INTRODUCTION

Monitoring of the alcohol intake is important in a wide range of situations, beginning from the necessity of exclusion of drunken drivers and machinists, through early identification of harmful drinking habits, to the necessity of following abstinence and relapses in patients undergoing treatment for alcohol addiction (Bean et al., 2005; Dahl et al., 2011), as well as estimation of the amount of alcohol drunk or abstinence of the patients during treatment programs. As people often underreport the amount of alcohol intake or even deny alcohol ingestion, indicators more objective than self-report are necessary (Waszkiewicz et al., 2010a; Dahl et al., 2011; Northcote and Livingston, 2011). A simple and single detection of ethanol level in breath or blood is insufficient in the detection of alcohol dependence or heavy drinking. Although combined tests, like early detection of alcohol consumption (EDAC) and an analysis of a panel of 10–56 routine laboratory tests, are considered useful in detection of heavy drinking (Harasymiw and Bean, 2001, 2007; Helander, 2003; Harasymiw et al., 2004, 2005; Bean and Harasymiw, 2011), more specific tests seem to be much more convenient. Determination of the ethanol derivatives of extended excretion time, like ethyl glucuronides and sulfates, fatty acid ethyl esters and phosphatidylethanol derivatives, is considered as they are sensitive and specific indicators of recent alcohol ingestion (Dahl et al., 2002; Helander and Beck, 2004, 2005; Ermi et al., 2007; Halter et al., 2008; Helander and Zheng, 2009; Helander et al., 2009a,b; Palmer, 2009; Wåszkiewicz et al., 2010b; Hastedt et al., 2012; Lees et al., 2012). Although useful, such tests also generate false-positive as well as false-negative results. The reliable and sensitive test confirming prolonged alcohol consumption is based on the level of carbohydrate-deficient transferrin (CDT), the serum glycoprotein with oligosaccharide chains truncated as a result of the disease (Anton et al., 1996; Arndt, 2001; Hock et al., 2005; Bortolotti et al., 2006). In CDT, the amount of sialic acid is lowered when compared with physiological glycoprotein. Anyway, methods that enable reliable and objective information about the amount and regularity of drinking, as well as novel biomarkers complementary to the currently used tests, are strongly demanded (Ermi et al., 2007; Hannuksela et al., 2007; Neumann et al., 2008; Niemelä and Alatalo, 2010).

The other problem is the choice of the appropriate biological material for testing. Blood tests are still the most popular, but there are some attempts to exploit other materials, especially non-invasively obtained. Ethyl glucuronides, for example, may be efficiently determined in urine or hair (Helander et al., 2009a, 2010; Hoiseth et al., 2010). The other excreted fluid underexploited so far is saliva, which may be especially useful for screening procedures. The composition of saliva reflects not only pathologies within the oral cavity, but also systemic disorders, as numerous protein components of the saliva originate from the blood. Thus the saliva proteome is being analyzed recently as a potent source of disease biomarkers (Hu et al., 2006; Helmerhorst and Oppenheim, 2007; Loo et al., 2010; Pfaffe et al., 2011; Xie et al., 2011; Rosa et al., 2012; Wåszkiewicz et al., 2013).

Apart from the proteome composition, posttranslational modifications of the proteins are worthy of attention and detailed analysis. Among them glycosylation is especially important (Sondej et al., 2009; Wåszkiewicz et al., 2011, 2012a). CDT assays confirm the impact of heavy drinking on the glycosylation pathway. This is, however, not the only glycopeptide, which may be altered as a result of the pathological processes. Fucose amount and location in the oligosaccharide...
structures should be regarded equally important (Guile et al., 1998), similarly to the truncation of O-glycans leading to the expression of T and Tn antigens.

In this study we aimed to analyze the expression of the common glycoepitopes, and thus the fucosylation, α2,3 sialylation and truncation of O-glycans in the salivary glycoproteins of alcohol-dependent patients, after chronic alcohol intoxication, followed by 7 weeks of abstinence.

MATERIALS AND METHODS

Patients and samples
Alcohol-dependent patients admitted for detoxification to an alcohol unit of the Psychiatric Hospital at Chorzow were consecutively included in the study. The samples of mixed saliva were taken at the moment of hospitalization and after 7 weeks of treatment from 31 patients (5 women, aged 30–47 years and 26 men, aged 26–64 years). The time period of alcohol dependence varied from 2 to 30 years, with an average value of 14 years (median 10 years). Daily alcohol intake varied from 75 to 700 g of ethanol (mean 225 g, median 200 g). Withdrawal symptoms were managed with isotonic saline, glucose or multivitamins, and lactulose. The samples were collected after informed consent of the subjects. The study has been approved by local Bioethics Council (R-I-003/289/2005).

Saliva samples were centrifuged at 2500 × g directly after collection to remove all the cell components. The supernatant was stored frozen at −70°C until used.

Protein concentration
Protein concentration was determined with Coomassie Brilliant Blue G250 according to the Bradford (1976) method, applying bovine serum albumin (BSA) as a standard.

Reference saliva
Reference saliva was prepared by pooling the constant amount of protein, 2 μg each, of the saliva samples of the healthy subjects. The reference saliva was used in all ELISA tests apart from patients’ and control samples for standardization of the procedure. It was also used for lectin-blotting experiments.

Lectin ELISA
Total expression of carbohydrate epitopes was measured with the direct lectin-ELISA method (Ferens-Sieczkowska and Kossowska, 2004). Six lectins with different carbohydrate specificities were chosen for the analysis. Thus, Aleuria aurantia lectin (AAL), Lotus tetragonolobus agglutinin (LTA) and Ulex europaeus agglutinin (UEA) are able to bind terminal fucose, with some preferences toward the type of monosaccharide linkage and location in the core or antennary part of the glycan (Yamashita et al., 1985; Yan et al., 1997; Loris et al., 2000; Zerfaoui et al., 2000; Haselhorst et al., 2001). Maackia amurensis lectin (MAA) specifically binds terminal sialic acid residues, whereas α2,3 binds to galactose (Knibbs et al., 1991). Abrus precatorius and Vicia villosa lectins (APA and VVL, respectively) are specific toward truncated O-glycans, binding T (GalGalNAc) and Tn (GalNAc) antigens, respectively (Tollefsen and Kornfeld, 1987; Puri et al., 1992; Wu et al., 2001, 2009). Detailed specificities of the lectins are shown in Table 1.

The wells of the microtiter plates (Nunc, Maxisorp) were coated with saliva samples containing exactly 400 ng of protein in PBS (15 mM phosphate-buffered saline, pH 7.4) and incubated overnight at room temperature (RT). The plates were then washed thrice with PBS and the still free binding sites were blocked with 1% BSA solution in PBS, by 2 h incubation at RT, on the rocking platform. Next, the plates were washed thrice with PBS-T (PBS containing 0.05% Tween-20) and incubated with biotinylated lectins (Vector Laboratories, Inc., USA) in the same buffer (1 h, 37°C). Lectins were diluted with 15 mM Tris-buffered saline (pH 7.4), and 0.05% Tween-20 (TBS-T) as follows: 5000 × VVL, 2000 × AAL, 1000 × the remaining ones. After another extensive washing (5 × TBS-T), the plates were incubated with extravidin-alkaline phosphatase conjugate for 30 min at 37°C, diluted 20 000 (LTA, UEA, MAA) or 50 000× (AAL, APA, VVL). After the incubation, the plates were once again extensively washed with PBS-T and the phosphatase reaction was developed with p-nitrophenyl phosphate as a substrate (1 mg/ml in 0.1 M Tris–HCl buffer (pH 9.5) containing 1 mM MgCl2). The reaction was stopped with 100 μl of 1 M NaOH per well and the absorbance was read at 405 nm (reference filter 630 nm) with a StatFax 20 microplate reader (Awareness Technology, Inc., USA). The samples were loaded in duplicates and the experiments were repeated twice.

Lectin reactivity was expressed in arbitrary units, defined as the ratio of the sample and the reference saliva absorbance values ([AU] = sample O.D./reference O.D.).

SDS–PAGE and western blotting
For the lectin-blotting experiments the pooled saliva samples were prepared similarly to the reference saliva of healthy subjects. Thus, the aliquots containing 2 μg of protein were pooled from each patient’s saliva collected at the time of hospitalization (S1) and after 7 weeks of treatment (S2).

The samples were denaturated with 1% SDS and 2.5% mercaptoethanol by heating for 5 min at 100°C. The constant amount of protein equal to 2.5 μg was applied to the gel lane. Electrophoresis was performed in 10% polyacrylamide gel

<table>
<thead>
<tr>
<th>Lectin source</th>
<th>Specificity</th>
<th>Table 1. Carbohydrate specificity of the lectins used in the study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aleuria aurantia</td>
<td>Fucose, preferentially α1,6 linked to GlcNAc in the core region of the glycan (Yamashita et al., 1985)</td>
<td></td>
</tr>
<tr>
<td>Abrus precatorius</td>
<td>Thomsen-Friedenreich antigen (Gal-GalNAc) and Gal-GlcNAc (Wu et al., 2001, 2009)</td>
<td></td>
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<tr>
<td>Maackia amurensis</td>
<td>Sialic acid α2,3 linked to galactose (Knibbs et al., 1991)</td>
<td></td>
</tr>
<tr>
<td>Vicia villosa</td>
<td>Tn antigen (single GalNAc) (Tollefsen and Kornfeld, 1987; Puri et al., 1992)</td>
<td></td>
</tr>
<tr>
<td>Lotus tetragonolobus</td>
<td>Fucose in the antennary part of the glycan, preferentially Le^a^Le^b^ antigens, the presence of sialic acid disturbs binding (Yan et al., 1997; Haselhorst et al., 2001)</td>
<td></td>
</tr>
<tr>
<td>Ulex europaeus</td>
<td>Fucose in the antennary part of the glycan, preferentially bifucosylated Le^a^Le^b^ antigens (Loris et al., 2000; Zerfaoui et al., 2000)</td>
<td></td>
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...
Salivary glycoproteins of alcohol-dependent patients

Lectin structure seems to be also slightly decreased, with no α-altered fucosylation most probably due to the core (1,6-linked monosaccharide). This decrease was found to be statistically relevant with healthy subjects (0.83 ± 0.15 AU vs 0.96 ± 0.09, respectively). This decrease was found to be statistically relevant (**P** < 0.05) was considered significant.

### RESULTS

Protein content in the saliva samples varied from 0.5 to 5.15 mg/ml and did not differ between patients’ (average 1.89 ± 1.25 mg/ml) and control (1.91 ± 1.2 mg/ml) groups (**P** > 0.05).

The reactivity of saliva proteins with different lectins is shown in Table 2, and the scatter of the values within groups in box-whisker diagrams in Fig. 1.

Fucose expression measured with AAL was lower in the salivary glycoproteins of alcohol-dependent people compared with healthy subjects (0.83 ± 0.15 AU vs 0.96 ± 0.09, respectively). This decrease was found to be statistically relevant (**P** = 0.0002). As the LTA reactivity remained unchanged, the altered fucosylation is most probably due to the core α1,6-linked monosaccharide. The expression of bifucosylated Le^a^ structure seems to be also slightly decreased, with no statistical relevance, in the group of alcohol-dependent people, when compared with healthy subjects. In this part of the analysis non-secretor subjects, with no UEA reactivity, have been skipped; thus the group of alcohol-dependent patients was limited to **n** = 25, and controls to **n** = 12. Both groups were also distinguished by the expression of MAA-reactive, thus α2,3-linked sialic acid, which was lowered in alcohol-dependent patients. The difference occurred statistically significant (**P** = 0.024). Another disease-related alteration of glycosylation profile regarded the expression of T-antigen in O-glycans. This was also lowered in the alcohol abuse group (0.80 ± 0.27 vs 0.99 ± 0.357 in the control group, **P** = 0.038). Such a difference was not observed in the expression of Tn antigen, the single terminal GalNAc residue, recognized with VVL.

The results of the analysis of total expression of the lectin recognized glycoepitopes indicated on three glycan structures, which seem to be different in the saliva of alcohol-dependent from healthy people: AAL, MAA and APA. To obtain a more detailed insight into these features in particular glycoproteins present in the saliva, we used lectin detection, following the electrophoretic separation of the samples. Pooled saliva of the patients abusing alcohol was prepared in the same way as the reference one, using the samples collected at the time of hospitalization and after 7 weeks of treatment. Lectin-probed blots are presented in Fig. 2, and the results of densitometric analysis in Table 3.

Ten glycoprotein bands were shown to react with the lectins used for the analysis, differing in their molar masses from 86 to 13 kDa. In most of them ‘reach’ glycoepitopes, reactive with many lectins, were found (gp 57: all six lectins, gp 13: five out of six lectins, gp 28: four out of six). In other bands the glycoepitopes were found to be more selective and reacted with a single lectin (gp 76 and 70: LTA, gp 40: APA and slightly AAL, gp 31: APA).

Expression of the fucose residues was once again studied with three lectins: AAL, LTA and UEA, similarly to the ELISA tests. In the bands, which are AAL-, but not LTA- and UEA-reactive, fucose is supposed to be located in the core region of N-glycans, thus bound with α1,6 linkage. This is observed in gp 62. In contrast, gsp 28 and 13 bind LTA and UEA, but not AAL, indicative of α1,2,3,4,1 linked fucose, thus located in the antennary part of N-glycans and/or in O-linked oligosaccharides. In some of the analyzed glycoproteins the reactivity with lectins was found to be in the group of patients abusing alcohol and the reference group. AAL binding in gp 86 was almost twice increased in patients abusing alcohol when compared with the healthy subjects, and three times lowered in gp 40.

The alterations in antennary fucose are more prominent. At least a 2-fold increase of LTA-binding was observed in gp 86, gp 76, gp 70 and gp 13. In gp 86 the increase of UEA binding was 3.6 times until treatment.

### Table 2. Lectin reactivity of the salivary glycoproteins of alcohol-dependent and healthy people

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Alcohol dependent, <strong>n</strong> = 31</td>
<td>0.83 ± 0.145</td>
<td>0.76 ± 0.400</td>
<td>0.87^a^ ± 0.577</td>
<td>0.80 ± 0.269</td>
<td>0.72 ± 0.425</td>
<td>0.63 ± 0.301</td>
</tr>
<tr>
<td>Controls, <strong>n</strong> = 21</td>
<td>0.96 ± 0.093</td>
<td>0.75 ± 0.525</td>
<td>1.18^a^ ± 0.570</td>
<td>0.99 ± 0.349</td>
<td>0.89 ± 0.431</td>
<td>0.839 ± 0.299</td>
</tr>
</tbody>
</table>

Arbitrary units [AU] were defined as the ratio of the sample and the reference saliva absorbance values ([AU] = sample O.D./reference O.D.).

^a^Secretors only, **n** = 25.

^b^Secretors only, **n** = 12.
occurred over 30-fold and over 20-fold in gps 28 and 13, respectively.

Comparison of these values to those observed after 7 weeks of treatment was also found to be interesting. In some glycoproteins the saliva collected after 7 weeks of treatment has shown a tendency for a correction of disease-related alterations. Thus, in UEA-reactive gps 86 and 28 the significant increase of fucose expression has been corrected after the treatment and UEA binding was similar to that of the reference group.

Similar correction was observed in gp 67. In LTA reactivity, this tendency to return to the reference level of lectin binding was observed to a lower extent; thus fucose expression was only partially corrected in gp 76 and gp 13, similarly to AAL reactivity in gp 86.

Different expression of α2,3-linked sialic acid in the analyzed samples was observed in gp 84. The band was weak in the control group and also after treatment, while it was strong at the moment of hospitalization the binding of the lectin

Fig. 1. Lectin reactivity of saliva glycoproteins of alcohol-dependent and healthy subjects. 1: control subjects, 2: alcohol-dependent patients. Boxes represent 25–75% quartiles, whiskers: the range of non-outliers, filled squares, median; filled circles, outliers; asterisks, extreme.
Intensive increase of the MAA reactivity in patients abusing alcohol and its decrease as a result of abstinence were also observed in the 28 kDa band. The next two lectins bind truncated O-glycan structures, known as T (APA reactive GalGalNAc) and Tn (VVL reactive GalNAc) antigens. Six glycoproteins expressed APA reactivity and the relevant difference between healthy and alcohol abuse groups was observed in gp 31, together with a tendency to correct the alterations after the treatment. A similar pattern of alterations was found in VVL-reactive gps 28 and 13.

In the other glycoproteins no alterations were observed among the studied groups or the differences were relatively small (UEA-reactive gp 56, LTA-reactive gps 67, 57 and 28, AAL-reactive gp 57, MAA-reactive gp 57 and APA-reactive gps 86 and 62). Probably some of them (gp 56) are generally less sensitive for the glycosylation changes, as there is no difference in the reactivity with five out of six lectins. In the others the alterations observed at the time of hospitalization were found permanently through the period of treatment (VVL-reactive gps 62 and 57, LTA-reactive gp 86). In some cases the changes absent in the group abusing alcohol were detected later, after the abstinence period (MAA-reactive gps 67 and 62).

The total glycosylation changes detected in the ELISA tests are not directly reflected in the alterations observed in western blotting. This is especially visible in AAL reactivity, which is significantly decreased in patients abusing alcohol when measured in the whole saliva, but increased in the main glycoproteins and when total reactivity units were summarized in western blots. This probably reflects the impact of the glycosylation of high molecular weight mucins on the total value obtained in the ELISA tests. These glycoproteins, not penetrating the SDS–PAGE, were not included in the western blot analysis. This also shows that the glycome of the saliva, both in physiological and pathological conditions, is highly dynamic and, in different glycoproteins, not only the extent but also the directions of the alterations may be different.

**DISCUSSION**

Among salivary glycoproteins most interest is focused on the glycosylation of mucins. This is rather intelligible, as the...
percentage of glycoproteins found in saliva is estimated to be ~20% (Pfaffe et al., 2011). Their abundant carbohydrate moieties (up to 50% of mass) are able to form clusters with high avidity toward bacterial as well as immune cells. Thus mucin clusters of glycan act as mediators of the interaction between the epithelium of the oral cavity and the pathogens settled within it, forming the first line of non-specific host defense (Thornton et al., 1999; Gabriel et al., 2005; Pfaffe et al., 2011). It was reported that salivary MUC7 is rich in sLeα epitopes, also in a sulfated form, which is a typical adhesion epitope (Karlsson and Thomsson, 2009). Glycans of salivary glycoproteins competitively inhibit the adhesion of Candida albicans to epithelium of the oral cavity (Everest-Dass et al., 2012).

Monosaccharides composing sLeα (and related antigens) and directly mediating the interaction are antennary fucose and α2,3-bound sialic acid. In our study we have observed a decrease of total fucosylation in the saliva of alcohol-dependent people. A similar tendency was also noted for MAA-reactive sialic acid. Lowered APA reactivity (which occurred statistically significant) may suggest generally lower glycosylation, as the lectin binds galactose, the common and abundant component of both N- and O-glycans. Therefore salivary glycoproteins of alcohol-dependent people may be generally less glycosylated, which may lead to the weakening of this first line of defense, and increased susceptibility for infections via the epithelium of the oral cavity. The detrimental effect of chronic alcohol drinking on the salivary antioxidant and immune glycoproteins, e.g. decreased output of lactoferrin, immunoglobulin A and lysozyme (Waszkiewicz et al., 2012b,c,d,e), is known.

The other salivary glycoproteins, less abundant than mucins and bearing either N-glycans, or both N- and O-glycans, are therefore hidden in the shadow of mucin-origin glyocalyx. Their impact on host–pathogen interaction probably is not very significant; however, their glycan profiles may be altered due to the disease process. Especially N-glycans are worthy of interest, as the N-glycosylation pathway is much more complex than mucin O-glycans, and subjected to strict control mechanisms, thus may also be more sensitive to disease-related disturbances. The direct evidence for the disorders of N-glycosylation pathway in alcohol-dependent people comes from the well-established fact of the truncation of transferrin glycans and the accepted application of CD-transferin as a biomarker of prolonged alcohol consumption. To get a closer look of such glycans, we separated the samples in 10% polyacrylamide gel for SDS–PAGE before lectin-binding analysis. Thus we can observe the glycoproteins of smaller sizes [molecular weight (MW) up to 100 kDa], as mucins do not enter the gel and do not screen less abundant glycoproteins.

The salivary glycome has been studied recently and some glycoproteins bearing N-glycans have been identified in the studies of Ramachandran et al. (2006) and explored in detail in a further study by the same team (Sondej et al., 2009). The studies connect the precise MS analysis of the peptide structures with the comparison of the reactivity of glycans with as many as 15 lectins, differing in their binding specificity. As a result these authors have prepared a basic catalog of N-glycoproteins present in human saliva. The availability of such a database is particularly useful when glycan profiles of diseased and healthy subjects are being compared in a search of potential disease biomarkers.

In our study we have limited the number of lectins, but the chosen six ones are characterized with relatively narrow glycan specificity, to avoid or at least limit less specific interactions with the enormously diverse spectrum of glycans present in the biological material. Also transcription of the lectin reactivity to particular oligosaccharide structure is easier to perform. The chosen lectins enabled analysis of fucosylation and sialylation, the expression of the two monosaccharides engaged in the formation of sLeα, Leβ structures, participating in carbohydrate–protein interaction also in vivo. Apart from N-glycans, we have also analyzed truncated O-glycans, expressing T and Tn antigens.

The main questions asked in this study were: (a) Are there any glycan structures able to distinguish saliva glycoproteins originated from healthy and alcohol-dependent individuals? (b) If so, which glycoproteins are the most efficient carriers for such marker glycoepitopes? For such a pilot study, it is more convenient to analyze pooled samples from larger groups of subjects, as with this approach we are able to minimize the effect of inter-individual variations of glycosylation profiles, and simultaneously emboss features that are related to the disease and should be similar within the group. Moreover, densitometric western blotting analysis is only semi-quantitative, and thus does not seem reliable for statistical analysis of individual samples. In accordance with expectations, the glycosylation profile observed in the western blotting analysis was found to be more complex than that previously seen in the lectin-ELISA tests. In most of glycoproteins detected with lectins, disease-related alterations of the glycan profiles have been observed. Moreover, the tendency to correct the glycosylation has been observed after an abstinence period of 7 weeks in alcohol-dependent patients.

Once again antennary fucosylation and sialylation showed glycoepitopes to be the most sensitive to the alterations accompanying the disease process. Truncated O-glycans, exposing uncovered GalNac, determined with VVL, also seemed worthy of interest. In the serum transferrin of people abusing alcohol truncated oligosaccharides lack mainly terminal sialic acid. A similar feature was also reported for O-glycans of apolipoprotein E (Waszkiewicz et al., 2012a). In our investigation, we observed the increase of reactivity with the lectins. In LTA it concerns four protein bands, most probably including transferrin (76 kDa). The effect may result, at least in part, from diminished sialylation, as the lectin binds fucosyl only in the absence of sialic acid. For the O-glycan-specific lectins, the elevated binding may also result from the uncovering of the inner glycoepitopes because of SA release, but in APA and VVL reactivity it was observed only in a single glycoprotein. MAA-reactive α2,3-linked SA, the less abundant one, may also be masked with α2,6-linked monosaccharide in the control subjects, but accessible for the lectin when the total amount of SA is limited. The other situation should be considered for UEA reactivity. This lectin prefers bifucosylated Leα antigens, and this glycoepitope may be formed only in the absence of sialic acid, as both monosaccharides compete for the binding sites on the galactose residue. Two facts should be underlined as a conclusion. First, carbohydrate–protein interactions possible in such an altered microenvironment must be completely different from that in the saliva of healthy people. And the second, glycosylation changes do not affect all the glycoproteins in the same way.
Table 4. Comparison of MW values of lectin-reactive glycoproteins and the glycoproteins identified in human saliva

<table>
<thead>
<tr>
<th>Lectin-reactive bands MW [kDa]</th>
<th>Identified salivary glycoproteins</th>
<th>(Ramachandran et al., 2006)</th>
</tr>
</thead>
<tbody>
<tr>
<td>86</td>
<td>83.3 kDa polymeric IgG receptor precursor</td>
<td>83.3 kDa myeloperoxidase precursor</td>
</tr>
<tr>
<td>76</td>
<td>77 kDa transferring precursor</td>
<td>76.7 kDa CEA-related CAMS precursor</td>
</tr>
<tr>
<td>67</td>
<td>69 kDa afamin precursor</td>
<td>65.3 kDa galectin-3-binding protein precursor</td>
</tr>
<tr>
<td>57</td>
<td>57.8 kDa α-amylase precursor</td>
<td>57.8 kDa clusterin precursor</td>
</tr>
<tr>
<td>40</td>
<td>37.6 kDa α2 globin chain C region</td>
<td>53.9 kDa Ig α3 chain C region</td>
</tr>
<tr>
<td>45</td>
<td>45.2 kDa haptoglobin precursor</td>
<td>45.2 kDa haptoglobin chain precursor</td>
</tr>
<tr>
<td>39</td>
<td>39. kDa α1 HS gp precursor</td>
<td>39. kDa α1 HS gp precursor</td>
</tr>
<tr>
<td>38</td>
<td>38.9 kDa leucine rich α gp precursor</td>
<td>38.9 kDa leucine rich α gp precursor</td>
</tr>
<tr>
<td>38</td>
<td>38.2 kDa B2gp1 precursor</td>
<td>38.2 kDa B2gp1 precursor</td>
</tr>
<tr>
<td>31</td>
<td>34.2 kDa Zn-α2 gp precursor</td>
<td>34.2 kDa Zn-α2 gp precursor</td>
</tr>
<tr>
<td>28</td>
<td>22.8 kDa neutrophil gelatinase associated lipocalin precursor</td>
<td>22.8 kDa neutrophil gelatinase associated lipocalin precursor</td>
</tr>
<tr>
<td>23</td>
<td>23 kDa metalloproteinase inhibitor precursor</td>
<td>23 kDa metalloproteinase inhibitor precursor</td>
</tr>
<tr>
<td>27</td>
<td>27 kDa short palate lung and nasal epithelium carcinoma-associated protein precursor</td>
<td>27 kDa short palate lung and nasal epithelium carcinoma-associated protein precursor</td>
</tr>
<tr>
<td>13</td>
<td>14.2 kDa extracellular gp lacritin</td>
<td>14.2 kDa extracellular gp lacritin precursor</td>
</tr>
<tr>
<td>16</td>
<td>16.6 kDa prolactin inducible protein precursor</td>
<td>16.6 kDa prolactin inducible protein precursor</td>
</tr>
<tr>
<td>16</td>
<td>16.3 kDa cystatin-related epididymal spermatogenic protein precursor</td>
<td>16.3 kDa cystatin-related epididymal spermatogenic protein precursor</td>
</tr>
</tbody>
</table>

As the lectin-binding glycoproteins were described only with their MW, we tried to compare these values to the glycome database created by Ramachandran et al. (2006) (Table 4). Based on this comparison, and the availability of the appropriate antibodies, we can suggest some salivary glycoproteins, which seem worthy of detailed glycosylation studies in alcohol-dependent patients. Among them we can list transferrin (which is not surprising), α-amylase, clusterin, haptoglobin and also both heavy and light chains of immunoglobulins. Another area for further studies seems to enfold low molecular weight glycoproteins, so far neglected in their role as potential carriers of disease-altered glycoepitopes and rather difficult to be identified nowadays. In our previous studies we have found significant alterations in the disease-related glycosylation profile within glycoproteins in the molecular weight range from 30 to 15 kDa, in patients with lung cancer and joint diseases (Kossowska et al., 2005; Ferens-Sieczkowska et al., 2007). It seems possible that identification of glycoproteins in this range of MW may be a general challenge for disease-related glycosylation studies. Further studies should be focused on the identification of glycoproteins indicated here by their MW values, through immunodetection at the outset and finally with MS, and next, analysis of the glycosylation profile of a particular glycoprotein in the large groups of patient/individual samples, using lectin-ELISA or similar techniques. Although there are clear advantages of our study, the limitation may be the fact that the present findings were obtained by contrasting rather extreme groups (social drinkers vs alcohol drinkers consuming 75–700 grams per day).

The glycosylation profile alterations, suggested in this paper, may be used by studies aimed at following time-related changes of the glycosylation profiles during the abstinence of alcohol-dependent people, monitoring their abstinence, and also finding out if the glycosylation profiles reflect the amount of alcohol intake and/or the duration of alcohol abuse.

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