heterogeneous glycans with specific linkage of monosaccharides to specific amino acids within glycoprotein. Numerous biological functions have been associated with protein glycosylation, including cell–cell signaling, protein stability, protein folding, protein localization, and immune response, tumour initiation and metastasis. Also, protein glycosylation plays important role in maintaining health and in diseases. [2] Protein glycosylation occurs in the endoplasmic reticulum and Golgi apparatus involving multiple enzymatic steps.

Study of modification of proteins by glycosylation is challenging for following reasons:

- No template for the glycome: Protein sequences are primary gene products but glycan structures are not encoded directly in the genome and are secondary gene products. A few percent of known genes in the human genome are dedicated to producing the enzymes and transporters responsible for the biosynthesis and assembly of glycans.
- Structural diversity: Glycans are produced by a set of competing and sequentially acting enzymes such as glycosidases and glycosyltransferases, thus representing numerous combinatorial possibilities.
- Glycan-macro-heterogeneity: Glycan structures on glycoconjugates may vary by monosaccharide/ oligosaccharide symmetry, length, linkages, number of antennae and composition at glycosylation site occupancy.

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- Glycan-micro-heterogeneity: at any particular glycan attachment site on a given protein synthesized by a particular cell type, a range of variations might be found in the structures of the attached glycan called “glycoforms”, each constituting a distinct molecular species. In other words, site specific glycan structures at the same glycosylation site.

Glycosylation types

Glycosylation is characterized by various glyosidic linkages, including N-, O- and C-linked glycosylation, glypiation (GPI anchor attachment) and phosphoglycosylation (Table 1). However, major protein glycosylation can be found in two main types: N glycosylation and O glycosylation. The complex assortment of glycosylation enzymes comprise an intricate assembly line for glycan maturation from the ER through the Golgi. The localization, dynamics, interactions, regulation and substrate competition of these enzymes within the ER and Golgi play crucial role in glycosylation pathway.[3]

N-Glycosylation involves the attachment of a glycan chain to the amide end of an asparagine residue in an Asn-X-Ser/Thr sequon, where X can be any amino acid except proline. The glycoprotein is correctly folded, after the glycan attachment; therefore, N-glycosylation influences the tertiary structure and the stability of the glycoprotein. N-Glycans are produced at first in the endoplasmic reticulum on a lipid (dolichol) to yield ultimately a high mannose structure with a triglucose terminus. This structure is then transferred to a nascent polypeptide chain where it guides the protein folding process. When folding is complete, the glycan structure first losses the triglucose structure and a stepwise process disassembles the high mannose structure and rebuilds a variety of structures in the Golgi. Typically, N- glycans have a common core consisting of two N-acetylglucosamine residues attached to three mannose residues. This core structure may be extended using multiple substitutions to form different branching patterns as well as large number of linkages. [4] Mechanistic pathway of formation of N glycan is shown in figure 1.

Table 1 : Different types of glycosylation

N-linked	:	Glycan binds to amino group of Asparagine (Asn-X-Ser, X≠Pro) in the ER
O-linked	:	Glycan binds to hydroxyl group of Serine/Threonine in the ER, Golgi, cytosol and nucleus
C-linked	:	Mannose binds to the indole group of Tryptophan
Glypiation	:	Glycan core links phospholipid and protein
Phosphoglycosylation	:	Glycan binds to Serine via phosphodiester bond

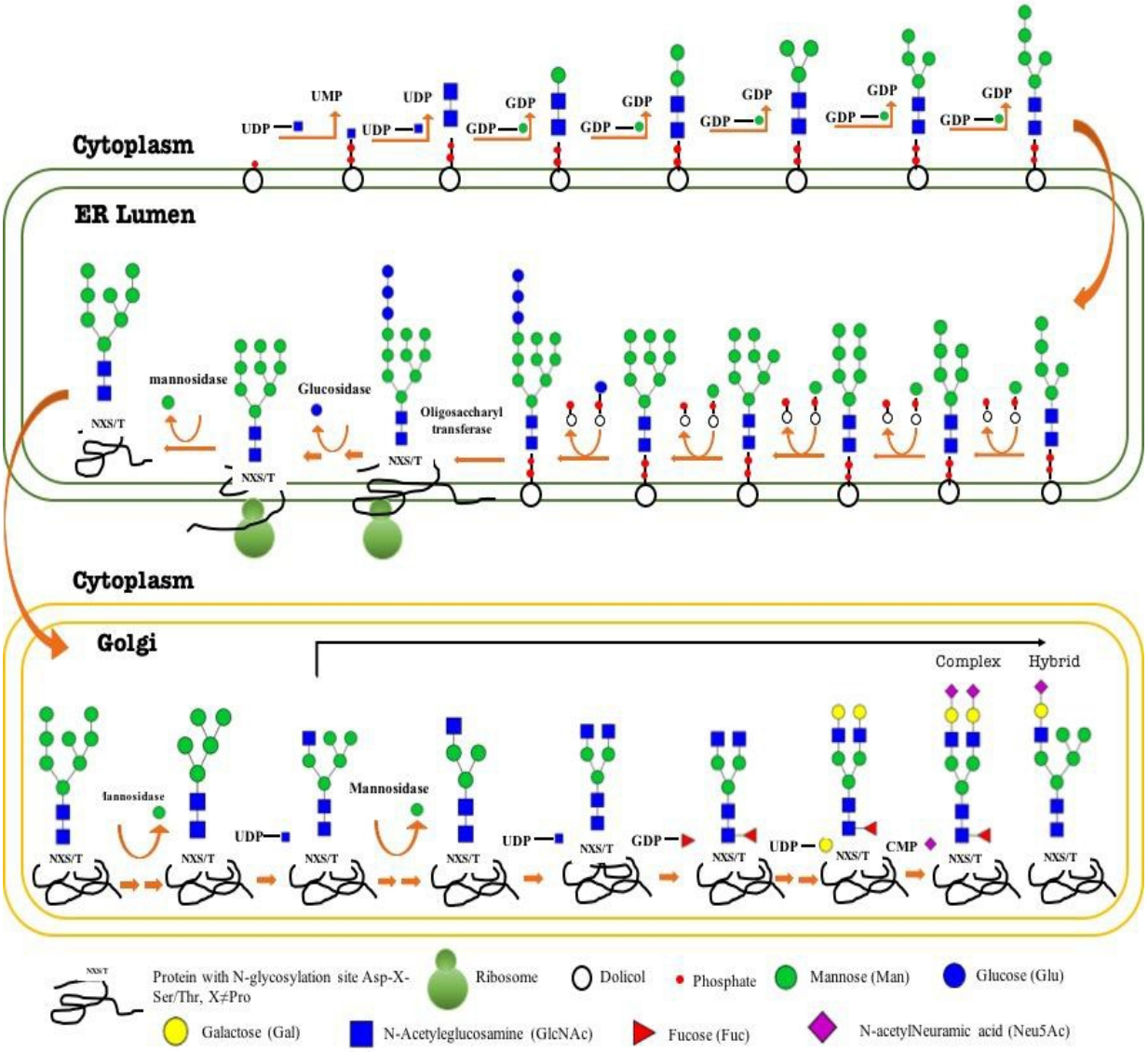

Figure 1: Protein N-glycosylation in ER and Golgi.

On the other hand, O-glycosylation represents great challenge as it is complex, relatively less abundant, does not involve any consensus sequence but includes the modification of Serine or Threonine residues through the attachment of oligosaccharides and it occurs after protein folding. The O-linked glycans are built up in a stepwise fashion with sugars added incrementally. Eight common core structures of O-glycans are illustrated in figure 2.

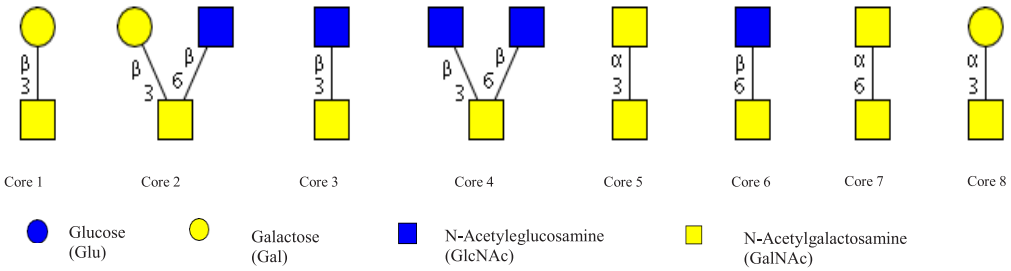

Figure 2: Eight common core structures of O-glycans

Glycomics and Glycoproteomics

Glycomics can be defined as study of the complete repertoire of glycans that a cell or tissue produces under specified conditions of time, location and environment. Glycoproteomics determines which sites on each glycoprotein of a cell are glycosylated and identification, quantitation of each glycan structure at each site on the heterogeneous glycoforms in the cell. [5] Mass spectrometry (MS) techniques such as high-performance liquid chromatography-electrospray tandem mass spectrometry (LC−ESI-MS/MS) and matrix-assisted laser desorption/ionization time-of-flight tandem mass spectrometry (MALDI-TOF MS/MS), coupled to a variety of dissociation techniques has become tool for analysis of sugars in the field of glycomics and glycoproteomics. Prior to MS analysis, various techniques such as purification, enrichment, chemical labelling, derivatization and release of glycan is performed for sequencing of complex oligosaccharide. [6]

Glycosylation in liver diseases in HBV infection

Hepatitis B virus (HBV), a partially double stranded DNA virus, is the major cause of chronic infection, liver cirrhosis and hepatocellular carcinoma. There are about 40–45 million HBV carriers in India.[7] India lies in the intermediate endemic zone of HBV infection leading to more than 50% cases of HCC. [8]

HBV envelop contains three distinct glycoproteins (L, M, S), the N-glycosylation of which is necessary for HBV release. Yu D et al have concluded that N-glycosylation changes in the major hydrophilic region of HBV surface contribute to immune escape due to decreased binding affinity of anti-HBs antibodies.[9] Mehta A et al have shown that with the advent of modern proteomic and glycomic methodologies, fucosylation, increased branching and increased sialylation have been identified in serum proteins of HCC patients either by direct glycan sequencing or lectin based methods. Similar findings are found directly in HCC tissue suggesting that these glycan changes may play a role in tumour formation and development. [10]

Study done by Qin Y et al have facilitated the discovery of biomarkers for HCC during its early stages based on precise alterations of N-linked glycans in saliva. The proportion of hybrid N-glycans was the highest (72.4%) and complex N-glycans was the lowest (27.6%) in cirrhotic patients. Tetra-antennary structures were increased in both cirrhosis (34.5%) and HCC (27.3%) patients groups. The proportion of fucosylated N-glycans were increased and sialylated N-glycans were decreased in HCC group when compared with other groups.[11] Purified carbohydrate chains from HCC against liver tissue showed chains with high-mannose levels in liver pathological conditions.[12]

Most serum glycoproteins are from hepatic origin, so it is inferred that liver diseases associated with abnormal glycosylation can display significant changes in glycoproteins. β1,6-GlcNAc branched N-glycan was found to be increased significantly in sera of HCC patients and 11 PHA-L reactive glycoproteins with significantly changed N-glycosite occupancy were identified, which were associated with cell migration, invasion and adhesion through p38 MAPK and NF-κB signaling pathway.β1,6 GlcNAc branching of N-glycans might be a metastasis-promoting glycoform which needs further investigation. [13] Core fucosylation of serum Vitronectin has been reported at Asn86 in HCC patients. Hybrid type and fucosylated N-glycans of vitronectin were increased in HCC

patients due to suppression of alpha mannosidase in Golgi apparatus in HCC. Also, sialylation and O-glycosylation in Hemopexin were increased in HCC [14]. Several liver secreted Acute phase proteins such as α-1-antitrypsin, transferrin, haptoglobin, complement factor C3, fetuin A, serum paraoxonase 1, ceruloplasmin are glycosylated in advanced liver disease stage which play important roles in immunity processes in response to tissue injury and infection. The distribution of altered glycoforms could have important implications for receptor-mediated responses involved in the progression of viral hepatitis-related liver diseases. [15] Major changes in glycosylation associated with different liver diseases stages in HBV infection is shown in the figure 3.

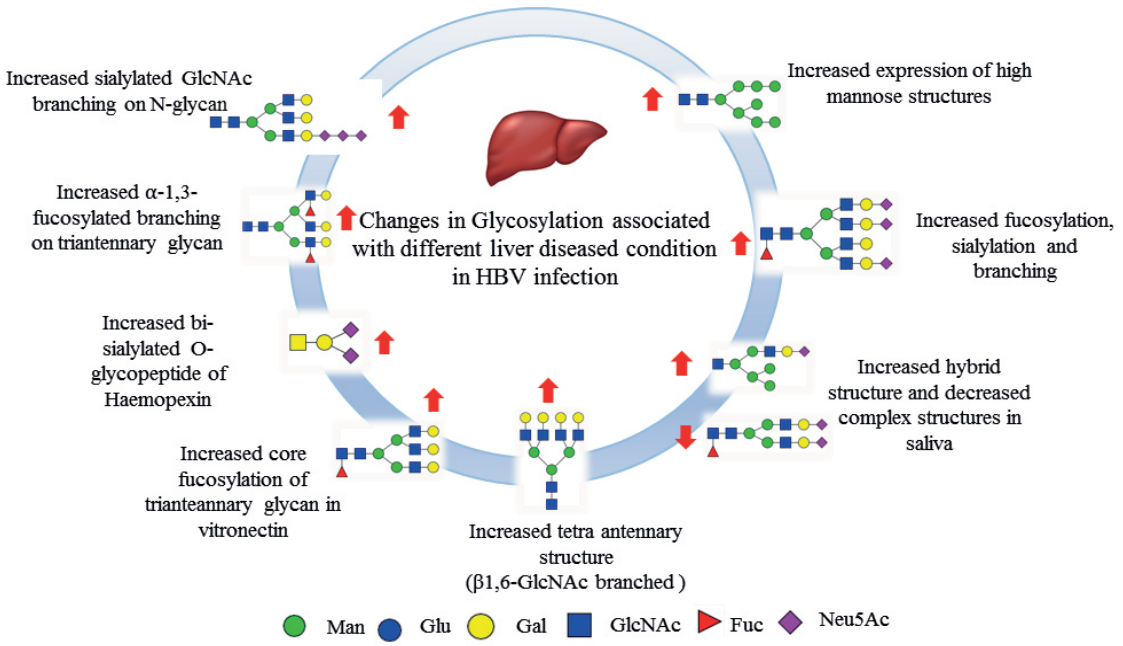

Figure 3 : Changes in glycosylation associated with different liver diseased condition in HBV infection

Indian Scenario

Chaerkady R et al identified 34 N-glycosylated peptides from lectin affinity enriched proteins from HCC tissue samples using LC-MS/MS. Majority of the confirmed N-glycosylation sites were found in proteins upregulated in HCC such as hemopexin, clusterin, and ceruloplasmin, haptoglobin. [16] Mondal G et al investigated enhanced fucosylation of α1-acid glycoprotein in the sera of chronic hepatitis B and hepatitis B cirrhosis patients and compared with healthy controls using high performance anion exchange chromatography and ELISA with fucose binding aleuria aurantia lectin. There was no apparent change in sialylation and branching of α1-acid glycoprotein in chronic hepatitis in comparison to controls. [17] There are only few published reports available. Glycomics and Glycoproteomics approach in liver diseases due to viral infection is still in infancy in India.

Experience at ICMR-NIIH

In a study at NIIH Mumbai, patients (N=90) aged between 18-65 years were recruited from the Department of Gastroenterology, King Edward Memorial Hospital, Mumbai and were categorized

into three groups namely chronic hepatitis B, liver cirrhosis and hepatocellular carcinoma based on guidelines of the Asian Pacific Association for the Study of the Liver. Healthy controls (N=30) aged between 18-65 years were included in this study with no history of HBV, HCV and HIV. Liver function tests, including total protein, albumin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, total and direct bilirubin were measured using Cobas C111. HBV serology, HBV DNA viral load was performed. Serum haptoglobin levels were measured using ELISA according to manufacturer's instruction. Glycopeptide mapping and glycan sequence variance analysis of Haptoglobin-beta were performed at Centre for molecular platforms (C-CAMP), NCBS-TIFR, Bengaluru and full-scan MS spectra were acquired.

Haptoglobin protein expression was significantly correlated with different liver disease stages ($p<0.05$). HCC cases had lower protein expression as compared to other disease groups and controls. Here, it was found that site N184: MVSHH**N**LTTGATLINE, N207: NLFL**N**HSE and N241: VVLHP**N**YSQVD contained eight, nine and two glycoforms respectively having bi- and tri-antennary glycans. The quantitative results revealed that fucosylated structures at N184 increased in LC and HCC patients compared to that in chronic HBV patients and healthy controls.

Conclusion

As majority of serum glycoproteins are hepatic in origin, many studies have shown that protein glycosylation is commonly altered in different liver diseases which may provide new opportunities for identifying diagnostic/prognostic/therapeutic biomarkers. Glycan modification combined with protein expression profiling may be used for monitoring development and progression of liver disease.

References

1. Dell A, Galadari A, Sastre F, Hitchen P. Similarities and Differences in the Glycosylation Mechanisms in Prokaryotes and Eukaryotes. International Journal of Microbiology. 2010;2010:1–14.
2. Mechref Y, Muddiman DC. Recent advances in glycomics, glycoproteomics and allied topics. Analytical and Bioanalytical Chemistry. 2017;409(2):355–7.
3. Moremen KW, Tiemeyer M, Nairn AV.Vertebrate protein glycosylation: diversity, synthesis and function. Nature Reviews Molecular Cell Biology. 2012 ;13(7):448–62.
4. Ruhaak LR, Xu G, Li Q, Goonatilleke E, Lebrilla CB. Mass Spectrometry Approaches to Glycomic and Glycoproteomic Analyses. Chemical Reviews. 2018 Available from: http://pubs.acs.org/doi/10.1021/acs.chemrev.7b00732
5. Thaysen-Andersen M, Packer NH. Advances in LC–MS/MS-based glycoproteomics: Getting closer to system-wide site-specific mapping of the N- and O-glycoproteome. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics. 2014;1844(9):1437–52.

6. Banazadeh A, Veillon L, Wooding KM, Zabet-moghaddam M, Mechref Y. Recent advances in mass spectrometric analysis of glycoproteins: Liquid Phase Separations. ELECTROPHORESIS. 2017;38(1):162–89.

7. Asim M, Sarma MP, Kar P. Etiological and molecular profile of hepatocellular cancer from India: Hepatocellular cancer from India. International Journal of Cancer. 2013;133(2):437–45.

8. Arora A, Sharma P, Tyagi P, Singla V, Arora V, Bansal N, et al. Hepatitis B Virus Infection can Cause Hepatocellular Carcinoma in Less Advanced Liver Cirrhosis: A Comparative Study of 142 Patients from North India. Journal of Clinical and Experimental Hepatology. 2013;3(4):288–95.

9. Yu D-M, Li X-H, Mom V, Lu Z-H, Liao X-W, Han Y, et al. N-glycosylation mutations within hepatitis B virus surface major hydrophilic region contribute mostly to immune escape. Journal of Hepatology. 2014;60(3):515–22.

10. Mehta A, Herrera H, Block T. Glycosylation and Liver Cancer. In: Advances in Cancer Research 2015: 257–79.

11. Qin Y, Zhong Y, Ma T, Zhang J, Yang G, Guan F, et al. A pilot study of salivary N-glycome in HBV-induced chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Glycoconjugate Journal. 2017;34(4):523–35.

12. de Oliveira RM, Ornelas Ricart CA, Araujo Martins AM. Use of Mass Spectrometry to Screen Glycan Early Markers in Hepatocellular Carcinoma. Frontiers in Oncology. 2018;7.

13. Liu T, Shang S, Li W, Qin X, Sun L, Zhang S, et al. Assessment of Hepatocellular Carcinoma Metastasis Glycobiomarkers Using Advanced Quantitative N-glycoproteome Analysis. Frontiers in Physiology;8.

14. Clerc F, Reiding KR, Jansen BC, Kammeijer GSM, Bondt A, Wuhrer M. Human plasma protein N-glycosylation. Glycoconjugate Journal. 2016;33(3):309–43.

15. Zhang S, Cao X, Gao Q, Liu Y. Protein glycosylation in viral hepatitis-related HCC: Characterization of heterogeneity, biological roles, and clinical implications. Cancer Letters. 2017 ;406:64–70.

16. Chaerkady R, Thuluvath PJ, Kim M-S, Nalli A, Vivekanandan P, Simmers J, et al. 18O Labeling for a Quantitative Proteomic Analysis of Glycoproteins in Hepatocellular Carcinoma. Clinical Proteomics. 2008;4(3–4):137–55.

17. Mondal G, Chatterjee U, Das HR, Chatterjee BP. Enhanced expression of α1-acid glycoprotein and fucosylation in hepatitis B patients provides an insight into pathogenesis. Glycoconjugate Journal. 2009;26(9):1225–34.

सारांश

हिपाटायटीस संबंधित यकृत की बिमारियों में प्रोटीन ग्लायकोसिलेशन का कार्य

मनिषा पटवर्धन

ग्लायकोसिलेशन क्या है ?

मानव के शरीर में साधारणतः ७०% प्रोटीन पर 'ग्लायकोसिलेशन' प्रकिया होती हैं। इस प्रकिया एनजाईम के द्वारा उत्प्रेरक होती है। प्रोटिन का निर्माण अमीनो ॲसिड के एक दुसरे से जुड़ने से (Amino acid chain) होता हैं। विभिन्न प्रकार के ग्लायकन (Glycans) अमीनो ॲसिड से जुड़ते हैं। प्रोटीन ग्लायकोसिलेशन बहुत से जैविक कार्यों के लिए जरूरी होता है। कोशिकाओं का आपसी सपंर्क, प्रोटिन को स्थिरता दिलाना, प्रोटिन को सही ढंग से मोड़ना (folding), प्रोटिन को सही जगह पर स्थानीयकरण (localization), प्रतिरक्षा प्रतिसाद (immuneresponse), टयूमर (tumor)का निर्माण और फैलाव आदि सभी कार्यों में हिस्सा लेनेवाले प्रोटीन ग्लायकोसिलेशन से गुजरते हैं। प्रोटीन ग्लायकोसिलेशन स्वास्थ्य बनाए रखने के लिए महत्त्वपूर्ण भूमिका निभाता हैं।

ग्लायकन की परिभाषा

ग्लायकोकॉन्ज्युगेट जैसे ग्लायकोप्रोटीन , ग्लायकोलिपीड में मौजूद कार्बोहायड्रेट को ग्लायकन कहते हैं। रक्तरस (serum) में रहनेवाले ज्यादातर ग्लायकोप्रोटीन यकृत में बनते हैं। यकृत की बिमारियॉं और विभिन्न ग्लायकन के स्तर का सबंध , बिमारी की तीव्रता दर्शाती हैं। अल्फा-फिटोप्रोटीन को 'लिवर कॅन्सर' का निशान (Biomarker) माना जाता हैं। फिर भी इसके स्तर में वृद्धि होना, बिमारी की पुष्टी नहीं करना। यकृत की विभिन्न बिमारियों में HBV का प्रादुर्भाव सिद्ध हो चुका हैं।

हिपाटायटीस B विषाणुसंक्रमण और यकृत में ग्लायकोसिलेशन प्रक्रिया

हिपाटायटीस B विषाणु डबल स्ट्रॅंडेड (doubleStranded)डी.एन. ए.(DNA)विषाणु हैं। यकृत की कई बिमारियॉं इस विषाणु के सकंमण से होती हैं। ग्लायकन में बदलाव टयुमर बनवाने में कारगज होते हैं। ऐसी आशंका वैज्ञानिक बता चुके हैं। हिपेटोसेल्युलर कार्सिनोमा में इसे साबित किया गया हैं। यु.डी.(Yu D et.al)और उनके साथियों ने इस पर अनुसंधान किया। हिपेटोसेल्युलर कार्सिनोमा (HCC) में मरीज के लार में मौजूद ग्लायकन की मात्रा जॉची गयी। मरीज और स्वस्थ लोगो के मुकाबले इन मरीजो में पाये गये ग्लायकान की मात्रा काफी अलग थी। HCC रूग्णों में β1, 6–GluNACनामक N-glycanकाफी ज्यादा मात्रा में पायी गयी. अब तक किए हुए अनुसंधान में सबसे महत्वपूर्ण ग्लायकान N-linked glycan प्रकार की होने का प्रमाण मिला हैं।

N-linked glycan का परिचय

N-linked glycan कोशीकाओं में आपसी संपर्क बनाने में मदद देते है। टयुमर से ग्रस्त कोशिका विकृत N linked ग्लायकान बनाती हैं। कोशिका में बने प्रोटीन को योग्य प्रकार से मोड़ना (folding), तृतीयक भंरचना, स्थिरता महत्वपूर्ण कार्य हैं। N-linked glycanइस कार्य को नियंत्रित करने का काम करते है। इनकी (N-linkedglycan) निर्मिती कोशिका के मौजूद एन्डोप्लाज्मिक रेटिक्युलम (ER) में होती हैं. अगर इनकी निर्मिती में कुछ बाधा आती हैं तो विकृती पैदा होती हैं।

आय सी एम आर - एन आय आय एच में अध्ययन

वहूकालिन हिपाटायटीस बी (chronic hepatitis B), लिव्हर सिरोसिस (Liver cirrhosis) और हिपेटोसेल्युलर कार्सिनोमा (HCC), के ९० रूग्णों के अध्ययन में शामिल किया गया। इनकी उम्र १८-६५ वर्ष थी। इनके साथ ही तुलना के लिए ३० स्वस्थ प्रकृती के लोग थे। सभी सहभागियों में लिव्हर फंक्शन टेस्ट (LFT), प्रोटीन अल्बुमिन की मात्रा, अस्पार्टेट ट्रान्सअमायनेज

(AST), अलानीन ट्रान्सअमायनेज (ALT), अल्कलाइन फॉस्फोटेज, बिलिरूबीन की मात्रा, आदि प्रचलों की (parameter) जॉच की गयी । एलियजा (ELISA) तंत्र से सीरम हप्टाग्लोबिन (serum haptoglobin) की मात्रा जॉची गयी । विकसित आण्विक तंत्र उन्नत प्रौद्योगिकी का उपयोग कर ग्लाय्कोपेप्टाइड मॅपिंग, ग्लायकान सेक्वेन्स वेरिएंस अनालिसिस के उपयोग से हप्टोग्लोबीन – बीटा का अध्ययन किया गया ।

विभिन्न प्रकृत की बिमारियों में हप्टाग्लोबिन प्रोटीन की मात्रा अलग अलग पायी गयी बिमारी की तीव्रता, अवस्था और हप्टाग्लोबिन की मात्रा का प्रत्यक्ष सबंध पाया गया । लिव्हर सिरोसिस (LC) और हिपेटोसेल्युलर कार्सिनोमा (HCC),में इन्ही बिमारियों में N 184 ग्लायकान की मात्रा बढ़ी हुई थी । बहुकालिन एच बी व्ही (chronic HBV) और स्वस्थ सहभागीयों में इसकी मात्रा कम थी ।

निष्कर्षः

रक्तरस में मौजूद अधिकतर ग्लायकोप्रोटीन (serum glycoprotein) यकृत में बनते हैं । प्रोटीन ग्लायकोसिलेशन एक अनिर्वाय कार्य हैं । यकृत की बिमारियों में प्रोटीन ग्लायकोसिलेशन की प्रकिया बदलने से ग्लायकान में भी बदलाव आते हैं, जो बिमारी का अन्देशा निदार्शेत करते हैं । ग्लायकान का अध्ययन फायदेमंद सावित हो सकता हैं क्योंकि बिमारी की अवस्था, तीव्रता, भविष्य में बिमारी में होनेवाले बदलाव को जल्दीं मॉपा जा सकेगा ।

The EQAS workshop 2018 round I was conducted by National Reference Laboratory of HIV testing (ICMR-NIIH) on 5th July and participants were from Mumbai and MP.

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