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Features of Histological Changes in the Diaphragm 14 Days After the Use of Low-Pressure Pneumoperitoneum

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ABSTRACT

The establishment of an optimal level of intra-abdominal pressure during laparoscopic surgery represents a considerable challenge for surgeons, with the most commonly used range being 12 to 15 mmHg, which allows for a sufficient working space. The aim of this work was to study the morphological features of the lumbar, costal and tendon parts of the diaphragm under low-pressure pneumoperitoneum modelling and long-term structure recovery. The experiment was performed on sexually mature rats of the reproductive period (6-7 months), weighing (230.0±20.0) grams, in the amount of 30 animals. The animals were divided into two experimental groups. The difference in the groups was in the timing of diaphragm sampling. In the first group, the diaphragm was sampled immediately after five hours of pneumoperitoneum, and in the second group 14 days after the simulated pneumoperitoneum. Histological examination of the microsections after five hours revealed destructive changes in all structural components of the diaphragm. Muscle fibres exhibited a compact arrangement, frequently with blurred boundaries, and a loss of differentiation from the stroma. Myofibrils undergo a transformation, becoming undulated, whilst transverse striations are fragmented or absent. The stroma exhibits thickening perivascularly, with adipose tissue representing this structure. The veins and capillaries are full of blood, and there is a distributed leukocytosis. In the arterioles, there is evidence of wall oedema, endothelial desquamation and moderate blood filling. The tendon part loses its compactness. The collagen fibres of the tendons were severely disintegrated, disintegrated and underwent lysis, and there was often detection of haemorrhages and reactive inflammation. After 14 days, the muscle fibres of the diaphragm exhibited an equidirectional, parallel and compact arrangement, and their thickness varied slightly. The nuclei were oval-round in shape, with a characteristic sublemmal arrangement. The endomysium was found to be thin, and was composed of a limited number of collagen fibres, among which hemocapillaries, filled with blood to a moderate degree, were visualised. The perimysium is more pronounced, especially around veins and arterioles, and a greater proportion of collagen fibres in the tendons are parallel to each other and retain their integrity. Low-pressure pneumoperitoneum for 5 hours leads to structural changes in the diaphragm. These changes are reversible, as demonstrated by the complete recovery of all diaphragm parts 14 days after the application of 5 mmHg pneumoperitoneum.

1. Introduction

The establishment of the optimal level of intra-abdominal pressure during laparoscopic surgery represents a considerable challenge for surgeons. The most commonly utilised option is a pneumoperitoneum within the range of 12 to 15 mmHg, a choice that facilitates adequate working space and effortless instrumentation [1-3]. Nevertheless, research has indicated the existence of numerous complications associated with the use of carbon dioxide (CO_2) insufflation, including hypercapnia, subcutaneous emphysema, pneumothorax and pneumomediastinum [4], [5]. Furthermore, it has been demonstrated that physiological cardiopulmonary changes occur during abdominal insufflation. These changes do not affect the intraoperative performance of patients without concomitant pathology, but are observed in patients with impaired cardiac or pulmonary function [6], [7]. Furthermore, shoulder pain has been identified as a frequent postoperative complaint following laparoscopic surgery [4]. Numerous randomised clinical trials have demonstrated that pneumoperitoneum pressure of less than 10 mmHg is effective in reducing shoulder pain following laparoscopic cholecystectomy in comparison with standard pressure [8], [9]. Recent scientific data analysis of the intraoperative and postoperative periods indicates that low intraperitoneal pressure (7 mmHg) minimises the adverse effects on the surgical peritoneal environment during CO2 pneumoperitoneum compared to standard pressure (12 mmHg) [10- 13]. Consequently, the reduction of pressure while maintaining an adequate intra-abdominal view is considered optimal for laparoscopic surgery.

It is interesting for the study to evaluate the effect of low pressure pneumoperitoneum (7 mmHg) on the structure of the diaphragm, and to assess how the picture changes in the long term after the creation of PP. It is evident that a significant proportion of surgeons operate with a standard pressure of 12 mmHg, despite the fact that a considerable number of laparoscopists continue to work at 15 mmHg. However, the present study opted for 7 mmHg, a decision that is in accordance with the prevailing trend of reducing abdominal pressure during laparoscopy. It is noteworthy that there are no explicit pressure recommendations for insufflation, nor any clear guidelines on the benefits of low pressure [5], [14], [15]. The primary endpoint of this study was the morphological structure of the diaphragm. The majority of authors who published on low blood pressure focused on the functional state of the diaphragm, its excursion, the state of the respiratory system, and the presence of postoperative pain (phrenic symptom). Under normal circumstances, intrathoracic pressure and diaphragm contraction overcome gravity and facilitate the movement of blood into the right atrium from the inferior vena cava. In the case of diaphragmatic structure disorders, this mechanism becomes disrupted. In a meta-analysis on low blood pressure, [3] reported on eight randomised clinical trials with shoulder pain [3].

The diaphragm is a vital organ in mammals that serves as the main muscle for breathing. While the external intercostal muscles assist in inhalation, the diaphragm serves as the primary respiratory muscle. Thus, diaphragmatic weakness can interfere with normal respiratory function. Acute diaphragmatic abnormalities cover a diverse range of relatively rare pathological conditions that disrupt the normal structure and function of the diaphragm. Such structural compromise can lead to tearing and rupture of the diaphragm, resulting in potential respiratory distress, especially in the event of trauma. Furthermore, diaphragmatic dysfunction can contribute to cardiopulmonary distress and exacerbate associated injuries. The function of the diaphragm is to facilitate respiration by contracting to increase the size of the chest cavity and decrease intrathoracic pressure, thus enabling the lungs to expand and fill their alveoli with air. The diaphragm is a dome-shaped muscle and tendon that serves as the primary respiratory muscle and is essential to the breathing process. It is a fibro-muscular sheet that has a convex upper surface that forms the floor of the chest cavity and a concave lower surface that forms the roof of the abdominal cavity [16], [17]. Low-pressure laparoscopy is a valid alternative to standard laparoscopy and has been shown to help reduce the manifestations of postoperative diaphragmatic dysfunction.

2. Aim of the work

Was to study the morphological features of the lumbar, costal and tendon parts of the diaphragm under low-pressure pneumoperitoneum modelling and long-term structure recovery.

3. Subjects and Methods

3.1 Study design and participation

The experiment was performed on sexually mature rats of the reproductive period (6-7 months), weighing (230.0±20.0) grams, in the amount of 30 individuals. The subjects were maintained in an accredited vivarium at the I. Horbachevsky Ternopil National Medical University. The experiments were conducted during daylight hours in a room maintained at temperatures of (20-22) °C, relative humidity of (60-80) %, and illumination of 250 lux. The induction of anaesthesia was achieved by administering an overdose of Thiopental sodium at a dose of 75 mg/kg of animal body weight, via intraperitoneal injection. Subsequent to this, the diaphragm was sampled. The experimental animals were divided into two experimental groups (EG). The distinction between the groups was the timing of diaphragm sampling. In the first group, the diaphragm was sampled immediately after five hours of pneumoperitoneum, and in the second group, 14 days after the simulated pneumoperitoneum [18].

3.2 Anesthesia and Carbon dioxide pneumoperitoneum

In both the first and second EGs, the animals were anaesthetised by the intramuscular injection of a combination of Ketamine (90 mg/kg) and Xylazine (10 mg/kg). Following the induction of general anaesthesia, the anterolateral abdominal wall was shaved. A skin incision was made with a length comparable to the diameter of the Veress Needle, which was inserted into the abdominal cavity and connected to the KARL STORZ electronic laparoflator 264300 20 (Fig. 1) and low-pressure pneumoperitoneum was modelled [19]. The following parameters were set on the device: intra-abdominal pressure - 5 mmHg; carbon dioxide supply rate - 0.1 litre per minute; duration - five hours. It is noteworthy that a pressure level of 5 mmHg in rodents corresponds to a pressure of 7-8 mmHg in humans [20].

Figure 1. Pneumoperitoneum formed by carbon dioxide (insufflator on the right, fixed animal on the left)

3.3 Histology

After taking a macro specimen of the diaphragm, the lumbar, rib, and tendon parts were separated and placed in separate tubes with a 10% solution of neutral formalin. The material was then dehydrated in increasing concentrations of ethyl alcohol and embedded in paraffin. Histological sections of 5-7 µm thickness were cut from each paraffin block on a microtome and, after deparaffinisation, stained with haematoxylin and eosin and Masson's stain.

3.4 Ethical principles

All experimental manipulations on laboratory animals were performed in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes of 18.03.1986, Council of Europe Directive 2010/63/EU and the Law of Ukraine "On Protection of Animals from Cruelty".

4. Results

4.1 Results obtained in the first experimental group

Histological examination of the microsections after 5 hours showed destructive changes in all structural parts of the diaphragm. The muscle fibres retained a compact arrangement. However, their borders were often blurred and differentiation from the stroma was lost. The sarcoplasm was heterogeneously stained - intensely eosinophilic areas of hypercontraction alternated with hypochromic areas with fragmentation, vacuolization, deep sarcoplasmic breakdown and myocytolysis. The myofibrils changed their course to become spherical and the transverse striations were fragmented or absent. In addition to these changes, we observed changes of a compensatory nature - fibre hypertrophy. The stroma is thickened perivascularly and is represented by fatty tissue. he veins and capillaries are full-blooded, and there is a distributed leukocytosis (Fig. 2).

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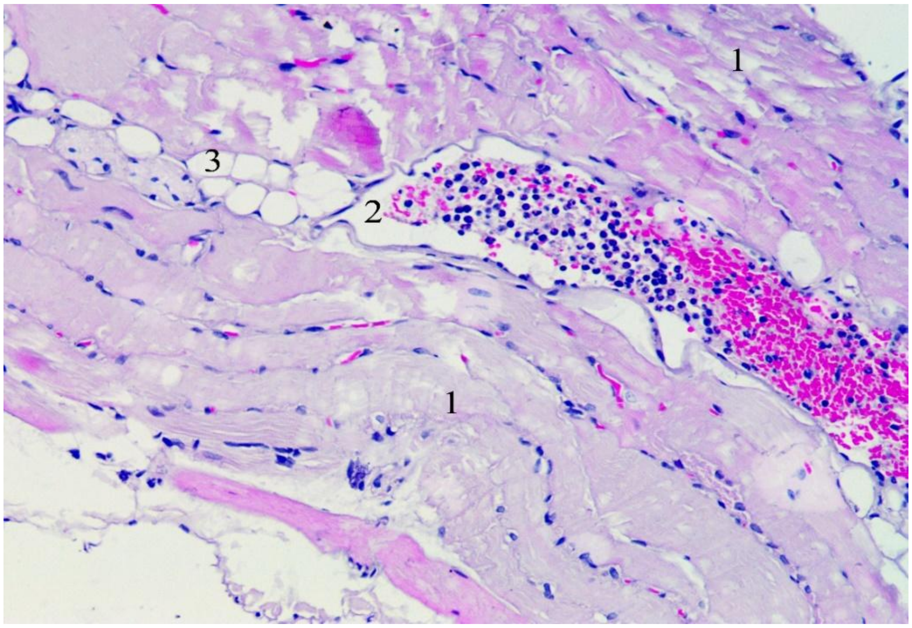

Figure 2. Morphology of the costal part of the diaphragm after 5 hours. Pneumoperitoneum: 1 - muscle fibres vacuolate and disintegrate, 'merge' due to lysis of cell membranes; 2 - redistribution of blood in the vein with the effect of distributive leukocytosis; 3 - fatty infiltration of the perimysium. Hematoxylin and eosin staining. × 200.

Similar changes with stromal oedema were observed in the lumbar part of the diaphragm. Fibres dissociated by oedema were atrophic. In the arterioles, there was wall oedema, endothelial desquamation and moderate blood filling. Perivascularly, there was increased collagen formation in the oedema-loosened stroma, and cellular infiltrates with predominantly macrophages and lymphocytes and fibroblasts (Figures 3, 4).

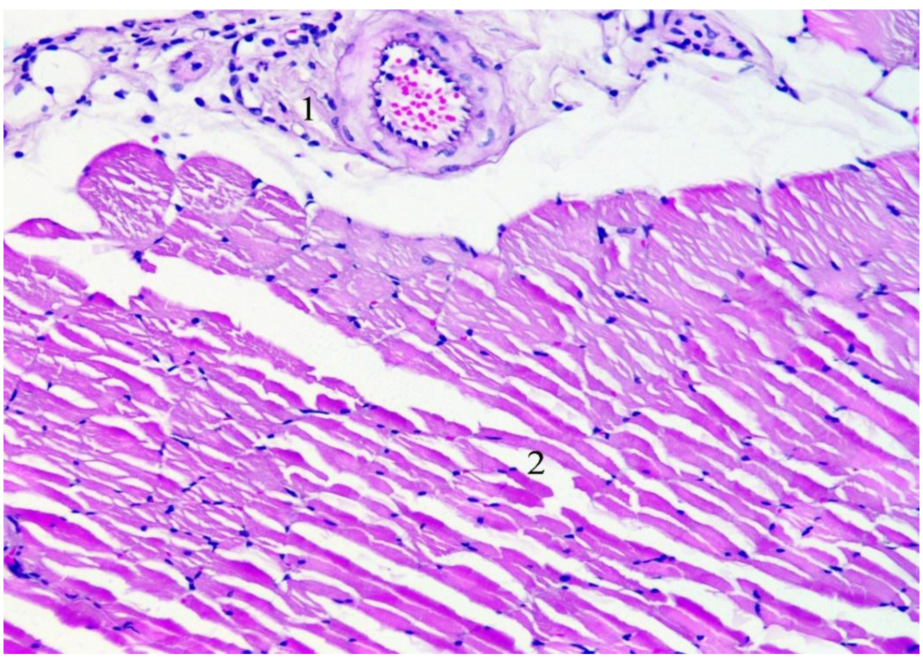

Figure 3. Longitudinal section of the lumbar part of the diaphragm after 5 hours of pneumoperitoneum: 1 - perivascular connective tissue is thickened due to collagen fibre proliferation and contains cellular infiltrate; 2 - stromal edema and muscle fibre atrophy. Hematoxylin and eosin staining. × 100.

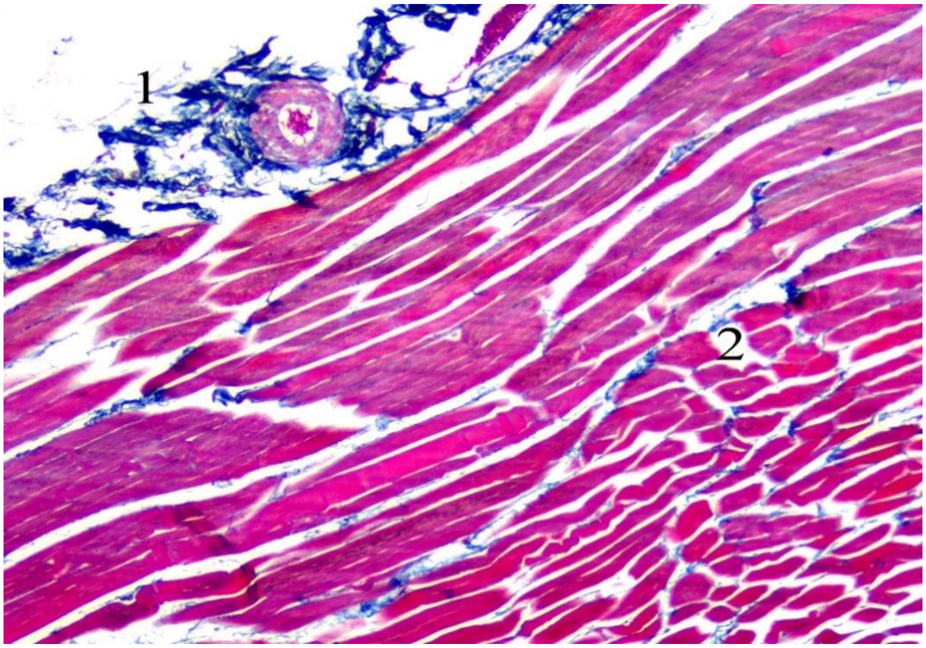

Figure 4. Cruses of diaphragm after 5 hours of pneumoperitoneum: 1 - proliferation of collagen fibres around the vessel in the perimysium; 2 - foci of increased collagen formation in the endomysium. Masson's stain. ×100.

At this stage, the tendons lose their compactness and the basic substance content increases. The collagen fibres of the tendon are highly degraded, disintegrate and undergo lysis. Haemorrhage and reactive inflammation are often seen (Fig. 5).

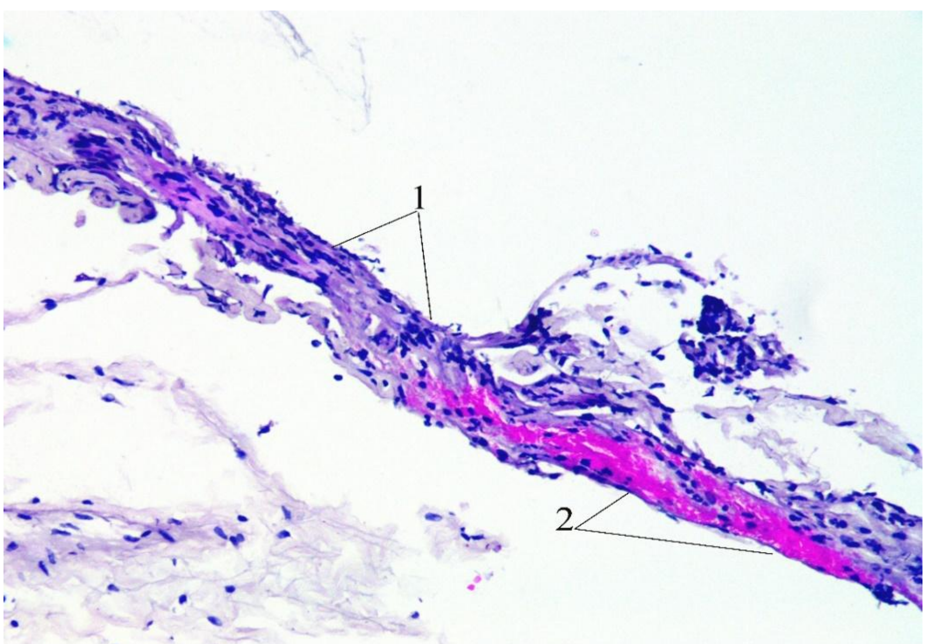

Figure 5. Fragment of rat diaphragm tendon after 5 hours of pneumoperitoneum: 1 - Intense inflammatory cell infiltration in areas of collagen fibre damage; 2 - Hemorrhage. Hematoxylin and eosin staining. × 100.

4.2 Results obtained in the second experimental group

Following a thorough analysis of the results obtained in the II EG 14 days after the creation of pneumoperitoneum of 5 mmHg for 5 hours, it was established that the diaphragm muscle fibres exhibited

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unidirectional, parallel and compact characteristics. Their thickness exhibited slight variability, and the nuclei were found to be oval-round in shape, predominantly homogeneous in colour, and had a sublemmal localisation characteristic of them. A small number of nuclei within the same field of view were displaced towards the fibre centre. The uneven eosinophilic staining of the sarcoplasm was due to its partial heterogeneity, which manifested as thinning and overstriation, and reflected the functional activity of the diaphragm. Uniform and clear transverse striation additionally indicated structural integrity (Fig. 6).

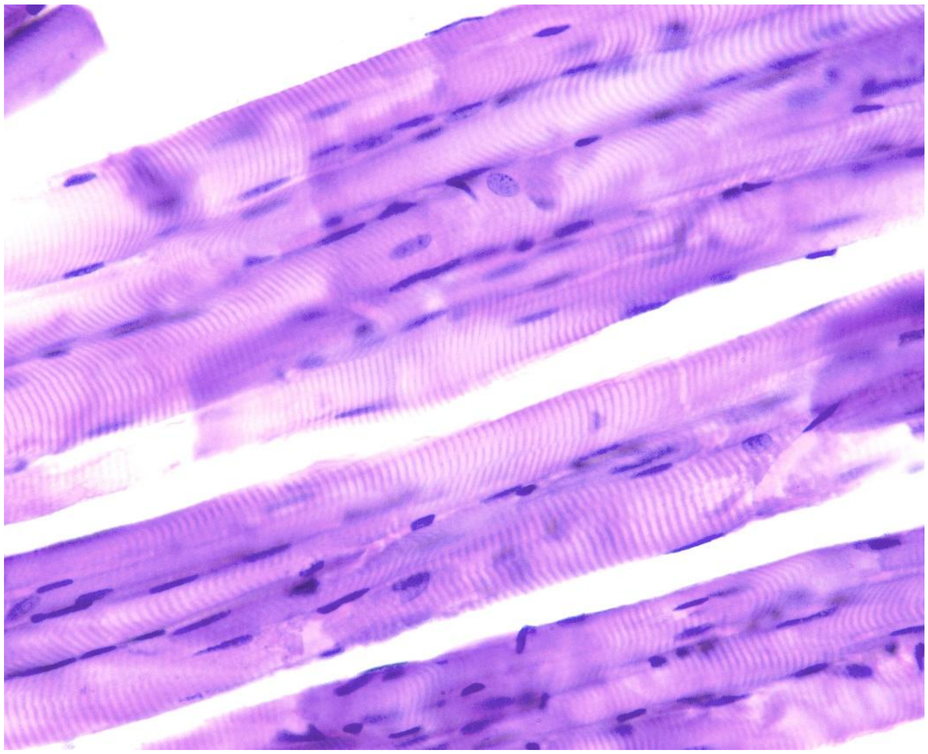

Figure 6. Longitudinal section of the rat diaphragmatic rib chassis after 14 days, showing clear visualisation of transverse striation. Hematoxylin and eosin staining. × 400.

The endomysium is characterised by a thin arrangement of collagen fibres, interspersed with moderately blood-filled haemocapillaries. The perimysium is more pronounced, particularly in proximity to veins and arterioles. Thickening of the stromal component due to proliferation and thickening of collagen fibrils was also observed. The presence of adipose tissue, connective tissue cells and lymphocytes was characteristic. Venules were typically full of blood (Fig. 7), and the walls of arterioles and small-calibre arteries exhibited a distinctive structure. In all cases, a proportion of damaged endothelial cells was detected, along with evidence of oedema, exfoliation and vacuolation of smooth muscle cells.

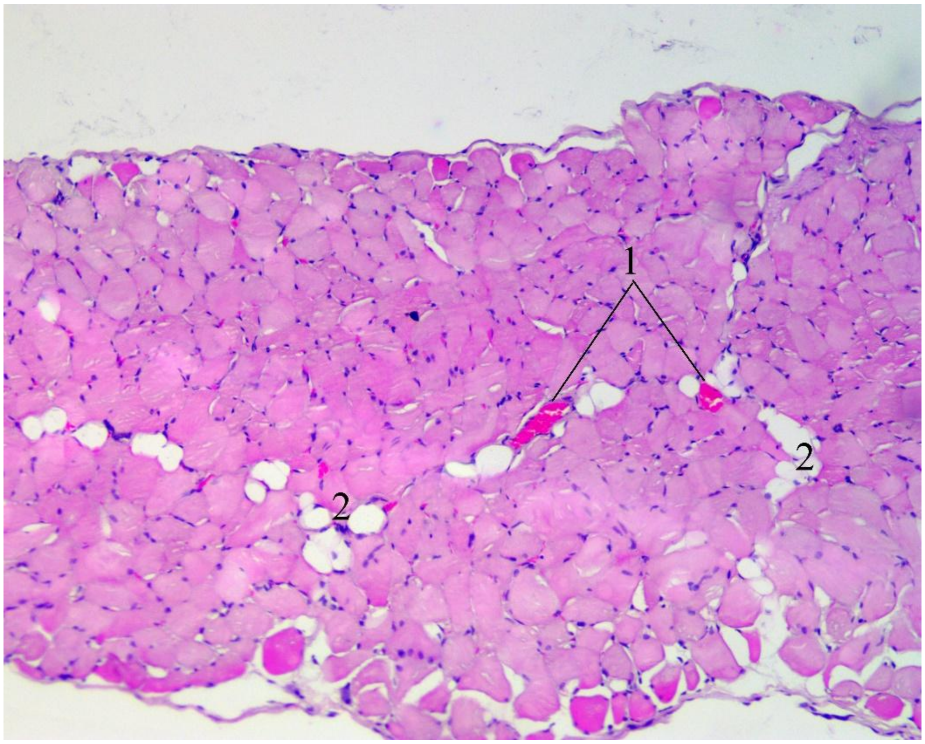

Figure 7. Transverse section of the costal part of the rat diaphragm after 14 days, muscle fibres are compactly arranged with typical nuclei localisation: 1 - full blooded capillaries and venules; 2 - small foci of adipose tissue. Hematoxylin and eosin staining. × 200.

The muscle fibres in the lumbar part of the diaphragm exhibited a series of comparable structural characteristics. The presence of transverse striation was observed, with myofibrils demonstrating compact arrangement. The vascular system was distinguished by the presence of venous haemorrhage, accompanied by indications of blood stasis within haemocapillaries and zacapillary venules. These findings indicated the potential for the development of stromal oedema surrounding the vessels. Additionally, a minimal amount of adipose tissue was present (Fig. 8).

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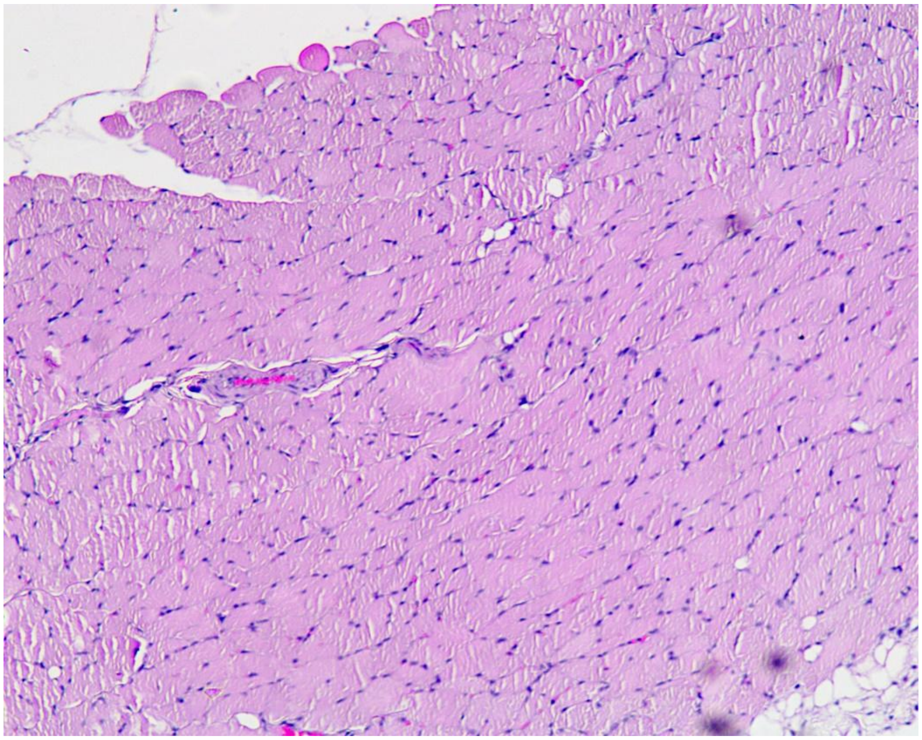

Figure 8. Lumbar part of the rat diaphragm after 14 days. Compact, ordered arrangement of muscle fibres. The nuclei are clearly visualised in the sublemmal spaces. Small fatty inclusions in the endo- and perimysium. Hematoxylin and eosin staining. × 100.

The majority of tendon collagen fibres exhibit a parallel arrangement, thereby preserving their structural integrity. In addition, fibroblast cells are present in minimal numbers between the fibres (see Fig. 9).

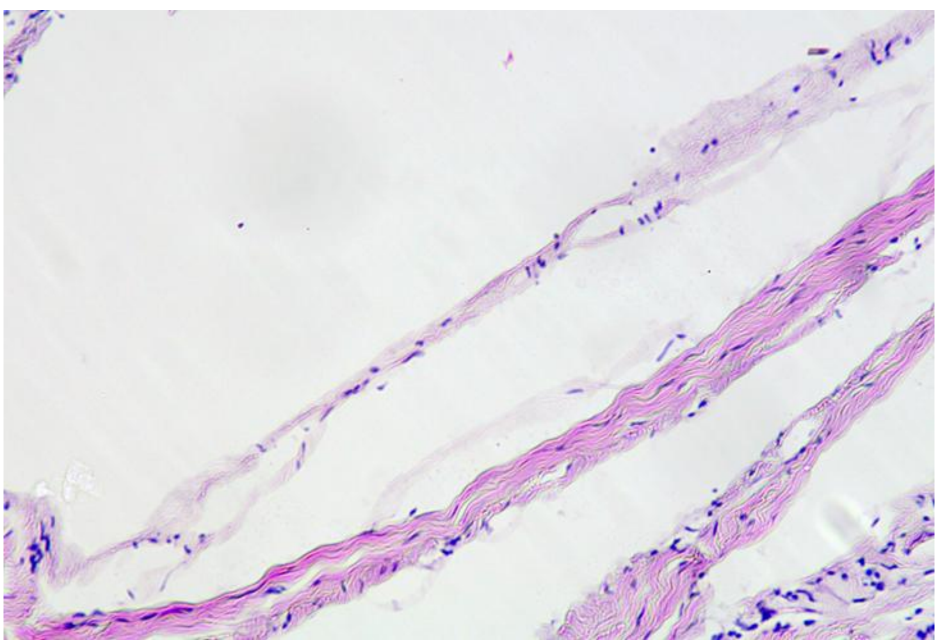

Figure 9. Compact arrangement of collagen fibres in the tendinous part of the rat diaphragm after 14 days. Hematoxylin and eosin staining. × 100.

5. Discussion

The findings of the present study demonstrate the clear advantage of using a low-pressure pneumoperitoneum in laparoscopic surgery, provided that adequate intra-abdominal space is available for the manipulation of instruments during all stages of surgery. The experiment was performed on healthy animals without concomitant pathology, and it was found that intra-abdominal pressure leads to changes in the diaphragm. It should be noted that the data show that the diaphragm restores its normal structure after 14 days. In the presence of concomitant pathology (cardiovascular, pulmonary), the use of such a pressure level is justified, which will contribute to a better postoperative period, which confirms the practical and clinical significance of this work. The results emphasise the opinions of other authors who recommend the use of lower pressure during laparoscopic surgery [1- 3], [6]. The present study suggests that low-pressure pneumoperitoneum is a safe procedure. Its surgical complication and open conversion rates are comparable to those of standard pressure pneumoperitoneum [8], [10]. The duration of the operation was found to be clinically indistinguishable between the groups, as reported by the authors [8], [9].

Increased pressure within the abdominal cavity has been demonstrated to reduce abdominal perfusion pressure [5], thus impairing perfusion to the intra- and extra-abdominal organs and, in particular, to areas such as the intestinal mucosa. Venous compression has been shown to increase venous pressure, which leads to congestion, intestinal oedema, and translocation of gastrointestinal bacteria.

The pliability of the abdominal cavity is subject to limitations, and it is unique to each patient. Consequently, pressure-volume curves cannot be easily predicted. The objective is to use the lowest possible pressure to accomplish the surgical task without compromising the surgical outcome. Clinical importance lies in caution and awareness of the point at which higher pressures do not increase the working space, but only increase the pressure [11].

6. Conclusion

The present study investigates the structural changes to the diaphragm that result from low-pressure pneumoperitoneum for a duration of five hours. The changes observed included blurring of muscle fibre boundaries and a loss of differentiation with the stroma. Additionally, myofibres underwent an undulating transformation, and transverse striations were visualised. Arterioles exhibited signs of wall oedema, endothelial desquamation and moderate blood filling, in addition to cell infiltrates. The tendon part lost its compactness, the content of the main substance increased, and collagen fibres were highly disintegrated, disintegrated and lysed. These changes are reversible, as demonstrated by the results 14 days after the application of pneumoperitoneum 5 mmHg, which showed complete recovery of all parts of the diaphragm.

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