Biochemical study of dermatan sulfate glycosaminoglycan in adult male patients with Nyhus type II inguinal hernia

Estudo bioquímico do glicosaminoglicano dermatam sulfato em homens adultos portadores de hérnia inguinal tipo II de Nyhus

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A B S T R A C T

Objective: To compare the amount of the dermatan sulfate glycosaminoglycan between male patients with Nyhus type II inguinal hernias and subjects without inguinal hernia, aged between 20 and 40 years. **Methods**: Two groups were formed: One with 15 male patients with Nyhus type II inguinal hernia and aged between 20 and 40 years with ASA risk I and II, and a control group of ten individuals, also males between 20 and 40, who had died up to 24 h before. We excluded female patients, diabetic patients with connective tissue disease, smokers and surgical risk ASA III and IV. We resected a sample of 1 cm□ of the transversalis fascia in the middle of the inguinal trigone, and 1 cm□ of the anterior sheath of the rectus abdominis muscle in the groin for the quantification of dermatan sulfate glycosaminoglycans by densitometry after agarose gel electrophoresis. **Results**: The amount of dermatan sulfate showed no statistically significant difference between patients with inguinal hernia and individuals without inguinal hernia in both the transverse fascia (p = 0.108) and anterior sheath of the rectus abdominis muscle (p = 0.292). **Conclusion**: There was no difference in the amount of the dermatan sulfate glycosaminoglycan among patients with Nyhus type II inguinal hernias and subjects without inguinal hernia in adult males.

Key words: glycosaminoglycans. Dermatan sulfate. Extracellular matrix. Inguinal hernia.

INTRODUCTION

The connective tissue is characterized by a cellular component, whose the main cell is the fibroblast, and an intercellular material called extracellular matrix. This, in turn, consists of a ground substance and fibers. The ground substance is made of complex glycosaminoglycans (GAGs) and proteins that are currently referred to as proteoglycan and a group of structural glycoproteins also called multifunctional proteins. The fibers are represented by collagen, elastic and reticular fibers[1-3].

The extracellular matrix was stigmatized as just a passive framework, which supported the tissues on an inert way. Currently, it is recognized as a dynamic environment in which tissues organize, exchange information and differentiate[4]. In the extracellular matrix, collagen fibers are responsible for tissue resistance, the elastic fibers and proteoglycans by the elasticity, and multifunctional proteins or glycoproteins by tissue adhesiveness[5].

The degeneration of collagen is reported by many authors as the main factor in the genesis of inguinal hernia[6-10]. The decrease in collagen I and increased in collagen III in the dense connective tissue are weakening factors that favor the emergence of hernia[11].

There is very intense interaction between the components of the connective tissue, to the point that changes in one of these components disrupt its harmony, compromising tissue function[12-14].

The decorin proteoglycan with dermatan sulfate glycosaminoglycan is crucial in fibrillogenesis and arrangement of collagen fibers in the extracellular matrix of the connective tissue. It is believed that decorin is a key regulator of collagen fibrillogenesis and is associated, in its disability, with the weakness of fascia, tendons and skin fragility. The deficiency of decorin is also associated with the Ehlers-Danlos syndrome[15].

This study aims to quantify and compare the dermatan sulfate glycosaminoglycans in the transversalis

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fascia and anterior sheath of the rectus abdominis between male patients aged 20 to 40 years with inguinal hernia Nyhus type II and those without an inguinal hernia.

METHODS

Casuistry

All patients were operated at the Department of General Surgery of the St John Baptist Hospital of the School of Medical Sciences of Volta Redonda. This study was approved by the Ethics Committee at the Federal University of São Paulo under No. CEP 1406/08.

Two groups were so arranged: group I – 15 male patients, aged between 20 and 40 years, with Nyhus type II inguinal hernia and risk ASA I and II; group II – control: ten fresh male cadavers with period of death less than 24 hours, aged between 20 and 40 years, without inguinal hernia. We excluded females, patients aged below 20 years and over 40, diabetics, patients with connective tissue disease, smokers and surgical risk ASA III, IV and V.

Collection procedures

During surgery to treat inguinal hernia in group I, 1 cm^2 fragments were removed both from the transversalis fascia in the middle portion of the inguinal triangle (Hasselbach's triangle) and from the corresponding anterior sheath of the rectus abdominis muscle.

During the autopsy in group II, after assuring that there was no inguinal hernia on either side, a fragment of 1 cm^2 of the transversalis fascia of the inguinal triangle and of the anterior sheath of the rectus abdominis muscle were removed.

Extraction of glycosaminoglycans

The samples were washed in phosphate buffered saline (PBS). After this process, they were shredded without being separated, fixed in 100% acetone and refrigerated. They were later taken to the laboratory of Molecular Biology, Department of Biochemistry, Federal University of São Paulo - Paulista Medical School (UNIFESP - EPM).

The fragments were removed from the acetone and subjected to drying in an oven at 50° C, resulting in a ketonic powder, which was individually weighed on an analytical scale. The dry powder was subjected to proteolysis by maxatase 4mg/ml (Sigma, St Louis, MO, USA) in 0.05 M Tris-HCl buffer, pH 8.0, 1M NaCl, in the proportion of 1g ketonic powder for each 20 ml of buffer, stirring. The solution was kept overnight at 60° C. On ice, 90% trichloroacetic acid was added to the 10% final concentration for precipitation of nucleic acids and peptides for 20 min at 4° C. Then the material was centrifuged at 5000r for 20 minutes at room temperature, the precipitate being discarded. The supernatant was added to two volumes of methanol for precipitation of glycosaminoglycans, at 20° C (freezer) for 18 to 24 hours. A new 5000r centrifugation was repeated for 20 minutes at room temperature, discarding the supernatant. The precipitated material containing GAGs was dried in an oven and re-suspended in distilled water at a rate of 5mg ketonic powder to 10ml of distilled water. Finally, the compounds were identified by agarose gel electrophoresis and quantified by densitometry[16-18].

Identification and quantification of glycosaminoglycans

Identification of sulfated glycosaminoglycans was performed by comparing the electrophoretic migration of the samples with those of known standards and purified. These same standards were used for the quantitative determination of compounds by densitometry at 525 nm. We used the Quick Scan 2000 densitometer Win - Helena Laboratories – Beaumont, Texas, USA – for this purpose.

The standards used were chondroitin sulfate extracted from whale cartilage; dermatan sulfate extracted from bovine intestinal mucosa; and bovine lung heparan sulfate. All have been purified in the Laboratory of Molecular Biology, UNIFESP.

Comparisons were made of the amount of dermatan sulphate and total glycosaminoglycans between transversalis fascia and anterior sheath of the rectus abdominis muscle in the control group and the inguinal hernia group separately. This was meant to assess if there was a difference and if it was a localized or systemic change.

The second analysis was made in the transversalis fascia to compare the amount of dermatan sulfate between individuals with inguinal hernia and individuals without it. Another analysis was performed to compare the amount of dermatan sulfate in the anterior sheath of rectus abdominis muscle in individuals with inguinal hernia and in individuals without it.

Statistical analysis

We conducted the following statistical tests: a) non-parametric test of Wilcoxon, used to compare two dependent or paired samples; it was applied to compare the amount of glycosaminoglycans between the locations transversalis fascia and rectus abdominis' sheath for the same patients; b) nonparametric Mann-Whitney, used to compare two independent samples; it was used to compare the amount of glycosaminoglycans between individuals with inguinal hernia and individuals without it. For all statistical tests the level of significance was set at alpha <0.05 or 5%.

RESULTS

There was no statistical difference between the amount of dermatan sulphate and total glycosaminoglycans in the transversalis fascia and anterior sheath of the rectus abdominis musle in the groups separately (Table 1).

There was no statistical difference between the amount of dermatan sulphate and total glycosaminoglycans in the transversalis fascia of patients with hernias and the control group (Table 2).

There was no statistical difference between the amount of dermatan sulphate and total glycosaminoglycans in the sheath of the rectus abdominis muscel between the control group and patients with hernia (Table 3).

DISCUSSION

The strong and significant interaction of glycosaminoglycans with collagen fibrillogenesis is a widely known fact.

In the dense connective tissue (fascia, tendons and aponeurosis) there is a proteoglycan called decorin that has, connected to its core protein, a single chain of glycosaminoglycan, which may be more often a dermatan sulfate or chondroitin sulfate. The proteoglycan decorin / dermatan sulfate represents 90% of the proteoglycans in the dense connective tissue and it is the one which has greater ability to interfere with collagen fibrillogenesis by inhibiting the radial growth of the fibril[19,20].

Proteoglycans dermatan sulfate and chondroitin sulfate interact strongly with collagen I, both through the glycosaminoglycan chain and through the protein core, inhibiting collagen fibrillogenesis[21].

A study showing the interaction between proteoglycans and collagen in rat tendon found that the chondroitin sulfate proteoglycan is related to the formation of thinner collagen fibers, present in immature tendons, and that dermatan sulfate proteoglycan is related to the thicker collagen fibers present in mature tendons. It also demonstrated that the increase of chondroitin sulfate proteoglycan leads to thinner collagen fibers, similar to what happens with the corneal tissue. In the mature phase of the tendon, the concentration of chondroitin sulfate proteoglycan decreases and the one of dermatan sulfate increases, leading to a predominance of thick fibers[22].

Through an electron microscopy study that examined the arrangement between the glycosaminoglycans and collagen fibers in tendons, it was found that the glycosaminoglycans are present on the outside, around the collagen fibril, and not within it. It was concluded that there is a strong and specific interaction between glycosaminoglycans and collagen in the dense connective tissue[23].

Studies performed a breach in the gene responsible for synthesis of proteoglycan decorin dermatan

Table 1 – Comparison of the amount of dermatam sulfate between the transversalis fascia and anterior sheath of the rectus abdominis muscle in groups separately.

Dermatan Sulfate	Without hernia	With hernia
Sheath > fascia	6	8
Sheath < fascia	4	7
P value	0.799	0.394

Wilcoxon test.

Table 2 - Comparison the amount of dermatam sulfate between control and inguinal hernia groups in the transversalis fascia.

Glicosaminoglycans	Control	Hernia	p value Mann-Whitney
Dermatam Sulfate			0.108
Average	3.6	2.9	
Standard deviation	1.1	0.92	
Min – max	1.86 – 5.18	1.41 – 5.07	

Table 3 - Comparison of the amount of dermatam sulfate between the control group and patients with inguinal hernia in the sheath of the rectus abdominis muscle.

Glycosaminoglycans	Control	Hernia	p value Mann-Whitney
Dermatam Sulfate			0.292
Average	4.07	3.16	
Standard deviation	1.85	0.63	
Min – max	2.11 – 7.97	1.74 – 4.12	

sulfate in rats, comparing them to a control group, founding that rats with the absence of decorin had different diameters and size of fibrils, alternating thick and thin fibers, opposite to the uniformity found in the control group. They had also irregularly shaped fibers, in contrast to the circular form of the control group. These changes have an important influence on the strength of the tissue. They concluded that decorin plays a fundamental role in the formation and ordering of collagen fibrils in the extracellular matrix, and what changes it can result in alterations in the architecture of the extracellular matrix, with consequent loss of tensile function of the dense connective tissue[15].

We know the importance of decorin dermatan sulfate proteoglycan in fibrillogenesis and arrangement of collagen fibers in the extracellular matrix and its consequent role in tissue resistance. The core protein of proteoglycans binds to collagen fibrils and the glycosaminoglycan chains are connected with the chain of the neighboring proteoglycan glycosaminoglycan. This arrangement gives mobility to the glycosaminoglycan chains that can align orthogonally or parallel to the long axis of collagen fibrils, rearranging during stress, ensuring the mechanical communication of the fibrils and distributing the received strength throughout the tissue[24,25].

Glycosaminoglycans have a key role in the modulation of collagen fibrillogenesis and organization of the extracellular matrix. It is hypothesized that because of this close relationship and functional mechanics of glycosaminoglycans with collagen, it would be possible, in theory, that changes in concentration of glycosaminoglycans might compromise the collagen fibers of the extracellular matrix of the connective tissue of the transversalis fascia, and therefore, cause the weakening of the tissue and appearance of inguinal hernia.

When the pathogenesis of inguinal hernia is studied, four factors must be analyzed: peritoniovaginal conduct persistence, failure of the musculo-fascial defense mechanisms of the inguinal canal, degeneration of connective tissue and triggering factors.

The degeneration of connective tissue is the main factor in the origin of inguinal hernia, a fact confirmed in clinical practice with the use of prostheses for its treatment. The use of prostheses, which in reality deals with connective tissue disease, reduced the recurrence of inguinal hernia to less than 1%[26-28].

In the extracellular matrix of dense connective tissue collagen is primarily responsible for tissue resistance. However, the matrix does not have only a support function. Today we know that it has complex roles, such as adhesion, differentiation, division, migration and cellular recognition, and information transmission between cells. There is intense interaction and harmony among the members of the extracellular matrix, and changing one component may compromise the function of the other and therefore determine tissue functional loss[4,12,29,30].

Recent studies on the pathogenesis of inguinal hernia analyzed mainly collagen. With results still controversial in humans, the alteration of the collagen fibers is the main factor in the weakening of connective tissue, but other components of the extracellular matrix could be causing the change of collagen fibers. Studies point to this fact by verifying changes in components of the extracellular matrix other than the collagen fibers in patients with inguinal hernia[14]. We then proposed to study, within the extracellular matrix, the glycosaminoglycans dermatan sulfate constituent of decorin proteoglycan, which has a primary role in collagen fibrillogenesis, in a specific group of hernias, Nyhus type II, and in younger individuals in which the effects of the extracellular matrix degeneration are less evident. Despite not finding statistically significant difference in the amount of glycosaminoglycans between patients with inguinal hernia and the control group, the role of glycosaminoglycans in the extracellular matrix is not only represented by their concentration. Changes in the molecular structure, such as alterations in the site of sulfation, for example, may also compromise their function[31]. Thus, further studies are needed to clarify whether there is change in the molecular structure of glycosaminoglycans that could alter their function and thus collagen fibrillogenesis.

The authors believe that for an individual to develop indirect inguinal hernia it is necessary a persistence of a patent peritoniovaginal conduct along with failure of the defense mechanisms in the inguinal canal, caused by the degeneration of the connective tissue, which causes the abdominal contents to protrude through the deep inguinal orifice, associated with triggering factors, which are conditions that increase intra-abdominal pressure.

In conclusion, there was no statistically significant difference between the amount of the dermatan sulfate glycosaminoglycan in men aged between 20 and 40 years with Nyhus type II inguinal hernia and those without an inguinal hernia.

RESUMO

Objetivo: *Comparar a quantidade do glicosaminoglicano dermatam sulfato entre pacientes homens, portadores de hérnia inguinal tipo II de Nyhus e, indivíduos sem hérnia inguinal, com idade entre 20 e 40 anos.* ***Métodos:*** *Foram constituídos dois grupos. Um de 15 pacientes do sexo masculino com hérnia inguinal tipo II de Nyhus e idade entre 20 e 40 anos, com risco ASA I e II, e um grupo controle com dez indivíduos, também do sexo masculino entre 20 e 40 anos, que morreram em período de até 24 h. Foram excluídos os pacientes do sexo feminino, diabéticos, portadores de doença do tecido conjuntivo, tabagistas e com risco cirúrgico ASA III e IV. Foi retirada uma amostra de 1cm² da fáscia transversal na parte intermediária do trígono inguinal, e 1cm² na bainha anterior do músculo*

reto abdominal na região inguinal correspondente e quantificados os glicosaminoglicanos dermatam sulfato por densitometria, após eletroforese em gel de agarose. ***Resultados:*** *A quantidade de dermatam sulfato não apresentou diferença estatisticamente significante entre os pacientes com hérnia inguinal e os indivíduos sem hérnia inguinal, tanto na fáscia transversal (p=0,108) quanto na bainha anterior do músculo reto abdominal (p=0,292).* ***Conclusão:*** *Não se encontrou diferença na quantidade do glicosaminoglicano dermatam sulfato entre os pacientes portadores de hérnia inguinal tipo II de Nyhus e indivíduos sem hérnia inguinal em homens adultos.*

Descritores: *Glicosaminoglicano. Sulfato de dermatana. Matriz extracelular. Hérnia inguinal.*

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