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# URINARY FREE OLIGOSACCHARIDES OF PATIENTS WITH LEUKAEMIA

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Free oligosaccharides (FOS) in urine of patients with different types of leukaemia and those of practically healthy people were studied using high-performance liquid chromatography (HPLC) after anthranilic acid labelling to evaluate the potential of these compounds for diagnostics and therapy monitoring. The labelled sugars were separated into neutral and charged oligosaccharides with QAE Sephadex (Q25-120) chromatography. The FOS profiles of normal urine were not identical in different samples, but all the samples had some common features. Urine FOS of the patients showed individual profiles containing different amounts of FOS species and were completely distinguished from those in normal urine. This individuality was provided mostly due to neutral species as HPLC profiles of charged FOS were almost identical among the samples.
***Keywords:*** free oligosaccharides, HPLC-profiles of glycans, urine, leukaemia.

## INTRODUCTION

The analysis of glycan structural alterations in glycoconjugates is becoming increasingly important in respect to search of new biomarkers in cancer, quality control of cancer therapy, and the development of new drugs and could be used in addition to genetic, gene expression and proteomic analyses. The observed alterations in glycosylation in cancer have led to clinical trials in which glycans on cancer cell-surface proteins are targeted. These new approaches to cancer treatment include immunotherapy and carbohydrate-processing inhibitor-based strategies. Compounds that mimic glycans involved in the metastatic dissemination of cancer are also actively sought. These glycodynamic approaches are contributing to our increased understanding of the underlying biology that is responsible for the development, progression and metastasis of cancer [1].

Cancer-associated alterations of glycan in glycocojugates or in their products of degradation have been found on the cell surface, in serum proteins and in urine components. New cancer glycobiomarkers or anticancer drug targets are being sought mostly among glycoproteins or proteoglycans and although cytosol free oligosaccharides in different cells are under intensive investigations [2,3,4] detailed analyses of their structures neither in serum nor in urine and their relevance to cancer alterations have not yet been carried out. Moreover, among all free N-glycans only high mannose-type is being studied so far and little is known about complex-type glycans in cell cytosol or in any biological liquid. At the same time even one of the first look at free oligosaccharides of

this type [5] allowed to find differences in some cells among the various cancer cell lines and to show a new direction for seeking of specific biomarkers for differentiated diagnostics, individual control and treatment.

As urinary samples can be collected in large amounts in a non-invasive fashion and they are the sources of informative compounds [6, 7], usage of urine has obvious advantages compared to serum or tissue. It means that any time it is possible to obtain enough amounts of samples without any disturbance of the patient that is vitally important in cases of cancer. That is why a detailed investigation of free oligosaccharides in urine must be promising addition to cancer glycobiomarkers obtained so far [8].

The main objective of this investigation was studying of free oligosaccharides in urine of patients with different types of leukemia to estimate prospects of their clinical and therapeutic usage.

**MATERIALS AND METHODS**

Urine samples (n=35) of patients with erythremia, subleukemic myelosis, hypoplastic anaemia, myelodysplastic syndrome with transformation, acute lymphatic leukaemia and acute myelomonocytic leukaemia were collected in SI "Dnepropetrovsk Medical Academy". The urine samples of practically healthy volunteers (n=20) were obtained in the Glycobiology Institute of Oxford University and in Dnepropetrovsk clinics. The samples were collected in accordance with the requirements of the Ethics Committees.

Partial sterilization and stabilization of urine with sodium aside (0.01%) were used for shipping and storage.

Protocols for preliminary purification of free glycans from urine were published by Alonzi D.S. et.al. [9] and were developed during further studies. The purification comprised filtration through a syringe with Millex-LH,0.45 µm, filter (Millipore Corp., USA) to take proteins away and porous graphitized carbon column (PGC from Thermo Electron, Runcorn, UK) chromatography to remove glucose.

Free oligosaccharides were analysed using high-performance liquid chromatography (HPLC) after anthranilic acid (2-AA) labelling [10, 11].

Free carbohydrates were labelled with 2-AA as described by Neville D.C.A. et.al.[10] with some modifications. For purification after 2-AA labelling the columns Spe-ed SPE Cartridges Amide-2, (Applied Separations, USA) were used after pre-equilibration with 1ml acetonitrile, 1ml $H_2O$, 1ml acetonitrile. Samples were loaded in the mixture of acetonitrile and water (97:3 v/v). The columns were washed with the mixture of acetonitrile and water (95:5 v/v) and the labelled oligosaccharides were eluted with 1.5ml water and separated by normal-phase HPLC (Water, UK) using a 4.6x250-mm TSK gel-Amide 80 column (Anachem, Luton, Beds, UK).

For more detailed studies 2-AA – labelled sugars were separated into neutral and charged oligosaccharides with QAE Sephadex (Q25-120) chromatography by eluting the neutral FOS with 0.5M acetic acid and the charged FOS with 0.5 ammonium acetate [11].

Glucose unit (GU) values were determined following comparison with a 2-AA-labelled glucose oligomer ladder derived from a partial hydrolysate of dextran as an external standard [10].

The data were collected and processed using Empower software, Waters Millennium, Waters Empower, Peak Time, Microsoft Office Excel 2003/2007, Microsoft Power Point 2003/2007.

**RESULTS AND DISCUSSION**

The concentration of urinary free oligosaccharides in the samples studied (Figure 1) varied over a wide range – from about 14000 pkmol/ml to about 90000 pkmol/ml – with the mean value 41410.71±3363.253 pkmol/ml and was significantly different ($p>0.01$) from that in the control group. All the samples of the patients could be subdivided into two groups ($p>0.05$): with a higher concentration **(**65871.85±3333.05 pkmol/ml) and with a lower one (30708.96±1826.65 pkmol/ml). The control samples also included two groups with different ($p>0.05$) concentrations of free oligosaccharides (37901.09±2846.16 pkmol/ml and 16466.3±1085.28 pkmol/ml) and the total mean value 23968.47 ±2658.67 pkmol/ml. In both cases the group with the higher concentration comprised about 30% of the samples.

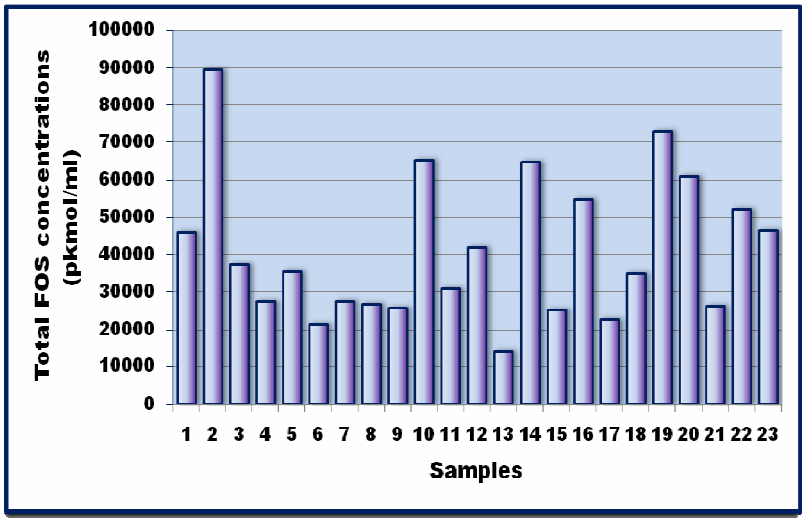


Fig.1. Total concentration of urinary free oligosaccharides of patients with leukaemia.

We were interested to analyze urinary free oligosaccharides consisting of 4 and more monosaccharide residues, therefore subjected to analysis of the chromatograms in the correspondent time interval from 20 to 44 min. HPLC chromatograms of the urine samples of practically healthy volunteers were not absoltely identical but similar enough to be used as control profiles. Chromatographic profiles of the patients' urinary free oligosaccharides were patient-specific with the most similar segment in the time interval from 26 to 44 min (Fig.2).

To simplify the comparison and analysis of the chromatograms we used two reference chromatographic peaks representing in the control profiles (Fig.2 A) as the main ones. The

position of the first of them corresponded to 5.87± 0.07GU (Fig.2C), the position of the second correlated with 8.76±0.05GU (Fig.2D). It means that the oligosaccharides in the first peak consist of 5-6 monosaccharide residues and those in the second peak comprise 8-9 monomer residues. In the figure 2 the positions of the reference peaks are shown by broken lines.

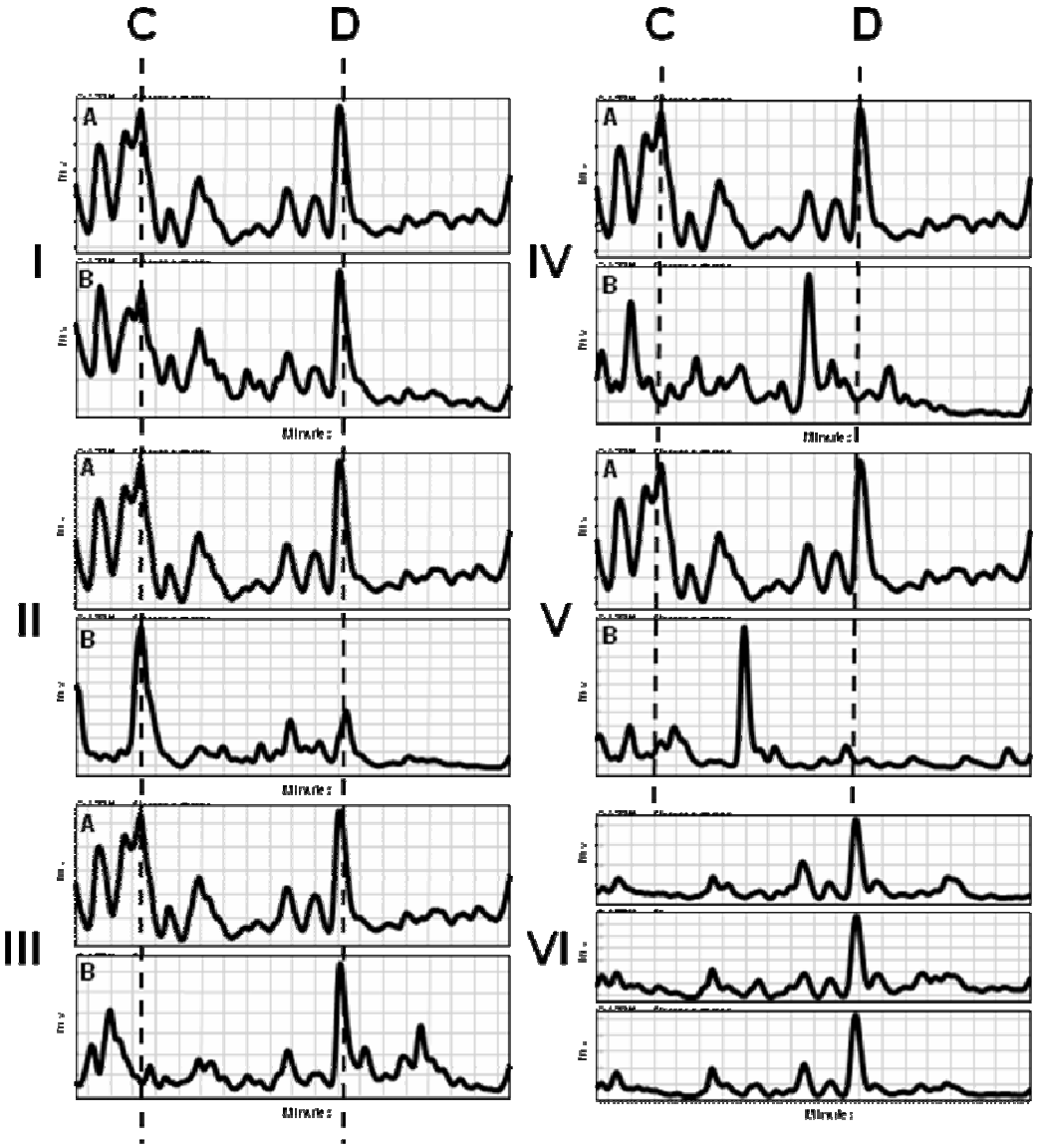


Fig.2. HPLC-profiles of urinary free oligosaccharides of patients with leukaemia. A – control; B – sample; C, D – position of the main reference chromatographic peaks; I, II, III, IV,V – HPLC-profiles of the total FOS in different samples; VI – HPLC-profiles of the charged FOS in different samples.

According to the structure of the HPLC-profiles in the mentioned interval all the samples can be divided into 5 groups (Fig.2. I, II, III, IV, V). Most of the samples were in the first group (Fig.2. I) with the chromatograms almost identical to the control in this interval. In the samples of groups II and III the first or the second reference peak was decreased. In some samples the reference peaks were not represented and a different main peak appeared in a different position (Fig.2. IV,V). Any correlation with a particular type of leukaemia was not found.

The separation of the urinary free oligosaccharides into neutral and charged fractions revealed that individual special characteristics of the HPLC-profiles were determined by the neutral fractions of the samples. The charged fractions were almost identical in all the samples (Fig.2.VI), included the only main peak with 8.76±0.05GU and a lot of minor species in the time interval from 26 to 44 min. This abundance of the minor peaks distinguished the charged fractions of the patients' urinary free oligosaccharides from the control.

## CONCLUSIONS

1. It was the first time HPLC spectra of urinary free oligosaccharides of patients with different types of leukaemia were obtained and compared with glycans spectra of practically healthy donors.
2. It was shown that HPLC-profiles of total fractions were patient-specific rather than disease-specific.
3. The neutral fractions of the samples comprised of the most species of the total fractions and determined HPLC-profiles' individuality.
4. The charged fractions of urinary free oligosaccharides of patients with different types of leukaemia were very similar and differentiated the patients' samples from those of practically healthy volunteers.
5. Analysis of urinary free oligosaccharides can be promising for searching of new cancer biomarkers.

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**Письменецкая И.Ю. Свободные олигосахариды мочи больных лейкемией / И.Ю. Письменецкая, С.Ю. Колыхалова, Т.Д. Баттерс //** Ученые записки Таврического национального университета им. В.И. Вернадского. Серия «Биология, химия». – 2014. – Т. 27 (66), №4. – С.69-74.

Для оценки перспективности использования в диагностике и мониторинге лечения различных видов лейкемий свободные олигосахариды (СО) мочи больных и здоровых волонтеров были исследованы высокоэффективной жидкостной хроматографией (ВЭЖХ) после маркирования гликанов антраниловой кислотой. Маркированные гликаны разделяли на фракции нейтральных и заряженных олигосахаридов хроматографией на QAE- Сефадексе (Q25-120). Спектры СО в норме не были полностью идентичны, но обладали характерными общими чертами. Спектры СО мочи больных были индивидуальны, состояли из разного количества пиков и полностью отличались от нормы. Эта индивидуальность связана, в основном, с разнообразием нейтральной фракции, так как ВЭЖХ-спектры заряженных СО были почти идентичны в различных образцах.

***Ключевые слова:*** свободные олиосахариды, ВЭЖХ-спектры гликанов, моча, лейкемия.

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