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Research Article

Expression of VEGF in Fallopian Tubes in Ovarian Ischemia-Reperfusion

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Abstract

Aim: Our aim was to investigate expression level of VEGF in tuba uterine in ovarian ischemia-reperfusion (I/R) by immunohistochemical techniques.

Material and Methods: 30 Sprague Dawley female rats were categorized into three groups. Sham group: The abdomen was opened and closed without any treatment. Ischemia (I) group: 1-hour ischemia was allowed to create ischemic injury. Ischemia-reperfusion (I/R) group: 1-hour ischemia and then 3-hour reperfusion was allowed to create I/R injury.

Results: MDA and MPO levels were increased after ischemia and IR while GSH content was decreased. Histological scores of follicular degeneration, inflammation, hemorrhage were high in I and IR groups. Normal histology of tuba uterine was observed in sham group. In I and IR group, degenerated cilia, desquamative epithelial cells impaired basement membrane leukocytes infiltration, apoptotic nuclei, vascular dilatation, thrombosis and inflammation and adenoma were observed. VEGF expression was mainly in sham group. In I and IR group, endothelial cells, adenoma structures, vessels, macrophage and inflammatory leukocyte cells and fibroblast cells showed positive VEGF expression.

Conclusion: IR damage affected inflammation and angiogenesis, changes in implantation.

Keywords: VEGF, ischemia-reperfusion, fallopian tubes, histopathology

INTRODUCTION

Insufficient oxygenation of tissues is called ischemia and delivery of oxygen to ischemic tissue called reperfusion. During the IR injury, cell damage occurs irreversibly. Generally, IR cause more damage than ischemia itself in tissues because of imbalance in oxidant and antioxidant system of cells. IR causes elevation of reactive oxygen species and nitrogen species, release of cytokines, induces inflammatory pathway. To overcome this situation, cells scavenge free radicals and produce antioxidant enzymes. This system is not always successful, so medicinal plants could be an alternative to help the restoration of tissue homeostasis (1,2). Ovarian IR is a common clinical emergency with 2.7% incidence. It mainly affects the women of reproductive age. Early diagnosis and treatment are critical in preventing infertility (3).

Tuba uterine consists of a pair of thin tubes 10-12 cm long that extend from the upper part of the uterus to the surface of the ovary. Its role is to provide a suitable environment for fertilization and to transport the ovum from the ovary to the uterus. Anatomically, it consists of 4 parts: infundibulum, ampulla, isthmus, intramural (4,5). Histologically, it consists of three layers: tunica mucosa, tunica muscularis, and tunica serosa. Occasionally, ovarian IR damage is seen in cases involving the tuba uterine. Therefore, IR has effects on tuba uterine and its associated structures (6,7).

In this study, we aimed to investigate VEGF immune expression on fallopian tubes tissue in ovarian ischemia-reperfusion.

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MATERIAL AND METHOD

Experimental Design

All experimental protocol was approved by the Dicle University Local Animal Ethics Committee (2022/02). Sprague Dawley female rats (weighing 200-250 g) were bought and housed in separate cages at 23±2°C, 12 hours light/12 hours dark period at 45-55% humidity, and were fed with standard pellet and water. Vaginal smear was analyzed under microscope to observed estrous cycles of rats at once in every 6-12 hour. 30 female rats were selected in estrus cycle. All experimental procedure was conducted under general anesthesia with injection of ketamine and xylazine. Rats were divided into three groups (10 rats per group) and the following procedures was applied to the groups.

Biochemical Analysis

Tuba uterine tissues were process for malondialdehyde (MDA) levels and glutathione peroxidase (GSH-Px) activities. Tissue samples were homogenized in physiologic saline solution at 10% ratio. All protocols were conducted on ice using homogenizer. Samples were spinned at 2000 rpm for 10 min in a centrifugation vehicle. Supernatant was collected for further analysis. 340 nm absorbance value were selected in spectrophotometry. MDA were expressed nmol/g and performed by Draper et al (9). GSH-Px were done by Paglia et al (10). GSH-Px were expressed as U/g protein. MPO level in tuba uterine tissues were calculated by Hillegas et al (11). MPO is expressed as U/g tissue.

Histological tissue processing

At the end of experiment, animals were sacrificed and fallopian tubes were dissected. The tuba uterine tissues were taken into formalin solution, dehydrated in increasing alcohol series, soaked in xylol solution and incubated in paraffin wax at 58°C. samples were put into paraffin blocks and 4 µm sections were cut and stored for hematoxylin eosin staining.

Immunohistochemical examination

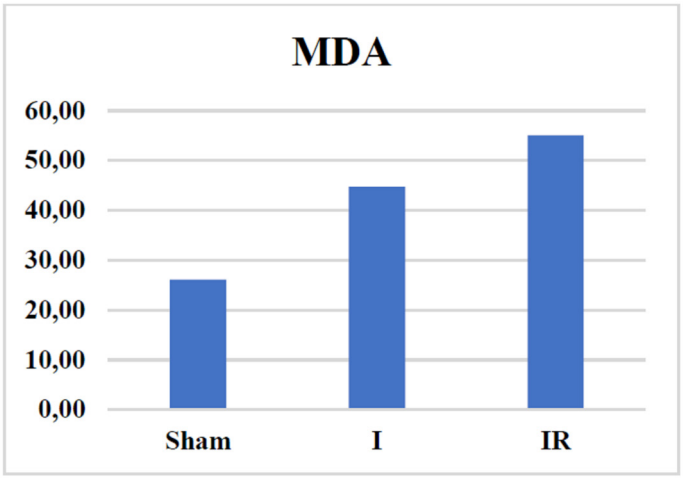
Uterine sections were cleared in xylol solution, dehydrated in alcohol and cleared in distilled water. Epitope retrieval was inducted by EDTA (ethyl diamine tetra acetic acid) solution (pH: 8.0) for 15 minutes in a microwave oven at 90°C. After sections were cooled down, they were rinsed in phosphate buffered saline (PBS) three times for 5 minutes. 3% hydrogen peroxide (H_2O_2) was dropped on slides to block endogen peroxidase activity. After washing in PBS, sections were incubated with rabbit polyclonal VEGF antibody (catalog no: A43269, AFG Bioscience, US) overnight at + 4°C. Sections were dipped into PBS and biotinylated antibody solution () was dropped onto slides for 14 minutes. Sections were reacted with streptavidin peroxidase solution was (ThermoFischer, US) for 15 minutes. After PBS washing, diaminobenzidine (DAB) chromogen was used to observe color change for maximum 10 minutes. Reaction were stopped with PBS solution and sections were stained with hematoxylin dye. Slides were analyzed under light microscope.

Statistical Analysis

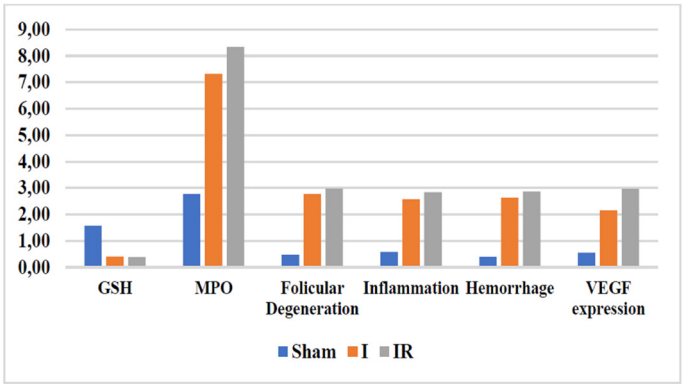
For statistical analysis, IBM SPSS Statistics 25.0 (IBM Inc, Chicago, IL, USA) will be used with a computer program. First, normality tests will be applied to the data and it will be checked whether the data are normally distributed. Kruskal Wallis test (non-parametric) will be used for comparison between independent groups, and if there is a difference, Mann Whitney U test will be used for paired comparisons. The data of this study will be given as mean ± standard error. A value of P <0.05 in all tests will be considered statistically significant.

RESULTS

Statistical analysis of biochemical and histochemical parameters was shown in Table I. MDA and MPO levels were higher in I and IR group than sham group. Histological scores of follicular degeneration, inflammation, hemorrhage were significantly higher in I and IR group than sham group. GSH content was the significantly lower in I and IR group compared to sham group. Table 1 was shown in Figures 1a and 1b.



1-a



1-b

Figures 1a-1b. Graphics of biochemical and histochemical parameters

Table 1. Biochemical and histological parameters of sham, I and IR groups

Parameter	Groups	n	Median (Min-Max)	P value
MDA	Sham	10	26.12 (10.33–42.84)	*p<0.001 **p<0.001
	I	10	44.69 (35.34–54.25)	
	IR	10	55.05 (40.68–68.58)	
GSH	Sham	10	1.57 (1.15-1.98)	*p<0.001 **p<0.001
	I	10	0.40 (0.23-0.72)	
	IR	10	0.38 (0.28–0.70.)	
MPO	Sham	10	2.78 (1.75–3.57)	*p<0.001 **p<0.001
	I	10	7.32 (4.45–9.46)	
	IR	10	8.34 (6.48–10.63)	
Follicular degeneration	Sham	10	0.50 (0.00-1.00)	*p<0.001 *p<0.001
	I	10	3.00 (1.00-3.00)	
	IR	10	3.00 (1.00-3.00)	
Inflammation	Sham	10	0.00 (0.00-1.00)	**p<0.001 *p<0.001
	I	10	3.00 (1.00-3.00)	
	IR	10	3.00 (1.00-3.00)	
Hemorrhage	Sham	10	0.00 (0.00-1.00)	*p<0.001 *p<0.001
	I	10	3.00 (1.00-3.00)	
	IR	10	3.00 (1.00-3.00)	
VEGF expression	Sham	10	0.50 (0.00-1.00)	**p<0.001 *p<0.001
	I	10	2.00 (1.00-3.00)	
	IR	10	3.00 (1.00-3.00)	

* sham vs I; **vs IR

In the control group, the epithelial structure and basement membrane were preserved in the transversal section of the tuba uterine. Connective tissue cells are solitarily distributed. Cells and fibers were detected in regular structure. The circular muscle fibers in the lamina propria were regular. The submucosal area was seen regularly (Figure 2a).

In the ischemia group, it was found that the cilia structure was completely lost in the transversal section of the tuba uterine, and desquamative epithelial cells were shed towards the lumen. The basement membrane structure was impaired, and leukocytes were present in the lamina propria. Crypt structures showed desquamative degenerative changes. Apoptotic nuclei are present. Crypts resembling adenoma were detected in some areas. Inflammation was markedly increased and the number of neutrophils and eosinophils was high (Figure 2b).

In the ischemia-reperfusion group, complete disappearance of the epithelial structure was observed. Vascular dilatations and thrombosis are evident in the connective tissue areas, and there is a dense solitary distribution of inflammatory cells around the vessel. Muscle structure is hyperplastic (Figure 2c).

In the control group section, it was observed that the epithelial structure was clearly preserved, the cryptic structures were regular, and the lamina propria, muscular and vascular structures were smooth. Positive VEGF expression was observed in the crypts, but negative VEGF was detected in endothelial cells (Figure 2d). In the section of the tuba uterine belonging to the ischemia group, vessel dilatation and degenerated basement membrane structure were observed in the lamina propria and muscular layer. Expression of VEGF in endothelial cells was positive. VEGF positive expression was detected in some adenoma structures, macrophage and inflammatory leukocyte cells and fibroblastic structures (Figure 2e). Thrombosis in dilated vessels and loss of integrity in the basement membrane were observed in ischemia-reperfusion sections. VEGF expression was positive in endothelial cells, crypts, inflammatory cells and macrophage cells, vascular structures (Figure 2f).

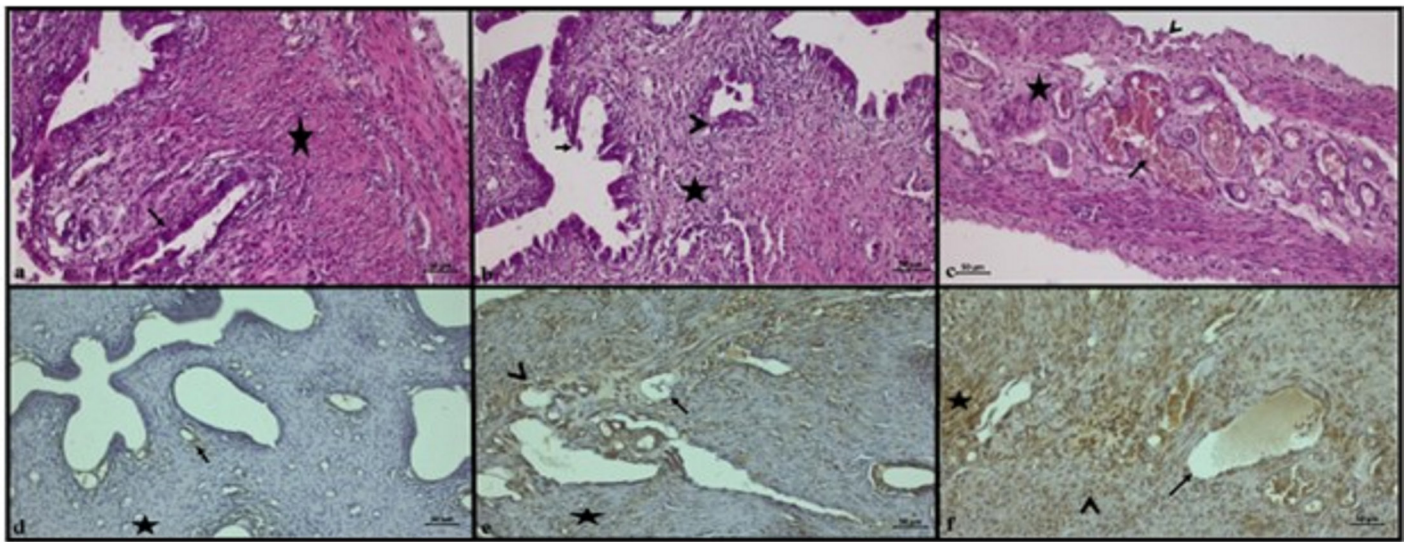


Figure 2. Sham group: Regular basement membrane (black arrow), solitary connective tissue cells, regular muscle fibers (star) (Figure 2a); Ischemia group: degenerated cilia and (arrow) basement membrane, leukocytes (star), adenoma (arrowhead) (Figure 2b); IR group: Degenerated epithelium (arrowhead), thrombosis (arrow), solitary inflammatory cells (asterisk) (Figure 2c); Sham group: positive VEGF expression in crypts (arrow), negative VEGF expression (asterisk) in lamina propria and endothelial cells (Figure 2d); Ischemia group: positive VEGF in dilated vessels (arrow), adenomas (arrowhead), macrophage and fibroblast cells (star) (Figure 2e); IR: positive expression of VEGF in endothelial cells (arrow), macrophage cells (star) and plasma cells (arrowhead) of dilated vessels (Figure 2f)

DISCUSSION

Ovarian ischemia reperfusion causes many pathologies in ovarian tissues, as well as remote organs such fallopian tubes, uterine and cervix. Aktaş et al studied ovarian IR and found that intense fibrosis, vascular dilatation and congestion, stromal inflammation in ovarian tissues after IR (12). Peker et al investigated IR injury in ovarian tissues and revealed that IR caused edema, inflammation, congestion, degenerated follicles, and cells with pyknotic nuclei (13). Eser et al graded the ovarian tissues histologically after IR injury and recorded that the histological scores of ovarian tissues with IR was lower than sham group (14). In our study, tuba uterine histology was normal with regular epithelial structure and basement membrane. Connective tissue and muscle fibers were normal (Figure 2a). In the ischemia group, degenerated cilia structure and desquamative epithelial cells were observed. Increased leukocytes were seen in the lamina propria. Many apoptotic bodies were present. Crypts resembling adenoma were detected in some areas. Inflammation was high (Figure 2b). Severe histopathology was observed in IR group with complete disappearance of the epithelial cells, dilatated vessels and intense inflammatory cells (Figure 2c).

Vascular endothelial growth factor, acronym for VEGF, involves in angiogenesis and promote the angiogenic activities. It is a growth factor having mitogenic and anti-apoptotic effect on endothelial cells (15). There are several members of VEGF family such as VEGFA, VEGFB, VEFGFC, VEGFD etc. A study conducted by Ersoy et al investigated the expression of VEGF in ovarian and uterine tissues. The authors found that VEGF expression was increased compared to sham group in both ovarian and uterine tissues after IR (16). Deger et al studied rat ovarian torsion detorsion model to investigate the VEGF expression in ovarian tissues. They recorded that VEGF immune activities were increased in ovarian degenerated follicles (17). Parlakgumus et al investigated expression level of VEGF in rat ovarian torsion model. The authors found that VEGF expression was significantly lower in IR group than in sham group with lower vascularization (18-20). In our study, in the control group section, VEGF expression was mainly negative (Figure 2d). In the ischemia group, VEGF expression was positive in endothelial cells, adenoma structures, macrophage and inflammatory leukocyte cells and fibroblastic structures (Figure 2e). In IR group. VEGF expression was positive in endothelial cells, crypts, inflammatory cells and macrophage cells, vascular structures (Figure 2f).

CONCLUSION

It has been thought that IR damage caused cilia loss and endothelial dysfunction occur in the tubal epithelium, leading to both an increase in inflammation and a change in angiogenesis, eventually affecting implantation.

Limitations and future perspectives

The animal number is small and may be led to a bias in the results. The conclusion can be supported with other molecular techniques quantitatively. IR injury is a crucial emergency and may lead to infertility. A therapeutic approach to VEGF may be an alternative for treatment. More large-scale experimental study should be further conducted.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *All experimental protocol was approved by the Dicle University Local Animal Ethics Committee (2022/02).*

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