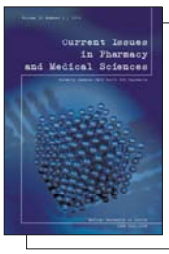

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Serum heparan sulfate concentration in healthy subjects

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ABSTRACT

Heparan sulfate is one of the essential components of extracellular matrices and plasma membrane. They take part in the interaction with protein ligands, which affects the metabolism, transport, information transfer, support and regulation in all organ systems. The aim of the study was to examine the physiological HS level in the serum of the healthy subjects, as well as to check if this level is age- and sex- related. The research was conducted on 30 healthy subjects: 16 women and 14 men, aged from 23 to 65 years, with the average of 30 years. Quantificational determination of HS was performed with the use of Heparan Sulfate ELISA kit. Concentrations of total cholesterol, HDL-cholesterol and triglycerides were measured with the use of standard enzymatic methods. The LDL-cholesterol was analyzed by direct method, and non-HDL-cholesterol was calculated. Based on the conducted research, it can be concluded that higher HS serum concentrations were observed in the group of healthy men compared to women group, and in the older ≥30 year-old group. The determination of serum HS concentration should consider gender and age differences in the result interpretation. Changes in HS serum concentrations are not correlated with the changes in the lipid profile parameters. For complete determining of existing statistical correlations between HS concentration, age, gender and the lipid profile parameters study results require confirmation on a greater number of cases.

Keywords: heparan sulfate, glycosaminoglycans, proteoglycans, ageing process, lipids profile

INTRODUCTION

Heparan sulfate (HS) is glycosaminoglycan (GAG) which consists of repeating disaccharide units composed of alternating N-acetylated or N-sulphated glucosamine units, and glucuronic or iduronic acid. The HS chains are connected to core proteins in the Golgi apparatus, using tetrasaccharide and then forms proteoglycans (PG). During their synthesis, they undergo a series of processes, such as deacetylation, sulphation and epimerization, which give them great structural heterogeneity of chain length, and the extent of sulphation and epimerization within the modified segments [1]. HS can appear in three spaces: cell surface, intracellular and extracellular matrix (ECM). Cell surfaces HS/HSPG act as receptors and bind a wide variety of ligands, such as chemokines, growth factors, infectious agents, enzymes, anticoagulants, and ECM components, thereby modulating their distribution, concentration and biological activity [3].

HS have essential roles in many systems: muscular, skeletal, nervous, digestive, urinary, circulatory and immune, as well as in embryological development and wound repair [10]. The bestknown HS ligand interaction, important for coagulation and inflammation, is the binding of antithrombin III [9]. Some HS, such as perlecan and agrin, contribute to the negative charge in the glomerular basement membrane (GBM). Changes in the concentration or the sulfation of HS may be associated with the development of nephropathy [8]. The luminal surface of vascular endothelial cells is covered by glycocalyx layer consisting of GAG, among which HS constitutes more than 50% of the total GAG. This layer behaves as an adhesion and transport barrier for water and larger solutes and plays essential roles in the mechanotransduction of shear stress [11]. The glycocalyx is involved in immune reactions and inflammatory processes, and mediates the release of nitric oxid [4]. Interaction of lipoproteins with HS is important for the cellular uptake and turnover of lipoproteins, in part by enhancing the accessibility of lipoproteins to lipoprotein receptors and lipase [6].

Sulphated GAGs, including HS were indentified as constituents of PrP^{Sc} plaques in the brains of Creutzfeldt-Jakob disease. Glipican-1(HSPG) interacts with both PrP^{C} and PrP^{Sc}, facilitating their conversion [5].

Although not all of the aspects of the HS role in these processes have been examined so far, correct interpretation of the results of the HS concentration requires a compilation of HS reference values. Thus, the aim of the

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study was determination of the physiological HS concentration in the serum of the healthy subjects, and examination of the presumptive dependence on the age and gender. In addition, it has been checked whether there is a correlation between the lipid profile parameters and HS serum concentration.

MATERIALS AND METHODS

The research was conducted in 30 healthy subjects: 16 women and 14 men, aged from 23 to 65 years, with the average of 30 years. Subjects who have coexisting disorders (diabetes, acute inflammatory disease, kidney, liver and bone disease, malignancy) which might have an influence on changes in HS serum concentration were excluded from the study. The study group was divided into subgroups, according to the gender and age, where 30 years of age was the border value. Blood samples were drawn after 14 hours of fasting, and laboratory tests were performed in serum. Quantitative determination of HS was performed with the use of Heparan Sulfate ELISA kit, (Seikagaku). HS concentration was expressed as HS serum concentration – µg/ml of serum. Routine laboratory parameters of lipid profile were determined using Roche analyzer by Roche kits. Concentrations of total cholesterol, HDL-cholesterol and triglycerides were measured with the use of standard enzymatic methods. The LDL-cholesterol was analyzed by direct method and non-HDL-cholesterol was calculated using the formula:

non-HDL-Cholesterol = Total cholesterol – HDL-cholesterol

Body Mass Index (BMI) was calculated using the formula:

$$BMI = \text{body mass [kg]} / \text{height [m}^2\text{]}$$

The results of the conducted studies were statistically analyzed using basic parameters of the descriptive statistics, and the data was expressed as the arithmetic mean ± standard deviation (SD), median, and quartile. Students' T test for independent variables was used for comparison between the groups. Non-parametric data was compared using the Mann-Whitney U test. Partial Spearman correlation coefficient was used to establish the association between serum HS concentrations and lipid parameters. All hypotheses were verified at the significance level of $p<0.05$. Statistical analyses were conducted using the Statistica program ver.10 (StatSoft, Polska).

RESULTS

The serum HS concentration and lipid profile parameters: total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, non-HDL-cholesterol, characteristics of examined men and women groups are shown in Table 1.

Table 1. The results of serum HS concentration and lipid profile parameters in healthy female and male groups

Parameters	Women group n = 16		Men group n=14		(p<0.05)
	Median	Quartile	Median	Quartile	
HS (µg/ml)	2.19	2.16/3.22	4.57	4.13/5.83	p=0.000006
T-Chol (mg/dl)	151.72	139.17/190.29	158.94	138.42/182.58	NS
LDL-Chol (mg/dl)	84.54	73.19/109.46	93.03	72.59/107.86	NS
HDL-Chol (mg/dl)	54.49	48.72/59.64	43.24	41.20/54.50	NS
non HDL-Chol (mg/dl)	98.92	81.78/131.35	113.17	99.00/147.43	NS
TG (mg/dl)	66.53	53.38/83.99	69.41	58.58/135.28	NS

Results have shown that HS concentration in women group (2.19 µg/ml) was significantly decreased when compared to the men group (4.57 µg/ml), (p=0.000006). No statistically significant differences in the range of the parameters of the lipid balance between the group of men and women were observed. In both groups, the obtained values of the lipid profile are within the recommended volume. The arithmetic mean of BMI in the healthy women and men was equal to 21 and 24 accordingly.

While evaluating the serum HS concentration in the healthy subjects, some statistically significant age-dependent differences were found: lower levels were observed in the <30 year-old group – 2.26 µg/ml, compared to 4.54 µg/ml in ≥30 year-old examined subjects, (p=0.0132) (Fig.1).

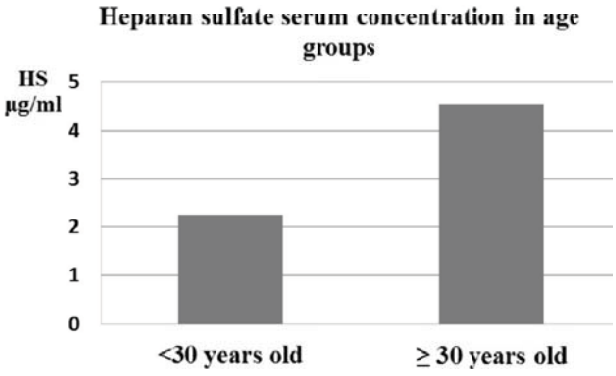

Fig. 1. The results of HS concentration in healthy subjects, depending on age (p=0.0132)

We did not observe any significant correlations between HS serum concentration and total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol, and non-HDL-cholesterol serum concentrations, as assessed by Spearman correlation method.

DISCUSSION

HS is one of the essential components of extracellular matrices and plasma membrane. Research over the past decades has demonstrated their importance in development and normal physiological processes. HS take part in

the interaction with protein ligands, which affects the metabolism, transport, information transfer, support and regulation in all organ systems [1]. Disturbances in the metabolism of HS were confirmed in the pathogenesis of mucopolisaccharidosis, atherosclerosis, osteoarthritis, emphysema and heart disease [6, 7, 9, 10, 12, 13].

In the majority of the available studies, HS is examined in the tissue samples or in the cell culture models. Undoubtedly, the quantitative examination of HS in the blood, especially in the serum, would be the most clinically useful. Nevertheless, it is connected with some analytical impediments resulting from the GAG isolation from the biological material, which always causes the loss of some of the examined analyte in the pretreatment procedure. In addition, multitude of the measurement techniques hinders or even prevents comparison of the results obtained using different procedures. Increasingly more frequently used, enzyme-linked immunosorbent assay (ELISA) techniques create perspectives of more widespread HS determination in the body fluids. Valid interpretation of the results obtained from the ill patients requires the compilation of the reliable reference values. Hence, the aim of the research conducted in this study was to examine the physiological HS level in the serum of the healthy subjects, as well as to check if this level is age- and sex- related.

Because HS is a common component of the connective tissue, the ageing process may influence the HS serum concentration. The first ageing changes in the skin and brain appear around 20-30th year of life, therefore the border value in the age groups division is 30 years in this examination. It has been proved that as the patient's age increases, the total GAG level in the tissues and organs decreases. This should be reflected by the changes of their concentration in the body fluids, such as serum, urine or synovial fluid. Similar phenomenon is taken under consideration in case of the concentration of individual GAG in the tissues. However, the changes in the concentration of the individual GAG, including HS, do not have to occur unidirectionally. What is more, Feyzi et al. proved that those ageing changes also have qualitative character and are connected with the increase in 6-O-sulfation, which causes enhanced binding of aorta heparan sulfate to platelet-derived growth factor [2].

The present study evaluated the value of serum concentrations of HS in healthy subjects. In the immunoenzymatic ELISA analysis, the median HS concentration in the healthy subjects, in the age group <30 years was equal to 2.26 μg/ml, while in the age group ≥30 years it was equal to 4.54 μg/ml and those differences had statistical importance.

What is more, comparative analysis has shown significant statistical differences in the HS concentrations between the groups of men and women, which were equal to 4.57 μg/ml and 2.19 μg/ml accordingly. This may be connected to the increased muscle and bone mass in men, and consequently increased connective tissue, as well as to the hormonal differences in men and women.

Changes relating to the HS in the structure of the vessels and ECM occupy a prominent place in the bibliography, although only several works concern HS in serum. In the available bibliography, it is possible to find works, in which the control group has been formed to examine HS concentrations in the impaired subjects. Tomatsu et al., who conducted such examinations on the patients suffering from mucopolisaccharidosis and mucopolidosis, formed the control group comprising 450 healthy subjects, divided into 5 age groups: 0-5, 5-10, 10-15, 15-20 and over 20 years old. In the children during puberty, the HS level was higher, probably due to the intensive processes in the connective tissue [12]. The arithmetic mean of the results obtained in the group over 20 years old was equal to 5.5 μg/ml, and was close to the values presented in this study. The examined material did not include serum from the children; the youngest person in that group was 23 years old, which justifies slightly lower HS concentrations than in the own results. It is worth mentioning that the small differences in the compared results may be because Tomatsu performed his examination in the plasma, while in the own work, it was done in the serum. In turn, Yousif et al. formed a group of 10 subjects aged 22-50 while determining serum HS in cirrhosis [14]. The serum HS determined using ELISA technique was equal to 6.5 μg/ml. It has to be emphasized that the control group in the quoted study was small and defined only in terms of liver disorders exclusion. It was not specified whether the subjects suffered from any other diseases, e.g. connected with the connective tissue, which may affect HS concentration. The advantage of the comparative analysis is the fact that in both studies the same measurement technique has been used (ELISA), which minimizes the risk of any changes resulting from the analytical procedure.

The significant feature of the ageing process is the atherosclerotic alternations, which occur as a cause of increased serum lipoproteins level, or hypertension. The deposition of cholesterol-rich lipoproteins promotes the formation of GAG-lipoprotein complexes, and consequently foam cells [9, 13]. This metabolic aspect was evaluated by setting a correlation between the HS concentration and lipid metabolism parameters, i.e. total cholesterol, LDL, HDL, non-HDL cholesterol and triglycerides. In the examined group, no correlation between the level of those parameters and HS serum concentration was observed. Nonetheless, it has to be emphasized that all the lipid metabolism parameters in the examined subjects were within the desired values, and BMI was correct. In the available bibliography there is a lack of studies

which could serve as a reference point for the changes occurring in the concentration of serum HS, in proportion to the lipid parameters.

Taken these observations together, HS serum concentration showed a significant association with age and gender. These results are the initial data and potentially useful diagnostic tool, which should be followed by the more precise and extended observations, involving a larger number of healthy subjects divided into age groups.

CONCLUSION

Based on the conducted research, it can be concluded that higher HS serum concentrations were observed in the group of healthy men compared to women group, and in the older, ≥30 year-old group. The determination of serum HS concentration should consider gender and age differences in the result interpretation. Changes in HS serum concentrations are not correlated with the changes in the lipid profile parameters. For complete determining of existing statistical correlations between HS concentration, age and gender, study results require confirmation on a greater number of cases.

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