ORIGINAL ARTICLE

CONTENTS

Morphological features of the costal part of the diaphragm induced by carboxyperitoneum: an experimental study

Myroslav Yu. Kritsak, Tetiana I. Gargula, Oleh B. Yasinovskyi, Svitlana O. Rosolovska, Oksana S. Karashivska, Nataliia O. Davybida

I.HORBACHEVSKY TERNOPIL NATIONAL MEDICAL UNIVERSITY, TERNOPIL, UKRAINE

ABSTRACT

Aim: To investigate the morphological changes in the costal part of the diaphragm following the induction of pneumoperitoneum at 10 mmHg for varying durations in a rat model, using objective methods.

Materials and Methods: To create the model of the experiment, 50 sexually mature rats aged 5-6 months and weighing (225.0±20.0) grams were used. The control group underwent a puncture of the abdominal wall with a Veress needle, which was maintained for a period of five hours. This was done to ascertain whether such an intervention would result in histological changes to the diaphragm. In the main groups, carboxyperitoneum was created at a pressure of 10 mmHg.

Results: The microscopic appearance of this type of muscle was observed in the preparations obtained from the intact group of animals. The sarcoplasmic sections obtained after the created pneumoperitoneum showed destruction of the basal membranes of muscle fibres in most cases. However, in some instances, muscle fibres with preserved sarcolemma were also observed, although damage was evident through the disintegration of sarcoplasm into fragments, partial lysis, vacuolisation and eosinophilic degeneration. Some myofibres became uncoiled and deformed. The stroma increased in oedema, the volume of adipose tissue and the number of collagen fibres, mainly around the vessels, and haemorrhagic infiltration was also noted. The intensity of these changes was found to depend on the duration of pneumoperitoneum.

Conclusions: The creation of pneumoperitoneum by carbon dioxide results in morphological and structural alterations to the costal part of the diaphragm. The extent of these changes is directly proportional to the duration of intra-abdominal pressure.

KEY WORDS: pneumoperitoneum, diaphragm, rats, histology

Wiad Lek. 2025;78(7):1245-1252. doi: 10.36740/WLek/204997 DOI

INTRODUCTION

Laparoscopy is a widely used surgical technique in modern medicine. It offers a number of advantages over other surgical procedures, including rapid tissue recovery, reduced postoperative pain, less damage to wound tissue, shorter hospitalisation periods and a quicker return to work [1, 2]. Since the advent of laparoscopy in the late 1980s, numerous studies have documented the effects of carbon dioxide-induced pneumoperitoneum (carboxyperitoneum (CP)), demonstrating cardiorespiratory changes, hypercarbia, hormonal changes, and as an effect on cancer cell survival [3, 4]. The majority of these studies use small animals, namely rats, due to their ease of use and simplicity. In these studies, CPs with pressures ranging from 3 to 20 mmHg are used [5-9]. For humans, surgeons use a standard operating pressure of 15 mmHg, which generally provides good visualisation of the abdominal cavity.

The growing use of laparoscopic surgical techniques has sparked significant interest in experimental research, particularly in relation to organ damage caused by increased intra-abdominal pressure. However, there is a limited body of literature examining the impact of CP on the diaphragm. This highlights an unaddressed question regarding the influence of CP pressure on the organs and systems of the body, including the respiratory muscle.

AIM

To investigate the morphological changes in the costal part of the diaphragm following the induction of pneumoperitoneum at 10 mmHg for varying durations in a rat model, using objective methods.

MATERIALS AND METHODS

All work on experimental animals was carried out in accordance with the relevant legislation, including the Law of Ukraine 'On Protection of Animals from

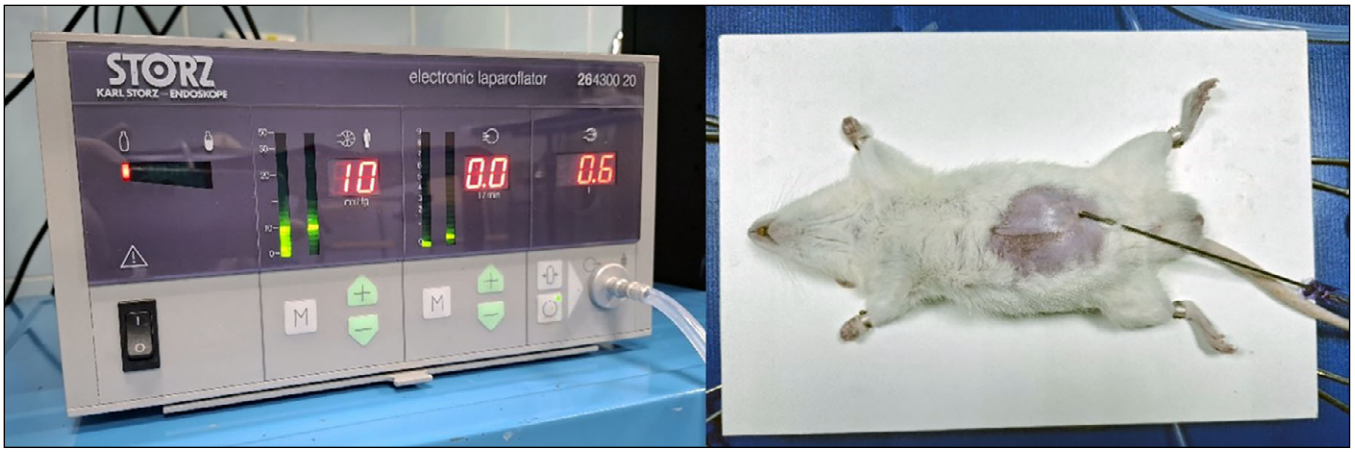

Fig. 1. Created carboxyperitoneum at 10 mmHg (left - insufflator, right - experimental animal)

Table 1. Distribution of individuals into experimental groups

Group of animals	Characteristics of the group
Intact group (n = 10)	Taking the costal part of the diaphragm, which was considered normal
Control group (n = 10)	Taking the costal part of the diaphragm after anaesthesia and laparocentesis
I the main group (n = 10)	Taking the costal part of the diaphragm after pneumoperitoneum at 10 mmHg for 1 hour
II the main group (n = 10)	Taking the costal part of the diaphragm after pneumoperitoneum at 10 mmHg for 3 hours
III the main group (n = 10)	Taking the costal part of the diaphragm after pneumoperitoneum at 10 mmHg for 5 hours
Total animals – 50	

Cruelty', the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of 18 March 1986, and Council of Europe Directive 2010/63/EU. To create the model of the experiment, 50 sexually mature rats aged 5-6 months and weighing (225.0±20.0) grams were used. The rats were sourced from an accredited vivarium at the I Horbachevsky Ternopil National Medical University. Table 1 presents the division of animals into groups. The experiments were conducted in the morning indoors at a relative humidity of (60-80) %, temperature of (18-20) °C and illumination of 200 lux. The animals were euthanised by an overdose of Thiopental sodium at a rate of 75 mg/kg of body weight intraperitoneally.

Prior to laparocentesis, all animals were anaesthetised with a combination of Ketamine Solutions (90 mg/kg) and Xylazine (10 mg/kg), administered via intramuscular injection. The animals in the intact group were not subjected to any additional treatment. In the control group, the abdominal wall was punctured with a Veress needle and maintained for a period of five hours to ascertain whether such an impact on the body would result in histological alterations to the diaphragm. In the main groups, carboxyperitoneum was created at the level of 10 mmHg, according to the copyright certificate for the work No. 126409 of 16 May 2024 [10], with the KARL STORZ electronic laparoflator 264300 20. The insufflator was used to set the appropriate level of pressure in the abdominal cavity and the rate of carbon dioxide delivery at 0.1 l/min for 1, 3 and 5 hours, respectively, according to the number of the main group (Fig. 1). This pressure level was set based on the findings of a literature analysis, which indicated that this pressure in rats produces comparable changes to the pressure of 15 mmHg in humans, which is regarded as a standard pressure [11, 12]. During the anaesthesia and the creation of a pneumoperitoneum, spontaneous breathing was maintained throughout the experiment.

After the experiment, the rat diaphragm was sampled in accordance with the copyright certificate for the work No. 126059 of 29 April 2024 [13]. The preparation of the macro sample of the diaphragm is illustrated in Fig. 2.

Following the separation of the costal part of the diaphragm, it was placed in a 10% solution of neutral formalin. Following the fixation of the selected material, it was dehydrated in ethyl alcohols of increasing concentration and embedded in paraffin. Histological sections 5-7 µm thick were made from each paraffin block on a microtome, which were stained with hematoxylin and eosin after deparaffinisation.

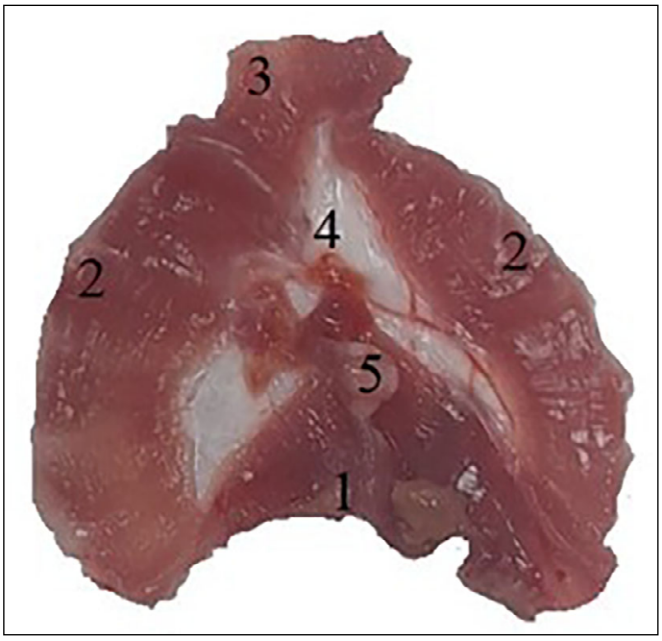

Fig. 2. Removed macrosection of the diaphragm: 1 – crus of the diaphragm; 2 – costal part; 3 - sternal part; 4 - tendon part; 5 - esophagus

RESULTS

The costal part of the diaphragm is composed of transversely striated muscle fibres that form a dense layer, which serves as the basis for its structure. The microscopic appearance of this type of muscle can be observed on the slides prepared with a longitudinal section of the specimen. The variation in fibre size and thickness is not significant, and the sarcoplasm itself has a light eosinophilic colour. The relationship between the dyes was heterogeneous, and the sarcoplasm was often heterogeneous as well. The nuclei, which were elongated-oval in shape, were located under the sarcolemma and were not always oriented parallel to the fibre in a uniform manner. The transverse striation, which took the form of straight or arcuate stripes on the longitudinal sections, varied. There were few fibrocytes present in the stroma, which was formed by blood-filled capillaries (Fig. 3).

Upon examination of a cross-section of the specimen, the muscle fibres are predominantly polygonal or oval-rounded in shape. Each muscle fibre is surrounded by thin collagen fibres, which are clearly visible, and the fibre bundles are also surrounded by a dense connective tissue layer, the perimysium. The presence of fibroblasts and capillaries in the connective tissue is minimal (Fig. 4).

It should be noted that the preparations obtained from the control group animals did not differ histologically, indicating that this type of exposure does not affect the structure of the diaphragm.

In the main groups, the creation of CP with an intra-abdominal pressure of 10 mmHg resulted in pathological changes in the selected areas of the diaphragm. Consequently, the muscles exhibited a partial loss of compactness due to perimysial and endomysial oedema, particularly in regions with adipose tissue and cellular infiltrates, following the initial hour of CP. With regard to the nuclei, they lost their monomorphism and were observed in regions that were not characteristic of their typical localisation. The sarcoplasm was found to be highly heterogeneous. Areas of compaction were

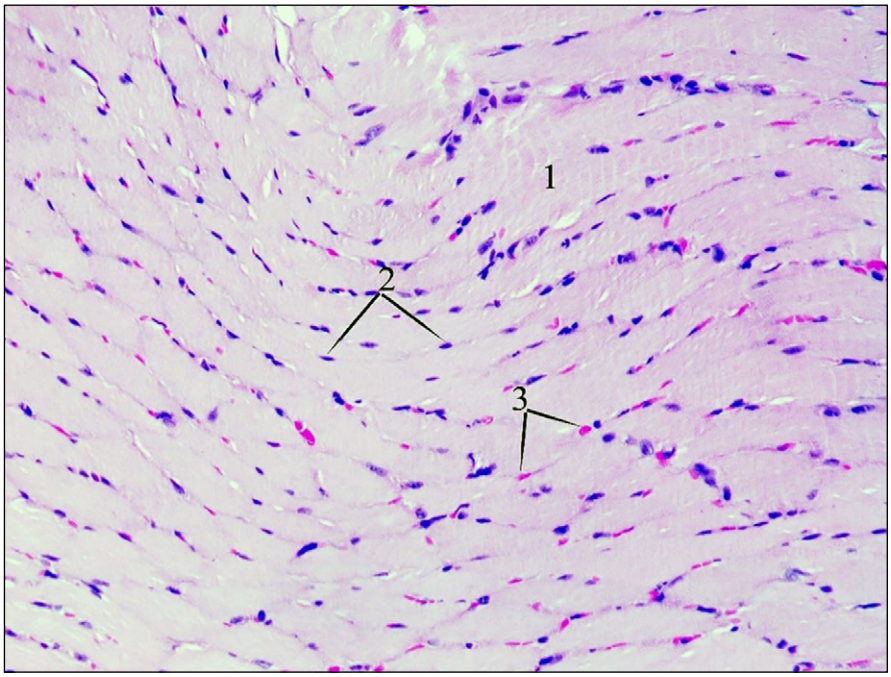

Fig. 3. Morphological structure of the costal part of the diaphragm of an intact animal: sarcoplasm (1); nuclei (2); capillaries located in the endomysium (3). Hematoxylin and eosin staining. × 200

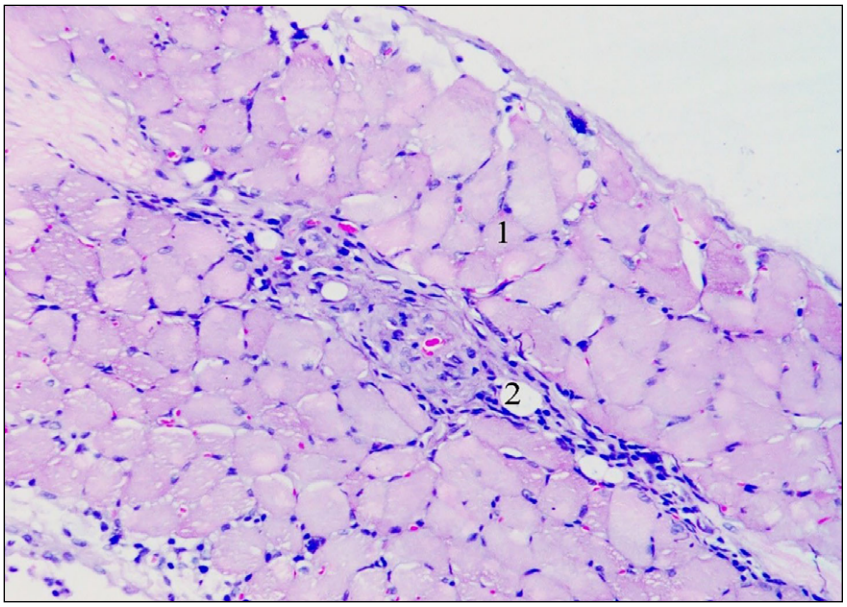

Fig. 4. Morphological structure of the costal part of the diaphragm of an intact rat in a transverse section. Sublemmal arrangement of nuclei (1), perimysium with a localised vascular bundle (2). Hematoxylin and eosin staining. × 200

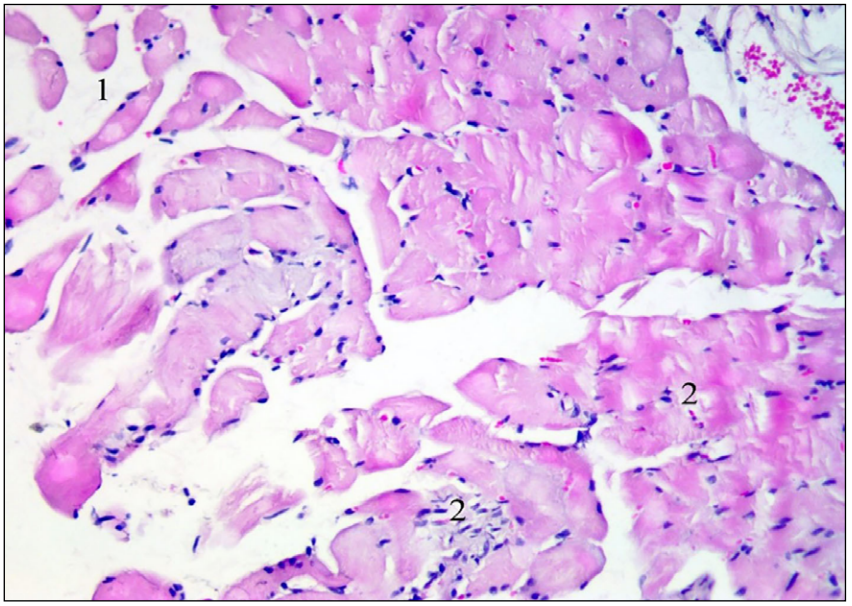

Fig. 5. Morphological structure of the costal part of the diaphragm one hour following the induction of pneumoperitoneum. Connective tissue oedema (1), fiber destruction and cellular infiltration (2). Hematoxylin and eosin staining. × 200

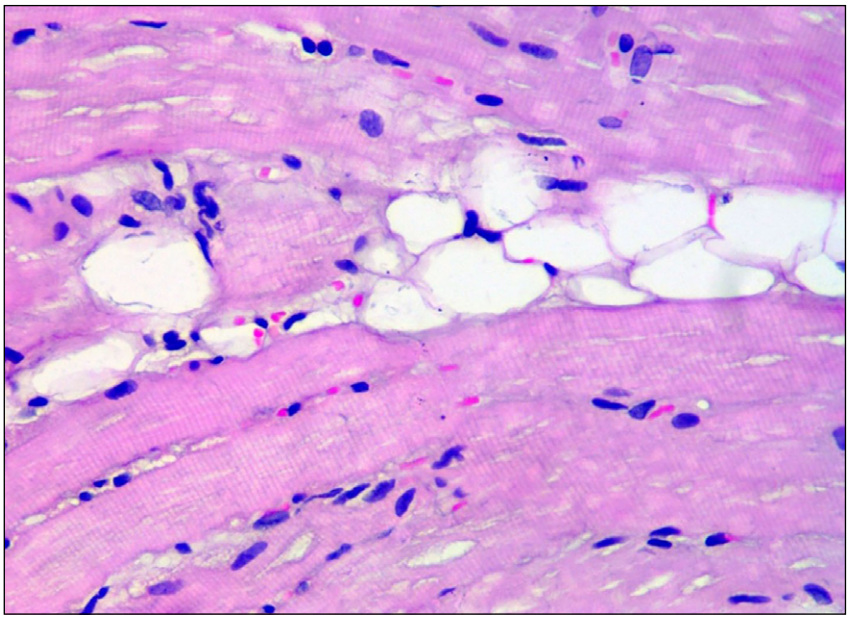

Fig. 6. Transverse striated muscles of the rat diaphragm 1 one hour following the induction of pneumoperitoneum.The transverse striation of muscle fibres is preserved. Fatty infiltration of the perimysium. Hematoxylin and eosin staining. × 400

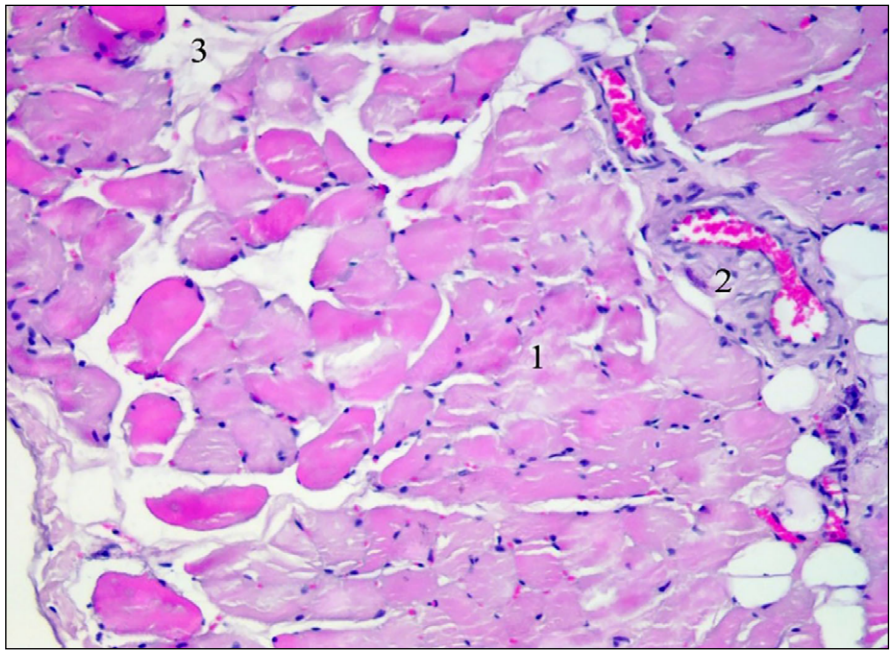

Fig. 7. Morphological structure of the costal part of the diaphragm three hours after pneumoperitoneum. Disintegration of basal membranes and fibres themselves (1), collagenisation of the perivascular stroma (2) and its oedema between fibres (3). Hematoxylin and eosin staining× 200

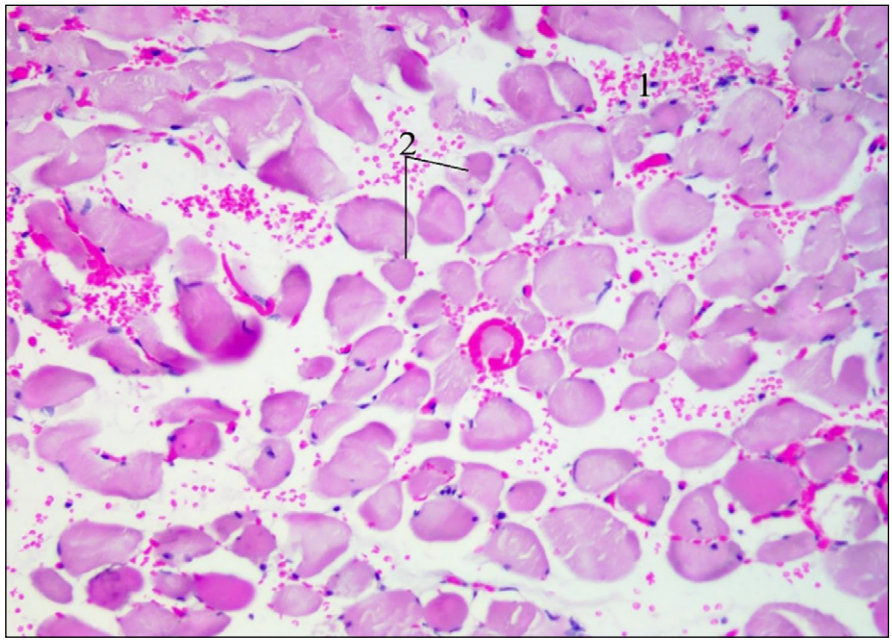

Fig. 8. Transverse section of the costal part of the rat diaphragm 5 hours after pneumoperitoneum. Diffuse haemorrhagic infiltration of the endomysial connective tissue combined with edema (1), atrophy of muscle fibres (2). Hematoxylin and eosin staining × 200

replaced by liquefaction, disintegration and, on occasion, cytolysis. This was accompanied by a change in the orientation of the fibres themselves (Fig. 5).

However, in conjunction with these alterations, there were also compact muscle groups that demonstrated minimal change and preserved, well-visualised transverse striations (Fig. 6).

Three hours after CP, degenerative changes in the sarcoplasm were observed, accompanied by the destruction of the basal membranes of muscle fibres. In these areas, single or grouped preserved pyknotic nuclei and macrophages were identified. In most cases, the muscle fibres had preserved sarcolemma, but showed signs of damage - sarcoplasmic disintegration into fragments, partial lysis, vacuolisation and eosinophilic degeneration. Some myofibrils are uncoiled with their deformation. Oedema, the volume of adipose tissue and the number of collagen fibres increase in the stroma, especially around the vessels (Fig. 7).

After 5 hours of CP, the histological picture described at the previous stage appeared, with an increase in the volume of muscle fibre deformation, fragmentation and lysis, indicating a sharp decrease in the contractile ability of the diaphragm. In transverse sections, muscle fibres became rounded and their diameter decreased, indicating signs of muscle atrophy, which in turn led to the appearance of cellular inflammatory infiltrates. A distinctive feature was the presence of haemorrhagic

infiltration. In the stroma at this stage, in addition to oedema and haemorrhage, the proportion of adipose and collagen fibres increased (Fig 8).

DISCUSSION

The diaphragm is a dome-shaped structure that separates the thoracic and abdominal cavities. It is the main respiratory muscle and is innervated by the phrenic nerves, which arise from the nerve roots from C3 to C5, and consist mainly of fatigue-resistant slow type I myofibres and fast-twitch type IIa myofibres. Its mechanical action is best understood by considering its anatomy and attachment to the chest wall. The diaphragm is attached to the lower chest in an area called the adductor zone. During diaphragmatic contraction, the contents of the abdominal cavity are displaced caudally, the pressure in the abdominal cavity increases at the point of contact and the lower chest expands. The created pneumoperitoneum prevents the diaphragm from contracting, due to the pressure created in the abdominal cavity [14]. The effect of carbon dioxide and intra-abdominal pressure on the human body during laparoscopic surgery is not well understood, especially on the morphological structure of the abdominal organs [15-18].

The diaphragm is the main breathing muscle. Diaphragm dysfunction is an underestimated cause of breathing difficulties and can be caused by a variety of factors, including surgery, trauma, tumour and infection [16, 17]. The sudden increase in abdominal volume also stretches the diaphragm and stiffens the chest wall, as shown in studies in dogs and pigs with their heads up. Thus, pneumoperitoneum can cause stiffening of the abdominal wall and diaphragm, while increasing pleural pressure and compressing the lungs [15].

In this study, the morphology of the costal part of the diaphragm of experimental animals subjected to 10 mmHg pneumoperitoneum for different durations was studied to see how this parameter affects the morphology of the muscle. There are several experimental studies on this subject, which have also investigated the effects of intra-abdominal pressure in pigs and rats, using higher pressures than those used in human clinical practice, since 8-10 mmHg has been established in rats as the pressure corresponding to the standard human pressure of 15 mmHg. [5-7, 11, 15] Thus, this is the first study to show whether 10 mmHg pneumoperitoneum can cause morphological changes in the diaphragm.

Most of the experiments investigating morphological changes after pneumoperitoneum focus on intra-abdominal organs. Intra-abdominal pressure of 10 mm Hg for 60 minutes in rats increased oxidative stress in intestinal tissue [3, 4]. Another study evaluated the effect of insufflation at 4, 8 or 12 mm Hg on kidney and liver blood flow, testicular structure, and liver regeneration rate [9, 11]. A decrease in renal blood flow was observed during CO2 inflation, but this decrease was only significant in the group infused at 8 and 12 mmHg. In terms of portal hepatic blood flow, a pneumoperitoneum of 12 mmHg was sufficient to cause a significant decrease in portal hepatic blood flow [11]. The data of our study indicate a haemorrhagic infiltration of the muscular part of the diaphragm, capillaries in the endomysium are full of blood, arterioles in the perimysium contain plasma or a small number of erythrocytes. The venous connection was observed to be full of blood. Comprehensive morphological data on the diaphragm are not yet complete. In the present study, we summarise the beginning of the morphological description of the diaphragm and describe the state of the morphological features under the influence of CP. In addition, we correlate how the morphological structure of the costal part of the diaphragm changes with the duration of pneumothorax.

CONCLUSIONS

The creation of pneumoperitoneum with carbon dioxide leads to morphological and structural changes in the costal part of the diaphragm, the severity of which is directly related to the duration of intra-abdominal pressure. Histological samples showed deformation of muscle fibres, their fragmentation and lysis. In cross-section, the fibres were rounded and slightly reduced in diameter, a sign of atrophy. Sometimes there were areas of inflammatory infiltration and haemorrhagic infiltration. In the stroma, oedema and sometimes haemorrhage were observed at different times, and the percentage of adipose and collagen fibres increased. This provides the basis for further research to determine the correlation between the level of pressure and the duration of laparoscopic surgery.

PROSPECTS FOR FURTHER RESEARCH

The next work will investigate the effect of lower pressure carboxyperitoneum, namely 5 mmHg, on the morphology of the diaphragm and the structure of the diaphragm 14 days after pneumoperitoneum. How does this respiratory muscle recover, are there any residual changes in it?

REFERENCES

1. Cianci P, Restini E. Management of cholelithiasis with choledocholithiasis: Endoscopic and surgical approaches. World J Gastroenterol. 2021;27(28):4536-4554. doi:10.3748/wjg.v27.i28.4536. DOI
2. Dikmen K, Dikmen A, Bostanci H et al. The effect of pneumoperitoneum on intravascular fibrinolytic activity in rats. Turk J Med Sci. 2016;46:1528-1533. doi: 10.3906/sag-1501-53. DOI
3. Strobel O, Wachter D, Werner J et al. Effect of a pneumoperitoneum on systemic cytokine levels, bacterial translocation, and organ complications in a rat model of severe acute pancreatitis with infected necrosis. Surg Endosc. 2016;20(12):1897–1903. doi: 10.1007/s00464-005-0417-x. DOI
4. 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. DOI
5. Liu Y, Wang M, Zhu Y, Chen J. Effect of carbon dioxide pneumoperitoneum on acid-base balance during laparoscopic inguinal hernia repair: a prospective randomized controlled study. Hernia. 2021;25(5):1271-1277. doi:10.1007/s10029-020-02292-6. DOI
6. Zhang YW, Li Y, Huang WB et al. Utilization of deep neuromuscular blockade combined with reduced abdominal pressure in laparoscopic radical gastrectomy for gastric cancer: An academic perspective. World J Gastrointest Surg. 2023;15(7):1405-1415. doi:10.4240/wjgs.v15.i7.1405. DOI
7. Gunusen I, Akdemir A, Gurel C et al. The Effects of Different Pressure Pneumoperitoneum Models Created By Standard or Heated-Humidified CO2 Insufflation on Ovary and Peritoneum: an Experimental Study in Rats. Reproductive Sciences. 2022;29:1197–1208. doi: 10.1007/s43032-022-00878-2. DOI
8. Birch DW, Dang JT, Switzer NJ et al. Heated insufflation with or without humidification for laparoscopic abdominal surgery. Cochrane Database Syst Rev. 2016;10(10):CD007821. doi:10.1002/14651858.CD007821.pub3. DOI
9. 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. DOI
10. Kritsak M, Rosolovska S et al. Sposib modeliuvannia eksperymentalnoho karboksyperytoneumu u shchura. [Method of modeling experimental carboxyperitoneum in rats.] Svidotstvo pro reiestratsiiu avtorskoho prava na tvir. № 126409:2024. (Ukrainian)
11. Mayer S, Peukert N, Gnatzy R et al. Physiologic Changes in a Small Animal Model for Neonatal Minimally Invasive Surgery. J Laparoendosc Adv Surg Tech A. 2018;28(7):912-917. doi:10.1089/lap.2018.0075. DOI
12. Bao JM, Lin JP, Yu X et al. 2018;98(26):2088-2091. doi:10.3760/cma.j.issn.0376-2491.2018.26.006. DOI
13. Kritsak M, Slabyi O et al. Sposib vidboru eksperymentalnoho materialu diafrahmy bilykh shchuriv. [Method of selecting experimental material for the diaphragm of white shchuriv]. Svidotstvo pro reiestratsiiu avtorskoho prava na tvir. № 126059:2024. (Ukrainian)
14. Buzkovaa K, Mullera M, Raraa A et al. Ultrasound detection of diaphragm position in the region for lung monitoring by electrical impedance tomography during laparoscopy. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2018;162(1):43-46. doi: 10.5507/bp.2018.005. DOI
15. Wirth S, Biesemann A, Spaeth J, Schumann S. Pneumoperitoneum deteriorates intratidal respiratory system mechanics: an observational study in lung-healthy patients. Surg Endosc. 2017;31(2):753-760. doi:10.1007/s00464-016-5029-0. DOI
16. 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. DOI
17. Lessa T, Abreu D, Bertassoli B, Ambrósio C. Diaphragm: A vital respiratory muscle in mammals. Ann Anat. 2016:205(3):122-127. doi: 10.1016/j.aanat.2016.03.008. DOI
18. Kocjan J, Adamek M, Gzik-Zroska B et al. Network of breathing. Multifunctional role of the diaphragm: a review. Adv Respir Med. 2017;85(4):224-232. doi: 10.5603/ARM.2017.0037. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

CORRESPONDING AUTOR

Myroslav Yu. Kritsak
I.Horbachevsky Ternopil National Medical University
1 Maidan Voli, 46001 Ternopil, Ukraine
e-mail: kricakmy@tdmu.edu.ua

ORCID AND CONTRIBUTIONSHIP
Myroslav Yu. Kritsak: 0000-0003-1020-3584 A B C D F
Tetiana I. Gargula: 0000-0003-3335-0501 C D E F
Oleh B. Yasinovskyi: 0000-0002-5121-3140 B E F
Svitlana O. Rosolovska: 0000-0003-4768-3905 B D E F
Oksana S. Karashivska: 0000-0002-9017-640X B E F
Nataliia O. Davybida: 0000- 0002-7746-4829 B E F

A – Work concept and design, B – Data collection and analysis, C – Responsibility for statistical analysis, D – Writing the article, E – Critical review, F – Final approval of the article

RECEIVED: 28.11.2024
ACCEPTED: 12.05.2025