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Enhanced expression of α1-acid glycoprotein and fucosylation in hepatitis B patients provides an insight into pathogenesis

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Abstract Altered glycosylation and concentration of α1-acid glycoprotein has been known to be related to the pathogenesis of the hepatic diseases. The present study investigated enhanced fucosylation of AGP in the sera of chronic hepatitis B (HBV-CH) and hepatitis B cirrhosis (HBV-LC) patients by high performance anion exchange chromatography and by ELISA using fucose binding *Aleuria aurantia* lectin. The concentration of AGP determined by ELISA using monoclonal anti-human AGP (mAb-AGP) showed high level of AGP in HBV-CH and HBV-LC patients. This was further judged by association constant (K_A) measured by surface plasmon resonance analysis. There was no apparent linkage variation of sialic acid among different patient groups when tested with two sialic acid binding lectins *viz*., *Maackia amurensis* agglutinin (MAA, NeuAc α2-3-) and *Sambucus nigra* agglutinin (SNA, NeuAc α2-6-) respectively. There was no change of oligosaccharide branching in HBV-CH in comparison to controls whereas a slight change was observed in HBV-LC using ConA. The above results suggest that the changes in concentration of AGP and fucosylation have a prognostic value of hepatitis diseases and it could be possible to use AGP as diagnostic marker besides clinical examination and routine laboratory investigation.

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Abbreviations

AAL	*Aleuria aurantia* lectin
AGP	α1-Acid glycoprotein
HBV	Hepatitis B virus
HBV-CH	Chronic viral hepatitis B
HBV-LC	Viral hepatitis B cirrhosis
HCC	Hepatocellular carcinoma
HPAEC-PAD	High performance anion exchange chromatography pulse amperometric detection
MAA	*Maackia amurensis* agglutinin
SD	Standard deviation
SNA	*Sambucus nigra* agglutinin
SPR	Surface plasmon resonance

Introduction

Human α_1-acid glycoprotein (AGP, orosomucoid) is an acute phase serum glycoprotein of molecular weight 41–43 kDa and contains a highly branched N-linked glycan [1, 2]. Compared to other human serum glycoproteins AGP contains high amount of sugar (45%) including large amount of sialic acid, giving rise to acidic isoelectric point (pI) 2.8–3.8. AGP is also present in polymorphonuclear leukocytes (PMN), which synthesize a specific glycoform of AGP with

higher molecular weight compared to serum AGP (50–60 kDa vs 41–43 kDa) due to the presence of highly fucosylated and sialylated polylactosamine units on its five N-linked glycans. AGP possesses five N-linked complex type heteroglycans, which may be present as di-, tri-, or tetraantenary structures [3] and the glycosylation sites are occupied at asparagines 15, 38, 54, 75 and 85. There is an increase in concentration of AGP from two- to five- fold and a change in glycan profile during acute and chronic inflammation, pregnancy, estrogen treatment, cancer, liver diseases, autoimmune disease like rheumatoid arthritis and graves disease [4–10]. During pathophysiologic state there is a change in the oligosaccharide chain of the relative proportions of di-, tri-, and tetraantennary glycans with α (1→3)-fucosylated structures. In diseased condition sialylation occurs resulting in monosialylated or disialylated diantennaric glycan chain. Hepatic glycosylation process of acute-phase proteins is strongly dependent on the interplay of cytokines (IL-6, IL-1β and TNF-α), growth factors, and hormones involved in inflammatory processes [11]. Increased fucosylation of serum glycoprotein has previously been reported in patients with liver disease [12, 13]. To analyze fucosylation in AGP in the sera of patients undergoing liver biopsy a novel sandwich lectin immunoassay was made [14].

The present investigation monitors the alteration of AGP glycoform in chronic hepatitis B (HBV-CH) and hepatitis B cirrhosis (HBV-LC) patients' sera with respect to that of AGP of healthy individuals by estimating the concentration of AGP by ELISA and surface plasmon resonance (SPR) analysis by measuring association constants (K_A). This study also presents the change in fucosylation of AGP determined by high performance anion-exchange chromatography (HPAEC) and by ELISA using fucose binding *Aleuria aurantia* lectin. Change of sialic acid linkage was assayed by using two sialic acid specific lectins, *viz*., *Maackia amurensis* agglutinin (MAA) and *Sambucus nigra* agglutinin (SNA), respectively, and branching using Concanavalin A are also described herein.

Materials and methods

Materials

α1-Acid glycoprotein (AGP), *Maackia amurensis* agglutinin (MAA), *Sambucus nigra* agglutinin (SNA), Concanavalin A, rabbit monoclonal antihuman AGP, N-glycosidase-F, ProteoPrep Blue Albumin depletion kit, biotin 3-sulfo-*N*-hydroxysuccinimide ester, Extravidin were purchased from Sigma (USA). *Aleuria aurantia* lectin (AAL) was procured from Vector Laboratories. Dithiothreitol (DTT) and iodoacetamide were obtained from Fluka. CM5 sensor chip, surfactant P20, amine-coupling kit containing *N*-ethyl-*N*″-3-dimethylaminopropyl carbodiimide hydrochloride (EDC), *N*-hydroxysuccinamide (NHS), ethanolamine hydrochloride were purchased from BIACore, AB, Uppsala, Sweden. All other materials and chemicals used were of analytical grade and obtained from commercial sources.

Serum samples

Sera from 16 chronic hepatitis B (HBV-CH) patients were collected from the Liver Clinic, Department of Gastroenterology, Institute of Post Graduate Medical Education and Research, Kolkata and those from 16 hepatitis B cirrhosis (HBV-LC) patients were kindly supplied by Prof. Y. K. Chawla, Post Graduate Institute of Medical Education and Research, Chandigarh. Sera from eight age and sex matched healthy individuals served as control. All serum was stored at −20°C. Informed consent was obtained from each patient and healthy individual. Ethical committee of the Institute approved the study.

Protein estimation

The protein in the sera of both patients and healthy individuals was estimated by the method of Lowry *et al.* [15].

Removal of albumin and IgG

In order to visualize the low abundant protein in serum, both albumin and IgG were removed by the ProteoExtract™ albumin removal kit before 2D gel electrophoresis. ProteoPrep Blue Albumin Depletion Medium (400 µl) was transferred to a spin column and centrifuged at 10,000 rpm for 10 s to remove the storage solution. After this, 400 µl ProteoPrep Blue Equilibration buffer (pH 7.8) was added to the column and was centrifuged at 10,000 rpm for 10 s and buffer was discarded from the collection tube. The above process was repeated. Serum sample (150 µl) was added to the top of the column, and was incubated at room temperature for 10 min. The serum absorbed in the column, was centrifuged at 10,000 rpm for 1 min and unbound albumin depleted serum was collected in the collection tube. Albumin and IgG depleted serum before applying to 2D gel electrophoresis was further added to the top of the column and the process was repeated.

Identification of AGP by 2D gel electrophoresis

Two-dimensional polyacrylamide gel electrophoresis was performed using the immobilized pH gradient system [16]. The sera from HBV-CH, HBV-LC patients and healthy individuals (protein: 300 µg) were mixed separately with denaturing buffer [8 M urea, 2% CHAPS (*w*/*v*), 50 mM Tris, 65 mM dithiothreitol and 0.5% (*v*/*v*) ampholine]. The

samples were then loaded into the strip holder, and the 13 cm IPG strips (pH 3–10) were placed into the strip holder and transferred into the isoelectric focusing (IEF) system. IEF (Multiphor II horizontal system, Amersham Biosciences) was carried out using a multistep protocol (14 h for rehydration, 3 h at 500 V, 2.5 h at 4,500 V and finally to reach at 18,000 VhT). All steps were carried out at 20°C. After IEF, the gel strips were subjected to a two-step equilibration for 10 min. The first step was with an equilibration buffer containing 8 M urea, 50 mM Tris-HCl (pH 8.8), 2% (*w*/*v*) sodium dodecyl sulphate (SDS), 1% DTT, and 20% glycerol and then in the same buffer containing 2% (*w*/*v*) iodoacetamide for another 10 min. IPG strips were then transferred to 10% SDS-PAGE and sealed with molten 1% agarose containing bromophenol blue dye. The electrophoresis was carried out at constant current (30 mA) in the buffer consisting of 250 mM glycine, 25 mM Tris and 0.1% SDS. The second dimension electrophoresis was performed using a vertical gel slab (Ruby).

Database search to identify AGP

The spot obtained by 2D gel electrophoresis of the serum samples was compared using Swiss 2D-PAGE database (SWISS-2DPAGE/PLASMA_HUMAN) to identify AGP.

Immunoblotting

After 2D gel electrophoresis the proteins from the gel were electroblotted [17] on to nitrocellulose membrane for 5 h at 80 mA and 40 V by using Tris-glycine buffer containing 25% methanol. The membrane was stained with Ponceau-S to confirm the complete transfer of proteins and was washed off with distilled water. The unoccupied site of the membrane were blocked by incubation with 1% BSA in phosphate-buffered saline (PBS, pH 7.0) containing 0.05% Tween-20 (PBST) for 1 h at room temperature. The membrane was washed thrice with PBST and incubated with rabbit anti-human monoclonal AGP (1:5000). After washing the membrane with PBST, 0.04% diaminobenzidine (DAB) containing 0.01% H_2O_2 in sodium acetate buffer (pH 5) was added to develop the AGP spot.

Enzymatic release of N-linked oligosaccharides from AGP

N-linked oligosaccharides were released from serum AGP using peptidyl *N*-glycosidase F (PNGase-F). AGP spot identified by 2D gel electrophoresis of different patient groups as well as healthy individuals' sera was cut and destained. To it was added 1 µl denaturing buffer containing 5% (*w*/*v*) SDS and 0.4 M DTT and the spot was heated on boiling water bath for 10 min. After cooling, 2 µl reaction buffer (0.5 M sodium phosphate, pH 7.5), 2 µl of 10% Nonidet P-40 and 1 µl of PNGase-F were added and was incubated at 37°C for overnight. The supernatant containing the N-linked oligosaccharides was concentrated in the Speed-Vac. The conditions were found to be adequate for complete *N*-deglycosylation of thyroglobulin, transferrin, ovalbumin, and fetuin [18].

HPAEC-PAD analysis of N-glycans

The released oligosaccharides were passed through cation exchange-resin (Dowex-50 W). For quantitative analysis of neutral and amino sugars, released oligosaccharides were hydrolyzed by 2 M TFA for 16 h and 4 M HCl for 6 h separately [19]. After removal of acids by evaporation, the hydrolyzates were mixed with 200 µl of milli Q water, and 20 µl of it was injected to HPAEC-PAD (Dionex DX 500) for glycan analysis of AGP. Monosaccharide analysis was carried out using the Dionex DX 500 chromatography system on a CarboPac PA-1, (4×250 mm) column. The HPAEC system consisted of an advanced gradient pump GP40, an eluant degas module (EDM-2) for pressuring the eluants with argon was used for analysis of monosaccharides and pulsed electrochemical detector (PED-40), equipped with a gold electrode and $Ag/AgCl_2$ reference electrode. The resulting chromatographic data was integrated using a PC based Oracle 2 data acquisition system.

Triple pulsed amperometry was used in the following pulse potentials and duration (for monosaccharides analyses) t_1=500 ms, E= +0.10 V; t_2=100 ms, +0.60 V; t_3=0.50 ms, −0.6 V; the response time of the PAD was set to 5 s.

The eluant was prepared by suitable dilution of 50% NaOH solution with 0.22 µm filtered 18 mQ deionized water. All eluants were vacuum degassed before use. The analysis of monosaccharide was carried out at an isocratic 18 mM NaOH at a flow rate of 0.8 ml/min. Column was washed with 200 mM NaOH for 30 min followed by a 30 min equilibration with 18 mM NaOH to yield highly reproducible retention time. Sample was injected via a Rhoedyne 7725/9725 valve equipped with a 20 µl sample loop.

Quantification of AGP by ELISA

Concentration of AGP in serum samples was measured by ELISA [20]. Each well of a 96 well microtiter plate (NUNC) was coated with 100 µl mAb-AGP (1 µg/well) in buffer (0.01 M Na_2CO_3 and 0.035 M $NaHCO_3$, pH 9.6). The plates were left for 24 h at 4°C, washed with 100 µl 0.01 M PBS, pH 7.4, containing 0.05% Tween- 20 and incubated with 100 µl of PBS containing 1% BSA at room temperature for 1 h. The plates were washed as before. Diluted sera of different patient groups and healthy individuals (100 µl) were added to each well and incubated at 25°C for 2 h. After washing, 100 µl of HRP-anti-AGP

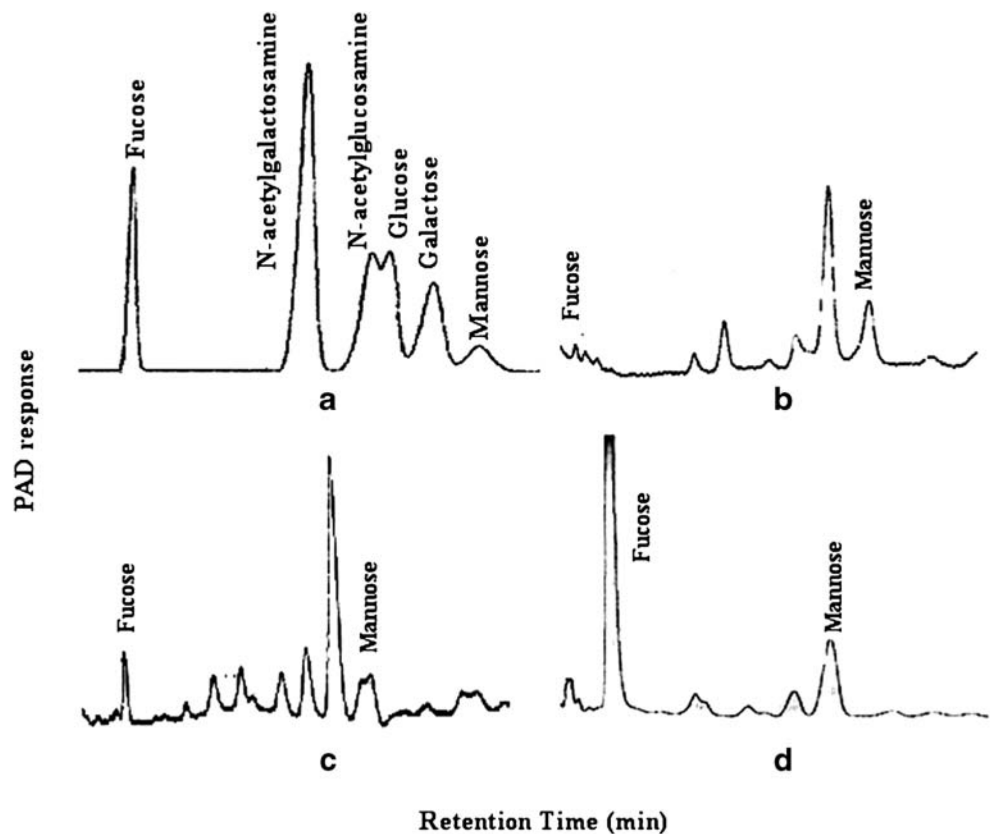

Fig. 1 HPAEC-PAD analysis of monosaccharides released enzymatically from N-linked glycan in AGP. **a** Standard monosaccharides profile. Peak 1: fucose, 2: *N*-acetylgalactosamine, 3: *N*-acetylglucosamine, 4: galactose, 5: glucose, 6: mannose. **b** Healthy individuals, **c** HBV-CH and **d** HBV-LC patients

rabbit serum (1:5,000) were added to each well followed by incubation at 25°C for 2 h. To each well 0.1% *O*-phenylenediamine dihydrochloride (OPD) (100 µl), and 0.05% H_2O_2 in 0.05 M citrate phosphate buffer, (pH 5.0) were added. The plates were left for 20 min at room temperature. The absorbance of each well was measured at 492 nm in an ELISA Reader after adding 50 µl of 1.5 (M) H_2SO_4 to the wells. Similarly, different concentrations of AGP were added to each well separately and the rest of the experimental steps were followed as before. AGP concentration in each sample was calculated using standard curve prepared by AGP. All experiments were done in triplicate and data presented are their mean values.

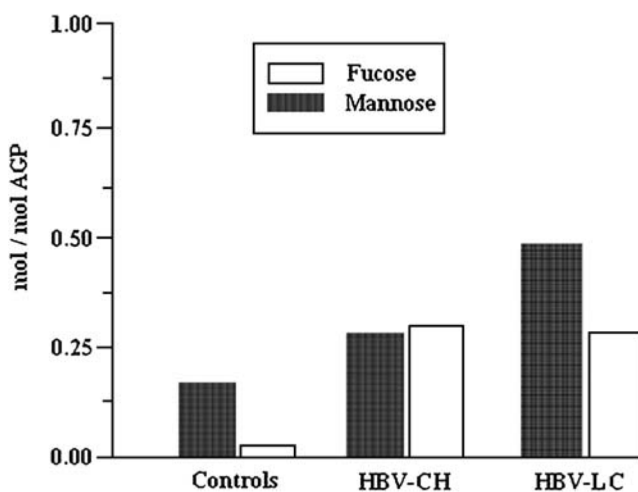

Fig. 2 The fucose and mannose content of AGP from HBV-CH, HBV-LC patients and healthy individuals analyzed by HPAEC. Results are expressed as mole of monosaccharide per mole of AGP hydrolyzed (MW 43 kDa)

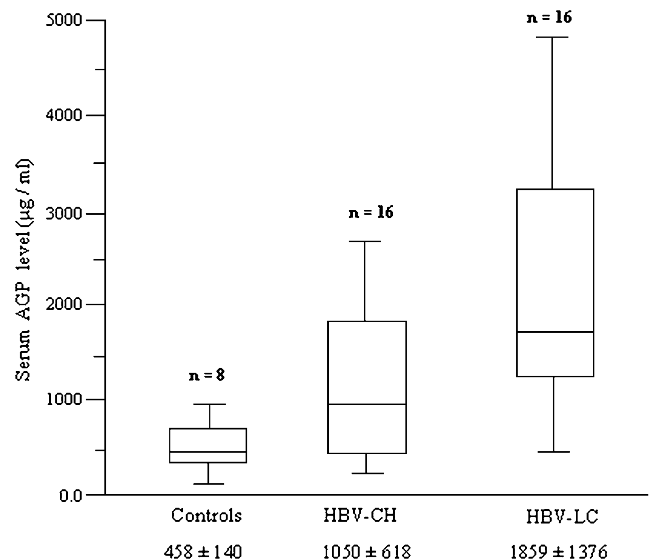

Fig. 3 Box-plot presentation of serum AGP levels among HBV-CH, HBV-LC patients and healthy individuals. Each *horizontal line* within box indicates the mean value of AGP. Data is presented as mean ± SD

Reactivity of serum AGP with lectins

ELISA was performed using two sialic acid specific lectins, *viz*., *Maackia amurensis* agglutinin (MAA, specificity: NeuAcα 2-3Galβ1-4GlcNAc) and *Sambucus nigra* agglutinin (SNA, specificity: NeuAcα2-6Galβ1-4GlcNAc) to differentiate between α 2-3- and α 2-6-linked sialic acid present in N-linked glycans of AGP [21]. The wells of microtiter plates were coated with 100 µl of mAb-AGP. To each well diluted sera of different patients, the AGP concentration of which adjusted to 500 ng/ml, was added and assay procedure was followed as before except addition of rabbit mAb-AGP-HRP conjugate; instead 100 µl of biotinylated MAA and SNA (1:100 in PBS), respectively, were added, incubated for 1 h followed by addition of 100 µl of streptavidin (1:1,000 in PBS). The rest of the procedure was same as before. The ratio of absorbances at 492 nm of AGP recognized by MAA and SNA was calculated (MAA/SNA ratio). All experiments were done in triplicate and data presented are their mean values.

Branching of AGP glycan

To establish alteration in the antennary glycan chain of AGP during disease state ELISA was performed with ConA [22] following the same procedure as above except addition of rabbit mAb-AGP-HRP conjugate; instead 100 µl of HRP-conjugated ConA (1:5,000) were added to each well and incubated for 2 h at 25°C. All experiments were done in triplicate and data presented are their mean values.

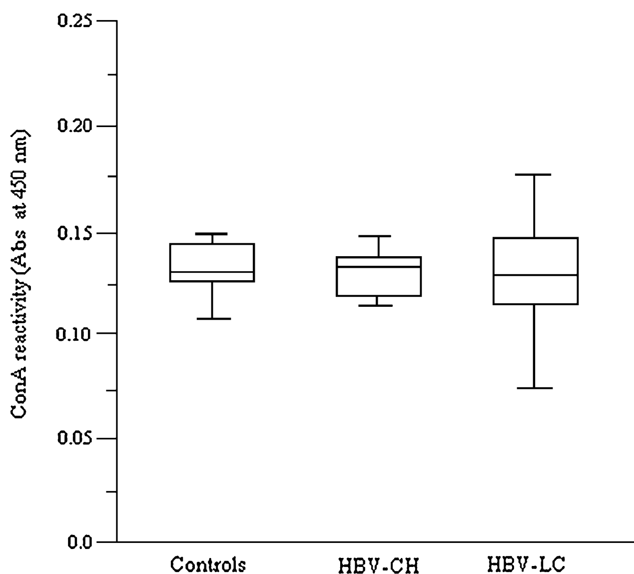

Fig. 5 Relative occurrence of diantennary glycoforms of AGP in serum between HBV-CH, HBV-LC patients and normal individuals. Absorbance is directly related to ConA binding

Determination of fucosylation in AGP

A lectin sandwich immunoassay was used to determine the change of fucosylation in AGP using fucose-specific *Aleuria aurantia* lectin (AAL) [14]. Following the same procedure as above, 100 µl of biotinylated AAL (1:100 in PBS) and 100 µl of streptavidin-HRP conjugate (1:1,000 in PBS) were used. All experiments were done in triplicate and data presented are their mean values.

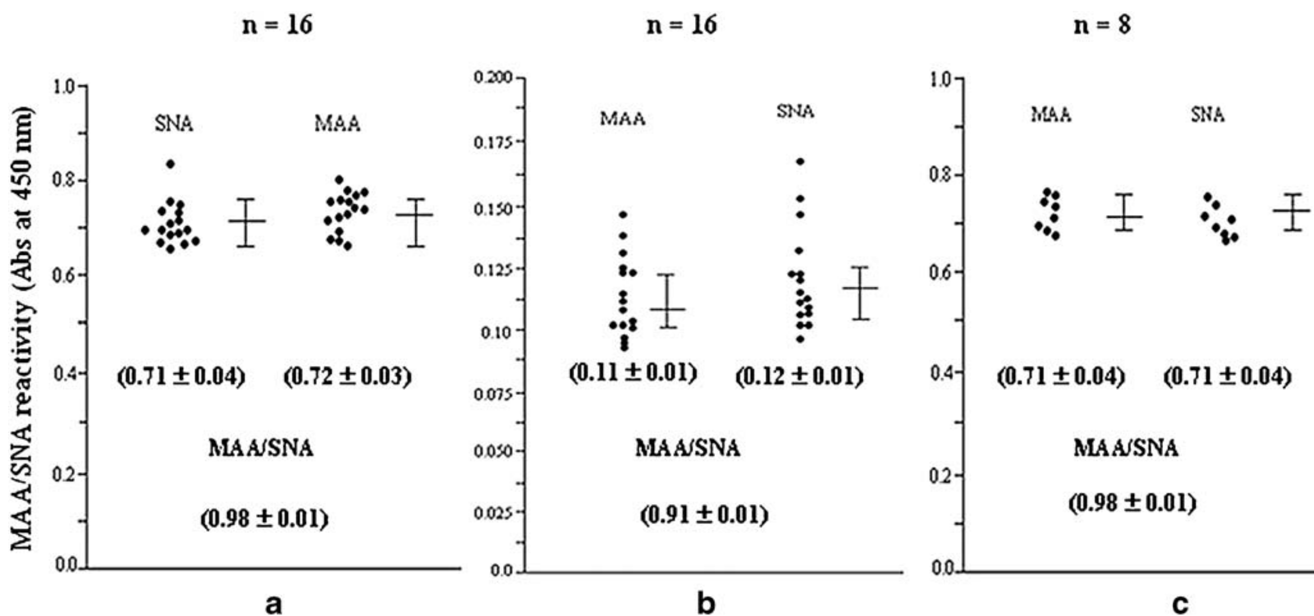

Fig. 4 Graphical presentation of MAA and SNA reactivities with AGP from **a** HBV-CH, **b** HBV-LC patients and **c** healthy individuals

Binding studies of serum AGP by SPR analysis

The binding studies were carried out using the BIAcore 2000 SPR apparatus (BIAcore AB, Uppsala, Sweden) at 25°C. Surface plasmon resonance (SPR) based affinity biosensor was used to measure association constant of AGP in patients' and normal individuals' sera. The response was recorded as a sensorgram representing the SPR signal measured in resonance units (RU) over time [23]. After equilibration with 5 mM HEPES-buffered saline (pH 7.4) and 0.005% surfactant P-20, the surface of the sensor CM 5 chip was activated with a 1:1 mixture of 0.1 M NHS and 0.1 M EDC. There after monoclonal mAb-AGP (30 μg/ml) was immobilized in 10 mM sodium acetate buffer (pH 5.4) to capture AGP in sera at a flow rate of 5 μl/min for 30 min and unreacted groups were blocked by injection of 1.0 M ethanolamine (pH 8.5). The association rate constants were determined by passing different concentrations of protein (100, 200, 300, 400, 500 μM) in the sera of different patients and normal individuals over immobilized mAb-AGP at a flow rate of 2 μl /min Injection time for samples was 4 min, corresponding to 8 μl. After every cycle the sensor chip was regenerated by passing 50 mM HCl for 1-min pulse. Kinetic parameters were calculated by BIA evaluation software version 3.0.

Statistical analysis

The STATISTICA 6.0 computer program was used for statistical analysis. The results are presented as mean ± SD. The statistical analysis was performed by unpaired Student's *t* test. For the statistical significance two-tailed *p* value of less than 0.05 was considered significant.

Results

Glycan analysis

Alteration of the monosaccharide content of AGP can occur in a disease-dependent manner; therefore the constituent sugars were analyzed using HPAEC-PAD analysis. Figure 1a–d show the results. Individual monosaccharides were separated due to slight differences in p*K*a arising from the position of the individual hydroxyl groups. Chromatographic runs of a range of concentrations of each monosaccharide reveals a linear response in HPAEC in terms of increasing area under each peak for increasing monosaccharide concentration. Determination of the equation of the line for each monosaccharide standard concentration versus the corresponding chromatographic peak area allows one to calculate the amount of monosaccharide released from a known amount glycoprotein. The quantity of monosaccharide was expressed as mole of monosaccharide per mole of AGP hydrolyzed. The relative change of fucose residue to mannose is presented in Fig. 2, which illustrates that the level of fucosylation was significantly higher in most of the HBV-CH and HBV-LC patients as compared to healthy individuals.

Serum AGP levels

Serum AGP levels in HBV-CH ($n=16$), HBV-LC ($n=16$) patients and in healthy control group ($n=8$) were assayed by ELISA. Figure 3, which shows that the levels of AGP in the patient groups were significantly high ($p<0.05$) compared to that of healthy control group. In patient groups HBV-CH and HBV-LC the latter showed higher amount of AGP. Very high AGP levels (>1,000 μg/ml) were found in some patients, whereas some showed less AGP concentration than that of controls. Very few normal individuals showed higher AGP than patients.

Reactivity of AGP with sialic acid specific lectins

AGP in patients sera reacted with SNA and MAA, which indicated that AGP was rich in sialic acid, which was glycosidically linked through α 2-6 (SNA) or α 2-3 (MAA) fashion. No significant differences were found in the reactivities between MAA and/or SNA in HBV-CH, HBV-LC patients and controls. Although very few patients have shown some alteration but the overall differences between healthy individuals and HBV patients were not significant (Fig. 4a–c).

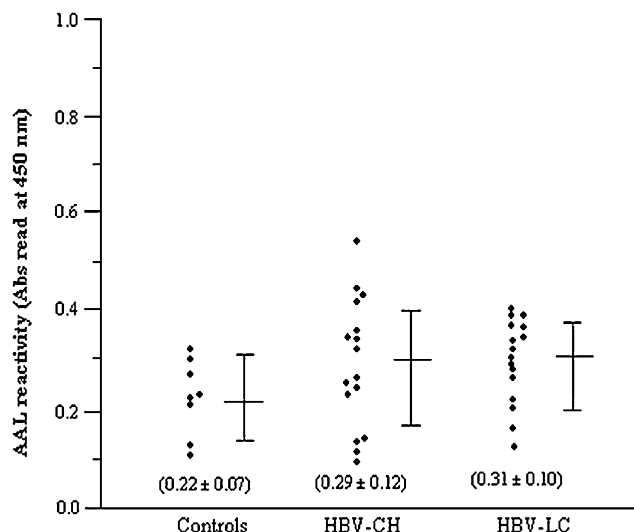

Fig. 6 Relative change in fucosylation of AGP of HBV-CH, HBV-LC patients and controls. The mean of AAL reactive AGP in HBV-CH and HBV-LC patients was significantly higher than that of controls ($p<0.05$)

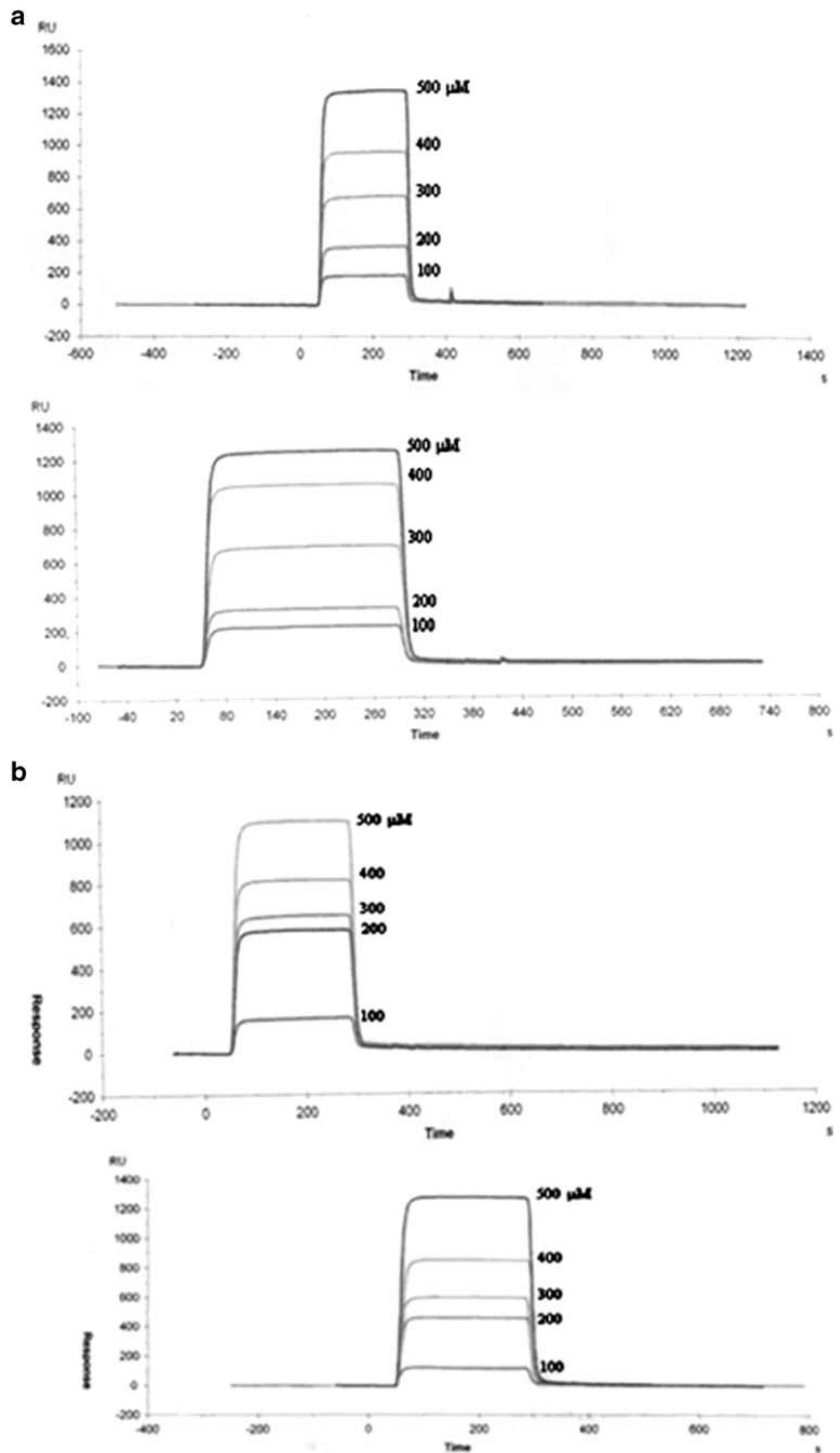

Fig. 7 Sensorgram depicting the binding between immobilized antihuman anti-AGP (mAb-AGP) with serum AGP. Diluted sera was injected over antibody conjugated CM5 sensor chip. Various concentrations of protein (100, 200, 300, 400 and 500 μM) in diluted sera were injected onto antibody-conjugated sensor chip at a flow rate of 2 μl /min. **a** Two HBV-CH patients and **b** two healthy individuals

Change in the degree of branching

The antennaric pattern of AGP was investigated by ELISA using ConA to investigate any possible change in the oligosaccharide chains on AGP that could be correlated with disease state and thus used as a prognosis of the disease. The absorbance at 450 nm was an indirect measure of the extent of ConA binding to AGP. The result showed no change of branching in HBV-CH in comparison to controls whereas a slight change was observed in HBV-LC (Fig. 5). Three patients showed less absorbance than normal individuals indicating changes in the branching which might give an insight into the progression of liver disease (data not shown).

Fucose concentration

Alteration of fucose concentration in AGP of patients determined by ELISA using *Aleuria aurantia* lectin, demonstrated that both HBV-CH and HBV-LC patients showed significantly ($p<0.05$) higher fucosylation than that of healthy controls (Fig. 6). This result corresponded to that of HPAEC analysis.

AGP concentration by SPR analysis

SPR analysis showed that the association constant (K_A) of captured AGP response unit (RU) was correlated with AGP concentration in serum (Fig. 7a, b). It was found from Table 1 that HBV patients' sera showed more strong binding with rabbit monoclonal antihuman-AGP than controls as evidenced by association constant (K_A); that is the higher the K_A value, the higher was the concentration of AGP. However, very few patients were found to have lower association constants than the normal individuals and vice versa.

Table 1 Relative association constant (K_A) of AGP from patients' and normal individuals' sera to immobilized monoclonal anti AGP on CM 5 chip by SPR analysis

Samples	K_A (M^{-1})
HBV-CH 1	4.24×10^{6}
2	8.22×10^{10}
3	1.37×10^{8}
4	1.22×10^{9}
5	9.01×10^{7}
6	3.06×10^{7}
7	1.71×10^{7}
8	7.71×10^{9}
9	5.05×10^{10}
10	4.19×10^{6}
11	2.77×10^{11}
12	1.69×10^{8}
Normal 1	3.13×10^{7}
2	1.34×10^{7}
3	1.55×10^{8}
4	6.75×10^{9}
5	5.79×10^{6}
6	7.16×10^{6}

Discussion

Most of the serum proteins are glycosylated which undergo changes in glycosylation in their oligosaccharide chains during the progression of the disease. Such glycosylation change observed in patients with various pathologic conditions [24]. The present study was undertaken to develop an assay system, which would be capable of measuring serum AGP with the sensitivity, specificity and reproducibility. Elevation of AGP concentration was reported for differential diagnosis of liver cirrhosis and hepatocellular carcinoma (HCC) [25]. Alteration of serum glycoproteins in alcoholic and cirrhotic patients was observed by two-dimensional gel electrophoresis [26]. Alteration in glycosylation of AGP denotes correlation to the severity of liver diseases. It was reported that 10–20% of acute hepatitis B (HBV) cases progressed to chronic hepatitis (HBV-CH), in which 30–40% of the HBV-CH cases later advanced to liver cirrhosis (HBV-LC). Subsequently, 2–8% of the patients with HBV-LC developed hepatocellular carcinoma (HCC) (National Cancer Institute's report, Korea, 2005). This information indicates the importance of monitoring progression of hepatic diseases which can decide the mode of treatment and the prognosis.

There are common diagnostic procedures to differentiate patients with various types of hepatic disease such as clinical examination, routine laboratory analyses, liver enzymes assay (Alanine aminotransferase, ALT; Aspartate aminotransferase, AST; Alkaline phosphatase and γ-Glutamyltranspeptidase) and many others. Liver function tests are very non-specific and give limited information. Enhancement of AGP expression and alteration of its oligosaccharide chain occur in a disease-specific manner. There is a need for accurate biochemical markers for early diagnosis of liver diseases. A clinical study with a large number of diverse serum specimens is necessary to validate serum AGP as a marker for diagnostic aid for liver disease. The outcome of these studies would determine whether serum AGP concentration may be a useful parameter not only as a diagnostic aid, but also to assess the disease status of patients. Increase of fucosylation has been observed in various serum proteins. For example, AGP fucosylation in ascitic fluid of a patient with LC [27], haptoglobin (Hp) fucosylation in alcoholic liver disease [28, 29], fucosylation in LC [30], fucosylation of α-fetoprotein and other serum glycoproteins in HCC [31] were reported. AGP fucosylation is considered as an important marker in accurate

diagnosis of liver diseases. Therefore, estimation of AGP concentration by sandwich immunoassay, the change of fucosylation by ELISA and HPAEC were adopted to consider AGP as a diagnostic marker.

From the foregoing results it is evident that the concentration of AGP of different groups of patient sera was found to have significantly increased in comparison to healthy individuals'; however, there was exception in few patients. HPAEC results showed significant changes in fucosylation level in AGP in most of the patients groups. These two results substantiated prognosis of the disease. Change in sialic acid linkages in transferrin (Tf) was considered an important factor in liver diseases [32]. However, no significant change in sialic acid linkage in AGP was noted in our case. No change in the oligosaccharide branching of AGP was observed in patients by using ConA lectin. Fucosylation of AGP is considered as an important biomarker in accurate diagnosis of liver diseases. In our study high amount of fucose content was found in HBV-CH and HBV-LC patients. This study suggests that the assessment of serum AGP concentration could help in the diagnosis and the prognosis of hepatic disease which may give insight into the proper treatment of liver disease. The addition of AGP value to the conventional liver function test could enhance the diagnostic accuracy of HBV-CH and HBV-LC.

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