MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
SUMY STATE UNIVERSITY
ACADEMIC AND RESEARCH MEDICAL INSTITUTE

Eastern Ukrainian Medical Journal

116, Kharkivska st., Sumy 40007, Ukraine
e-mail: eumj@med.sumdu.edu.ua
eumj.med.sumdu.edu.ua
ISSN: 2663-5909 (print)/2664-4231 (online)

How to cite: Dzyubanovsky I, Kritsak M, Dzyubanovsky O, Gargula T, Burak A. The morphology of the structural parts of the diaphragm 14 days after application of standard intra-abdominal pressure in laparoscopic surgery: an experimental study. *East Ukr Med J*. 2025;13(4):1120-1130. https://doi.org/10.21272/eumj.2025;13(4);1120-1130

ABSTRACT

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THE MORPHOLOGY OF THE STRUCTURAL PARTS OF THE DIAPHRAGM 14 DAYS AFTER APPLICATION OF STANDARD INTRA-ABDOMINAL PRESSURE IN LAPAROSCOPIC SURGERY: AN EXPERIMENTAL STUDY

Introduction. In the course of laparoscopic surgery, carbon dioxide is injected into the abdominal cavity in order to create a working space through pneumoperitoneum. The increase in intra-abdominal pressure leads to an upward displacement of the diaphragm, increased airway pressure and decreased chest wall compliance, and a decrease in lung volume.

Materials and Methods. The experimental study was performed on 30 mature rats, which were divided into two groups of 15 animals each.The animals underwent the creation of a pneumoperitoneum of standard pressure in laparoscopy under general anaesthesia for a period of 5 hours.In the first group, the diaphragm was sampled immediately after 5 hours.In the other group, the diaphragm was sampled 14 days after the created pneumoperitoneum. The costal, lumbar, and tendon parts of the diaphragm were taken separately and placed in 10% formalin for further histological examination.

Results. Following a 5-hour pneumoperitoneum, observations were made of muscle deformation, fragmentation and lysis in muscle parts, and an increase in fibre heterogeneity. In transverse sections, muscle fibres became rounded and reduced in diameter. In response to the damage, cellular inflammatory infiltrates appeared, and a distinctive feature was the presence of haemorrhagic infiltration. In the lumbar region, significant changes were observed in the arterial vessels: the endothelium was exfoliated and freely located in bloodless lumens. The arterial wall exhibited thickening, the boundaries between its layers became indistinct, and the myocytes manifested a vacuolated appearance. The presence of oedema and polymorphonuclear cell infiltrates, as well as proliferating connective tissue, was observed in

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the perivascular area.In the tendon part, the lesion area exhibited multiple foci of destruction, which were infiltrated by lymphocytes and macrophages, accompanied by oedema of the underlying substance.

The data obtained from the second group revealed a heterogeneous morphological picture, characterised by areas of normal structure and areas exhibiting partial loss of muscle layer compactness. The sarcoplasm was found to be heterogeneous, with foci of oedema and disintegration, and transverse striations in these foci were not visualised. The nuclei retained their typical location within the skeletal muscle. The connective tissue of the endomysium and perimysium exhibited uneven thickness, attributable to edematous loosening and proliferation. Perivascular fibrosis was pronounced, and the haemocirculatory bed was observed to be uniformly filled with blood. Small-calibre arteries contained a limited number of red blood cells or were found to be empty. A similar heterogeneity was observed in the tendons, which exhibited both an ordered compact arrangement of collagen fibres and areas of loosening and fragmentation.

Conclusions. The results obtained allow the conclusion to be drawn: 14 days after the implementation of pneumoperitoneum, there is an incomplete structural recovery of the muscle and tendon component of the diaphragm of experimental animals. This is evidenced by replacement fibrosis in areas of muscle fibre damage, an increase in the amount of adipose tissue in the stroma, and a loosening of collagen fibres. These changes cannot lead to diaphragmatic dysfunction.

Keywords: Laparoscopy, pneumoperitoneum, intra-abdominal pressure, diaphragm, histology, rats, experimental model.

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МОРФОЛОГІЯ СТРУКТУРНИХ ЧАСТИН ДІАФРАГМИ ЧЕРЕЗ 14 ДНІВ ПІСЛЯ ЗАСТОСУВАННЯ СТАНДАРТНОГО ВНУТРІШНЬОЧЕРЕВНОГО ТИСКУ В ЛАПАРОСКОПІЧНІЙ ХІРУРГІЇ: ЕКСПЕРИМЕНТАЛЬНЕ ДОСЛІДЖЕННЯ

Вступ. Під час лапароскопічної хірургії в черевну порожнину вводять вуглекислий газ з метою створення робочого простору завдяки пневмоперитонеуму. Підвищення внутрішньочеревного тиску призводить до зміщення діафрагми вгору, підвищення тиску в дихальних шляхах і зменшення податливості грудної стінки, зменшення легеневого об’єму.

Матеріали і методи. Експериментальне дослідження виконано на 30 статевозрілих щурах, яких розділено на дві групи по 15 особин. Тваринам проводили створення пневмоперитонеуму стандартного тиску в лапароскопії під загальною анестезією протягом 5-ти годин. У першій групі відбір діафрагми здійснювали відразу після проходження 5-ти годин. У іншій групі відбір діафрагми проводили через 14 днів після створеного пневмоперитонеуму. У діафрагмі відбирали окремо реброву, поперекову частини та сухожилковий центр, які поміщали у 10 % формалін для подальшого гістологічного дослідження.

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Результати. Через 5 годин пневмоперитонеуму у м’язових частинах відмічалась деформація м’язових волокон, їх фрагментація та лізис, зростала гетерогенність волокон – на поперечних зрізах м’язові волокна ставали округлі і зменшені в діаметрі. У відповідь на пошкодження виникали клітинні запальні інфільтрати. Відмінною особливістю була присутність геморагічної інфільтрації. У поперековій частині суттєвих змін зазнавали артеріальні судини – ендотелій злущувався і вільно знаходився у безкровних просвітах. Стінка потовщувалася, межі її шарів стиралися, міоцити мали вакуолізований вигляд. Навколо судин прослідковувалися набряк та поліморфні клітинні інфільтрати та проліферація сполучної тканини. В сухожилковому центрі, зона ураження представляла собою множинні осередки деструкції інфільтровані лімфоцитами та макрофагами поряд із набряком основної речовини.

Дані отримані у другій групі показали неоднорідну морфологічну картину. Поряд із ділянками звичайної будови виявляли часткову втрату компактності м’язового пласту. Саркоплазма неоднорідна з осередками набряку і розпаду. Поперечна посмугованість в таких осередках не візуалізується. Ядра зберігали характерну для скелетних м’язів локалізацію. Сполучна тканина ендомізію і перимізію нерівномірної товщини частково за рахунок набрякового розрихлення, частково – її проліферації. Найбільше виражений периваскулярний фіброз. Гемоциркуляторне русло кровонаповнене рівномірно. Артерії дрібного калібру містять незначну кількість еритроцитів або порожні. В сухожилковому центрі також неоднорідна картина – поряд із впорядкованим компактним розташуванням колагенових волокон виявлялися ділянки їх розрихлення та фрагментації.

Висновки. Результати дозволяють зробити висновок, що через 14 днів після застосування пневмоперитонеуму відбувається неповне структурне відновлення м’язового та сухожильного компоненту діафрагми піддослідних тварин у вигляді замісного фіброзу в місцях пошкодження м’язових волокон та зростання кількості жирової клітковини в стромі, розрихлення колагенових волокон. Дані зміни не можуть призводити до порушення функції діафрагми.

Ключові слова: Лапароскопія, пневмоперитонеум, внутрішньочеревний тиск, діафрагма, гістологія, щурі, експеримент.

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LIST OF ABBREVIATIONS

CO_2 – carbon dioxide
PP – pneumoperitoneum
LS – laparoscopic surgery
AC – abdominal cavity
IAP – intra-abdominal pressure
LC – lung capacity
RG – research group

INTRODUCTION

In the course of laparoscopic surgery (LS), carbon dioxide (CO_2) is injected into the abdominal cavity The introduction of carbon dioxide (CO_2) into the peritoneal cavity through insufflation creates a working space within which surgeons are able to monitor and perform interventions.

The intra-abdominal pressure (IAP) during pneumoperitoneum (PP) generally exceeds the threshold for intra-abdominal hypertension syndrome (IAHS), i.e. 12 mmHg, which is considered the standard pressure in the laparoscopic surgery (LS) [1]. The abdominal cavity (AC) is comprised of rigid (spine, thorax and pelvis) and pliable (antero-lateral abdominal wall, pelvic muscles and diaphragm) anatomical boundaries.It has been demonstrated that the abdomen exhibits inherent anisotropic behaviour during a PP, which is manifested by the difference in the ability to expand in different directions [2, 3, 4]. It is generally accepted that there is an initial phase in which the marginal increase in the volume of the AC in accordance with the applied pressure, i.e. abdominal pliability, has a linear dependence [5]. In accordance with the established principles of biomechanics, the materials under consideration are subject to deformation until they reach their maximum tensile capacity. The elastic behaviour of the abdominal cavity is determined by the pressure-volume ratio of the working space [4, 5, 6]. It has been demonstrated that at lower inspiratory pressures, the abdomen expands with minimal resistance, and each increase in pressure results in a greater increase in volume. Conversely, at higher insufflation pressures, the ease of abdominal expansion is reduced, and further increases in pressure will result in a decrease in the rise in intra-abdominal volume [7].

Growth IAP leads to an upward displacement of the diaphragm, elevated airway pressure and decreased chest wall compliance reduction lung capacity (LC) [3]. Diaphragmatic displacement can be partially compensated for by applying sufficient positive pressure during mechanical ventilation. A substantial body of preclinical research has examined the impact of IAP within the range of 5 to 25 mmHg on PAP. These studies have demonstrated that the rate of abdominothoracic transmission, defined as the proportion of abdominal pressure that is transferred to the thorax for maximum pressure, ranges from 40% to 50% in animal models and from 20% to 62% in human physiological studies [5].

The implementation of high pressure during PP has been shown to have deleterious effects on organ perfusion, impede mechanical ventilation, and result in postoperative pain and prolonged postoperative rehabilitation, particularly in patients who also have comorbidities [8, 9]. In order to achieve the most favourable surgical conditions, the insufflation pressure setting should be based on a calculation of whether the benefits of increasing the working space volume outweigh the disadvantages of using a higher insufflation pressure.

CO2 insufflation has been demonstrated to be associated with a sharp deterioration in haemodynamics during surgery. Elevated IAP has been shown to cause an increase in venous return resistance, which has been found to lead to a significant increase in central venous pressure, thereby disrupting blood flow in the inferior vena cava basin [9]. There is evidence to suggest that the use of PP for LS has systemic effects on the renal system, with the potential to contribute to acute renal injury or postoperative renal dysfunction. Specifically, when the pressure exceeds 10 mmHg, PP has been shown to reduce renal blood flow, leading to renal dysfunction and temporary oliguria. The effect of PP on the kidneys is a result of both the direct effect of increased intra-abdominal pressure and indirect factors such as carbon dioxide uptake, neuroendocrine effects and tissue damage due to oxidative stress [10].

The focus of this study is the state of the respiratory muscle during LS, with particular reference to the effect of PP on it. There are few scientific studies that investigate the function of the diaphragm during laparoscopic surgery, and even fewer that examine its morphological structure. However, existing studies suggest that PP disrupts the function of the diaphragm, with this disruption being directly related to the duration of the latter [11, 12]. It has been demonstrated that stretching the diaphragm can result in impaired trophism of the diaphragmatic nerve, which can consequently lead to postoperative pain in the supraclavicular region and respiratory dysfunction. However, it has been observed that the utilisation of low pressure or laparolifting in the LS has been shown to reduce the frequency of these manifestations [13].

The aim of this study was to investigate the morphological features of the diaphragmatic parts in the application of PP and how the structure of this respiratory muscle is restored 14 days after the creation of PP.

MATERIALS AND METHODS

For the experimental study, 30 healthy, mature rats, aged between six and seven months and weighing (230±30) grams, were selected. The animals were divided into two research groups (RG) of 15 animals each. All experimental animals were subjected to simulated PP, with a level of IAP of 10 mmHg for a period of five hours. According to the literature, this pressure corresponds to a similar pressure of 12–15 mmHg in the human body, which is the standard pressure in the LS [13]. This IAP has been demonstrated to induce similar alterations in vital signs in both rodents and humans. Why was this time period chosen? Every year, the volume of surgical interventions for pulmonary hypertension is expanding and requires more time for PP storage. The modelling of the PP was carried out in accordance with the copyright certificate for the work № 126409 [14]. CO2 was injected using a KARL STORZ electronic laparoflator 264300 20 insufflator (Fig. 1). This device automatically insufflates gas in order to maintain IAP at the desired level. The device was set to a pressure reading of 10 mmHg.

Figure 1. *Simulated pneumoperitoneum at 10 mmHg*

Prior to the creation of the PP, the animals were anaesthetised. The animals were not fed for 12 hours prior to the first injection, but were provided with free access to water. Two hours before anaesthesia, water was withdrawn. The premedication consisted of Xylazine at a dose of 10 mg/kg administered 15 minutes prior to the main drug, followed by Ketamine Solutions at a dose of 90 mg/kg.The animals were then fixed in a supine position on a table.

Following a five-hour exposure to the created PP, the animals were euthanised with diaphragm sampling, but only in I RG. Rats from II RG were returned to their cages after the created PP for this time and were maintained on a standard diet for a period of 14 days, after which the material for the study was collected. The experimental animals were killed by intraperitoneal injection of high doses of Thiopental sodium at a rate of 75 mg/kg body weight. The diaphragm samples were obtained in accordance with the stipulated copyright certificate for the work № 126059 [15]. The costal, tendon and lumbar parts of the diaphragm were separately selected from the macro preparations and placed in separate tubes with a 10% neutral formalin solution (Fig. 2). Following fixation, the histological material was dehydrated in ethyl alcohols of increasing concentration and embedded in paraffin. Histological sections of 5-7 µm thickness were made from each paraffin block on a microtome, which were stained with hematoxylin and eosin and alcian blue after deparaffinisation.

All phases of the experimental study were conducted within the vivarium of I.Horbachevsky Ternopil National Medical University. The work with animals was conducted in the morning, in an indoor environment, with temperatures ranging from 20 to 22°C and relative humidity levels between 60 and 80%. Throughout the entire period of the animal experiment, the provisions of the Law of Ukraine 'On Protection of Animals from Cruelty', the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of 18.03.1986, and the Council of Europe Directive 2010/63/EU.

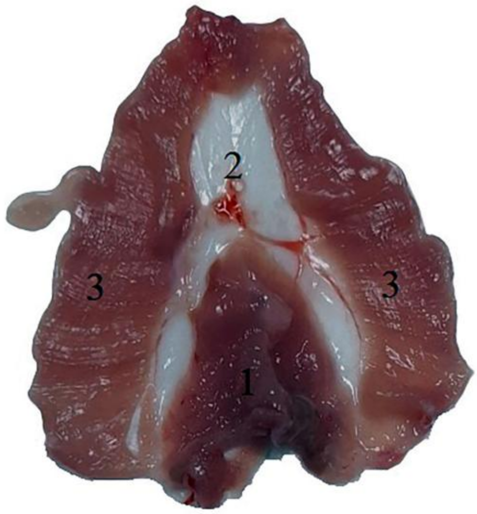

Figure 2. *Selected preparation of the rat diaphragm: 1 – lumbar part; 2 – tendon part; 3 – costal part*

RESULTS

In the I RG, following a period of 5 hours of PP duration, signs of a pathological process within the structural components of the diaphragm were observed. This included deformation of muscle fibres, their fragmentation and lysis, which indicated a significant decrease in the contractility of the diaphragm.The heterogeneity of fibres increased, with muscle fibres becoming rounded and reduced in diameter on transverse sections, indicating signs of partial atrophy of the diaphragmatic muscle. In response to the observed damage, the development of cellular inflammatory infiltrates was observed, with a distinctive feature being the presence of haemorrhagic infiltration.At this stage, the proportion of adipose tissue and collagen fibres increased in the stroma, along with oedema and haemorrhage (Fig. 3).

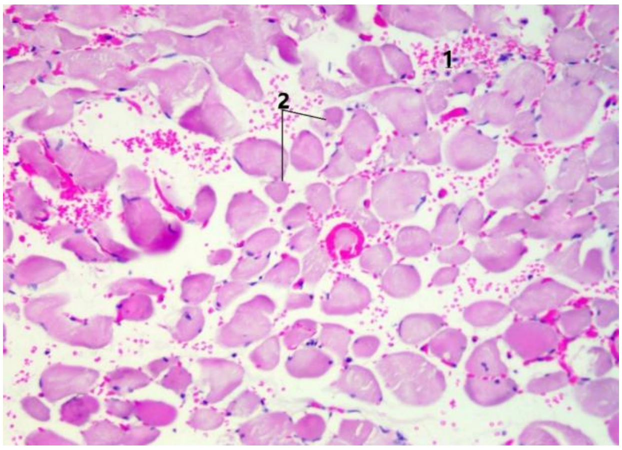

Figure 3. *Transverse section of the costal part of the diaphragm: 1 – diffuse haemorrhagic infiltration of the endomysial connective tissue combined with edema; 2 – atrophic changes in muscle fibres. Hematoxylin and eosin staining. × 200*

Haemorrhages were more prevalent and severe in the cruses of diaphragm, with erythrocytes infiltrating the endomysium. Another notable feature was the presence of haemorrhagic seepage of necrotic fibres (Fig. 4). The arterial vessels underwent significant changes, including the exfoliation of the endothelium and the presence of erythrocytes in bloodless lumens.

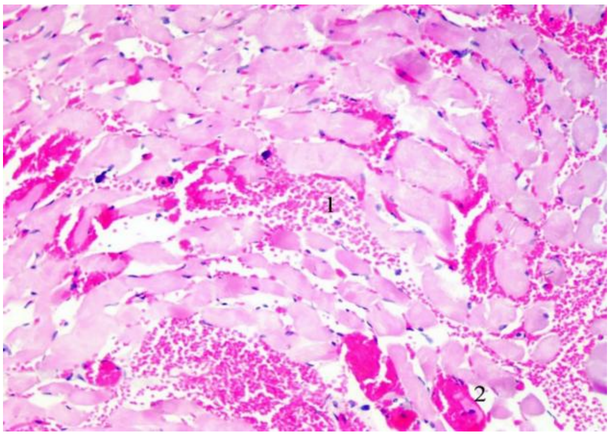

Figure 4*. Morphology of the lumbar part of the diaphragm: 1 – diffuse haemorrhages; 2 – haemorrhagic soaking of necrotic fibres. Hematoxylin and eosin staining. × 200*

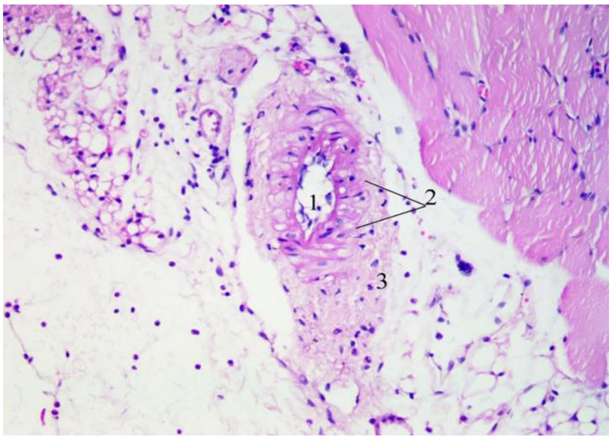

Figure 5. *Histological structure of the lumbar part of the diaphragm: 1 – endothelial desquamation; 2 – vessel wall; 3 – oedema, proliferation of collagen fibres in the perivascular space. Hematoxylin and eosin staining. × 200*

The vessel walls exhibited thickening, with the boundaries between its layers becoming indistinct, and myocytes displayed a vacuolated appearance. Oedema and polymorphic cellular infiltrates, as well as connective tissue proliferation, were observed in the perivascular areas (Fig. 5). It is noteworthy that such alterations in small arterial vessels were observed in all the studied cases within this experimental series.

In the tendon part, the lesion area consisted of multiple foci of destruction infiltrated by lymphocytes and macrophages along with oedema of the underlying substance. Alcian blue staining did not reveal signs of systemic disorganisation of collagen fibres, in particular mucoid swelling, which excluded the presence of an autoimmune factor in this case (Fig. 6, 7).

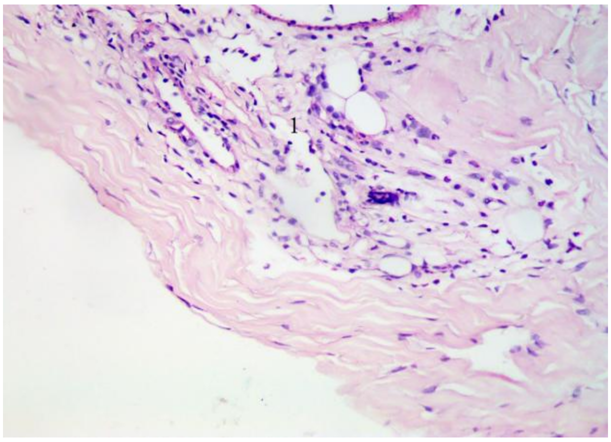

Figure 6. *Morphological picture of the tendon part of the diaphragm: 1 – area of collagen fibre destruction, oedema and cellular infiltration. Hematoxylin and eosin staining. × 200*

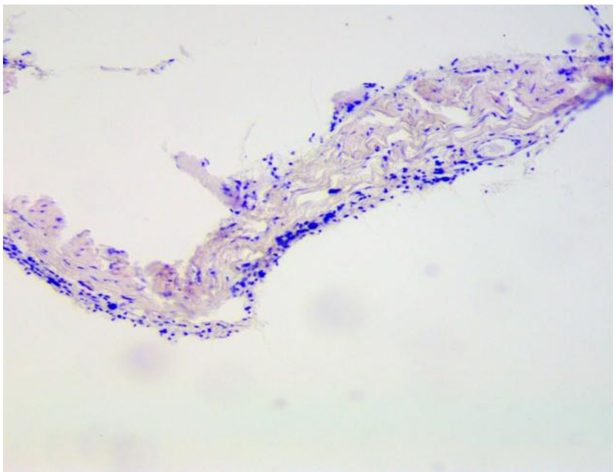

Figure 7. *Structure of the diaphragmatic tendon under alcian blue staining. × 100*

Microscopic analysis of the muscular part of the diaphragm in the II RG 14 days after the created PP for 5 hours revealed a heterogeneous morphological picture, both in different cases and within one case. Alongside areas exhibiting normal structure, a partial loss of muscle layer compactness was detected, and occasionally, fibre orientation was disturbed. The sarcoplasm was heterogeneous, with foci of oedema and disintegration, and transverse striations in such foci were not visualised. The nuclei retained the localisation characteristic of skeletal muscle. The connective tissue of the endomysium and perimysium was of uneven thickness, partly due to edematous loosening and partly to its proliferation, and perivascular fibrosis was most pronounced.Occasionally, maturing granulation tissue was found in the areas of previous muscle damage (Fig. 8).

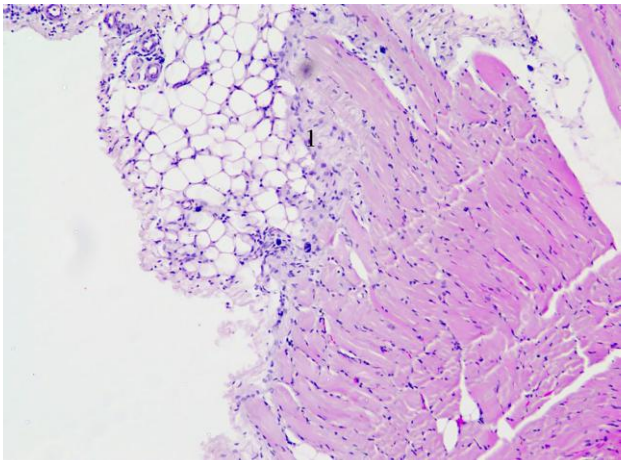

Figure 8. *The costal part of the diaphragm 14 days after the creation of the PP: 1 – replacement fibrosis and proliferation of fatty tissue in the area of muscle damage. Hematoxylin and eosin staining. × 100*

The haemocirculatory system is evenly filled with blood. Small-calibre arteries contain a small number of erythrocytes or are empty. A small proportion of endothelial cells are exfoliated, and subendothelial oedema and swelling of smooth muscle cells are also detected. The adventitia is thickened with proliferating collagen fibres. In the lumbar part of the diaphragm, similar changes were noted (Fig. 9).

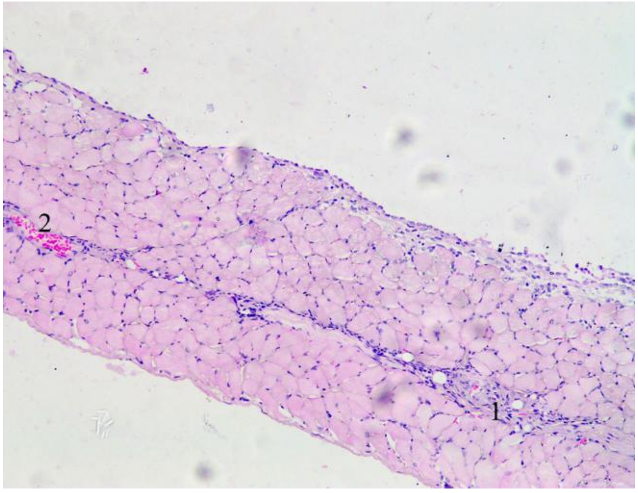

Figure 9. *The lumbar part of the diaphragm 14 days after the created PP: 1 – perivascular sclerosis; 2 – small-calibre vein full blood. Hematoxylin and eosin staining. × 100*

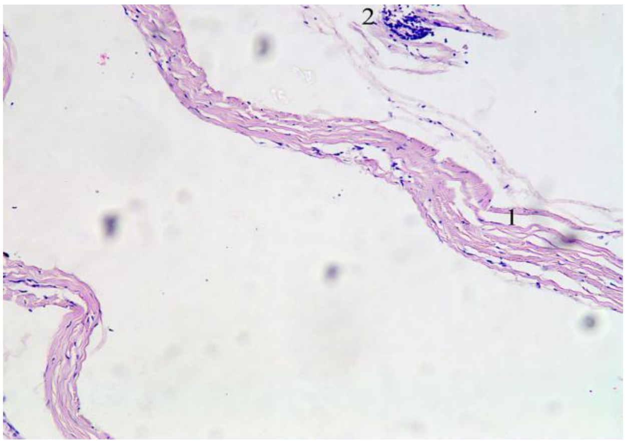

Figure 10. *Tendinous part of the rat diaphragm 14 days after the creation of the PP: 1 - partial loss of collagen fibre compactness; 2 - foci of cellular infiltration. Hematoxylin and eosin staining. × 100*

The tendon part exhibited a heterogenous configuration, characterised by a well-ordered, compact arrangement of collagen fibres. However, this was interspersed with areas of fibre dissolution and fragmentation. Histiocytes and lymphocytes were observed among fibroblasts (Fig. 10).

DISCUSSION

In recent years, there has been a discernible tendency to broaden the indications for LS, thereby concomitantly increasing the volume of surgical intervention. This, in turn, has led to an increase in the duration of the operation itself and an increase in the time of exposure to PP on the body as a whole. The expansion of indications for LS also applies to elderly patients with not always compensated comorbidities. Consequently, our study of the respiratory muscle is highly relevant. This underscores the significance of our experimental study for practical medicine, aiming to elucidate the microstructural alterations in the diaphragm. It is noteworthy that this experiment characterises the changes occurring under standard pressure in the abdominal cavity, and we will further emphasise the outcomes observed when utilising low pressure in laparoscopy. In their scientific contributions, some authors have drawn parallels between standard IAP in LS and low pressure. The results come down to the fact that lower pressure provides a sufficient volume of the abdominal cavity for surgical manipulation with instruments, thus not affecting the blood circulation and respiratory system as much as the standard pressure [16, 17, 18]

The microscopic changes in the parts of the diaphragm in I RG are associated with the direct effect of IAP created by PP of standard pressure. This leads to the stretching of the fibrous structures of the tendon part, the occurrence of oedema, the fragmentation and lysis of muscle fibres due to overstretching when the diaphragm is displaced in the cranial direction. Disturbances at the level of arterioles and venules lead to impaired trophism during PP, which is manifested by haemorrhagic tissue seepage.In the studies of the diaphragm breathing function in robotic LS, it was found that the postoperative diaphragm function deteriorates significantly, which does not recover to the preoperative level after discharge. Elevated serum levels of Club Cell Protein (CC16) post-surgery are indicative of potential lung damage. Adverse effects may be associated with prolonged PP during laparoscopic surgery [19].

The study by P.S. Rustagi was based on a comparison of diaphragmatic excursions before and after major laparoscopic pelvic surgery under general anaesthesia in the Trendelenburg position. Ultrasound examination of diaphragmatic excursion and lung compliance was used to achieve this. The results showed a significant decrease in excursion and lung compliance. Age, duration of anaesthesia, and post-procedure pain were significant independent predictors [20].

A comparison of the results obtained with those of other authors is rendered difficult by the lack of information regarding the morphological structure of the diaphragm under the influence of IAP of generated CO_2 during PP. The majority of studies, as previously described, focus on the excursion of the diaphragm or the state of the diaphragm in macroscopic terms, as well as the state of the lungs. Consequently, it can be concluded that such morphological changes in such a volume may not occur in the human body. However, it has been demonstrated that PP, producing CO_2 similar to that in the lungs, leads to microstructural changes in the rat diaphragm. It is important to note that the animals in this study were not suffering from any concomitant pathologies, which raises the question of whether these changes would occur in the diaphragm in more severe cases of diseases such as mechanical jaundice (one of the most common diseases in LS), diabetes mellitus, metabolic syndrome, or subcompensated diseases of the cardiovascular and respiratory systems.The results obtained in the second study group indicate that there are residual changes in parts of the diaphragm that do not lead to significant functional impairment in the long term.

CONCLUSIONS

Pneumoperitoneum resulted in muscle fibre deformation, fragmentation and lysis, indicating a significant decrease in diaphragm contractility. In transverse sections, muscle fibres became rounded and reduced in diameter. In response to the injury, cellular inflammatory infiltrates with haemorrhagic infiltration were observed. Multiple foci of destruction infiltrated by lymphocytes and macrophages were seen in the tendons, accompanied by oedema of the underlying substance. The analysis of the study results indicates that 14 days following the application of pneumoperitoneum with carbon dioxide at a level of 10 mmHg, there is incomplete structural recovery of the muscle component of the diaphragm of experimental animals, manifesting as replacement fibrosis in the areas of muscle fibre damage and an increase in the amount of fatty fibre in the stroma. Furthermore, the presence of haemomicrocirculatory disorders contributes to the progression of ischaemic muscle damage. However, it is important to note that these changes do not inevitably lead to diaphragmatic dysfunction.

PROSPECTS FOR FUTURE RESEARCH

The study of the morphological structure of the diaphragm in pneumoperitoneum has been shown to hold great potential for enhancing our understanding of the impact of intra-abdominal pressure on respiratory function. The evaluation of alterations observed in response to varying pressure levels during laparoscopic surgery has the capacity to contribute to the improvement of the postoperative period for patients.

AUTHOR CONTRIBUTIONS

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FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ARTIFICIAL INTELLIGENCE DISCLOSURE

No artificial intelligence technologies were used during manuscript writing or editing.

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Received 12.02.2025 ***Accepted 18.03.2025***

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