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**MATRIX METALLOPROTEINASE-9 AS A BIOMARKER PREDICTING
MALE FERTILITY**

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Abstract

Early recognition of fertility after hernia repair surgery performed according to different methods is still a challenging task. The paper discusses the study of matrix metalloproteinase-9 (MMP-9) content in ejaculate after the hernia repair surgery. The clinical research study is based upon the results of inguinal hernia repair in 68 male patients at the age from 18 to 40. The elevated concentration of MMP-9 content in ejaculate is conclusively ascertained even at early cases of disease processes after the hernia repair surgery. High content of MMP-9 in the ejaculate of patients operated for the hernia repair shows that this enzyme takes part in the after surgery disease process. Along with that it is possible to say that not only the chemical composition but the implant surface morphology as well is an important characteristic that influences the body response to the synthetic material. The results of the study give reasons to consider the elevated content of MMP-9 in ejaculate the evidence of a long-continued inflammatory process. In the surgery performed with Desarda techniques with no synthetic implants introduced, the above said value is reduced many times. The detection of metalloproteinase (MMP-9) in ejaculate can be considered as a criterion for choice of hernia repair surgery for fertile men.

Keywords: inguinal hernia; matrix metalloproteinase; fertility.

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**МАТРИКСНАЯ МЕТАЛЛОПРОТЕИНАЗА-9 КАК БИОМАРКЕР
ПРОГНОЗИРОВАНИЯ МУЖСКОЙ ФЕРТИЛЬНОСТИ**

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Аннотация

Ранняя диагностика фертильности после герниопластики различными способами остается трудной задачей. Данная работа посвящена исследованию матриксной металлопротеиназы-9 (ММР-9) в эякуляте после герниопластики. Клиническое исследование базировалось на материале результатов хирургического лечения паховой грыжи у 68 пациентов мужского пола в возрасте от 18 до 40 лет. Установлено достоверно повышенная концентрация ММР-9 в эякуляте после герниопластики уже на начальной стадии патологического процесса. Высокие показатели ММР-9 в эякуляте у пациентов оперированных по поводу паховой грыжи демонстрирует участие этого фермента в патологическом процессе после операции. Вместе с тем можно говорить, что не только химический состав, но и морфология поверхности имплантата является важной характеристикой, определяемой реакцию организма на синтетический материал. Результаты исследования дает основание, что увеличение содержания ММР-9 в эякуляте можно говорить о наличии продолжительного воспалительного процесса. При оперативном лечении по методике Desarda без синтетических эндопротезов эти показатели в разы меньше. Определение металлопротеиназы (ММР-9) в эякуляте может служить критерием выбора герниопластики у репродуктивных мужчин.

Ключевые слова: паховая грыжа; матриксная металлопротеиназа; фертильность.

INTRODUCTION

WHO estimates that in developed countries, the fertility level of the population is up to 20% [1, 3]. In the Russian Federation, more than 15% of couples of reproductive age are infertile and this problem is undoubtedly of state importance [4]. According to the report of the Ministry of Health of the Republic of Dagestan in 2014, the incidence of infertility exceeds the average of 1.2 - 1.5 times. Such diseases as cryptorchidism, varicocele, inguinal hernia violate the reproductive function of men and 40 - 45% of infertility is due to male sterility (4). In the literature, there is no precise statistics on the percentage of infertility in men suffering from an inguinal hernia. In our country, a great number of operations on inguinal hernias are made. The introduction of synthetic materials in surgical practice in hernia repair has reduced the frequency of relapses up to 0.5 - 5% [5, 10]. In this regard, now the surgeons are facing a challenge of improving long-term results, including male infertility due to hernia carrier and hernia repair [1, 2, 8]. The studies revealed a negative effect as of hernia carrier on spermatogenesis and subsequent hernia repair [13, 29]. Various techniques for inguinal canal traditional plastics involve plastic inguinal canal local tissues with trauma, the movement of the elements of the spermatic cord. Internal or external inguinal ring is narrowed, the degree of narrowing is determined by the tip of the little finger of the surgeon and is therefore primitive. The spermatic cord is surrounded by a dense, stubborn scar tissue, which can also lead to disruption of the blood – and lymph drainage from testicles [2, 5]. In the postoperative period, when the plastic local tissues are likely to develop ischemic orchitis, is 2.5 - 3.2% [2]. Violation of blood-testis barrier caused by ischemia can also stimulate the production of sperm antibodies, followed by the development of autoimmune orchitis with testicular dysfunction contralateral testis. It should be recognized that the problem of surgical treatment of inguinal hernias in men of reproductive age is far from being resolved. If we consider that postoperative infertility usually begins unnoticed, it is characterized by a prolonged period, small and hidden symptoms, the diagnosis in the early stages is a difficult task. Therefore, the problem of early diagnosis of postoperative fertility is important. Over the past decade, due to the development of molecular biology, biotechnology and genetic engineering has made a significant progress in the study of gene expression control mechanisms involved in physiological and pathological processes of the human body [4, 7]. Taking into account the lack of study of hormonal

background after various options of prosthetic hernioplasty, it is of particular interest to study the activity of metalloproteinases [11, 12].

MAIN PART:

Objective. The study of the prognostic significance of the expression of MMP-9 in the ejaculate after prosthetic hernia repair.

Materials and methods of the research. This clinical research study is based upon the results of inguinal hernia repair in 68 male patients at the age from 18 to 40, hospitalized and operated by means of clinical bases of Continuing Medical Education and Professional Development of Dagestan State Medical University, National Interregional Multifield Hospital and Derbent Hospital during the period from 2010 to 2015. All the patients were engaged in the study with observance of the basic principles of Biomedical Ethics during the medical experiment performance. The Research Protocol was approved by the local Ethics Committee of the State-funded Educational Institution of Higher Vocational Education «Dagestan State Medical University».

All patients having inguinal hernia were separated into 3 groups:

1-st – 23 patients operated according to Lichtenstein.

2-nd – 23 patients operated according to Transabdominal Preperitoneal Hernia Repair method.

3-rd – 22 patients operated according to Desarda.

The control group included 5 patients undergoing their ejaculate diagnostics for infertility.

The criteria for the patients to be included into the selected subset are as follows:

– inguinal hernia detected using the Nyhus classification;

– elective surgeries for inguinal hernia;

– age of patients between 18 and 40.

112 patients who were hospitalized for the indicated period of time fulfilled the above said criteria.

The exclusionary criteria are as follows:

– relative or absolute contra indications to any type of Hernia Repair Surgery, foreseen for the present research (6 patients);

– history of surgeries for varicocele or hydrocele (2 patients);

– history of dysregulated spermatogenesis before the hernia progression (1 patient);

– detected cryptorchism (3 patients);

– refusal to participate or lack of informed consent statement for participate on at any stage of the study (44 patients).

Thus the selected subset of 68 patients corresponding to the inclusion criteria and not in conformity with exclusion criteria was formed.

Thus the 50 patient with unilateral hernia were identified and among them: 26 (38.2%) patients with sinister al hernia, 24 (35,3%) patients with dextral hernia and 10 (14,7%) patients with bilateral hernia of the total number of patients diagnosed with hernia. According to Nyhus classification of hernia types, 16 patients were diagnosed with the 1-st type, 22 patients – with the 2-nd type, 19 patients – with the 3-rd B type, and 11 patients – with the 4-th type.

All patients signed the informed consent statement for research study and the related therapy. All patients underwent a general clinical examination. 1 ml ejaculate samples were homogenized in Potter homogenizer in $\pm 4,1^{\circ}\text{C}$ Tris Buffer (pH 8,0), 0,02 M EDTA. After 2 minutes of the centrifuge process, using the D-24T centrifuge (Federal Republic of Germany) at 5000 rpm, the solvable forms of analyzed metalloproteinases-9 (MMP-9) were detected in supernatant fluid by enzyme-linked immune sorbent assay (ELISA) by applying conventional test-systems by R@DS (USA). The optical density measurement was conducted using the digital immune enzyme metric analyzer AT-858 (Chine) at 450 nm wave length. The calibration line construction and calculate on of concentrations of MMP-9 analyzed values were performed in accordance with linear regression conditions in logarithmic coordinates. The postsurgical examination and ejaculate sampling took place 20 days past the surgery.

The Research Protocol was approved by the local Ethics Committee of Dagestan State Medical University of the Russian Federation Ministry of Health. The study was conducted in accordance with the National Standard of Russia. Before including into the study program, all the patients and donors had given their voluntary informed consent. The statistical processing of data was performed in «Statistica 7,0» software using the single-factor analysis of variance (Fisher's variance ratio). The differences were considered significant at $p < 0,05$.

Results and their discussion. The results obtained as a part of the study point out to the fact that the most traumatic method of inguinal hernia repair is the inguinal canal posterior wall plastic surgery according to Lichtenstein, while it was not the case for surgeries according to Transabdominal Preperitoneal Hernia Repair and Desarda methods. The most severe hemodynamic dysregulations during the post-surgery period were detected in patients after the hernia repair according to Lichtenstein. Although there was also some blood circulation deficiency, but

the results were much better in the Transabdominal Preperitoneal Hernia Repair and Desarda methods than in the Lichtenstein method of hernia repair surgery. In the latter case, the average blood circulation speed decreased by 21% and maximum blood circulation speed decreased by 17.3% in 6 months after the hernia repair surgery. The augmentation of average values of resistance index decreased by 11% with hemodynamic changes in patients after the hernia repair surgery according to the Lichtenstein method evidencing the blood circulation difficulties over the spermatic cord blood vessels. The dynamic changes of the analyzed values in patients after the hernia repair surgery according to Desarda indicate that the testicular functions and hemodynamics are less affected by surgery traumas than they are in the first case. For Transabdominal Preperitoneal Hernia Repair surgeries the better blood flow recovery is incidental to the method of over inguinal access during the surgery. All surgery manipulations within the anteperitoneal space are less traumatic for the structural elements, because they are basically performed near the spermatic cord at its tension, but MMP-9 values are still high in 10 patients during the post-surgery period. During the surgery with the Lichtenstein method the operating surgeon must work in a close proximity with the spermatic cord and the most harmful effect causes the extensive touching of a wire mesh implant. This aggravates the inflammatory response of the spermatic cord structural elements leading to scarring of the structure of these elements. Thus it is possible to observe the loss of blood circulation intensity and persistent chronic inflammation associated with a high level of MMP-9 in the ejaculate fluid. In 20-25% of cases the hernia repair surgeries with implant installation are accompanied with microcirculatory disorders of the scrotum and testes tissues with partial testicular atrophy, 1.2-1.5 times drop of blood circulation, and very often with a decrease of basic values of spermogram and content of testosterone in the blood serum. About 40% of patients suffer from a cremasteric reflex. The spermogram values represented below (table 1) clearly demonstrate the change of the analyzed values for the comparative analysis. As it is seen from (table.1), the basic spermogram values in the group of patients operated according to Transabdominal Preperitoneal Hernia Repair and Desarda methods are virtually the same that their initial values and are much higher than the values of patients groups, operated according to the conventional method, where the decrease ($p < 0.05$) of sperm cells amount and sperm motility is detected. As for the MMP-9 values, the difference is even more pronounced (table. 2)

Table 1

Spermogram of patients affected with inguinal hernia depending on hernia repair surgery methods

Spermogram values of patients operated according to Lichtenstein, n=8.			
Spermogram values interpretation	Before surgery	After surgery	3 months past surgery
Normozoospermia	7	6	6
Asthenozoospermia, 1 st stage	0 (16;>21;>40)*	1 (28;30;>40)*	1 (20;27;>40)*
Teratozoospermia 1 st stage	0 (28;>40;>40)*	0 (20;>40;>40)*	1 (28;>41;30)*
Spermogram values of patients operated according to TAPP, n=8.			
Spermogram values interpretation	Before surgery	After surgery	3 months past surgery
Normozoospermia	6	6	5
Oligoasthenozoospermia 2 nd stage	1 (4.6; 30;>41)*	1 (3.8; 31;>41)*	0 (3.8; 25;>41)*
Spermogram values of patients operated according to Desarda, n=9.			
Spermogram values interpretation	Before surgery	After surgery	3 months past surgery
Normozoospermia	8	7	6
Oligoasthenozoospermia 2 nd stage	1 (4.8; 30;>41)*	1 (3.8; 32;>40)*	0 (3.9; 27;>42)*

Note: *- a mount of sperm cells (millionpcs.); active motile cells (%), within the morphological normal value (%)

It is noted that the MMP-9 content in the ejaculate of patients operated for inguinal hernia repair is higher if compared with the control group (Table.2). The average MMP-9 level in the control group is 58.5 ng/ml and the MMP-9 level in patients operated for inguinal hernia repair ranges from 128.8 to 160.5 ng/ml. Thus the content of MMP-9 in the ejaculate fluid of patients operated for inguinal hernia repair is 2.8 times ($p<0,05$) higher than this value of the control group. It is of particular significance for this research that the patients operated for inguinal hernia repair according to Transabdominal Preperitoneal Hernia Repair and Lichtenstein methods have a much higher level of MMP-9 in their ejaculate even at the beginning of an inflammatory process.

Table 2

MMP-9contentin ejaculate of examined patients (M±m)

Operated patients	s =	MP-9 contentM±m ng/ml
1stgroup	s = 8	150,1 ± 12,8
2ndgroup	s = 8	160,1 ± 10,5
3rdgroup	s = 9	128,8 ± 8,9
Control	s = 5	58,5

It is determined that the MMP-9 level of patients operated according to Lichtenstein and Transabdominal Preperitoneal Hernia Repair methods with installation of the synthetic implant is reasonably higher than the MMP-9 level in the ejaculate of patients operated according to Desarda method with no implant installed (table 2). High MMP-9 concentrations in the ejaculate of patients after surgeries for inguinal hernia repair may be the evidence of the participation of this enzyme in post-surgical pathological processes. Moreover it would be reasonable to say that not only the chemical composition but also the morphology of implant surface is an important characteristic determining the body response to the foreign material. The results of our research give reasons to consider the elevated content of MMP-9 in the ejaculate as the evidence of a long-continued inflammatory process during the post-surgical period. The results of the study may be the evidence of the active involvement of MMP-9 in the developing chronic inflammatory processes marked with higher motility with the examined patients 6 months after the surgery with synthetic implants installed. Thus we can suppose the participation of MMP-9 in unpronounced processes with a breach of the blood-testis barrier induced by the long-lasting testicle ischemia. The advantage of this rather simple and noninvasive diagnostics method is that it provides a possibility to detect the beginning of testicle ischemia and the initial stages of smoldering inflammatory processes when no spermatic changes can be detected or these changes are still unremarkable. It is reasonable to take into consideration the fact that the late male in fertility is a disease with a difficulty of establishing diagnosis at early stage after the hernia repair surgery including implant installation. Thus it is important to perform early diagnostics of testicular abnormalities to begin the therapy and prevent the irreversible abnormal testicular changes. For many patients with timely drug treatment it is possible to slow down the disease progression and prevent the infertility establishment.

CONCLUSION

1. There was revealed a high content of metalloproteinases (MMP-9) in the ejaculate in

patients after hernioplasty. The mean concentration of MMP-9 in the ejaculate control group was 58.5 ng/ml in patients after hernioplasty from 128.8 to 160.1 ng/ml.

2. MMP-9 level was significantly higher in patients' ejaculate synthetic endoprosthesis and lower pull hernioplasty in Desard procedure. This makes it possible to use the indicator of the level of MMP-9 as a marker for the screening method of diagnostics of the initial symptoms of infertility.

3. MMP-9 concentration in ejaculate when bridged hernia repair can be a criterion of progression of testicular ischemia.

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