Roles of glycoprotein glycosylation in the pathogenesis and effectiveness evaluation of the sodium hyaluronate treatment of an endemic osteoarthritis Kashin-Beck disease

Abstract:

Background/aim: We aimed to explore the roles of glycoproteins glycosylation in the pathogenesis of Kashin-Beck disease (KBD) and evaluated the effectiveness of sodium hyaluronate treatment.

Materials and methods: Blood and saliva were collected from KBD patients before and after the injection of sodium hyaluronate. Normal healthy subjects were included as controls. Saliva and serum lectin microarrays and saliva and serum microarray verifications were used to screen and confirm the differences in lectin levels among the three groups.

Results: In saliva lectin microarray, bindings to Sophora Japonica Agglutinin (SJA), Griffonia (Bandeiraea) Simplicifolia Lectin I (GSL-I), Griffonia (Bandeiraea) Simplicifolia Lectin I (EEL), Maackia Amurensis Lectin II (MAL-II), Sambucus Nigra Lectin (SNA), Hippeastrum Hybrid Lectin (HHL) and Aleuria Aurantia Lectin (AAL) were higher in the untreated KBD patients than in the control group. Increased levels for HHL, MAL-II and GSL-I in the untreated KBD patients discriminated them in particular from the treated ones. Jacalin was lower in the untreated KBD patients compared to the treated KBD and the normal groups. In serum lectin microarray, HHL and Peanut Agglutinin (PNA) were increased in the untreated KBD group in
comparison to the control one. AAL, Phaseolus vulgaris Agglutinin(E+L) (PHA-E+L) and Psophocarpus Tetragonolobus Lectin I (PTL-I) were lower in the untreated KBD patients compared to the treated KBD and the normal groups. Hyaluronate treatment appeared to normalize SNA, AAL and MAL-II levels in saliva, and HHL, PNA, AAL, PTL-I and PHA-E+L levels in serum. Saliva reversed microarray verification confirmed significant differences between groups in SNA and Jacalin, in particular, GSL-I levels, while serum reversed microarray verification indicated that HHL, PNA and AAL levels returned to normal level after the hyaluronate treatment. Lectin blot confirmed significant differences in HHL, AAL and Jacalin in saliva, and HHL, PNA, PHA-E+L and AAL in serum.

**Conclusion:** HHL in saliva and serum may be valuable diagnostic biomarker of KBD, and it may be used to follow-up of the hyaluronate treatment.

**Key words:** Kashin-Beck disease, carbohydrate chain, lectin microarray, serum, saliva

1. **Introduction**

Kashin-Beck disease (KBD) is an endemic osteoarthritis, which is characterized by chondrocyte necrosis and apoptosis, cartilage degeneration and extracellular matrix degradation. It is endemic in a crescent-shaped area extending from southeastern Siberia to northeast and southwest China. It affects approximately 690,000 people and poses a high risk to 10,584 million others residing in 366 counties within 14 provinces or autonomous regions of China, according to the 2010 Health Statistical Yearbook of China [1].
The capacity for hyaluronate synthesis is decreased in chondrocytes from patients with the KBD. Exogenous hyaluronate increases its synthesis in chondrocytes, which provides the foundation for the theory that the KBD patient should be treated with an intra-articular injection of hyaluronate. Compared with the normal control group, IL-1β and TNF-α levels were higher in the KBD group, while their expression levels decreased after administration of hyaluronate [2]. The levels of type II collagen and aggrecan mRNAs were also lower in the KBD group. After the administration of different doses of hyaluronate (100 and 500 mg/ml), their levels were significantly increased, so that 500 mg/ml treatment led to more significant effect [3]. Intra-articular injections of sodium hyaluronate effectively reduced knee pain caused by the KBD to a baseline value, measured by using the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) index [4].

Protein glycosylation plays a key role in a number of biological processes, such as development, maintenance of normal cellular functions, intercellular signaling, protein folding, protein-protein interactions, cellular differentiation, metabolism, and bacterial infections. About half of human proteins are considered to be modified with different glycosylation patterns [5]. Lectins are carbohydrate-binding proteins, which have been used to discriminate carbohydrate structures, including glycosaminoglycans, glycolipids and glycoproteins in a high-throughput manner, based on slight structural differences [6]. Oligosaccharides, which exist in human saliva, include amino sugars, galactose, sialic acids (Sia), xylose, fucose and mannose. Proteins, which perform many diverse
functions, are modified by their glycan parts of glycoproteins [7]. A lectin microarray is one of the main ways to investigate variable glycosylation, and it can test many different glycan bindings simultaneously [8].

The etiology of the KBD remains unclear. Its pathogenesis has not yet been fully defined. Currently, there are no diagnostic or treatment follow-up indicators available for the KBD based on glycoprotein levels. In this study, we supposed that the glycosylation change in the salivary and serous glycoproteins may involve the pathogenesis of the KBD, and play a role in guiding the treatment. Therefore, we aimed at revealing whether such glycosylation pattern differences are present in the saliva and serum of the KBD patients, and to investigate the role of knee joint injection of sodium hyaluronate on the glycosylation in the KBD patients. Lectin microarray was used to detect the expression patterns of glycosylations. Reversed microarrays and lectin blot were used to validate the result of lectin microarray. Finally, this study evaluated whether the identified potential lectins could be effective as diagnostic biomarkers for the KBD, and useful in the follow-up of sodium hyaluronate treatment of the KBD.

2. Material and methods

2.1 Whole saliva and venous blood samples collection

Patients with KBD (n=35, 20 males, 15 females, 61±5 years old) and normal control individuals (n=38, 20 males, 18 females, 60±6 years old) were recruited from Linyou and Yongshou counties in Shaanxi Province, China. All samples were excluded if the
persons showed genetic indications of bone or cartilage diseases, osteoarthritis or rheumatoid arthritis. Sodium hyaluronate (10 mg/ml, 2 ml) was injected into a knee joint of each patient with the KBD. Both venous blood and saliva were collected from the KBD patients before and two weeks after the injection (untreated KBD group, n=35; treated KBD group, n=35). Samples were also collected from the matched normal group (n=38). At least 2 h after the last intake of food, three milliliters of peripheral blood and three milliliters of unstimulated saliva were both collected. 0.9% saline was used to rinse participant’s mouth before saliva was collected. Protease inhibitor cocktail (1 µl/ml of saliva, Sigma, Germany) was added to the saliva immediately after collection. Then, the saliva was centrifuged, 12000 rpm, 30 min, 4°C. The supernatant was stored at -80°C. Venous blood sample was incubated at room temperature for 30 min. The serum was collected by centrifugation at 3,000 rpm for 5 min and then used immediately or stored at -80°C.

2.2 Saliva and serum lectin microarrays

Twenty samples of saliva or serum from both the untreated and the treated KBD groups were applied to the lectin microarray. In addition, twenty samples from the normal group were also applied to the lectin microarray. Samples from the untreated KBD, the treated KBD and normal groups were divided into subgroups by age (±5 years old) separately. The samples in each subgroup were pooled into one sample separately. Then, pooled samples were matched according to the gender constitution. Saliva and serum lectin microarrays were produced separately and incorporated 37 lectins (Sigma,
Germany), which will bind N- or O-linked glycans (As shown in Table 1). The arrangement of the lectin microarray is shown in figure 1. In brief, 37 lectins were dissolved in the manufacturer's recommended buffer and then spotted on the homemade epoxysilane-coated slides with Stealth micro-spotting pins (SMP-10B; TeleChem, Sunnyvale, CA) by a Capital smart microarrayer (CapitalBio, Beijing, China). Every lectin was spotted in triplicate on one slide. The protein labeled with Cy3 was diluted in 0.5 ml buffer containing 2% glycine, bovine serum albumin (BSA), and 0.1% Tween-20. Then, the mix was used to the lectin microarrays. Finally, incubation was performed in the chamber at 37°C, 3 h [9].

2.3 Statistical analysis of saliva and serum lectin microarrays

Three replicate slides of each sample were consistently analyzed. We obtained primary fluorescence intensity value of each lectin, then, each value was globally normalized by getting the ratio of each intensity value compared with the sum of 37 lectins values. The mean of three replicate ratios was the final fluorescence intensity value of each lectin. Subsequently, we separately compared the means of two groups (untreated KBD/treated KBD, untreated KBD/normal and treated KBD/normal). Fold changes ≥1.5 or ≤0.67 between the pairs indicated up- or down-regulation of each lectin, respectively.

2.4 Saliva and serum reversed microarrays verification

A reversed microarray analysis was used to verify the lectin microarray results. Salivary and serous protein samples from 15 untreated KBD patients, 15 KBD patients treated with sodium hyaluronate, and 18 normal individuals were spotted in triplicate on
microarray. Then, the Cy3-labeled lectins were applied to detect the specific glycan
structures in the salivary and serous protein samples, which were immobilized on the
slides. Lectins GSL-I, SNA and Jacalin were selected for saliva microarrays, and HHL,
AAL and PNA for serum microarrays. The slides were scanned using a Genepix 4000B
confocal scanner, and the acquired images were analyzed at 532 nm to detect Cy3.

2.5 Statistical analysis of saliva and serum microarrays verification

Significant differences between the untreated KBD, the treated KBD and the normal
groups were calculated using one-way ANOVA with SPSS statistics 19. Differences
were considered statistically significant for values of P<0.05.

2.6 Lectin blot analysis

In order to further confirm the different abundance of significant glycans, the SDS-
PAGE and lectin blotting analysis was performed with 3 lectins (HHL, AAL and
Jacalin) in pooled saliva group samples and 4 lectins (HHL, PNA, PHA-E+L and
AAL) in both pooled saliva group samples and pooled serum group samples of the
normal control, the untreated KBD and the treated KBD groups separately. In brief, the
samples were run on a 10% SDS-PAGE polyacrylamide resolving gel and a 3% stacking
gel. Afterwards, the proteins in the gels were transferred to a PVDF membrane
(Millipore, USA) with a wet transfer unit (Hoefer Scientific, USA) at 100 V, 1.5 h. Then,
the membrane was washed twice with TTBS (10 mmol/L Tris-HCl, 150 mmol/L NaCl,
0.05% Tween-20, pH 7.5) and blocked with Carbo-Free Blocking Solution (Vector,
Burlingame, CA) for 1 h at room temperature. The membrane was incubated with Cy5-
labeled (GE Healthcare, Buckinghamshire, UK) lectins (2 mg/l) at 4°C overnight in the dark. Finally, the membrane was scanned using a phosphorimager (Molecular Dynamics Inc. USA) [9].

3. Results

3.1 Differential lectins in saliva and serum lectin microarrays

Saliva lectin microarray showed significant differences in the fluorescence intensities of 8 lectins between the untreated KBD, the treated KBD and the normal groups (As shown in Figure 2). Stronger signals for SJA, GSL-I, EEL, MAL-II, SNA, HHL and AAL were observed in the saliva from the untreated KBD patients than from the treated patients with KBD and the normal group. Meanwhile, Jacalin signal was lower in the saliva from the untreated KBD patients and the treated KBD in comparison with the control group. After hyaluronate treatment, the levels of SNA, AAL and MAL-II appeared to return to approximately the normal level.

In the serum lectin microarray (As shown in Figure 3), significant differences in the fluorescence intensities of 5 lectins were observed between the untreated KBD, the treated KBD and the normal groups. Strong signals of HHL and PNA were typical for the samples of the untreated KBD group in comparison to the normal ones. Meanwhile, decreased AAL, PHA-E+L and PTL-I signals were observed in the serum samples of the KBD patients. Hyaluronate treatment appeared to normalize the levels of HHL, PNA, AAL, PHA-E+L and PTL-I.
3.2 Differential lectins in saliva and serum microarrays verification

Salivary protein microarray showed significantly stronger GSL-I signal in the untreated KBD patients (As shown in Figure 4). Significantly elevated SNA signal level in the untreated patients was normalized by the hyaluronate treatment, although the level still remained higher than in the control group (As shown in Figure 4). Saliva from the untreated patients had significantly lower level of Jacalin than the healthy controls (As shown in Figure 4).

Data from serum microarray verification showed that significantly increased levels of HHL and PNA signals in the untreated KBD group declined to the control level in the treated group (As shown in Figure 5). A significantly decreased level of AAL signal in the untreated KBD group increased to the control level in the treated group (As shown in Figure 5).

3.3 Lectin blot analysis

The different abundance of glycans among normal control (Health), the untreated KBD (UK) and the treated KBD (TK) groups were confirmed by the lectin blotting analysis with 3 lectins (HHL, AAL and Jacalin) in saliva samples and 4 lectins in serum samples (HHL, PNA, PHA-E+L and AAL). The results of the lectin blotting analysis showed apparent bands belonging to different molecular weight ranging from 15 to 250 kDa. The apparent bands were marked as L1-L10 (As shown in Figures 6 and Figure 7).

Among the selected lectins, HHL showed strong binding to two bands between 70-100 kDa (L1,L2), two bands between 25-50 kDa (L4,L5) and one band between 15-20 kDa
(L6) in the saliva of the UK group than the TK ones, and also showed stronger in TK group than healthy ones.

AAL showed strong binding to one band between 15 and 20 kDa (L6) in the saliva of the UK group than the TK and the healthy ones. On the contrary, Jacalin exhibited weaker binding to two bands between 100 and 250 kDa (L1, L2) and one band between 25 and 50 kDa (L6) in the saliva of the UK group than the TK ones, and also showed weaker in the TK group than the healthy one (As shown in Figure 6).

HHL showed stronger binding to one band 70 kDa (L4) in the serum of the UK group than the TK and the healthy ones. PNA showed stronger binding to one band 70 kDa (L1) in the serum of the UK group than the TK and the healthy ones. On the contrary, PHA-E+L exhibited weaker binding to one band 50-70 kDa (L5) in the serum of the UK group than the TK and the healthy ones. AAL also exhibited weaker binding to one band 70-100 kDa (L2) and one band 50-70 kDa (L3) in the serum of the UK group than the TK and the healthy ones (As shown in Figure 7). The results were coincident with the results from the lectin microarrays.

4. Discussion

Glycosylation is one of the most common posttranslational modifications of secreted proteins and plays a significant role in cell-cell interactions, cell adhesion, malignant transformation and metastasis [10]. It also has an important role in the molecular recognition events of the body. The glycosylation patterns of glycoproteins provide
clues about cell metabolism and the expression and function of oligosaccharides. Glycoproteins can exist in many glycosylated variants, and the distribution of different classes of oligosaccharide structures is most often specific for each glycosylation site [11]. Although serum is frequently used as a sample to analyze various biomarkers, saliva is also a good indicator of the plasma levels of various substances. Although both saliva and serum are samples from systemic sources, the components derived from tissues, such as cartilage, can accumulate in them. The extracellular matrices of tissues, such as the cornea, hyaline cartilage, and nucleus pulposus of the intervertebral disks, are rich sources of glycoproteins and proteoglycans (PGs) [12]. More importantly, shifts in the glycosylation patterns have been identified in diseases, such as in IgG of the serum of rheumatoid arthritis patients [13], which was later found to be responsible for the activation of complement system [14]. Lectins can be valuable tools in the diagnosis of the diseases, and even in discrimination of their stages [15], although the actual protein with altered glycosylation and/or its function would not be known.

In this study, the goal was to identify changes in glycosylation patterns in the KBD by using a group of lectins. Hyaluronate treatment has been reported to alleviate the symptoms of the KBD patients [4]. Therefore, we also screened whether the treatment would reveal lectin level, which are changed in the KBD, but normalized by hyaluronate therapy, since they could be useful in evaluating the efficacy of the treatment. As a result, the first screening experiments identified several lectins, which
had differential expression level in the KBD saliva and serum in comparison to the normal ones. Three of those (GSL-I, SNA and Jacalin) were selected for the verification microarray of saliva samples, and for serum samples (HHL, PNA and AAL). The carbohydrate chain Galα1-3-Gal is recognized by PNA, high mannose is recognized by HHL and GlcNAc is recognized AAL, Jacalin and PHA-E+L. The glycosylation pattern changes can have marked effects in the functions of the proteins. Previously, the levels of the Galα1,3-Gal antigen reduced in HT-transgenic porcine cartilage confered resistance to delayed rejection [16]. Expression levels of high mannose-type N-glycans were significantly increased at the later stages of mouse chondroprogenitor cell differentiation. On the other hand, the levels of some high mannose-type N-glycans were recently shown to be significantly decreased in both human OA cartilage and degraded mouse cartilage [17,18]. In developing rat embryos, the presence of numerous lectin binding affinities have been described with a general reduction of oligosaccharide structures during the development [19]. Primary OA chondrocytes are characterized by significantly higher levels of high mannose-type N-glycans and sialic acid-capped N-glycans compared to chondrocyte cell lines. In immortalized chondrocyte cell lines, the patterns of O- and N-linked oligosaccharides appear to be shifted toward reduced levels of high mannose-type and sialic acid-capped N-glycans, as well as increased levels of fucosylated O-glycosylation products [20]. An alteration in high mannose-type N-glycans was attributed to the expression of the Mgat1 gene, which regulates the expression of the key proteases.
matrix metalloprotease-13 and aggrecanase-2 during the cartilage degradation process [18]. Also the levels of high mannose-type N-glycans in primary human chondrocytes decrease in response to inflammatory cytokines IL-1β and TNF-α [21]. The GlcNAc-accelerated production of hyaluronate is associated with the induction of hyaluronate synthase-2, a key enzyme involved in hyaluronate synthesis [22].

Intra-articular hyaluronate injection has been proposed to have many therapeutic mechanisms of action in the OA knee, including anti-inflammatory effects, joint lubrication, shock absorption, chondroprotection, proteoglycan synthesis, and cartilage matrix alteration. At present, there are not good indicators how to follow biological effects of the treatment. Lectin analysis may be potential assay for the follow-up of hyaluronate treatment, not only for the KBD, but other arthritis treatments. Both in the saliva and serum samples, HHL signal was stronger in the patients with the KBD than in the normal controls, and normalized after hyaluronate treatment. HHL binding was the best marker to differentiate the KBD patients from the controls. It also differentiated between the untreated and the hyaluronate-treated group and, thus, might be a valid biomarker to evaluate the efficacy of the treatment. We plan to analyze the lectin levels in chondrocytes and to confirm diagnostic biomarker of the KBD in the next step. The method used to reveal the differences in the glycopatterns of human salivary and serous glycoproteins may provide pivotal information to help researchers understand other bone joint diseases. The selected lectins can only indicate changes in overall glycosylation pattern, but does not identify specific proteins involved. Therefore, the
effects of these changes on protein functions cannot be defined by these analyses. However, it does not reduce the value, which this type of assay can have in the diagnostics of the KBD.

References:


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15


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averted by transgenic expression of alpha1,2-fucosyltransferase. FASEB J 2003; 17(1):

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mannose type N-glycosylation in human and mouse osteoarthritis cartilage.

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GlcNAc and alpha-2,3-linked sialic acid determinants are associated with membrane
proteins of the more metastatic/aggressive cell lines. J PROTEOME RES 2014; 13(1):


Table. The structures of the carbohydrate chains recognized especially by the lectins.

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<th>Lectin</th>
<th>Full name of the lectin</th>
<th>Specificity carbohydrate chain</th>
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<tr>
<td>ACA</td>
<td><em>Amaranthus caudatus</em></td>
<td>Galβ1-3GalNAcα-Ser/Thr (T antigen), sialyl-T(ST) tissue staining patterns are markedly different than those obtained with either PNA or Jacalin</td>
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<tr>
<td>Abbreviation</td>
<td>Name</td>
<td>Specificity</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
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<tr>
<td>AAL</td>
<td><em>Aleuria Aurantia Lectin</em></td>
<td>Fucα1-6 GlcNAc (core fucose), Fucα1-3(Galβ1-4)GlcNAc</td>
</tr>
<tr>
<td>BPL</td>
<td><em>Bauhinia Purpurea Lectin</em></td>
<td>Galβ1-3GalNAc, Terminal GalNAc</td>
</tr>
<tr>
<td>BS-I</td>
<td><em>Bandeiraea simplicifolia</em></td>
<td>α-Gal, α-GalNAc, Galα-1,3Gal, Galα-1,6Glc</td>
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<td>ConA</td>
<td><em>Canavalia ensiformis</em></td>
<td>High Mannose, Manα1-6(Manα1-3)Man, αMannose, αGlc</td>
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<td>DBA</td>
<td><em>Dolichos Biflorus Agglutinin</em></td>
<td>αGalNAc, Tn antigen, GalNAcα1-3((Fucα1-2))Gal (blood group A antigen)</td>
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<tr>
<td>DSA</td>
<td><em>Datura stramonium</em></td>
<td>(GlcNAc) 2-4, polyLacNAc and LacNAc (NA3, NA4)</td>
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<tr>
<td>ECA</td>
<td><em>Erythrina cristagalli</em></td>
<td>Galβ-1,4GlcNAc (type II), Galβ1-3GlcNAc (type I)</td>
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<td>EEL</td>
<td><em>Euonymus Europaeus Lectin</em></td>
<td>Galα1-3(Fucα1-2)Gal (blood group B antigen)</td>
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<td>GNA</td>
<td><em>Galanthus nivalis</em></td>
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<td>GSL-I</td>
<td><em>Griffonia</em> <em>(Bandeiraea)</em></td>
<td>αGalNAc, αGal, anti-A and B</td>
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<td><em>Simplicifolia Lectin I</em></td>
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<tr>
<td>GSL-II</td>
<td><em>Griffonia</em> <em>(Bandeiraea)</em></td>
<td>GlcNAc and agalactosylated</td>
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<td><em>Simplicifolia Lectin II</em></td>
<td>tri/tetra antennary glycans</td>
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<td>HHL</td>
<td><em>Hippeastrum</em></td>
<td>High-Mannose, Manα1-3Man, Manα1-6Man, Man5-GlcNAc2-Asn</td>
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<tr>
<td>Jcalin</td>
<td><em>Artocapus integrifolia</em></td>
<td>Galβ1-3GalNAcα-Ser/Thr(T), GalNAcα-</td>
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Ser/Thr(Tn), GlcNAcβ1-3-GalNAcα-
Ser/Thr(Core3), sialyl-T(ST). not bind to Core2, Core6, and sialyl-Tn (STn)

LCA  *Lens Culinaris Agglutinin*  α-D-Man, Fucα-1,6GlcNAc, α-D-Glc

LEL  *Lycopersicon Esculentum (Tomato) Lectin*  (GlcNAc)$_n$, high mannose-type N-glycans

LTL  *Lotus Tetragonolobus Lectin*  Fucα1-3Galβ1-4GlcNAc,
     Fucα1-anti-H blood group specificity

MAL-I  *Maackia Amurensis Lectin I*  Galβ-1,4GlcNAc

MAL-II  *Maackia Amurensis Lectin II*  Siaα2-3Galβ1-4Glc(NAc)/Glc, Siaα2-3Gal, Siaα2-3, Siaα2-3GalNAc

MPL  *Maclura Pomifera Lectin*  Galβ1-3GalNAc, GalNAc

NPA  *Narcissus Pseudonarcissus Lectin*  High-Mannose, Manα1-6Man

PHA-E+L  *Phaseolus vulgaris Agglutinin(E+L)*  Bisecting GlcNAc, bi-antennary N-glycans, tri- and tetra-antennary complex-type N-glycan

PWM  *Phytolacca americana*  (GlcNAc)$_n$ and polyLacNAc

PSA  *Pisum Sativum Agglutinin*  Fucα-1,6GlcNAc,α-D-Man, α-D-Glc

PHA-E  *Phaseolus vulgaris Agglutinin(E)*  Bisecting GlcNAc, biantennary complex-type N-glycan with outer Gal

PTL-I  *Psophocarpus Tetragonolobus Lectin I*  GalNAc, GalNAcα-1,3Gal, GalNAcα-1,3Galβ-1,3/4Glc

PTL-II  *Psophocarpus Tetragonolobus*  Gal, blood group H, T-antigen
### Lectin II

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<th>Lectin</th>
<th>Name</th>
<th>Specificity</th>
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<tr>
<td>PNA</td>
<td><em>Peanut Agglutinin</em></td>
<td>Galβ1-3GalNAcα-Ser/Thr(T)</td>
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<td>RCA120</td>
<td><em>Ricinus Communis Agglutinin I</em></td>
<td>β-Gal, Galβ-1,4GlcNAc (type II), Galβ1-3GlcNAc (type I)</td>
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<td>SJA</td>
<td><em>Sophora Japonica Agglutinin</em></td>
<td>Terminal in GalNAc and Gal, anti-A and anti-B human blood group</td>
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<td>STL</td>
<td><em>Solanum Tuberosum (Potato) Lectin</em></td>
<td>Trimmers and tetramers of GlcNAc, core</td>
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<tr>
<td>SBA</td>
<td><em>Soybean Agglutinin</em></td>
<td>α- or β-linked terminal GalNAc, (GalNAc)n, GalNAcα1-3Gal, blood-group A</td>
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<td>SNA</td>
<td><em>Sambucus Nigra Lectin</em></td>
<td>Sia2-6Gal/GalNAc</td>
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<td>UEA-I</td>
<td><em>UlexEuropaeus Agglutinin I</em></td>
<td>Fucα1-2Galβ1-4Glc(NAc)</td>
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<td>VVA</td>
<td><em>Vicia Villosa Lectin</em></td>
<td>Terminal GalNAc, GalNAcα-Ser/Thr(Tn), GalNAcα1-3Gal</td>
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<tr>
<td>WFA</td>
<td><em>Wisteria Floribunda Lectin</em></td>
<td>Terminating in GalNAcα/β1-3/6Gal</td>
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<td>WGA</td>
<td><em>Triticum vulgaris</em></td>
<td>Multivalent Sia and (GlcNAc)n</td>
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**Figures and legends**
**Figure 1.** Layout of 37 lectins, the negative control (bovine serum albumin, BSA), and a positive marker contained in saliva or serum lectin microarray.
Figure 2. Salivary glycoprotein glycosylation patterns determined with Cy3-labeled lectins spotted onto a lectin microarray. UK: untreated KBD patients, TK: treated KBD patients, and Health: normal controls. a. Profiles of Cy3-labeled salivary proteins from the UK, the TK, and the healthy individuals bound to the lectin microarrays. The lectin microarrays revealed significant lectins marked with white rectangles. b. Significant differences in the lectin levels between the three groups. The bars show mean ± SD of three biological replicates from each group.
Figure 3. a. Serous glycoprotein expression levels were determined with Cy3-labeled lectins using a lectin microarray. UK: untreated patients with KBD, TK: treated patients with KBD and Health: normal controls. The lectin microarrays revealed significant lectins marked with white rectangles. b. Significant differences in the lectin levels between the three groups. The bars show mean ± SD of three biological replicates from each group.
Figure 4. Verification of the differences in the saliva levels of three lectins GSL-1, SNA and Jacalin. UK: untreated patients with KBD, TK: treated patients with KBD and Health: normal controls. a. Scanned pictures of Cy3-labeled lectins bound to the salivary protein microarrays. b. Box plot analysis of the salivary microarray data obtained from the three groups. Error bars show 95% confidence intervals for mean values. The P-values indicate the statistical significance of differences between groups. c. Scatter plot analysis of the salivary protein microarray data obtained from the three groups. Lines show mean ± SEM.
Figure 5. Verification of the differences in the sera levels of three lectins HHL, PNA and AAL. UK: untreated patients with KBD, TK: treated patients with KBD and Health: normal controls. a. Scanned pictures of Cy3-labeled lectins bound to the serous protein microarrays. b. Box plot analysis of the serous microarray data obtained from the three groups. Error bars show 95% confidence intervals for mean values. The P-values indicate the statistical significance of differences between groups. c. Scatter plot analysis of the serous protein microarray data obtained from the three groups. Lines show mean ± SEM.
Figure 6. Lectin blot analysis of the differential expressions of the glycopatterns in the saliva from three groups: Health: normal control; UK: untreated KBD and TK: treated KBD. The three groups of saliva pooled samples using 3 lectins (HHL, AAL and Jacalin). The apparent bands belonging to different molecular weights, ranging from 15 to 250 kDa, which were marked as L1-L8, respectively.
Figure 7. Lectin blot analysis of the differential expressions of the glycopatterns in the sera from three groups: Health: normal control; UK: untreated KBD and TK: treated KBD. The three groups of saliva pooled samples using 4 lectins (HHL, PNA, PHA-E+L and AAL). The apparent bands belonging to different molecular weights, ranging from 15 to 250 kDa, which were marked as L1-L10, respectively.