

# Effect of vitamin E on decreasing post-operative adhesion in rat uterine horn

Shahnaz Aram<sup>1</sup>, Fatemeh Mostajeran<sup>2</sup>, Azar Danesh Shahraki<sup>1</sup>, Shahriar Adibi<sup>3</sup>, Shekoofeh Shamloo<sup>4</sup>

<sup>1</sup> Associate Professor, Department of Obstetrics and Gynecology, School of Medicine, Isfahan University of Medical sciences, Isfahan, Iran. <sup>2</sup> Professor, Department of Obstetrics and Gynecology, School of Medicine, Isfahan University of Medical sciences, Isfahan, Iran. <sup>3</sup> DVM, Isfahan Research Clinical Hospital, School of Medicine, Isfahan University of Medical sciences, Isfahan, Iran. <sup>4</sup> School of Medicine, Isfahan University of Medical sciences, Isfahan, Iran.

**BACKGROUND:** Adhesions, as abnormal scar tissue, are common following gynecological operations and can cause chronic pelvic pain, infertility, poor outcomes of future surgeries, and intestinal obstruction. Oxygen free radicals are known as one of the main causes of adhesion formation. This study is to evaluate the effect of vitamin E on decreasing post operative adhesion in uterine horn of rats. **METHODS:** This experimental study was performed on 45 non-pregnant female rats randomly placed into 3 equal groups. In all groups after general anesthesia and sterile exploration, anti-mesenteric surface of the left uterine horn of the rats was traumatized using a single-polar couther. The right side was traumatized by a scalpel. Intra-peritoneal and intra-muscular vitamin E were administered in the 1st and 2nd groups, respectively, while in the control group only normal saline solution was poured onto the horns. Following 7 weeks recovery, second laparotomy was performed to grade the adhesions, if any. The dose of vitamin E for each rat was 10 IU (50u/kg). **RESULTS:** Vitamin E had no effect on preventing adhesion formation in the right uterine horn traumatized by scalpel. However, vitamin E administration in the left side (both intra-peritoneal and intra-muscular) traumatized by cautery, had a significantly preventive effect against adhesion formation. In comparison with intra-peritoneal administration, intra-muscular vitamin E had a greater protective effect. **CONCLUSIONS:** Considering vitamin E availability, cost-efficacy and safety, injected intra-muscularly before operations can prevent adhesion formation.

**KEYWORDS:** Adhesion, Vitamin E, Rat Uterine Horns, Peritoneum, Laparotomy

## BACKGROUND

Adhesions, as abnormal scar tissue, are common following gynecological operations and are seen during the healing processes [1] In some cases they can lead to some complications such as: chronic pelvic pain, infertility, poor outcomes of future surgeries, intestinal obstruction, and etcetera. Adhesion mechanism is known as an inflammatory continuous event during the healing process where the peritoneum is traumatized. Although, this inflammation occurs normally, in abnormal situations such as ischemia, fiber bands will remain and result in the formation of adhesion. This fact that peritoneum surrounds all organs in the pelvis -except ovaries in women- has led to high prevalence of adhesions following operations of this region.[1,2] The prevention and decreasing of adhesions is a controversial issue, and no decisive method has been substantiated. These solutions can be categorized in 3 subgroups of improving surgical methods, using barriers and administrating pharmacological agents. The first step to have less adhesion is to use less traumatizing surgical procedures. Laparoscopic operation was a great advance to minimize post-laparotomy adhesion, however; some adhesions have also been reported following laparoscopy.

Other simple surgical hints could be: cleaning talcum powder of the surgical gloves before manipulating peritoneum, using as few absorbable sutures as possible, not using rough sponges and etcetera. Barriers themselves consist of absorbable, non-absorbable and liquid barriers like Oxidized Regenerated Cellulose (ORC), Expanded Poly Tetra Fluoro Ethylene (EPTFE) and Crystalloids, respectively. But their efficacy was not consistent in all cases.[3-4] Different researches have studied several pharmacological agents in the prevention of adhesion formation. Basbug et al. (2004) showed the preventive effect of hyaluronic acid plus heparin on adhesion formation in rat uterine horn.[5] In the year 2007 the effect of melatonin on adhesion formation in the rat cecum and uterine horn was assessed by Ara et al.[6] It was documented that the use of melatonin in prior surgeries showed a significant lower amount of adhesion formation compared to the control group. In many studies oxygen free radicals are known as one of the main causes of adhesion formation; they may cause further tissue damage and therefore lead to more adhesion formation.[7] Vitamin E as an antioxidant vitamin plays an important role in the human health and reproductive systems and it is an essential nutrient for the fetus

**Address for correspondence:** Azar Danesh Shahraki, Associate Professor, Department of Obstetrics and Gynecology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran. E-mail: danesh@med.mui.ac.ir

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and infants' growth.<sup>[1-3]</sup> The antioxidant effects of vitamin E has been an interesting subject for researchers in this field during previous years .<sup>[8-10]</sup> In two separate studies efficacy of oral, subcutaneous and intra-peritoneal vitamin E in preventing post-operative adhesion formation were compared with control groups and positive results were obtained.<sup>[8,9]</sup> Due to the proven side effects of other pharmacological agents such as melatonin<sup>[6,11]</sup> and sodium carboxyl methyl cellulose<sup>[10]</sup> and of course their high prices and unavailability, it seems that vitamin E can be a beneficial choice. In another study conducted in 2004 on rats, intra-peritoneal (intra-peritoneal olive oil, the vehicle/diluent of vitamin E, intra-peritoneal vitamin E diluted in olive oil) injection of vitamin E was significantly more efficient in preventing adhesion formation in comparison with intra-muscular administration.<sup>[8]</sup>

The purpose of this study is to evaluate the effect of vitamin E on decreasing post-operative adhesion in rat uterine horn. The effect of different administration methods when rat's peritoneum is traumatized is also discussed.

## METHODS

This experimental study, with research project number 386271, was performed on 45 non-pregnant female rats with the mean weight of 200 grams, 6 months old and bought from Pastor Institute. They were kept in optimum temperature, fed with plet add lipitum, drank water freely with same environmental temperature, 12 hours in light (25watt) and 12 hours in the dark. All of the rats adapted to the environment 2 weeks before surgery. They were randomized into 3 groups, each consisting of 15 rats. Vitamin E was used in the form of 100 IU/ml ampoules. Administrated dosage for each rat was 10 IU equal to 0.1ml (50 U/kg). This study was approved in the Research Council and Ethics Committee of the School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

All the surgical procedures were performed by researchers. Chloroform and then halothane and oxygen mixture were used for general anesthesia before the operation. Soon after anesthesia and right before incision, their abdomens were shaved and prepped. An inferior-middle incision with the length of 3 cm was made using the sterile technique. Skin, muscle fascia and peritoneum were exposed and uterine horns were explored.

After this exploration, in all 3 groups, a 2 cm incision was made on the wide ligament of the left uterine horn and it was devascularized. Then 5 points of the anti-

mesenteric surface of the left uterine horn were traumatized using a single-polar couther with the power of 1 voltage. On the surface of the right horn and also on the peritoneal wall of that side, 5 scratches were made by scalpel. In this group 0.1 cc (10 IU) intra-peritoneal vitamin E was injected while in the second group the same dosage was administrated intramuscularly before making any incisions. In the control group normal saline solution was poured onto the uterine horns. In all groups monofilament surgical sutures were used to close incisions in two layers. Post-operation conditions were the same for all the cases. Following 4 weeks recovery, second laparotomy was performed to grade adhesion formation. In this study adhesion was defined as joining bands from the traumatized points to intestines, fascia and omentum. Vitamin E was purchased from the Zahravi Corporation in Tabriz.

Adhesion formation was measured in terms of severity as: Not resistant against resection (grade 0), Moderate resistant against resection (grade 0.5) and Resection just by sharp dissection (grade 1) and according to extent of adhesion formation as: No adhesion (grade 0), Adhesion in 25% of traumatized points (grade 1), Adhesion in 50% of traumatized points (grade 2) and Adhesion in all of the traumatized points (grade 3). For each horn one grade was recorded resulting in two grades for each rate. In other words, 30 scores were achieved for each group and the mean values were calculated.

### *Statistical analysis:*

Kruskal-Wallis test was used for between group comparisons and Mann-Whitney U test as the post-hoc test was adjusted for type I error. Wilcoxon sign rank test was used for within group comparisons. All statistical analysis was done using SPSS software, version 12. The P-value < 0.05 was considered as statistically significant.

## RESULTS

As shown in table 1, there was no statistically significant difference between group 1 and the control group in terms of adhesion grade. Moreover, the grade of adhesion was not significantly different between group 3 and the control group. These results implicate that vitamin E did not act as an effective agent in reducing the adhesion formation among the subjects whose right uterine horn had been scratched using scalpel. However vitamin E administration in the left uterine horn, damaged with cautery (both intra-peritoneal and intramuscular), had a significantly preventive effect against adhesion formation comparing to the control group ( $p < 0.01$ ). In groups 3 and 2 grade one of adhesion was

detected in 4 cases (33%) and 3 cases (20%), respectively, while for the control group this grade of adhesion was observed in 12 cases (80%) (Table 2). Based on these findings it can be concluded that vitamin E had a significant effect in decreasing adhesion formation or its grade. Mann-Whitney U test showed that in comparison with intra-peritoneal administration, intramuscular vitamin E had a greater protective effect ( $p < 0.05$ ). However, both groups had a significant difference in terms of adhesion compared to the control group ( $p < 0.001$ ). Within group comparisons based on Wilcoxon test revealed that control group ( $p < 0.001$ ) and Intra-peritoneal group ( $p < 0.1$ , marginally) differed significantly in terms of adhesion in the right uterine horn and left uterine horn. All rats were alive after the operation. No antibiotics were administered.

## DISCUSSION

Adhesion as an abnormal tissue is formed during the healing process and bonds areas that should not be connected. Such a response can occur following surgery, infection, trauma or radiation. Peritoneal involving operations including gynecological operations may lead to adhesion formation.<sup>[1]</sup> On the other hand, adhesions by themselves can cause pain and other complications like bowel obstruction, infertility, and etcetera.<sup>[1,2]</sup> In a well done surgery procedure with less unnecessary traumatized tissues and foreign bodies -such as sutures- these reactions can be highly prevented.

To prevent adhesions, it is assumed that surgeons understand and adhere to the principles of good

surgical technique at the time of the primary surgery, sometimes termed "microsurgical principles". These recommendations include: use of atraumatic instruments, use of fine and inert suture materials, careful tissue handling, prevention of desiccation, and meticulous hemostasis.<sup>[12]</sup> Surgical approach is an important factor, with laparoscopy being associated with less adhesion formation compared to laparotomy.<sup>[13]</sup>

In different studies various chemicals have been used during surgery to prevent adhesion formation, among which Dextrin and Sodium carboxyl methyl cellulose had better effects.<sup>[10]</sup>

Vitamin E has antioxidant, anti-inflammatory, and antifibrotic effects. In addition, it decreases collagen formation. In theory, it may decrease adhesion formation. In a similar study on rats, intra-peritoneal vitamin E administration during surgery had a significant preventive effect on adhesion formation.<sup>[8]</sup> In the study by Hemadeh et al. the difference in how vitamin E is used in rats (oral, subcutaneous or intra-peritoneal) was significant for oral consumption ( $p < 0.04$ ).<sup>[10]</sup>

In our study, the protective effect of vitamin E on adhesion was more significant when it was administered intramuscularly. In other words, with intramuscular vitamin E no adhesion was formed in approximately 33% of cases while the control group showed various grades of adhesion. The difference was not statistically significant for intra-peritoneal administration.

Intra-muscular vitamin E had greater effect in preventing adhesion formation of left uterine horn (traumatized

**Table 1. Comparison of the grade of right uterine horn adhesion formation among the studied groups**

Groups	Group 1 (Control group)		Group 2 (Intra-muscular vitamin E)		Group 3 (Intra-peritoneal vitamin E)	
	Number	%	Number	%	Number	%
Adhesion Grade						
0	13	(87)	11	(67)	15	(100)
1	2	(13)	4	(33)	0	(0)
2,3,4	0	(0)	0	(0)	0	(0)

The difference in adhesion formation compared to control group was not significant with P-value = 0.15 in group 2, and P-value = 0.35 in group 3.

**Table 2. Comparison of the grade of left uterine horn adhesion formation among the studied groups**

Groups	Group 1 (Control group)		Group 2 (Intra-muscular vitamin E)		Group 3 (Intra-peritoneal vitamin E)	
	Number	%	Number	%	Number	%
Adhesion Grade						
0	3	(20)	12	(80)	11	(67)
1	12	(80)	3	(20)	4	(33)
2,3,4	0	(0)	0	(0)	0	(0)

The difference in adhesion formation compared to the control group was significant with P-value = 0.001 in group 2, and P-value = 0.001 in group 3.

with cautery) in comparison with intra-peritoneal and control groups (20% vs. 33% and 80% adhesion formation, respectively). In the right uterine horn (traumatized with scalpel) neither intra-muscular nor intra-peritoneal vitamin E decreased adhesion. Surprisingly no adhesion was observed in the control group.

The cause of the stronger effect of vitamin E when administered intramuscularly compared to intra-peritoneal method, might be due to the fact that inflammatory processes start in the primary hours of operation and high levels of vitamin E at this time can stop these reactions. While, in the intra-peritoneal form, vitamin E is absorbed late, therefore, its ideal level to prevent the inflammatory processes is not achieved. The same mechanism may prevent fibroblast aggregation in the site of ulcer, scar tissue formation and adhesion.<sup>[11]</sup>

It seems that tissue damaged due to trauma forms free radicals which have critical role in adhesion formation. Moreover, because cauterization causes more damage than a surgical scalpel, cauterization probably leads to more adhesion formation.<sup>[14]</sup> As we know, cauterization damages tissues much more in comparison with surgical blade. Therefore, adhesions would occur more frequently and severely and maybe that is why we were not able to show the efficacy of Vitamin E in the scalpel group with a lower level of injury. The preventive effects of Met-RANTES, methylene blue and vitamin E, and vitamin E and human amniotic membrane on postoperative intra-peritoneal adhesion formation were studied in the rats.<sup>[15-17]</sup>

In our study, intra-muscular administration of vitamin E was the most effective way to reduce adhesions. Therefore, it might be concluded that the role of vitamin E in decreasing post-operative adhesion could differ based on the intensity of the damage and mode of administration, which calls for further studies. Today, we are still far from finding the ideal adhesion-preventing agent. There is still no adhesion-preventing substance, material, or barrier that is unequivocally effective. One can reduce the adhesion formation, but not prevent it entirely. In conclusion, considering vitamin E availability, cost-efficacy and safety in high doses it can be injected intra-muscularly before operations to prevent adhesion formation. Although, our study was performed on rats, due to vitamin E safety such a study can be conducted on higher populations of humans with longer follow-up durations to reach a definite conclusion.

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