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ORIGINAL PAPER

FEATURES OF HISTOLOGICAL CHANGES IN THE DIAPHRAGM 14 DAYS AFTER THE USE OF LOW-PRESSURE PNEUMOPERITONEUM

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Abstract

Introduction. The establishment of an optimal level of intra-abdominal pressure during laparoscopic surgery represents a considerable challenge for surgeons.
The objective of the study was to assess the morphological features of the lumbar, costal and tendon parts of the diaphragm under low-pressure pneumoperitoneum modelling and long-term structure recovery.
Materials and methods. The experiment was performed on 30 sexually mature rats of the reproductive period. 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.
Results. The histological examination of the microsections after five hours revealed destructive changes in all the 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 changes,

Résumé

Caractéristiques des modifications histologiques du diaphragme 14 jours après l'utilisation d'un pneumopéritoine à basse pression

Introduction. L'établissement d'un niveau optimal de pression intra-abdominale lors d'une chirurgie laparoscopique représente un défi considérable pour les chirurgiens.
L'objectif de l'étude a était d'évaluer les caractéristiques morphologiques des parties lombaires, costales et tendineuses du diaphragme sous modélisation du pneumopéritoine à basse pression et récupération de la structure à long terme.
Matériel et méthodes. L'expérience a été réalisée sur 30 rats sexuellement matures en période de reproduction. Dans le premier groupe, le diaphragme a été prélevé immédiatement après cinq heures de pneumopéritoine, et dans le second groupe 14 jours après le pneumopéritoine simulé.

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becoming undulated, whilst transverse striations are fragmented or absent. The veins and capillaries are full of blood, and there is a distributed leukocytosis. The tendon part loses its compactness. The collagen fibres of the tendons were severely disintegrated, and underwent lysis. After 14 days, the muscle fibres of the diaphragm exhibited an equidirectional, parallel and compact arrangement, and their thickness varied slightly. Greater proportion of collagen fibres in the tendons are parallel to each other and retain their integrity.

Conclusions. 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.

Keywords: laparoscopy, pneumoperitoneum, intra-abdominal pressure, diaphragm, rat.

List of abbreviations:
PP – pneumoperitoneum
CO_2 – carbon dioxide
EG – experimental groups
CC16 – Club Cell Protein

Résultats. L'examen histologique des microsections après cinq heures a révélé des modifications destructrices de tous les composants structurels du diaphragme. Les fibres musculaires présentaient une disposition compacte, souvent aux limites floues, et une perte de différenciation par rapport au stroma. Les myofibrilles subissent une transformation, devenant ondulées, tandis que les stries transversales sont fragmentées ou absentes. Les veines et les capillaires sont saturés de sang, et on observe une leucocytose distribuée. La partie tendineuse perd sa compacité. Les fibres de collagène des tendons étaient sévèrement désintégrées, puis lysées. Après 14 jours, les fibres musculaires du diaphragme présentaient une disposition équidirectionnelle, parallèle et compacte, et leur épaisseur variait légèrement. Une plus grande proportion de fibres de collagène dans les tendons sont parallèles les unes aux autres et conservent leur intégrité.

Conclusions. Un pneumopéritoine à basse pression pendant 5 heures entraîne des modifications structurelles du diaphragme. Ces modifications sont réversibles, comme le démontre la récupération complète de toutes les parties du diaphragme en 14 jours.

Mots-clés: laparoscopie, pneumopéritoine, pression intra-abdominale, diaphragme, rat.

Introduction

The establishment of the optimal level of intra-abdominal pressure during laparoscopic surgery represents a considerable challenge for surgeons. The most commonly used is a pneumoperitoneum (PP) 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 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 PP 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 CO_2 PP 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 to evaluate the effect of low pressure PP (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 majority of authors who published on low intra-abdominal 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



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

mechanism becomes disrupted. In a meta-analysis on low blood pressure, Ortenzi et al. 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.

The objective of the study was to assess the morphological features of the lumbar, costal and tendon parts of the diaphragm under low-pressure PP modelling and long-term structure recovery.

Materials and methods

The experiment was performed on 30 sexually mature rats of reproductive period (6-7 months), weighing (230.0±20.0) grams. The subjects were maintained in an accredited vivarium at the I. Horbachevsky Ternopil National Medical University, Ukraine. 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. Subsequently, 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 PP, and in the second group, 14 days after the simulated PP.

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. 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[18].

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

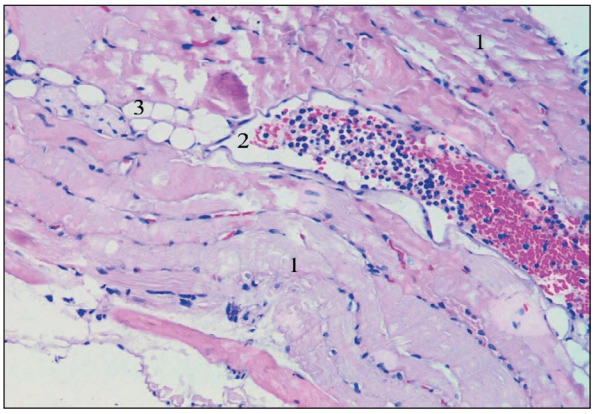


Figure 2. Morphology of the costal part of the diaphragm after 5 hours. PP: 1 – muscle fibres vacuolate and disintegrate, 'merge' due to lysis of cell membranes; 2 – redistribution of blood in the vein with the effect of distributive leukocytosis; 3 – fatty infiltration of the perimysium. Hematoxylin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

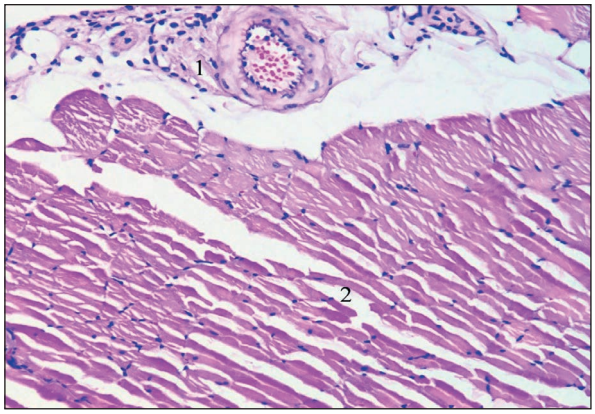


Figure 3. Longitudinal section of the lumbar part of the diaphragm after 5 hours of PP: 1 – perivascular connective tissue is thickened due to collagen fibre proliferation and contains cellular infiltrate; 2 – stromal edema and muscle fibre atrophy. Hematoxylin and eosin staining. × 100.

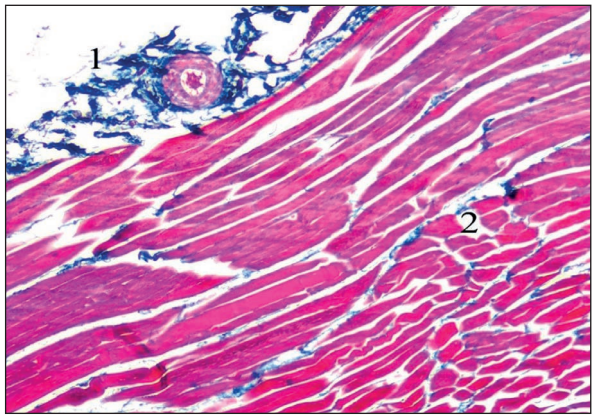


Figure 4. Cruses of diaphragm after 5 hours of PP: 1 – proliferation of collagen fibres around the vessel in the perimysium; 2 – foci of increased collagen formation in the endomysium. Masson's stain. ×100.

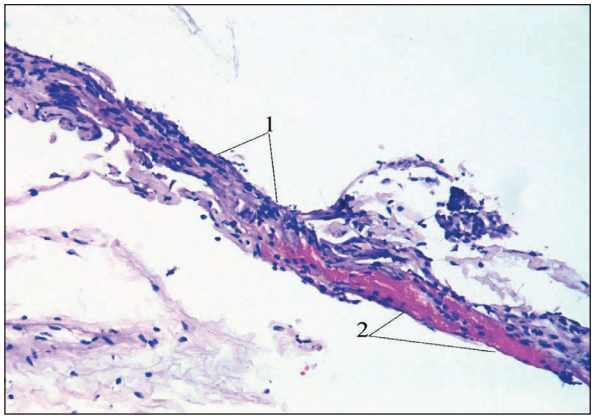


Figure 5. Fragment of rat diaphragm tendon after 5 hours of PP: 1 – Intense inflammatory cell infiltration in areas of collagen fibre damage; 2 – Hemorrhage. Hematoxylin and eosin staining. × 100.

on a microtome and, after deparaffinisation, stained with haematoxylin and eosin and Masson's stain.

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".

Results

The 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. The veins and capillaries are full-blooded, and there is a distributed leukocytosis (Fig. 2).

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

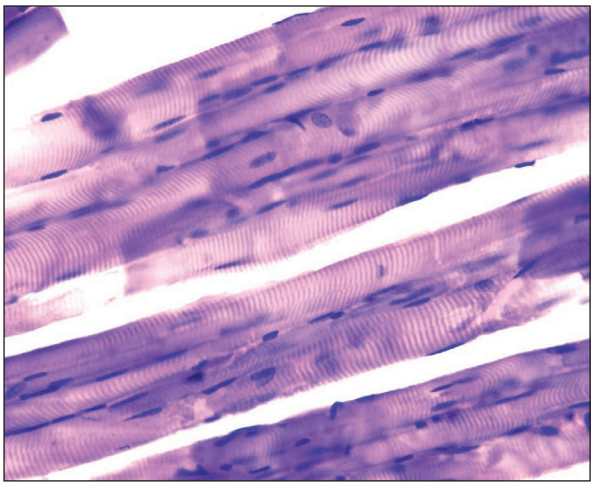


Figure 6. Longitudinal section of the rat diaphragmatic rib chassis after 14 days, showing clear visualisation of transverse striation. Hematoxylin and eosin staining. × 400.

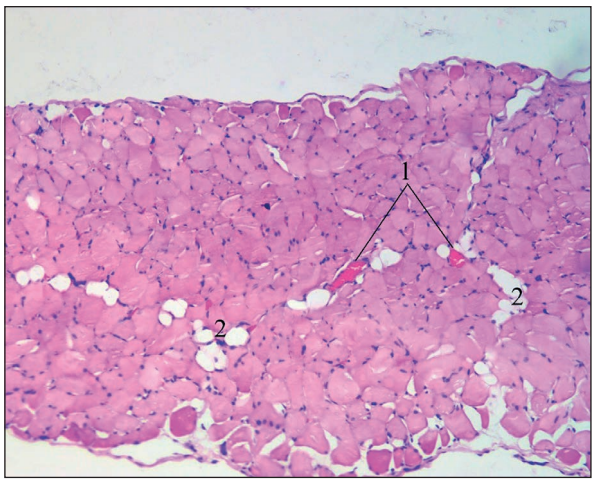


Figure 7. Transverse section of the costal part of the rat diaphragm after 14 days, muscle fibres are compactly arranged with typical nuclei localisation: 1 – full blooded capillaries and venules; 2 – small foci of adipose tissue. Hematoxylin and eosin staining. × 200/

predominantly macrophages and lymphocytes and fibroblasts (Fig. 3, 4).

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).

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

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,

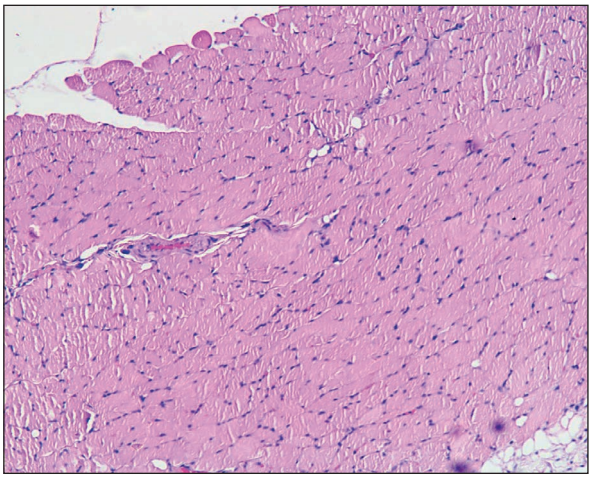


Figure 8. Lumbar part of the rat diaphragm after 14 days. Compact, ordered arrangement of muscle fibres. The nuclei are clearly visualised in the sublemmal spaces. Small fatty inclusions in the endo- and perimysium. Hematoxylin and eosin staining. × 100.

a proportion of damaged endothelial cells was detected, along with evidence of oedema, exfoliation and vacuolation of smooth muscle cells.

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).

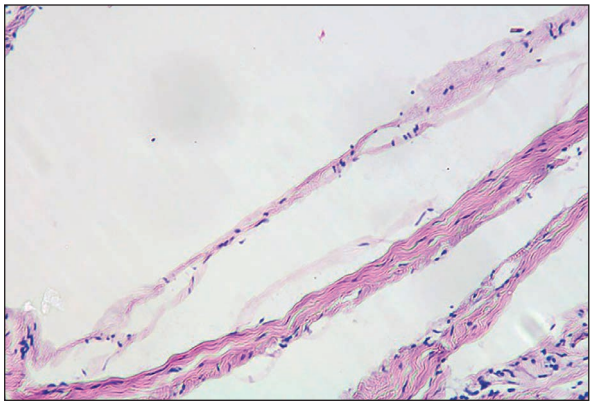


Figure 9. Compact arrangement of collagen fibres in the tendinous part of the rat diaphragm after 14 days. Hematoxylin and eosin staining. × 100.

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 (Fig. 9).

Discussion

The findings of the study demonstrate the clear advantage of using a low-pressure PP 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. 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 emphasize the opinions of other authors who recommend the use of lower pressure during laparoscopic surgery[1-3,6]. Our study suggests that low-pressure PP is a safe procedure. Its surgical complication and open conversion rates are comparable to those of standard pressure PP[8,10]. The duration of the operation was found to be clinically indistinguishable between the groups, as reported by the authors[8,9].

When analyzing the literature, there are sufficient data on the effect of PP on the diaphragm, the respiratory system as a whole, and other abdominal organs; however, there are no data on the histological structure of parts of the diaphragm after the creation of PP of various durations. Increased pressure within the abdominal cavity has been demonstrated to reduce abdominal perfusion pressure[5], thus impairing perfusion of the intra- and extra-abdominal organs and, in particular, of 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.

Most experiments that investigated morphological changes after PP focused on intra-abdominal organs. An intra-abdominal pressure of 10 mmHg for 60 minutes in rats increased oxidative stress in intestinal tissue[19]. Another study evaluated the effect of insufflation at 4, 8 or 12 mmHg on kidney and liver blood flow, testicular structure, and liver regeneration rate[20]. A decrease in renal blood flow was observed during CO_2 inflation, but this decrease was only significant in the group infused at 8 and 12 mmHg. In terms of portal hepatic blood flow, a PP of 12 mmHg was sufficient to cause a significant decrease in portal hepatic blood flow[21].

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. The 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].

The microscopic changes in the parts of the diaphragm in the first EG are associated with the direct effect of PP. 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 laparoscopic cholecystectomy, 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[22].

One study was based on a comparison of diaphragmatic excursions before and after major laparoscopic pelvic surgery under general anaesthesia in the Trendelenburg position, using ultrasound examination of diaphragmatic excursion and lung compliance. The results showed a significant decrease in excursion and lung compliance. Age, duration of anaesthesia, and post-procedure pain were significant independent predictors[23].

Conclusions

The present study investigated the structural changes of the diaphragm that result from low-pressure PP 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 PP 5 mmHg, which showed complete recovery of all parts of the diaphragm.

Authors' Contributions:

Conceptualization, methodology, design of the work M.K., wrote the manuscript, analysis M.K., T.G., project administration, draft preparation O.K., R.K., interpretation of data for the work, editing, validation V.T., I.I.

All authors have read and agreed to the published version of the manuscript.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law."

"All institutional and national guidelines for the care and use of laboratory animals were followed"

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References

1. Buda A, Di Martino G, Borghese M, et al. Low-pressure laparoscopy using the AirSeal System versus standard insufflation in early-stage endometrial cancer: a multicenter, retrospective study (ARIEL Study). *Healthcare (Basel).* 2022;10(3):531. doi:10.3390/healthcare10030531
2. Sroussi J, Elies A, Rigouzzo A, et al. Low pressure gynecological laparoscopy (7mmHg) with AirSeal® System versus a standard insufflation (15mmHg): A pilot study in 60 patients. *J Gynecol Obstet Hum Reprod.* 2017;46(2):155-158. doi:10.1016/j.jogoh.2016.09.003
3. Ortenzi M, Montori G, Sartori A, et al. Low-pressure versus standard-pressure pneumoperitoneum in laparoscopic cholecystectomy: a systematic review and meta-analysis of randomized controlled trials. *Surg Endosc.* 2022;36(10):7092-7113. doi:10.1007/s00464-022-09201-1
4. Tian H, Qi H, Xu X, Yu T, Lin X. Research hotspots and trends in postlaparoscopic shoulder pain from 2003 to 2023: A bibliometric analysis. *Heliyon.* 2024;10(4):e25846. doi:10.1016/j.heliyon.2024.e25846
5. Łagosz P, Sokolski M, Biegus J, Tycinska A, Zymlinski R. Elevated intra-abdominal pressure: A review of current knowledge. *World J Clin Cases.* 2022;10(10):3005-3013. doi:10.12998/wjcc.v10.i10.3005
6. Brusasco C, Germinale F, Dotta F, et al. Low intra-abdominal pressure with complete neuromuscular blockage reduces post-operative complications in major laparoscopic urologic surgery: a before-after study. *J Clin Med.* 2022;11(23):7201. doi:10.3390/jcm11237201
7. Albers KI, Polat F, Loonen T, et al. Visualising improved peritoneal perfusion at lower intra-abdominal pressure by fluorescent imaging during laparoscopic surgery: A randomised controlled study. *Int J Surg.* 2020;77:8-13. doi:10.1016/j.ijsu.2020.03.019
8. Neogi P, Kumar P, Kumar S. Low-pressure pneumoperitoneum in laparoscopic cholecystectomy: a randomized controlled trial. *Surg Laparosc Endosc Percutan Tech.* 2020;30(1):30-34. doi:10.1097/SLE.0000000000000719
9. Raval AD, Deshpande S, Koufopoulou M, et al. The impact of intra-abdominal pressure on perioperative outcomes in laparoscopic cholecystectomy: a systematic review and network meta-analysis of randomized controlled trials. *Surg Endosc.* 2020;34(7):2878-2890. doi:10.1007/s00464-020-07527-2
10. Hamid M, Mostafa OES, Mohamedahmed AYY, et al. Comparison of low versus high (standard) intraabdominal pressure during laparoscopic colorectal surgery: systematic review and meta-analysis. *Int J Colorectal Dis.* 2024;39(1):104. doi:10.1007/s00384-024-04679-8
11. Ott DE. Abdominal compliance and laparoscopy: a review. *JSLS.* 2019;23(1):e2018.00080. doi:10.4293/JSLS.2018.00080
12. Jalal NA, Abdulbaki Alshirbaji T, et al. Effects of intra-abdominal pressure on lung mechanics during laparoscopic gynaecology. *Annu Int Conf IEEE Eng Med Biol Soc.* 2021;2021:2091-2094. doi:10.1109/EMBC46164.2021.9630753
13. Kritsak MYu, Gargula TI, Slabyi OB, Yasinovskyi OB, Kuziv OV, Ihnatishchev MR. Characteristics of morphological changes in the lumbar part of the diaphragm in cases of obstructive jaundice and pneumoperitoneum. *Arch Balk Med Union*, 2025;60(1):62-68. doi.org/10.31688/ABMU.2025.60.1.06
14. Mazzinari G, Diaz-Cambronero O, Serpa Neto A, et al. Modeling intra-abdominal volume and respiratory driving pressure during pneumoperitoneum insufflation-a patient-level data meta-analysis. *J Appl Physiol.* 2021;130(3):721-728. doi:10.1152/japplphysiol.00814.2020
15. Sterke F, van Weteringen W, Ventura L, et al. A novel method for monitoring abdominal compliance to optimize insufflation pressure during laparoscopy. *Surg Endosc.* 2022;36(9):7066-7074. doi:10.1007/s00464-022-09406-4
16. Bains KNS, Kashyap S, Lappin SL. Anatomy, Thorax: Diaphragm. In: StatPearls. Treasure Island (FL): StatPearls Publishing; July 24, 2023.
17. McCool FD, Manzoor K, Minami T. Disorders of the diaphragm. *Clin Chest Med.* 2018;39(2):345-360. doi:10.1016/j.ccm.2018.01.012

18. Avital S, Itah R, Szomstein S, et al. Correlation of CO_2 pneumoperitoneal pressures between rodents and humans. *Surg Endosc*. 2019;23(1):50-54. doi:10.1007/s00464-008-9862-7
19. de Lacy FB, Taurà P, Arroyave MC, et al. Impact of pneumoperitoneum on intra-abdominal microcirculation blood flow: an experimental randomized controlled study of two insufflator models during transanal total mesorectal excision: An experimental randomized multi-arm trial with parallel treatment design. *Surg Endosc*. 2020;34(10):4494-4503. doi:10.1007/s00464-019-07236-5
20. Eva I, Rosario V, Guglielmo R, et al. Laparoscopic surgery: A randomised controlled trial comparing intraoperative hemodynamic parameters and arterial-blood gas changes at two different pneumoperitoneal pressure values. *Ann Med Surg (Lond)*. 2022;81:104562. doi:10.1016/j.amsu.2022.104562
21. Qi RZ, Li ZW, Chang ZY, et al. Clinical efficacy of total laparoscopic splenectomy for portal hypertension and its influence on hepatic hemodynamics and liver function. *World J Gastrointest Surg*. 2023;15(8):1684-1692. doi:10.4240/wjgs.v15.i8.1684
22. Xue S, Wang D, Tu HQ, et al. The effects of robot-assisted laparoscopic surgery with Trendelenburg position on short-term postoperative respiratory diaphragmatic function. *BMC Anesthesiol*. 2024;24(1):92. doi:10.1186/s12871-024-02463-3
23. Rustagi PS, Yadav A, Nellore SS. Ultrasonographic evaluation of diaphragmatic excursion changes after major laparoscopic surgeries in the Trendelenburg position under general anaesthesia: A prospective observational study. *Indian J Anaesth*. 2023;67(Suppl 4):S274-S280. doi:10.4103/ija.ija_643_23