THE EFFECT OF DIAPHRAGMATIC PERITONEAL LYMPHATICS ON PERITONEAL ADHESIONS: AN EXPERIMENTAL STUDY

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ABSTRACT

In this study, we examined the effect of diaphragmatic peritoneal lymphatic function on the formation of peritoneal adhesions. A two phased design was used in fifty-two Wistar albino female rats. In the first phase (n=12), the effects of diaphragmatic peritoneum damage model on the cecum and the terminal ileum were evaluated. In the second phase, the rats (n=40) were divided into two equal groups. The adhesion model was created only in the cecum and the terminal ileum in the first group, whereas the diaphragmatic peritoneal damage model was created in the second group together with the same adhesion model. The rats were sacrificed on day 10 postoperatively and the adhesions formed were graded. In the first group, adhesions were formed at grade 1 in 5 rats (25%), grade 2 in 11 rats (55%) and grade 3 in 4 rats (20%), whereas adhesions were formed at grade 1 in 2 rats (10%), grade 2 in 8 rats (40%) and grade 3 in 10 rats (50%) in the second group (χ^2: 21.65, p<0.0001). Diaphragmatic peritoneal lymphatic function reduced the number of adhesions and severity of the adhesions which occurred among intra-abdominal organs after laparotomy. These findings suggest that special care should be undertaken to avoid damaging the diaphragmatic peritoneum during abdominal surgery so as to reduce the incidence of peritoneal adhesions.

Peritoneal adhesions (PA) are a very serious problem following abdominal surgery occurring after 93-100% of abdominal surgical interventions (1,2). Although experimental and clinical studies on the prevention of PAs are considerable both in the number of studies and the variety of materials and techniques, no definitive solutions have yet been found (3,4).

Despite an accumulation of considerable data in recent years on the pathogenesis of PA formation, much remains unclear (5). The process causing PAs starts with damage to the monolayer of mesothelial cells forming the peritoneum. Either during this damage or during the process of repair initiated as a response to this damage, many particles, including necrotic tissue, inflammatory cells, fibrin, fibrin degradation products and collagen residues are deposited in the peritoneal cavity. Removal of these particles from the cavity is important because these residues cause insufficient or delayed repair and they can trigger the formation of foreign bodies and/or inflammatory reactions which ultimately cause formation of PAs (3-5).

Most of the residues are too large to be absorbed by blood capillaries and these large molecules and complexes are removed from the peritoneal cavity by peritoneal lymphatics. Diaphragmatic lymphatics play the key role in peritoneal cleansing by the lymphatic system (6-8). However, the role of diaphragmatic lymphatics in the removal of

residues within the peritoneal cavity following peritoneal damage, and its effect on PA formation, is unknown.

In this study, we also produced damage in the diaphragmatic peritoneum in the rats in which we created the adhesion model in the cecum and in the terminal ileum, thus preventing the absorption of lymphatics at these sites. We aimed to determine the correlation of impaired diaphragmatic lymphatic absorption with the formation of PAs by comparing rats with and without an intact diaphragmatic peritoneums.

MATERIALS AND METHODS

This study was performed at Istanbul University, Cerrahpasa Medical School, Experimental Animals Reproduction and Research Laboratory with the approval of the animal ethics board. The study was performed on 52 out-bred Wistar albino female rats with average weight 185±18g and average age of 5.5 months and was performed in two stages.

Stage 1

The aim of this stage was to define the results which the diaphragmatic peritoneal scraping model would provide alone as one of the models to be used in this study. Following overnight fasting, the rats (n=12) were left for 45-60 seconds in a jar containing ether to initiate anesthesia, which was sustained with 75mg/kg subcutaneous Ketamine.

After antisepsis was provided by povidone iodine, a 3 cm long incision was made along the vertical median line starting at the xiphoid. First, the triangular ligament connecting the liver to the diaphragm was cut and the liver, stomach, and spleen were reflected to expose the diaphragm. In a manner similar to the Allen-Raybuck procedure (9), all of the diaphragmatic peritoneum was scraped with dry gauze until the formation of diffuse petechial bleeding foci occurred (mechanical peritoneal damage).

Next, a gauze impregnated in 70% alcohol (chemical peritoneal damage) was applied for 30 seconds. Diaphragms were ruptured in two rats in this group while performing scraping and these subjects were excluded from the study. The anterior abdominal wall and the skin were closed separately by 000 propylene, using continuous suture technique. Following monitoring for 24 hours postoperatively, the rats were placed on a regular feeding shedule.

The rats were sacrificed by prolonged ether inhalation on the tenth postoperative day and the abdominal cavity was opened by performing a wide "reverse U" incision which exposed the entire peritoneal cavity. The diaphragm and adhesions in the entire abdominal cavity were graded according to the model defined in *Table 1*. Subsequently, diaphragms of all rats were resected for histopathological examination.

Stage 2

Forty rats were divided into two equal groups. Twenty rats in Group-1 (Adhesion Group) were administered anesthesia and the incision as described above, followed by entrance into the peritoneal cavity. Following five scrapings by dry gauze on the anterior and posterior surfaces of the cecum and 3 cm of the terminal ileum, the damaged areas were covered for 10 seconds by gauze impregnated with 70% alcohol (mild adhesion model) (3). The closure of incisions and postoperative procedures were performed as defined in Stage 1.

Twenty rats in Group 2 (Adhesion and Diaphragmatic Group) were given identical anesthesia and incision procedures followed by entrance into the peritoneal cavity. Following five scrapings by dry gauze on the anterior and posterior surfaces of the cecum and 3 cm of the terminal ileum, the damaged areas were covered for 10 seconds by gauze impregnated with 70% alcohol (mild adhesion model) (3). The closure of incisions and postoperative procedures were performed as defined in Stage 1.

TABLE 1
Adhesion Grade Model

Adhesion Grade Definition

0	No adhesion
1	Spontaneously separating adhesions
2	Adhesions separating by traction
3	Adhesions separating by dissection

TABLE 2
Results of Stage 1 (n=12)

Tissues Adhesions	Grade 1	Grade 2	Grade 3
Diaphragm-Hepatic	-	-	10
Diaphragm-Gastric	3	1	4
Diaphragm-Splenic	2	4	4
Hepato-Omental	2	4	-
Hepato-Gastric	3	2	-
Diaphragm-Omental	1	-	-

The rats in both groups were sacrificed by prolonged ether inhalation on the tenth postoperative day and "reverse U" incision was applied to all. The PAs observed were graded according to the adhesion staging model used in Stage 1.

The operating table was tilted 45 degrees so that the fluid in the peritoneal vacity would accumulate in the pelvic cavity. After aspirating the pooled fluid with a syringe, the fluid was measured. Subsequently, diaphragms (and all tissues adhered thereto, if any) and cecums as well as the terminal ileums (and all tissues adhered thereto, if any) of all rats were resected for histopathological examination.

RESULTS

Results of Stage 1

PAs were created at various levels between the liver, stomach, spleen and omentum by using the diaphragmatic peritoneal scraping model. These are shown in *Table 2*.

Adhesions covering the liver and the spleen developed in all rats. Although splenic adhesions were at various levels of severity, all hepatic adhesions were of grade 3. All PAs developing at this stage were limited to the upper abdomen, and PAs were not observed in the lower abdominal organs of any rats.

TABLE 3
Results of Stage 2 (n=40)

	Grade 0	Grade 1	Grade 2	Grade 3
Adhesion Group (n= 20)	-	5 (25%)	11 (55%)	4 (20%)
Adhesion and Diaphragmatic Peritoneum Scraping Group (n=20)	-	2 (10%)	8 (40%)	10 (50%)

TABLE 4
Amount of Peritoneal Liquid in the Groups

	No Liquid	<1ml Liquid	>1ml Liquid
Group 1	18 (90%)	2 (10%)	-
Group 2	11 (44%)	7 (35%)	2 (10%)

Results of Stage 2

Analyses of this stage were performed with GraphPad Prisma V.3 program. The Chi-square test was used in evaluating qualifiable values. The results were evaluated at $p<0.05$ significance level and within a confidence range of 95%. PAs resulting from models applied at this stage are shown in *Table 3*. There were no subjects in either group which failed to develop PAs. When PA grade distribution of groups were compared, it was observed that PA development was at a statistically significantly lower level in the adhesion only group ($\chi^2 = 21.65$, $p<0.0001$).

Free fluid was detected in 2 subjects in Group 1 (10%) and in the peritoneal cavity of 9 (45%) subjects in Group 2 (Fisher's, $p<0.05$). Quantities of free fluid found in the peritoneal cavity are shown in *Table 4*.

Histopathological Evaluation

Tissues were fixed in 70% alcohol, dehydrated, and immersed in paraffin. 5 µm sections were cut from the paraffin molds and stained with hematoxylin-eosin for examination. Histopathological examination revealed an invasive inflammatory reaction which was rich in polymorphonuclear neutrophils. In many sections, the peritoneum was totally adhering and liver was frequently seen *(Fig. 1)*.

DISCUSSION

PAs occur as the result of damage to the mesothelial cell monolayer forming the peritoneum. The damaged site first produces an exudate rich in fibrin, which within a few days forms bands between other peritoneal surfaces contacting this region (1,3,5).
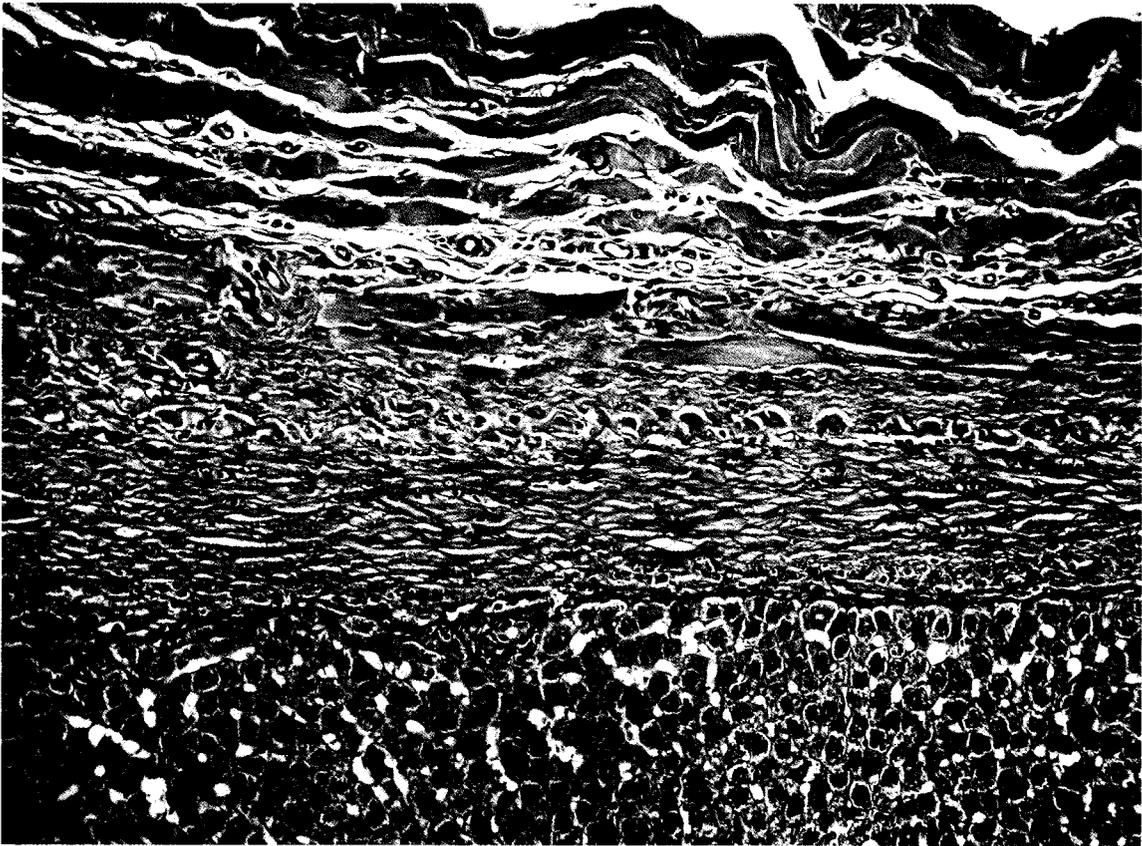
Fig. 1: Histopathology of scraped diaphragmatic peritoneum. Liver (1) and diaphragmatic striated muscle (3). Between these two components, a broad zone of fibrous tissue has developed (2). Note the progressive fibrosis of the diaphragmatic striated muscle and scattered mild lymphoid infiltration. (H&E, magnification of x100)

It is important that residues formed during the natural peritoneal healing process are eliminated from within the peritoneal cavity. If they continue to remain in the environment, they both impede the peritoneal healing process and trigger the growth of foreign body and/or inflammatory reactions, thus leading to more PA formation (3).

In this study, we examined the effect of absorption of residues within the peritoneal cavity. Residues within the peritoneal cavity may be removed in two ways: by blood capillaries and lymphatics. Blood capillaries have a low absorption capacity and can remove only substances with relatively smaller molecules (generally up to 20 micrometers) from the environment. Peritoneal lymphatics have a much higher absorption capacity and are the only method for elimination of all macromolecular residues, proteins, fats and carbohyrates (6,7,10).

Peritoneal lymphatics consist of two systems: the lymphatics of the diaphragmatic peritoneum and the lymphatics of the peritoneal lining the abdominal walls and visceral organs (10,11). Many studies have shown that the majority of peritoneal lymphatic absorption is through the diaphragmatic lymphatics (12-14).

Olin et al and Flessner et al applied similar methods on rats, and they showed that 66-75% of absorption from peritoneal cavity is by diaphragmatic lymphatics (15,16). Yuan et al found that the peritoneal

lymphatic drainage speed is 1.35ml/hr/kg and 88% of this was by diaphragmatic lymphatics (17). The functioning of diaphragmatic lymphatics following intraperitoneal injection of various dyes or particles have been demonstrated in detail by electron microscopic studies (18,19).

In line with these data, we designed our study on diaphragmatic lymphatics to reveal the relation between PAs and the elimination of residual materials in the peritoneal cavity. In the first stage, we perfomed only diaphragmatic lymphatic scraping on 10 rats, and we observed PAs resulting from this surgical procedure alone. We found that this surgical procedure caused PAs only among the upper abdominal organs of the diaphragm, liver, spleen, stomach, and omentum while the lower abdominal structures remained intact. Consequently, one of the lower abdominal organs would make an unbiased and objective comparison for the adhesion model in the second stage of the study. For these cases, we chose the cecum and terminal ileum, which have a wide surface, are easy to manipulate, and are frequently used in adhesion studies.

The adhesion model we used here is milder than the standard scraping model used in other PA studies (20) due to the fact that the scraping procedure was not continued until petechial bleedings emerged on the surface. Only five scrapings were performed with dry gauze. If we had applied the standard model, we would not have been able to discern more severe adhesions due to diaphragmatic damage, since this process causes severe PAs in all subjects.

Different classifications are used for grading of PAs (3-5). The grading defined by Evans classifies PAs as 0, 1, 2 or 3 according to the severity of adhesion on the serosal surfaces (4). In the clinic, the severity of this adhesion plays a considerable role in the development of complications due to PAs. Our main reason for preferring this classification is its adaptability to and practicality in clinical application. In the second stage, we applied the diaphragmatic lymphatic damage

model. This model is not new, having already been applied by Lill et al (21). In this study, we examined microscopically the diaphragmatic peritoneal areas in which we performed scraping, and observed that the integrity of the scraped diaphragmatic peritoneum was completely impaired and a severe inflammatory reaction developed (Fig. 1).

Comparing adhesion scores of the groups, we observed that PAs were at statistically significantly higher level in rats which were subject to diaphragmatic peritoneal scraping. In addition, a higher quantity of free fluid existed in the peritoneal cavity of this group compared to the group with intact diaphragmatic peritoneum (nine subjects versus two subjects).

The most probable way to explain these data is the failure of diaphragmatic lymphatic stomata, damaged due to diaphragmatic peritoneal scraping, to absorb residues in the peritoneal cavity. Consequently, residues accumulated within the peritoneal cavity increase PAs both by impairing the wound healing process and by causing foreign body reactions.

We also compared the total quantity of fluid within the peritoneal cavities of the subjects in the groups. Following this evaluation, we saw that free fluid existed in the peritoneal cavities of only two of the rats in Group 1, whereas free fluid existed in the peritoneal cavities of nine rats in Group 2 and the quantity was higher than 1 ml in two of the subjects.

Although the diaphragmatic lymphatic system is the most efficient way to eliminate residues in the peritoneal cavity, lymphatics on the peritoneal surface play a role in this process as well. In human biology, a general principle applies for several systems performing the same function in that damage to any one of these systems causes the others to increase their functions to compensate for the failing system. It is highly probable that this general rule also applies for elimination of residues from the peritoneal cavity. Consequently, the rate of absorption must

have been increased relatively in other peritoneal lymphatic systems and even in blood capillaries of rats in the group which was subject to diaphragmatic scraping.
If this theory is true, the significance of diaphragmatic peritoneal lymphatics in preventing formation of peritoneal adhesion will be much greater than the level shown in this study.

To emphasize the importance of the subject, studies must be made to evaluate quantitatively the relations between PAs, the lymphatic system and the peritoneal cavity. In addition, future studies need to be made to evaluate the functions of the peritoneal absorption systems individually to test the compensation mechanism theory.

It would be beneficial to act in due diligence to avoid any unnecessary damage to the diaphragmatic peritoneum, in order to cause fewer PAs during abdominal surgery. This important detail applies particularly for peri-diaphragmatic surgical procedures such as hiatal hernia repair and operations for proximal gastric or lower esophageal diseases.

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