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Prevention of Adhesion Following Hysterotomy Using Omentum in Rabbits

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Abstract

Adhesion formation is a major source of the postoperative threaten the life of the animals. The purpose of the investigation is the effect of autologous graft by using the omentum to reduce and prevention of adhesion throughout hysterotomy in rabbits. Study was applied on 20 local breed rabbits that divided randomly into 2 groups of 10 animals each. Aseptic surgical laparotomy and hysterotomy done for all rabbits, and the omentum were used in treatment group. Postmortem and microscopic examination showed the presence of mild to severe adhesion in control group. At the 14th postoperative days, the degrees of adhesions were graded as: grade (1) in two rabbits, and (2) in the remaining two rabbits. Also at 28th post operative days, different adhesions formed and were graded as: grade (1) in two rabbits, grade 1 and 3 in one rabbit each. Conversely, in treatment group, at 14th postoperative day, 4 rabbits shown adhesion grade (2) in each 2 rabbits. While at 28th postoperative day, grade 1 adhesion was found in 3 rabbits that exhibiting moderate band of adhesion. The results of 2 run time referred that adhesion extension increased in control group with the time. The average *p*- value for adhesion of extension in all rabbits of treatment group was 1.50±0.577 and 1.25±0.500 at day 14th and 28th respectively. Histological examination revealed significantly high score of fibrosis with infiltration of inflammatory cells especially in control group, with slightly infiltration of inflammatory cells within muscular and serosal layers in treatment group. In conclusion, we confirmed that the using of autologous graft especially the omentum leads to somewhat reduction and prevention of adhesion during postoperative hysterotomy in rabbit model.

Introduction

Generally, adhesion formation will be prevented using physical barriers in order to limit tissue apposition during the critical period of peritoneal healing, thereby reducing the development of fibrin matrix between tissue surfaces [10]. Following surgical operation, trauma, severe infection and deep injury, peritoneal adhesions are produced between structures in the abdominal cavity and pelvis in the form of bands of tissue (Yoon and Kohane, 2007). Omentum is easily accessible during pelvic surgery and considered as an excellent barrier to reduce adhesions so far. Omental tissues have been successfully used in thoracic, oncologic, vascular, and reconstructive surgery. On the other hand, high concentrations of thromboplastin for hemostasis in omentum and its atrophic effect on surrounding tissues, allowing a free graft to survive in the peritoneal cavity without impairment. Extensive vascularity, rapid angiogenesis capability and rapid capillary growth are the main important features of omentum. Another mode of preventing scar and adhesion formation is affixing of omentum into denuded surfaces of canine bowel and bladder. Drugs and barriers have been suggested to prevent adhesions [5, 8].

Materials and methods

Animals

Animals used throughout this research were 20 clinically normal adult, female local breed rabbits (does), weighting from 1.5 to 2 kg. The rabbits were acquired from a commercial supplier and kept for acclimatization under good laboratory conditions at an optimal room temperature of 22-25 °C at the teaching hospital/ College of Veterinary Medicine, University of Sulaimani. The study was conducted from April, 2017 to July, 2017. Rabbits were fed a high fiber diet with free access to water and food before and after surgical procedures.

Animal Grouping

Before operation, 20 rabbits were randomly assigned into 2 groups of 10 animals each as below:

Group one: Control group (CG)

Group two: Treatment group (TG)

Surgical procedure

Surgical operations were performed for all 20 rabbits with the same laparatomy procedure under a maximal aseptic precaution and under the effect of general anesthesia [14] using intramuscular injection of 50 mg/kg ketamine hydrochloride and 1.6 mg/kg xylazine hydrochloride [3, 6]. Ventral aspect of abdomen was clipped and shaved extending from xyphoid to pubic regions. Following evacuation of the bladder, the animal's abdomen was scrubbed with povidone iodine and draped in preparation for sterile surgery, while the animal was secured to the table in supine position. About 5 cm vertical midline infra umbilical incision was made until the peritoneal cavity was entered. Then, the uterus was identified and exteriorized. Surgical scalpel blade was used to make a 2 cm incision in one of uterine horns. Consequently, each hysterotomy was repaired with 2/0 catgut, in control group there was no omental covering over the hysterotomy site, and it was returned to the abdomen immediately after repairing of the hysterotomy. The uterine horn in each rabbit of treatment group was covered with an autologous free omental graft. The omentum was gently pulled down from the upper abdomen and exteriorized. Regarding, the free omental graft, blunt scissor was used to create an approximately 2 cm x 0.5 cm unattached piece of omentum; then this graft was placed over the hysterotomy and secured with interrupted stitches using 4/0 catgut. Enrofloxacine (5 mg/kg, subcutaneously) was administered daily for 3 consecutive days as a post operative care. In addition to the local wound care and application of the wound spray, oxytetracycline 10%, once a day for 5 successive days were applied. Finally, the skin stitches were removed at the 7th post operative day.

Gross examination and histopathological study

Euthanasia at the 14th and 28th post operative days, for 5 rabbits from each group with halothane over dosage was performed to investigate the post operative intraperitoneal changes as well as for tissue specimens' collections for histopathological study. Upon immediate opening of the carcass, the entire abdominal and pelvic cavities were fully explored and the macroscopic staging of adhesions was conducted. Simultaneously, the developed adhesions were graded blindly according to Kruskall-Wallis Scale (Table 1). Specimens were collected from the adhesion sites, preserved in 10% buffered formalin solution for further histopathological processing. The histological sections (5 - 6 mm) were stained with hemotoxylin and eosin and examined [11].

Table 1: Kruskall-Wallis method's scale used on the rabbits in control and treatment groups for the postmortem assessment of the postoperative adhesion.

Grade	Adhesion Description
0	No adhesion
1	Thin or narrow, easily separable adhesions
2	Thick adhesions limited to one area
3	Thick and widespread adhesion
4	Thick and widespread adhesion +adhesion of viscera to the anterior and posterior abdominal wall

Results

In this study, adhesions were assessed by both gross and histopathological evaluation on re-opening the abdominal cavity after scarifying the rabbits on post surgical day schedules 14th, 28th respectively. All rabbits unaware of the animal groups, being re-opened for performance of gross adhesion evaluation. Multiple tissue samples were taken from the rabbits and processed for histopathological analysis by an independent pathologist.

Postmortem examination

The suggested surgical procedures and the administration protocol were regularly performed by the animals. All laparotomy sites were sound, clean, and none of the tested animals faced incisional hernia or wound infection. Variable grades of adhesions were found in uterine horns with adjacent organs of abdominal cavity in all rabbits of CG and TG, at 14th and 28th post operatives (Table. 2). In control group, at the 14th postoperative days, the degrees of adhesions were graded as: grade (1) in 2 rabbits, and grade (2) in the remaining 2 rabbits. The adhesion showed fine filmy transparent bands of white thin fibrous adhesions between the colon and the uterus (Figure 1). Also, at 28th post operative days, different adhesion formed and were graded as: grade (1) in 2 rabbits, grade 1 and 3 in 1 rabbit each. Adhesion represented in CG with gross appearance of thick fibrous tissue formed between the uterus, colon and urinary bladder (Figures 2 and 3). Whilst, in treatment groups, lower grads of adhesion in rabbits were recorded. At 14th postoperative day, 4 rabbits showed adhesion grade (2) in each 2 rabbits. There was a fibrin tissue between the visceral of abdominal cavity and the uterus (Figure 4). On the other hand, at 28th postoperative day, grade (1) adhesion was found in 3 rabbits that exhibiting moderate band of adhesion (Figure 5). The statistical analysis (Table 3) showed significant lowering of adhesions in treated groups than normal control group. At the 14th postoperative day, the average p-value for extent of adhesion was 1.50±0.577, while at 28th postoperative day, was 1.75±0.957. The explanation of the results for the 2 run time referred to that the adhesion extension increased in control group with the time. Conversely, the average p- value for adhesion of extension in all rabbits of treatment group was 1.50±0.577 and 1.25±0.500 at the 14th and 28th postoperative days, respectively. In conclusion this means for the 2 run times, the formation and extension of adhesion in treatment group decreased gradually with the time.

Table 2: Scores for the grade of extension of adhesion for rabbits in both control and treatment group at the 14th and 28th postoperative days.

Groups	**No.**	**Scores at the 14th P.O. day**					**Scores at the 28th P.O. days**				
		0	1	2	3	4	**0**	**1**	**2**	**3**	**4**
Control group	10	0	2	2	0	0	**0**	**2**	**1**	**1**	**0**
Treatment group	10	0	2	2	0	0	**0**	**3**	**1**	**0**	**0**

Table 3: Statistical analysis for macroscopic evaluation of adhesion using Mann-Whitney U.Test.

Groups	Number	14th P.O.day N=4 (Mean ±SE)	28th P.O.day N=4 (Mean ±SE)
Control group	10	1.50±0.577	1.75±0.957
Treatment group	10	1.50±0.577	1.25±0.500

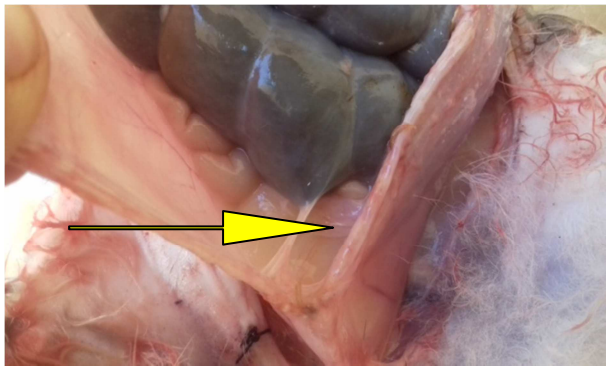

Figure 1: Gross photograph of a rabbit in CG, at the 14th P.O. day. Fine filmy transparent bands of white thin fibrous adhesions between the colon and uterus (score 1), as indicated by the yellow arrow.

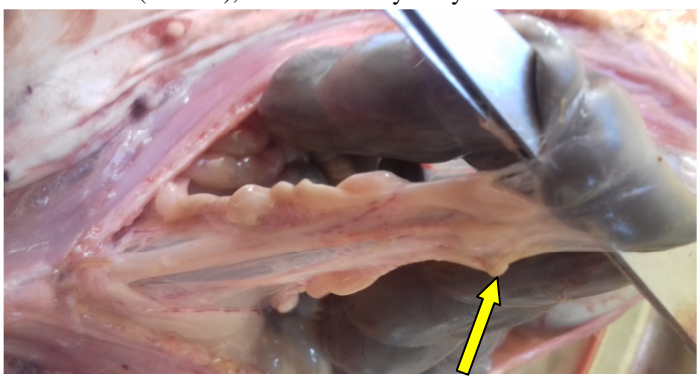

Figure 2: Gross photograph of a rabbit of CG on the 28th P.O. day with gross appearance of large adhesions between colon and uterus (score 2), as indicated by yellow arrow.

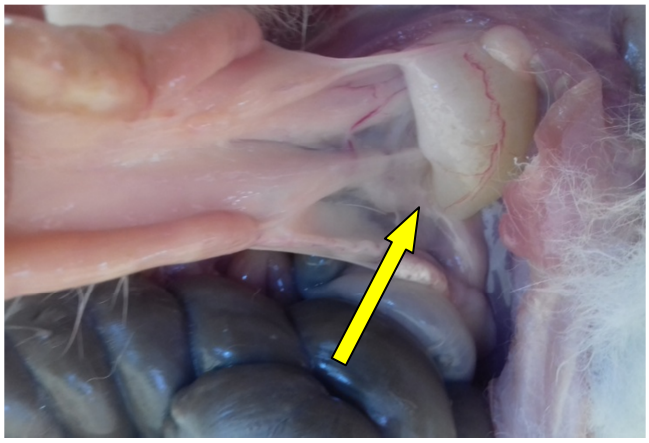

Figure 3: Gross photograph of a rabbit of CG on the 28th P.O. day with gross appearance of large adhesions between urinary bladder and uterus (score 2) , as indicated by yellow arrow.

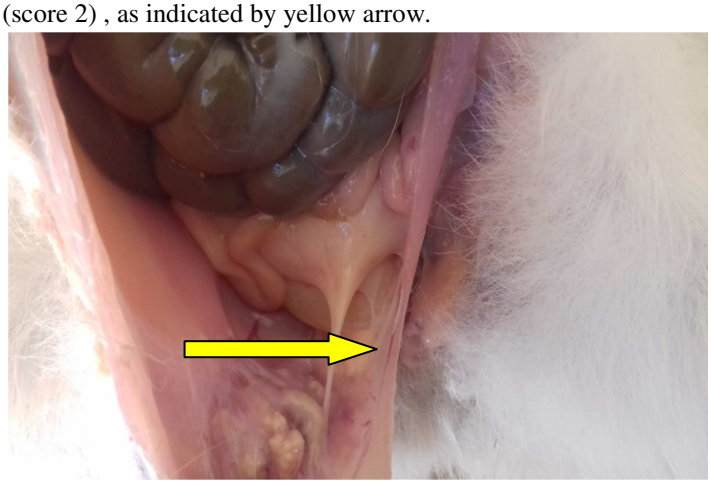

Figure 4: Gross photograph for a rabbit in TG on the 14th P.O. day shows score (1) adhesion (as indicated by yellow arrow) between the visceral abdominal wall and uterus.

Figure 5: Gross photograph of a rabbit in TG on the 28th P.O. day shows score (0) adhesion, because P.M. examination did not reveals gross signs of adhesion formation**.**

Histopathological Findings

The histopathological findings of adhesions differed significantly among both the CG and TG with respect to fibrosis, inflammation and vascular proliferation. The CG showed the highest scores for fibrosis, heavy infiltration of inflammatory cells and loss of integrity of the serosal surface (Figures 6 and 7). While the TG showed slight infiltration of inflammatory cells within the serosal layers, slightly normally appearing muscular and serosal layers of the uterus (Figures 8, 9, and 10).

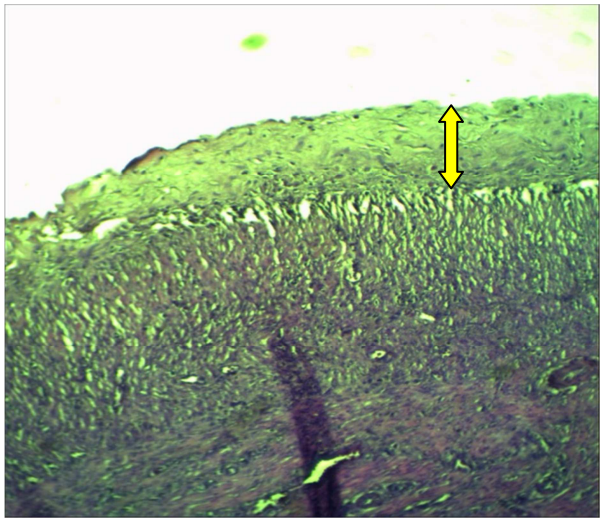

Figure 6: Histomicrographs of rabbit uterus in the CG on the 14th P.O. day. The presence of moderate to severe proliferated adhesive tissues extended outward (as indicated by yellow arrow), H&E (X100).

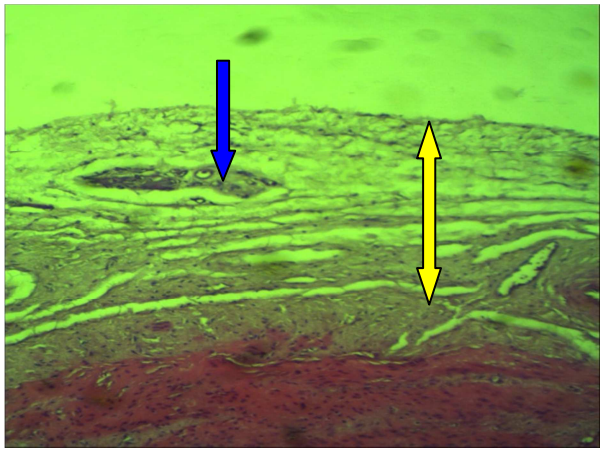

Figure 7: Histomicrographs of rabbit uterus in the CG on the 28th P.O. day. The presence of massive proliferated adhesive tissues extended outward (as indicated by yellow arrow), new capillary formation (indicated by blue arrow), H&E (X100).

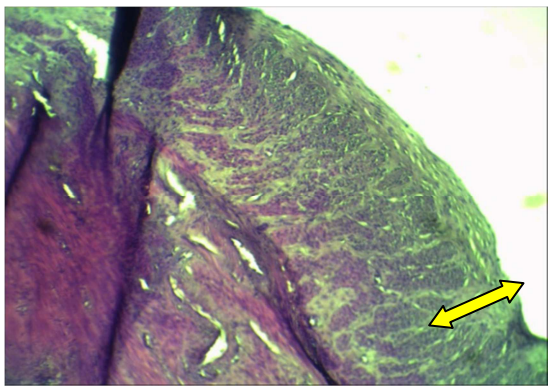

Figure 8: Histomicrographs of rabbit's uterus in TG on the 14th postoperative day. Marked lesser proliferation of adhesive tissues (as indicated by yellow arrow), H&E (X100).

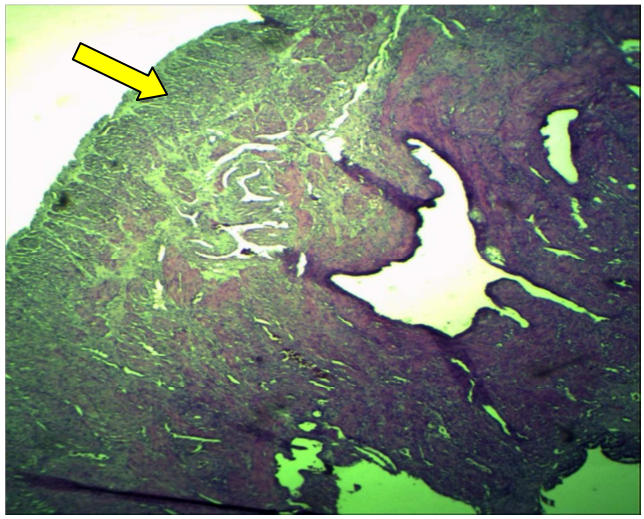

Figure 9: Histomicrographs of rabbit's uterus in TG on the 28th P.O. An extensive decrease in the proliferation of adhered uterus layers (as indicated by yellow arrow), H&E (X100).

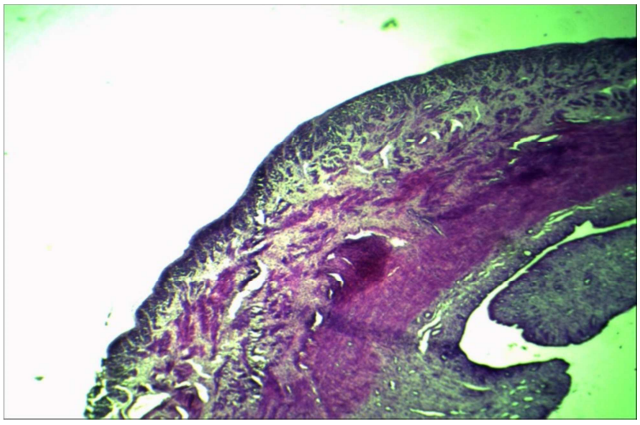

Figure 10: Histomicrographs of rabbit's uterus in TG on the 28th P.O. No adhesion formation; normal mesothelial layer, uterine muscle and endometrial layers, H&E (X100).

Discussion

Abdominal adhesions found during the postoperative period that generating a serious problem in general surgery, obstetrics and gynecology due to high morbidity and mortality levels. Various treatment modalities have been utilized with protective measures to prevent postoperative adhesion. In this respect, [4, 5] classified the agents that used for this purpose mainly into 2 groups as bearing local and systemic effects. Locally applied medicines prevent adhesions by blocking the contact between peritoneal surfaces or by their fibrinolytic effects. Systemic agents have more complex effects in the prevention of adhesions compared to local one. In this study, all CG rabbits had adhesions, indicating the predictability of the adhesion model.

Choosing rabbits, for the induction of adhesion, coincides in the present study with the essentials and importance of an animal model. Formation of adhesions begins during the inflammatory stage of healing, 24 to 48 hours after the injury, and the adhesions usually are well formed by the 5th to the 7th days after the injury [7]. In this study, the results of adhesion formation were not evaluated only by the presence or absence of fibrous adhesions, but also by the degree of their formation. The majority of methods for grading adhesions are based on the character of the adhesions; strong or weak and fibrinous or fibrous. Other methods including evaluation of the percentage of the traumatized area covered by adhesion; measurement of surface area involved; enumeration of adhesions present; or simply the presence or absence of adhesions [9].

Formation of postoperative adhesion in the peritoneal and pelvic cavities is a fibrotic tissue disorder developed as a result of excessive deposition of fibrin during normal wound healing. It is currently believed that a breakdown in the balance between fibrinogenesis and fibrinolysis during wound healing leads to this surgical complications [2]. Various medications were used locally or systematically for that purpose but still there is no safe and effective prophylaxis for intra-abdominal adhesions [13].

Omentum is an intra-abdominal fold of visceral peritoneum that is readily available, easy to apply, non-inflammatory, and inexpensive [1]. Autologous grafting reduced and increased healing as well as prevent adhesion throughout surgical operation in rabbits since it contains more epithelial cells than spontaneous healing [12]. In conclusion, with this experimental study we aimed to get some information about using autologous graft for reduction and prevention of adhesion during postoperative uterine surgery in rabbits.

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