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Are the collagen types and density in the wound healed after midline and transverse laparotomy different? An experimental study in mice

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Abstract

Background/Aim: Whether midline or transverse incision is superior in laparotomy is still controversial. The aim of this study was to investigate the total collagen amounts in wound healing after midline and transverse incisions and reveal whether there was a difference between these two laparotomy techniques.
Methods: Twenty-four BALB/c mice were randomly divided into three groups (control, midline, and transverse groups). Mice were sacrificed 28 days after the surgical procedures. The samples were examined histochemically in terms of total collagen density, Type I and Type III collagen rate and maturation index.
Results: Total collagen density, Type I collagen rate and maturation index were slightly insignificantly higher and Type III collagen rate was insignificantly lower in the transverse incision group compared to the midline group (P=0.486, P=0.541, P=0.336 and P=0.541, respectively).
Conclusion: The total collagen density, Tip I and Tip III collagen amounts, and maturation index of wounds healed after transverse and midline laparotomy are similar according to this experimental model.

Keywords: Laparotomy, Collagen, Wound healing, Maturation index

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Ethics Committee Approval
This study was conducted at Necmettin Erbakan University KONUDAM Experimental Medicine Application and Research Centre (Number:2019-06). Animal Experiments Ethics Committee approval was obtained before the study.

Conflict of Interest
No conflict of interest was declared by the authors.

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Introduction

Although laparoscopic procedures are increasingly used in abdominal surgeries, laparotomy procedures are still practiced frequently. Incisional hernias remain a problem for surgeons, with an incidence of 9% to 19% after laparotomy [1]. Although discussions about the ideal laparotomy closure technique are as important as the visceral surgery itself, a conclusion has not been made regarding the optimal suture material or the suture technique [1, 2]. It has been emphasized in many studies that transverse incisions are more advantageous in many aspects (e.g., pain, respiratory complications, incisional hernia risk, and early discharge and return to work) compared to midline incisions, however, midline incision is still the most preferred technique [3-5]. This is because a midline incision can be performed quickly and easily, and it can provide a wider surgical area by extending up and down depending on the need.

In the literature, there are many randomized controlled trials and meta-analyses comparing midline and transverse laparotomy procedures in clinical practice [6-9]. Although laparotomy closure techniques and suture materials are known to affect the tensile strength of the wound and the incidence of incisional hernia after laparotomy, whether the collagen content and collagen subtypes have any role in this effect have not been evaluated.

Type I and Type III collagen formations in the remodeling phase have a critical role on wound healing [10]. During scar formation, the first collagen type to occur is Type III collagen, with low tensile strength. The amount of Type III collagen, which is initially seen at a rate of 80%, decreases gradually during scar formation and is eventually replaced by Type I collagen with high tensile strength. The mechanical properties of scar tissue are determined by prolonged inflammation, which leads to dense and persistent weak Type III collagen [11]. The effect of suture techniques and suture materials on collagen synthesis and wound healing have been investigated in several experimental studies and both suture materials and suture techniques have been proven effective on Types I and III collagen production [12, 13].

The aim of this study was to investigate the total collagen amounts and the rate of Type I and Type III collagen synthesis in wound healing after midline and transverse incisions and reveal whether there was a difference between these two laparotomy techniques.

Materials and methods

This study was conducted at Necmettin Erbakan University KONUDAM Experimental Medicine Application and Research Centre. Animal Experiments Ethics Committee approval was obtained before the study. Institutional and national guidelines on the care and use of laboratory animals were followed and animal rights were protected in line with the principles of "Guide for the Care and Use of Laboratory Animals" [14]. All procedures were performed at Necmettin Erbakan University KONUDAM Experimental Medicine Application and Research Centre. A total of 24 BALB/c mice weighing 25 to 30 g were included in the study. General anesthesia was induced intraperitoneally with 2% xylocaine and 10% ketamine before the procedure. All mice were housed in groups of four in each cage under standard laboratory conditions. They were maintained at a constant room temperature of 20–21°C under a 12-hour light/dark cycle. The mice were given food and water ad libitum. The general condition and wounds of the mice were checked daily. No antibiotics were administered during the procedure. They were sacrificed by cervical dislocation after 28 days.

Surgical technique

The mice were randomly divided into three groups, each containing eight. The abdominal areas of the mice were shaved. The surgical area was sterilized with povidone-iodine. The suture length to wound length (SLWL) ratio of all suture materials was kept at 4:1 for the tensile strength of wounds to be equal in all mice [15]. All operations were performed by the same surgeon. The weight, general condition, and wounds of all mice were checked daily for 28 days.

No procedure was performed in the first group (control group, n=8) after anesthesia induction. The second group (midline group, n=8) underwent laparotomy along the linea alba with a 3 cm midline incision from the xiphoid (Figure 1). Peritoneum and linea alba were closed with 4*0 polypropylene nonabsorbable running suture (Prolene®, Ethicon GmbH& Co. KG, Norderstedt, Germany) and the skin was closed with 4/0 silk nonabsorbable interrupted sutures (silk® Doğsan Ltd. Co., Rize, Turkey). In the third group (transverse group, n=8), the laparotomy was performed with a 3-cm transverse incision at the umbilicus level. The peritoneum and musculoaponeurotic layer were closed with 4*0 polypropylene nonabsorbable running suture (Prolene®, Ethicon GmbH& Co. KG, Norderstedt, Germany) and the skin was closed with 4/0 silk nonabsorbable interrupted sutures (silk® Doğsan Ltd. Co., Rize, Turkey) (Figure 1).

Histochemical examination

All mice were sacrificed by cervical dislocation after 28 days. Then, the skin was removed, and an abdominal wall tissue of about 8 cm^2 (4x2cm) was totally excised with laparotomy lines remaining inside the incision. In the control group, an abdominal wall tissue of 8 cm^2, including the midline and transverse lines, was excised from the periphery of the umbilicus. The samples were fixed in 10% formaldehyde solution. A single well experienced pathologist who was blinded to the study design evaluated each specimen. Five-six mm thick sections obtained from two different layers of the wound were stained with hematoxylin/eosin, Masson Trichrome, and reticulin histochemical stains and then, evaluated under a light microscope (Figure 2).

Collagen fibers stained blue with the Masson Trichrome staining were evaluated as the sum of Type I and Type III collagen fibers (total collagen) (Figure 2). The density of these fibers was scored from one to five according to the following data [16].

Collagen (+): Collagen in the form of a single fiber.

Collagen (++): Collagen in the form of multiple fibers.

Collagen (+++): Collagen is more dense but loose.

Collagen (++++): Collagen covers the microscopic area but there are gaps between them.

Collagen (+++++): Collagen covers the microscopic area and has a very dense structure.

Figure 1: Images taken immediately after laparotomy and images taken on the 28th postoperative day A: 3-cm incision line identified at the level of the umbilicus before the transverse incision, B: Continuous closure of the fascia after transverse laparotomy, C: Healing in the midline incision line in the tissue sample taken 28 days after midline laparotomy D: 3-cm incision line identified at the midline before the midline incision E: Skin closed with single sutures after midline laparotomy, F: Healing in the transverse incision line in the tissue sample taken 28 days after transverse laparotomy.

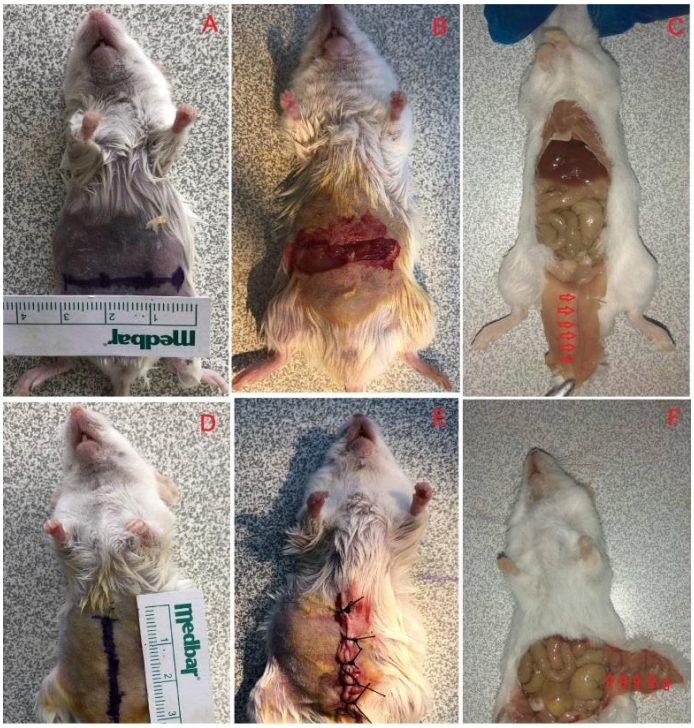
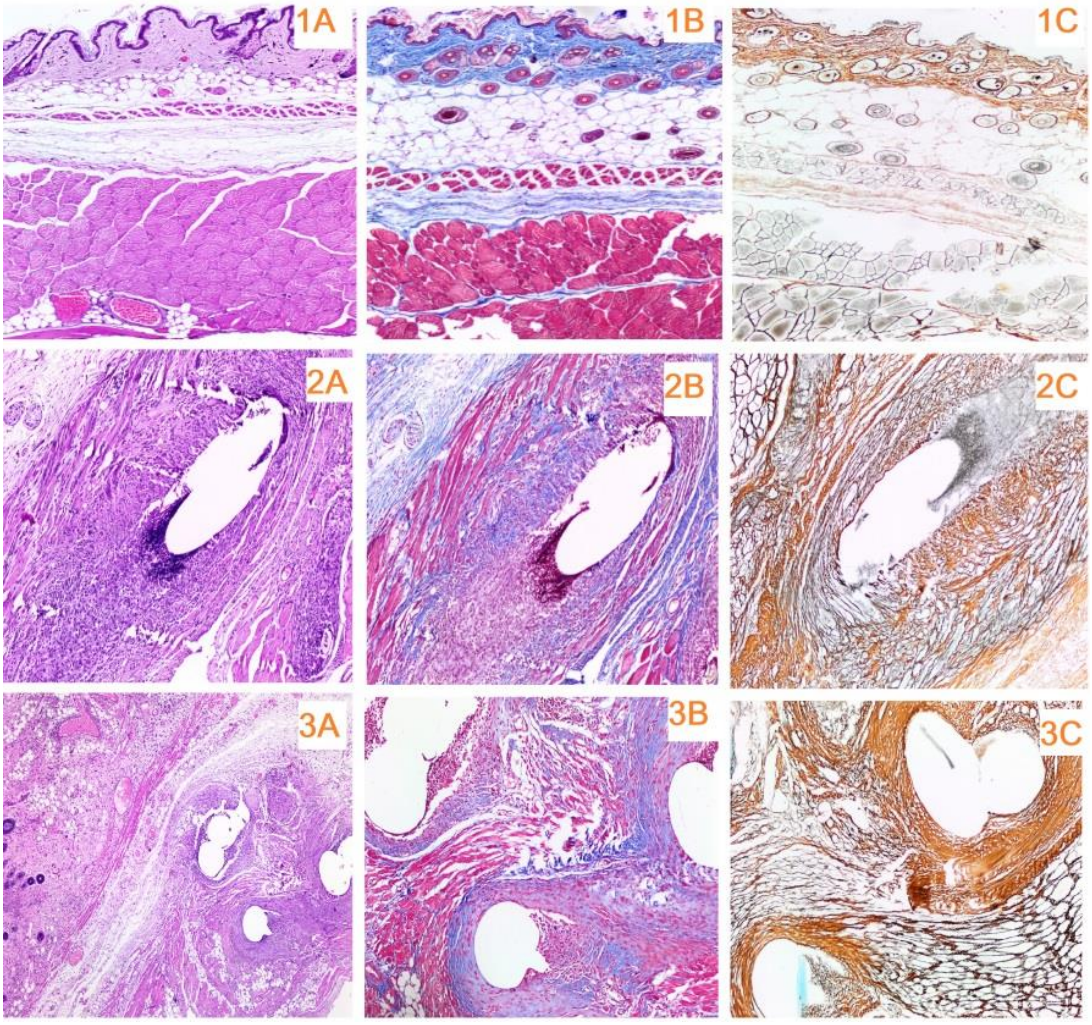


Figure 2: Histochemical examination images of the subjects 1A: Tortuous collagen bundles stained in pink color after hematoxylin-eosin staining in the dermis and fascia in the control group (x40), 1B: Tortuous total collagen density stained in blue color after MASSON-TRICHROME staining in the dermis and fascia in the control group (x40), 1C: Type I collagen fibers stained in color ranging from yellow to dark yellow after reticulin staining at a greater amount in the dermis and fascia in the control group (x40) 2A: Granulation tissue area, inflammatory cells, foreign body (suture material), giant cells, and collagen after hematoxylin-eosin staining in the transverse group (x100), 2B: Loose collagen fibers and granulation tissue area stained in blue color after MASSON-TRICHROME staining in the transverse group (x100), 2C: Type I (dark yellow) and type III collagen fibers (fine black fibers) together after reticulin staining in the transverse group (x100). 3A: Less collagen density and granulation tissue area in the dermis and fascia after hematoxylin-eosin staining in the midline group (x40), 3B: Tighter collagens (blue color) with spaces in between after MASSON-TRICHROME staining in the midline group (x100), 3C: Granulation tissue area with denser and thicker type I collagen fibers (yellow colored) and rare fine type III collagen fibers (black colored) after reticulin staining in the midline group (x100)



The fibers observed in the form of thin black lines with the reticulin histochemical staining indicated Type III collagen fibers. Other thicker lines, stained from yellow to dark yellow showed Type I collagen fibers (Figure 2). Measurements were semi-quantitative, and the rate of Type I and III collagens were recorded in percentage (%). Collagen I/Collagen III ratio was used to calculate maturation index [17].

Statistical analysis

Data were expressed as mean, standard deviation, minimum and maximum. Kolmogorov–Smirnov test was used to determine whether data were normally distributed. Since they were not, Tamhane's T2 post hoc test with ANOVA was used for group comparisons. Statistical analysis was performed using SPSS version 21.0 software (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, United States). A *P*-value <0.05 was considered statistically significant.

Results

Total collagen density

There was a slight increase in total collagen density in laparotomy groups (midline and transverse) compared to the control group, and the mean total collagen density in the transverse group was insignificantly higher than the other groups (P=0.248 and P=0.486 for the control and midline groups, respectively) (Table 1). Four positive total collagen density values were observed in three samples in the transverse group whereas a maximum of three positive total collagen density values were observed in other groups.

Table 1: Distribution and comparison of collagen density and collagen subtypes by groups

Collagen Data	Control Group Mean (SD) (Min-Max)	Midline Group Mean (SD) (Min-Max)	Transverse Group Mean (SD) (Min-Max)	*P*-value
Total Collagen density	2.12 (0.834) (1-3)	2.37 (0.744) (1-3)	3.00 (1069) (1-4)	0.901[a] 0.248[b] 0.486[c]
Type I Collagen rate (%)	98.12 (2.587) (95-100)	65.62 (20.604) (30-85)	76.87 (14.37) (60-90)	0.008[a] 0.012[b] 0.541[c]
Type III Collagen rate (%)	1.87 (2.587) (0-5)	34.37 (20.604) (15-70)	23.12 (14.376) (10-40)	0.008[a] 0.012[b] 0.541[c]
Type I/Type III collagen rate	69.62 (41.921) (19-100)	2.82 (1.875) (0.43-5.67)	5.145 (3.505) (1.5-9)	0.001[a] 0.001[b] 0.336[c]

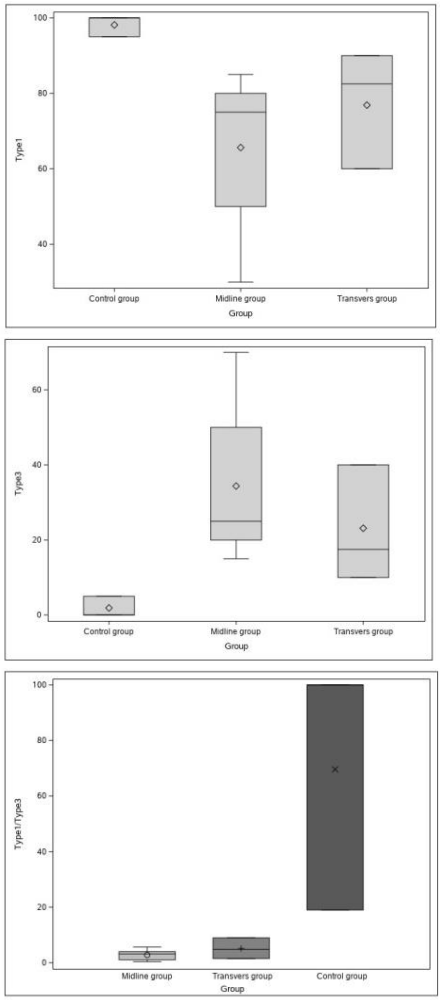
[a]: control group vs. midline group, [b]: control group vs. transverse group, [c]: midline group vs transverse group

Type I and Type III

The rate of Type I collagen, which was an indicator of mature collagen, was higher in the control group than in both the midline and transverse groups (P=0.08 and P=0.01, respectively) (Table 1). In the control group, the Type I collagen rate was 100% in half of the samples and 95% in the other half. Midline group had the lowest Type I collagen rate. Type I collagen was identified at a rate of 30% in one sample in the midline group, whereas at least one sample had 60% Type I collagen in the transverse group. Type I collagen rate was slightly higher in the transverse group than in the midline group (Table 1, Figure 3).

The rate of Type III collagen, which was an indicator of immature collagen, was lower in the control group than the other groups (P=0.008 and P=0.012 in the midline and transverse groups, respectively) (Table 1, Figure 3). Type III collagen rate was insignificantly lower in the transverse group than in the midline group (P=0.541) (Table 1, Figure 3).

Figure 3: Levels of type I and type III collagen concentrations and maturation index in groups



Maturation index

The maturation index was significantly higher in the control group compared to the laparotomy groups (P=0.01). The maturation index was higher in the transverse group than the midline group, but the difference was not statistically significant (P=0.336) (Table 1, Figure 3).

Discussion

In this experimental animal study, we compared collagen changes during wound healing after transverse and midline laparotomy incisions. Collagen is the most important component of mature connective tissue scar. During the tissue repair process, newly formed connective tissue containing capillaries and fibroblasts (granulation tissue) fills the gaps caused by dead cells and liquefaction of debris. The fibroblasts in this tissue produce collagen. Collagen is a family of structural proteins, the consistency, resilience, and durability of which vary depending on environmental influences and functional requirements. There are different types of collagen. Most of the collagen in the skin and tendons consists of Type I and Type III fibers. Therefore, other types of collagen can be excluded during the calculation of the total collagen amount in these areas.

Most of the studies conducted on laparotomy techniques so far have investigated suture techniques, suture materials, and wound tension [8, 16, 18, 19]. In an animal experiment, the bursting pressure of the two incisions after healing was analyzed and the pressure of the transverse incision was proven more durable [20]. However, the relationship with collagen is not

mentioned in this study. Although laparotomy closure techniques are known to influence the transverse tensile strength of healing after laparotomy surgeries and the incidence of incisional hernia formation, it has not been evaluated whether this effect is mediated by a direct influence of transverse and midline laparotomies on the collagen content and the collagen subtypes of the regenerating tissue. Therefore, this is the first study comparing the collagen amounts in wounds healed after midline and transverse laparotomy techniques.

The advantages of transverse and midline laparotomies are still controversial [8, 21]. Many studies have reported that the incidences of pulmonary and wound complications and incisional hernia are lower in the transverse laparotomy technique compared to the midline technique [4, 10, 22]. In these studies, the main reason for superiority has been attributed to the fact that the incision is parallel to the anatomical structures (muscle, vascular, nerve, etc.) in transverse incisions and it therefore causes less anatomical damage, ensuring that local wound healing is not adversely affected, and that minimal wound tension occurs since the incision is perpendicular to the major abdominal wall [23]. Short-term failures are often attributed to technical reasons, however, the most important factor determining wound durability in the long term is the density and type of collagen synthesis in the incisional region [19]. In the present study, it was aimed to investigate the differences between these two techniques in terms of collagen synthesis in wound durability rather than the technical and anatomical advantages of the transverse incision compared to midline incisions.

Parkinson et al. have reported that the density of the collagen determines the tensile strength of the structure [24]. Nevertheless, the rise in mean collagen fibril diameters in regenerating tissue occurs when the thin fibers of initially formed Type III collagen are replaced by the mature fibrils of Type I collagen, which, in conjunction with other collagen types, leads to definite tissue architecture and mechanical stability [25, 26]. In the present experimental study, total collagen density, which affects wound durability, Type I collagen synthesis, and maturation index were relatively high in the transverse incision technique whereas Type III collagen synthesis was relatively lower.

In a study by Höer et al., different suture techniques and materials used in laparotomy closures and SLWL ratio have been reported to affect both collagen synthesis and wound healing outcomes [19]. In the same study, a higher amount of collagen synthesis has been observed in wounds closed with an SLWL ratio of <4:1 and running sutures [19]. Therefore, all laparotomy wounds were closed with an SLWL of 4:1 using monofilament non-absorbable continuous sutures to ensure that our study was under optimal conditions.

Hydroxyproline analysis is considered the gold standard in measuring the amount of collagen in biological tissues [27]. However, hydroxyproline analysis has some limitations in terms of sensitivity, specificity, and accuracy, as well as difficulties of clinical approach [28, 29]. In an article, it has been reported that determining the amount and types of collagen by histopathological analysis is as effective as the hydroxyproline level [26]. Therefore, histochemical methods were used to determine collagen density and collagen types in the present study.

We believe that the present study will shed light on future clinical studies as a parameter to show the differences between these two incisions in terms of durability. We are aware that the disputable translation of results from animal experiments to human conditions can raise serious questions due to the different mechanical influences on the incision and genetic polymorphism influencing wound repair.

Conclusion

Although relative differences were observed in total collagen density, there were no significant differences between midline and transverse laparotomies. Type I and Type III collagen rates, total collagen density and maturation index were similar between both techniques.

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