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# ORIGINAL: Induction of Experimental Endometriosis in Rat: Evaluation of Systemic Inflammatory Response and Liver Tissue Changes

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**ABSTRACT**

**Introduction:** Nowadays non-alcoholic fatty liver disease (NAFAD) is considered as a serious problem in human societies. Recently, the possibility of an association between endometriosis and NAFAD has been considered. This study was designed to evaluate some general inflammation parameters and hepatic lesions during and after experimental endometriosis.

**Material and Methods:** In 20 female rats, the endometriosis model was induced by suturing parts of the uterine horn wall to the mesenteric gut. After four weeks, the rats in group I were euthanized and endometriosis cysts and some fragments of their liver were used for histopathological evaluation. At the same time, endometrial cysts were surgically removed in the rats of group II and they were kept for four weeks later. In addition, hematobiochemical evaluation was performed. In group II, similar evaluative investigations were performed 8 weeks after experimental surgery.

**Results:** Significant increase in triglyceride, LDL, AST, ALP, ALT and estrogen parameters was observed in this study ($P<0.05$), Whereas, total WBC count, lymphocyte, PCV and HDL level decreased significantly ($P<0.05$). In histopathological evaluation, induction of endometriosis was confirmed at the microscopic level, but no evidence of fatty liver or hepatic inflammation was found.

**Conclusion:** Despite notable changes in some hematobiochemical factors in rats with experimental endometriosis, there was no evidence of fatty liver and hepatic inflammation. Therefore, there may be no association between endometriosis and non-alcoholic fatty liver disease.

## Introduction

Endometriosis is a disease specific to women that causes chronic abdominal pain and infertility at reproductive age (1). Primates' models are used to mimic endometriosis in women. The rodent model is currently preferred because of the high costs. Since endometriosis occurs only in humans and non-human primates, therefore, endometriosis must be induced in animals such as rodents to study its symptoms (2). Inflammatory response related to the endometriosis is due to the role of inflammatory cells including the infiltration and activation of peritoneal macrophages and their secreted



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cytokines (3,4). It is clear that the process of inflammation caused by this anatomical disorder affects ovarian function. Additionally, local inflammation can lead to adhesion, angiogenesis and endometrial cyst formation (5). Nowadays, non-alcoholic fatty liver disease (NAFAD) is considered a serious issue due to obesity and metabolic abnormalities. However, little is known about the association between symptoms of NAFAD and endometriosis. NAFAD is the accumulation of fat in the liver without inflammation or damage to the liver cells. People with NAFAD also have other metabolic abnormalities, such as obesity, diabetes mellitus, and abnormal blood lipids (triglycerides, cholesterol, and phosphorlipids). In addition to the symptoms of NAFAD, inflammation, injury, swelling and the presence or absence of fibrotic tissue can also be seen in the liver, which is called NASH (Nonalcoholic steatohepatitis, a type of non-alcoholic fatty liver disease) which characterized by inflammation and damage to the liver cells (6). Recently, it has been shown that the occurrence of endometriosis in women (about 8%) is related to NAFAD (about 24%) and venous thromboembolism (7). In addition, within 5 years, 35% of women undergoing endometriosis had been reported to have NAFAD (8). Estrogen level during endometriosis is high and liver is a vital organ of the body for hormone clearance and detoxification. Following endometriosis, the levels of estrogen-related hormones increase dramatically in the body (9). Estrogen plays a key role in lipid metabolism and with endometrial removal the risk of developing NAFAD in women is greatly increased (10). It is therefore hypothesized that following endometriosis, the probability of tissue changing in the liver increases due to abnormal accumulation of fats in the liver. However, some aspects of endometriosis have a great deal to do with the immune system and inflammation. The present study will also help us understand the process of inflammation and immune factors in the liver during and after endometriosis. Therefore, experimental endometriosis is induced in rats, and surgical treatment is considered as their main treatment, and in assessing the hemato-biochemical changes of the animals, tissue changes in the liver are assessed.

# Methods

Ethical and executive aspects of this study were approved by the Research Council of the Department of Veterinary Clinical Sciences (170/1312).

### Preparation of samples

A total of 29 healthy adult female rats with an approximate age of 8-12 weeks and weighing 180-230 g were assigned to the current investigation. Of these, 9 rats were sacrificed humanly (time 0) and 20 rats were randomly divided into two equal groups in order to induction of endometriosis. Animals in the first group were euthanized 4 weeks after induction of endometriosis and evaluated for histopathological and hematobiochemical analysis. In the second group, the induced endometrial cysts were removed surgically and then the animals were kept for another 4 weeks. Finally, the animals were euthanized to assess histopathological lesions and inflammatory process of the liver as well as evaluation of hepatic enzymes activity and some blood and biochemical parameters.

### Surgery preparation and procedure

Experimental endometriosis was induced by implanting parts of the uterine horn at the mesenteric surface of the intestine (11). Following induction and maintenance of injectable anesthesia (intraperitoneally) by combination of ketamine (80 mg / kg) - xylazine (10 mg / kg) (12), and after aseptic celiotomy, longitudinal fragments harvested from the uterine horn and sutured to the mesenteric gut with 4.0 polyglactin 910 sutures to induce endometriosis and finally the abdomen closed routinely. Endometrial peritoneal cysts were developed for 4 weeks. At this stage, half of the rats were euthanized by overdose of anesthetics and considered as the first group. Subsequently, endometriosis cysts were removed after surgical celiotomy



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and rats were maintained again for 4 weeks and finally, after sacrificing the remaining rats, they were considered as the second group.

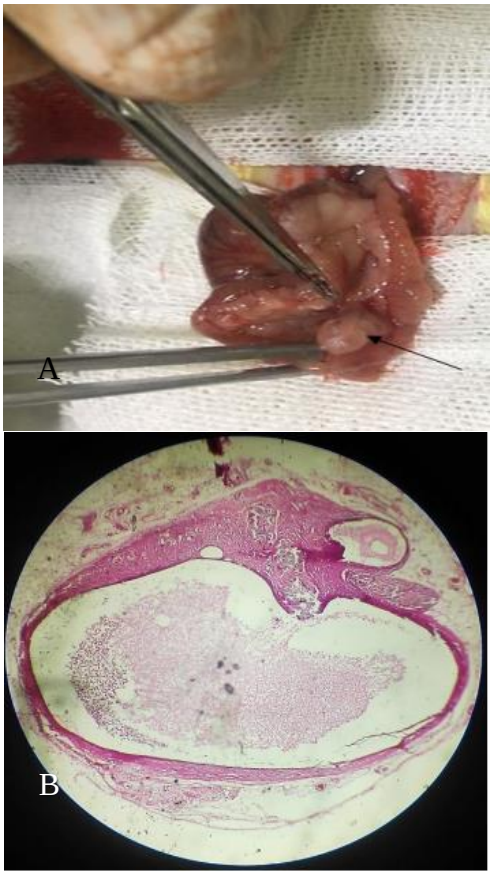


**Figure 1. (A) Induced endometriosis cyst in rat mesentery (black arrow); (B) A microscopic cross section of the uterine mesenteric graft showing endometrial cyst induction**

### Sampling, histological examination, and blood works

Time 0, week 4 and week 8 after experimental induction of endometriosis were considered for hematobiochemical and histopathological evaluation. Autoanalytical apparatus was used to determine estrogen, AST, ALT and ALP in serum. In addition, mean and total count of white blood cells, neutrophil to lymphocyte ratio, hematocrit (PCV), total protein, albumin, globulin, glucose, cholesterol, triglyceride, LDL, HDL and estrogen levels were analyzed. For histopathological evaluation, appropriate tissue samples which collected from the livers and endometrial cysts, were then fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm thickness, and stained with Masson's trichrome and hematoxylin-eosin staining for light microscopic examination (13).

### Statistical analysis

For hematological parameters analysis, descriptive statistics including the mean, standard deviation, median, minimum and maximum were calculated for all variables. The one-way ANOVA followed by Tukey's post hoc test were used for comparison of different parameters. The data were analyzed by SPSS software, version 22, and differences of $P<0.05$ were considered significant.

## Results

The formation of peritoneal endometriosis cysts after 4 weeks was confirmed in both macroscopic and microscopic examinations (***Figure 1***).

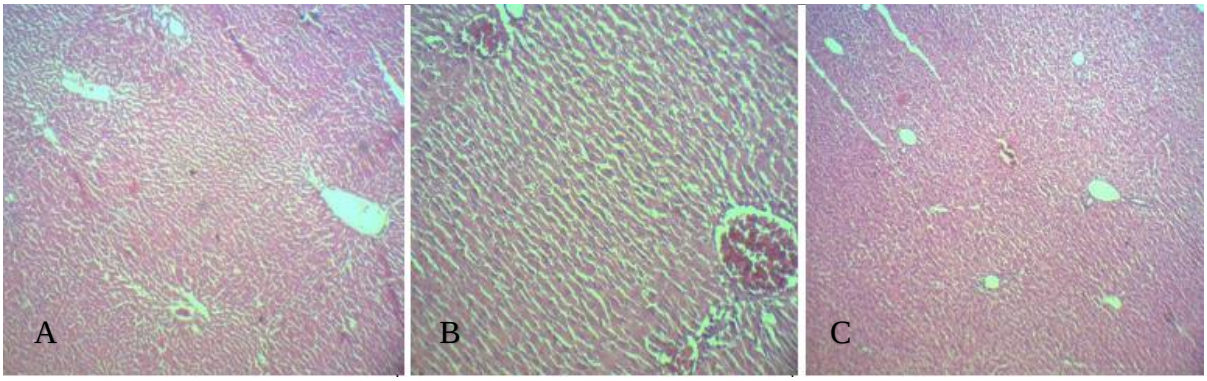


**Figure 2. Hepatic sections; (A) normal tissue at time 0; (B) The fourth week when endometriosis was induced experimentally (group 1); (C) Eighth week of study when endometrial cysts were surgically removed (group 2)**



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Microscopic examination of the animal's liver at the different times (Time 0 and weeks 4 and 8 after surgery) revealed no evidence of fatty liver (***Figure 2***). The presence of neutrophils, lymphocytes, and macrophages which are indicative of liver inflammation was not found in the histopathological sections. In addition, the ballooning hepatocytes as an independent factor in the diagnosis of NASH, cannot be seen on the histopathological examination.

Based on the ***Table 1*** and ***Table 2***, the results showed that the parameters of total WBCs, lymphocytes and PCV counts in the first and second groups were significantly decreased compared to the normal group at time 0 (baseline data) ($P<0.05$). The results also showed that the number neutrophils, monocytes and neutrophil to lymphocyte ratio in the fourth and eighth weeks (group 1 and group 2 respectively) were not significantly different when compared to the baseline data (time 0) ($P>0.05$). Total protein, albumin, globulin and glucose levels in the first and second groups were not significantly different from the time 0. It was also found that estrogen, LDL, ALP, AST and ALT levels increased significantly ($P<0.05$) in the groups 1 and 2 compared to the baseline data. The analyzed data showed that the cholesterol level in the first group was not significantly different from the baseline data ($P>0.05$), but it was significantly increased in the second group ($P<0.05$). Triglyceride level in the first group showed a marked increase ($P<0.05$) in comparison to the baseline data, while a significant decrease ($P<0.05$) was observed in the second group compared to the time 0. The results also showed that HDL level decreased significantly ($P<0.05$) in the groups 1 and 2 compared to time 0.

**Table 1. Comparison of the mean of some blood parameters at different times**

| Blood parameters | Time 0 (N=9) | Week 4 (N=10) | Week 8 (N=10) |
|---|---|---|---|
| Total WBCs ($10^9$/L) | 5133.33±331.45$^a$ | 3345±88.65$^b$ | 3735±191.64$^b$ |
| Lymphocyte ($10^9$/L) | 5133.33±331.45$^a$ | 2360±66.27$^b$ | 2652.1±201.67$^b$ |
| Neutrophil ($10^9$/L) | 1144.89±62.86$^a$ | 944.5±82.41$^a$ | 1029.1±68.52$^a$ |
| Monocyte ($10^9$/L) | 68.22±17.29$^a$ | 39.70±10.94$^a$ | 53.80±14.30$^a$ |
| N/L ratio | 0.31±0.03$^a$ | 0.40±0.04$^a$ | 0.41±0.04$^a$ |
| PCV | 0.48±0.01$^a$ | 0.39±0.01$^b$ | 0.43±0.02$^b$ |

Different superscript letters in each row indicates statistical significance.

**Table 2. Comparison of the mean of some biochemical parameters at different times**

| Biochemical parameters | Time 0 (N=9) | Week 4 (N=10) | Week 8 (N=10) |
|---|---|---|---|
| Total protein (g/dl) | 4.6±0.29 | 5.07±0.20 | 4.96±0.40 |
| Albumin (g/dl) | 2.81±0.21 | 3.41±0.24 | 3.87±0.46 |
| Globulin (g/dl) | 2.47±0.19 | 2.94±0.28 | 2.74±0.27 |
| Glucose (mg/dl) | 160.37±13.01 | 155.20±9.58 | 160.04±9.28 |
| Cholesterol (mg/dl) | 90.33± 12.89$^a$ | 161.50±13.95$^a$ | 136.80±15.77$^{ba}$ |
| Triglyceride (mg/dl) | 121.44±12.68$^a$ | 166.90±13.01$^b$ | 116±10.72$^{ac}$ |
| LDL (mg/dl) | 48.44±5.30$^a$ | 82.6±7.54$^b$ | 72.50±6.70$^b$ |
| HDL (mg/dl) | 45.55±2.79$^a$ | 32.20±2.98$^b$ | 32.00±3.27$^b$ |
| AST (IU/l) | 92.00±10.99$^a$ | 141.90±14.2$^b$ | 145.00±14.96$^b$ |
| ALP (IU/l) | 87.67±4.17$^a$ | 110.20±7.29$^b$ | 107.40±4.24$^b$ |
| ALT (IU/l) | 72.67±8.34$^a$ | 97.50±5.28$^b$ | 79.60±6.09$^{ab}$ |
| Estrogen (pg/ml) | 21.82±1.02$^a$ | 42.41±2.97$^b$ | 32.17±3.20$^c$ |

ALT (Alanine amino transferase); ALP (Alkaline phosphatase); AST (Aspartate amino transferase);
Different superscript letters in each row indicates statistical significance

## Discussion

In the present study, notable changes in some hematobiochemical factors in rats with experimental endometriosis was observed, however there was no evidence of fatty liver



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and hepatic inflammation. One of the mechanisms that may contribute to the development of endometriotic lesions is the response between the scattered endometrial tissue and the immune system. Abnormal cellular and humoral immune function and numerous cellular mechanisms play an important role in the development and progression of endometriosis (14).

In one study, a significant decrease in the number of B lymphocytes was observed in women with endometriosis (15), and another study suggests a significant decrease in total T lymphocyte levels in women with endometriosis (16). This is in agreement with the present study because, in the present study, rats with experimental endometriosis showed a significant decrease in lymphocyte levels in time zero and a decrease in monocytes at no significant time at time zero. In another study, no changes in resting monocyte activity were observed in women with endometriosis, but monocytes increased in response to different stimuli in these women (17).

It has been reported that the levels of Colony-stimulating factor of granulocyte and macrophages, which are a growth factor in stimulating stem cells to produce granulocytes and lymphocytes, did not change in women with endometriosis compared to controls (18).

In the present study, we observed an increase in lymphocyte and monocyte levels compared to the fourth week after surgical treatment of experimental rats with endometriosis (Lymphocyte levels decreased significantly after surgery (week 8) compared to time zero and monocyte levels decreased after surgery (week 8) compared to time zero). In one study, the level of white blood cells, especially neutrophil levels, was increased in women with endometriosis (19). This is in contrast to the present investigation because in the current study, a decrease in the total white blood cell count in animals with endometriosis was observed in the fourth week. The level of total white blood cells also increased after surgery and in the eighth week. Another study reported no change in the absolute number of polymorphonuclear neutrophils, but found a slight but significant decrease in their chemotactic index. (Because polymorphonuclear neutrophils in women with endometriosis showed decreased chemotactic properties in response to a stimulus compared to the control group) (20). These findings are in agreement with our results, because in our study there was no significant difference in neutrophil levels in rats with endometriosis after 8 weeks of surgery.

Neutrophil to lymphocyte ratio has been reported to be one of the diagnostic tests with a sensitivity and specificity of 60% (19). It is reported that this index is not a good indicator for determining the severity of endometriosis in patients with moderate to severe degrees of endometriosis and is only dependent on the patient's age. It has been reported that there is no significant difference between neutrophil to lymphocyte ratio in patients with endometriosis (19), which is similar to the present study (21). Increased hemoglobin and hematocrit levels are usually dependent on blood concentrations. It has been reported that the percentage of hematocrit decreased significantly in patients with endometriosis, which is similar to the results of the present study. In the current study, a significant decrease in hematocrit percentage was observed in the fourth and eighth weeks compared to time zero (baseline data). It has been suggested that very severe endometriosis is associated with lower blood concentrations, with impaired RBC regulation and iron metabolism. Some evidence suggests that iron metabolism is potentially involved in the pathogenesis of endometriosis (22, 23). Iron can produce species of free radicals that are capable of inducing cellular damage and altering gene expression by enhancing the inhibition of transcription factors associated with endometriosis pathogens, such as the NF-κB that activates B cells (23). There is ample evidence that oxidative stress plays a role in the pathophysiology of endometriosis. Peroxigenase-1 (PON-1) is an HDL-dependent enzyme that prevents LDL oxidation. PON-1 activity



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levels were significantly decreased in patients with endometriosis (mild disease level) and lipid hydroperoxide (LOOH) levels were significantly higher than controls. There was a significant decrease between PON-1 activity and disease stage. It has been reported that women with endometriosis show a significant decrease in PON-1 activity and HDL levels and an increase in the levels of LOOH, total cholesterol, triglycerides, LDL, and lipoproxygenases (24), which is in agreement with the present study.

In rats with endometriosis, there was also a significant decrease in HDL levels, a significant increase in cholesterol and LDL levels and an increase in triglycerides. In the present study, LDL increased significantly in the eighth week (after surgery) compared to time zero (baseline data). The results showed that the cholesterol parameter increased significantly at week 8 compared to time zero. The results showed that the triglyceride parameter in the eighth week had a significant decrease compared to time zero. The results also showed that the HDL parameter at week 8 had a significant decrease compared to time zero. Oxidative stress has been reported to be a contributing factor in the pathophysiology of the disease (25).

It has been reported that levels of superoxide dismutase and glutathione peroxidase in the peritoneal fluid of women with endometriosis were significantly reduced compared to the control group, both of which play important roles in the formation of free radicals and reactive oxygen species (ROS) (25). Studies have also shown that ROS may increase the growth and adhesion of endometrial cells in the peritoneal cavity, stimulating endometriosis formation and infertility. Therefore, in women with endometriosis there is a significant decrease in antioxidant levels and a significant increase in LOOH (25). PON-1 is primarily responsible for degradation of lipid peroxides before accumulation in LDL (26) and increased LDL uptake (27). PON-1 directly inhibits macrophage oxidative stress and thus reduces the ability of macrophages to activate superoxidase anions and LDL oxidation (28).

In addition, PON-1 inhibits macrophage cholesterol biosynthesis and protects HDL against lipid peroxidation (29). Inhibition of HDL oxidation by PON-1 preserves the anti-atherogenic effects of HDL in reverse cholesterol transfer (30). The antioxidant activity of HDL is also mediated by PON-1 (31). Finally, possibly increased inflammation and oxidative stress in the progression of endometriosis may explain the decrease in PON-1 activity in moderate to severe endometriosis.

Endometriosis is a hormone-dependent inflammatory disease that is usually associated with high levels of estrogen and abnormal levels of cytokines, whose expression is regulated by GATA-3 in lymphocytes. GATA-3 is a specific transcription factor of Helper-2 T cells that is expressed in endometrial epithelial cells in patients with endometriosis (32). Therefore, GATA-3 may regulate the expression of cytokines in endometrial cells in patients with endometriosis. Estrogen regulates GATA-3 expression in a dose- and time-dependent manner. Estrogen induces translocation of GATA-3 from the cytoplasm to the nucleus and may be involved in the development and progression of endometrial disease by regulating the secretion of cytokines in ectopic endometrial cells in patients with endometriosis. Estrogen levels in women with endometriosis have been reported to be very high (33). This is similar to the present study because high estrogen levels were recorded in the rats with endometriosis at weeks 4 and 8 (after surgery) compared to time zero (baseline data). IL-6 is a Th2 cytokine that induces endometrial cell translocation, localization, and growth in patients with endometriosis (34). IL-6 has been reported to be less expressed in estrogen in endometriosis. In contrast to estrogen, GATA-3 induces IL-6 expression and therefore increased IL-6 expression in local lesions of patients with endometriosis may be due to the interaction of GATA-3 and estrogen effects (35, 36). Estrogen decreases the regulation of IL-8 expression and therefore increased IL-8 in local lesions of



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patients with endometriosis may be due to the coordination between GATA-3 activities and estrogen. IL-10 has been reported to increase in peritoneal fluids in patients with endometriosis (37). IL-10 reduces cellular immunity and contributes to the mechanism underlying the development and endometriosis. Estrogen increases IL-10 expression (38).

AST, ALT, ALP, and albumin can be used to evaluate patients with or suspected liver disease. ALT and AST are widely distributed throughout the body. AST is found primarily in the heart, liver, skeletal muscles and kidneys, whereas ALT is found primarily in the liver and kidneys and has lower levels in the heart and skeletal muscles (39). In the present study, we observed a significant increase in AST and ALT values in the fourth and eighth weeks compared to the control group (baseline data). ALT activity is higher than AST in many types of liver disease with the exception of alcoholic hepatitis. It seems that several causes lead to increased AST activity in alcoholic hepatitis.

Alcohol increases the activity of mitochondrial AST in plasma, whereas in other cases hepatitis does not (40). In most forms of liver injury, hepatocyte activity is decreased in both systolic and mitochondrial AST, but alcohol only causes a decrease in systolic AST activity (41). Pyridoxine deficiency (vitamin B6) is common in alcoholic hepatitis, which reduces hepatic ALT activity (42) and alcohol induces mitochondrial AST release from cells without cellular damage (43). AST and ALT are measured by catalytic activity (44), which requires pyridoxal-5-phosphate (P-5′-P) for its maximum activity, while the effect of P-5′-P reduction on ALT, more than its effect on AST (45). AST and ALT were significantly decreased in patients with renal failure compared to healthy subjects and may be due to P-5′-P serum bonds which increased overall P-5′-values. P and P-5′-P depletion is released which eventually reduces enzymatic activity (46). ALP is involved in the transport of metabolites through the cell membrane and is found in placenta, ileum mucosa, kidney, bone, and liver (47, 48). Bone, liver, and kidney isoforms of ALP have a common protein-like structure encoded by similar genes (49) but differ in carbohydrates. Hepatic isoenzyme half-life is about 3 days (50). Cholestasis stimulates ALP synthesis by hepatocytes and bile salts release ALP from the cell membrane (51). Diseases other than liver diseases that affect ALP levels include hemolysis, pregnancy, smoking, bone disease, tumors, severe enteritis (in neonates), hypophosphatase, and oral contraceptives (52, 53). The present study showed a significant increase in ALP values in the fourth and eighth weeks compared to the control group (baseline data). Because of the profound association between elevated ALP and its hepatic origin and increased activity of other canalicular enzymes (such as GGT (gamma glutathione transferase)), measurement of GGT activity is a good marker of liver ALP but is not a good test for the detection of ALP in bone diseases (54). Albumin is the most abundant plasma protein produced by liver cells. The level of albumin production depends on several factors, such as the presence of amino acids, plasma oncotic pressure, concentrations of inhibitory cytokines (especially interleukin-6) and some hepatocyte activities (55). It has been reported that antibodies to albumin have been rare in patients with endometriosis, which is consistent with the present study because we observed a (non-significant) increase in albumin in the fourth and eighth weeks compared to the control group (baseline). A significant increase in serum albumin levels to dehydration is the long-term use of tourniquet for blood sampling or evaporation. The main causes of albumin depletion include: decreased protein (nephrotic syndrome, burns, decreased protein due to intestinal damage), increased albumin circulation (catabolic states, glucocorticosteroids), reduced protein intake (malnutrition, diets with Very low protein) and liver disease (55). Plasma albumin rarely decreases in acute hepatitis because it has a long plasma half-life but gradually decreases in chronic hepatitis-progressing cirrhosis.



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Albumin is also a marker for the prognosis of liver cirrhosis (55).

### Statistical analysis

Fatty liver disease includes all types of alcoholic fatty liver disease (ALD) and non-alcoholic fatty liver disease (NAFLD). NAFLD is associated with obesity, diabetes, and insulin resistance and is considered a manifestation of the liver in metabolic syndrome. Histopathology is the gold standard for evaluating the severity of liver injury in NAFLD and ALD. NAFLD has not always been associated with increased ALT or GGT (56). It should always be kept in mind that although overall fat intake is not associated with a risk of liver cirrhosis or liver cancer, it is associated with cholesterol (57). In patients with NAFLD, approximately 30% progress from non-alcoholic steatosis to non-alcoholic steatohepatitis (NASH). Of these patients with NAFLD, approximately 20% develop cirrhosis and 30-40% of those with cirrhosis have compensatory activity and die from liver injury over a 10-year period (58). On the other hand, the first and most common histological manifestations of ALD are steatosis. About 20-40% of patients with steatosis develop into alcoholic steato-hepatitis (ASH) (59). About 40% of patients with ASH develop cirrhosis (60). Histo-pathological analysis of liver biopsy is the gold standard and the only accurate method to evaluate the rate of steatosis, necrosis-inflammatory changes, and fibrosis in NASH, and thus distinguishes NASH from separate steatosis (61). Mostly lobular inflammatory infections are composed of neutrophils but usually lymphocytes and macrophages are also seen at histopathological stages. In practice, the presence of ballooned hepato-cytes is an independent issue in the diagnosis of NASH (not steatosis). No evidence of hepatitis and fatty liver was found in the present study and at histopathological sections of the liver at the fourth and eighth time points. In the present study, uterine implants were clearly seen in the cystic area after 4 weeks of surgery. Endometriosis cysts have been reported to be characterized by severe cell infiltration, edema, and persistent inflammatory lesions. Implants, proliferation, angiogenesis, and inflammation play key roles in the development and growth of endometrial lesions (62). In this study, an autologous rat model was used to induce endometrial surgery. Prostheses appear to play an important role in the progression of endometriosis (63), and in the implant tissue, proximal nerves have been found to be the best site for release of some mediators such as NGF, which in turn Participate in the development of pelvic pain caused by endometriosis (64). Drugs play an important role in the new angiogenesis process and other manifestations of endometriosis that guarantee oxygenation to the lesions (65). In endometrial lesions, both neutrophilic and eosinophilic myeloid cells are found at all stages of puberty, showing strong cytoplasmic responses to myeloperoxidase (66).

## Conclusion

In the present study, there was a significant increase in parameters such as triglyceride, LDL, AST, ALP, ALT and estrogen following induction of experimental endo-metriosis in rats. Whereas, white blood cell count, lymphocyte, PCV and HDL counts decreased significantly. However, there was no evidence of fatty liver and liver inflammation in histopathologic evaluation, although endometriosis was confirmed at macroscopic and microscopic levels. In other words, despite significant fluctuations in some hematobiochemical factors in rats with experimental endometriosis, no evidence of fatty liver and hepatic inflammation was observed. Therefore, according to the data obtained from this study, there may be no association between endometriosis and non-alcoholic fatty liver disease, and the initial hypothesis of this study regarding the occurrence of liver inflammation and non-alcoholic fatty liver disease following endometriosis cannot be confirmed.

## Ethical standards statement



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All investigational procedures used in this study were reviewed and approved by the Council of Department of Veterinary Clinical Sciences of the Shahrekord University (13990925; P/47/170).

### Conflicts of interest

The authors declare no conflict of interest.

### Authors' contributions

General design of study and performing of experiment: MJ, FS, ES, AB; Analysis of data and designing of graphs: MJ, MH; Interpretation of Histological section: MH; Writing primary draft: MJ; Manuscript critical revision: MJ, ES, AB.

## References

1. Bulletti C, Coccia ME, Battistoni S, Borini A. Endometriosis and infertility. The Journal of Assisted Reproduction and Genetics. 2010;27(8):441-7.
2. Grummer R. Animal models in endometriosis research. Human Reproduction Update. 2006;12:641-9.
3. Wu MH, Hsiao KY, Tsai SJ. Endometriosis and possible inflammation markers. The Journal of Minimally Invasive Gynecology. 2015;4(3):61-7.
4. Berkkanoglu M, Arici A. Immunology and endometriosis. American Journal of Reproductive Immunology. 2003;50(1):48-59.
5. Acién P, Velasco I. Endometriosis: A Disease That Remains Enigmatic. ISRN Obstetrics and Gynecology. 2013;242149.
6. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55:2005-23.
7. Moeini A, Machida H, Takiuchi T, Blake EA, Hom MS, Miki T, et al. Association of Nonalcoholic Fatty Liver Disease and Venous Thromboembolism in Women with Endometrial Cancer. Clinical and Applied Thrombosis/Hemostasis. 2017;23:1018-27.
8. Matsuo K, Gualtieri MR, Cahoon SS, Jung CE, Paulson RJ, Shoupe D, et al. Surgical menopause and increased risk of nonalcoholic fatty liver disease in endometrial cancer. Menopause. 2016;23:189-96.
9. Bulun SE. Endometriosis. The New England Journal of Medicine. 2009;360:268-79.
10. Palmisano BT, Zhu L, Stafford JM. Role of Estrogens in the Regulation of Liver Lipid Metabolism. Advances in Experimental Medicine and Biology. 2017;1043:227-56.
11. Paola RD, Fusco R, Gugliandolo E, Crupi R, Evangelista M, Granese R, et al. Co-micronized Palmitoylethanolamide-/Polydatin-Reduced Endometriotic Lesion regression in a rodent model of surgically induced endometriosis. Frontiers in Pharmacology. 2016;7:382.
12. Javdani M, Nafar M, Mohebi A, Khosravian P, Barzegar A. Evaluation of Leukocyte Response due to Implant of a Controlled Released Drug Delivery System of Chitosan Hydrogel Loaded with Selenium Nanoparticle in Rats with Experimental Spinal Cord Injury. Tabari Biomedical Student Research Journal. 2022;4(2):1-6.
13. Palmisano BT, Zhu L, Stafford JM. Role of Estrogens in the Regulation of Liver Lipid Metabolism. Advances in Experimental Medicine and Biology. 2017;1043:227-56.
14. Bruner-Tran KL, Mokshagundam S, Herington JL, Ding T, Osteen KG. Rodent Models of Experimental Endometriosis: Identifying Mechanisms of Disease and Therapeutic Targets. Current Women's Health Reviews. 2018;14(2):173-88.
15. Szyllo K, Tchorzewski H, Banasik M, Glowacka E, Lewkowicz P, Kamer-Bartosinska A. The involvement of T lymphocytes in the pathogenesis of endometriotic tissues overgrowth in women with endometriosis. Mediators of Inflammation. 2003;12:131-8.
16. Gagne D, Rivard M, Page M, Shazand



[ DOI: 10.32598/tbsrj.v4i4.10517 ]

K, Hugh P, Gosselin D. Blood leukocyte subsets are modulated in patients with endometriosis. Fertility and Sterility. 2003; 80:43-53.
17. Zeller JM, Henig I, Radwanska E, Dmowski WP. Enhancement of human monocyte and peritoneal macrophage chemiluminescence activities in women with endometriosis. American Journal of Reproductive Immunology. 1987;13:78-82.
18. Othman EE-D, Hornung D, Salem HT, Khalifa EA, El-Metwally TH, Al-Hendy A. Serum cytokines as biomarkers for nonsurgical prediction of endometriosis. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2008; 137:240-6.
19. Clark JM. The epidemiology of nonalcoholic fatty liver disease in adults. Journal of Clinical Gastroenterology. 2006; 40(1): S5-S10.
20. Garzetti GG, Ciavattini A, Provinciali M, Amati M, Muzzioli M, Governa M. Decrease in peripheral blood polymorphonuclear leukocyte chemotactic index in endometriosis: role of prostaglandin E2 release. Obstetrics & Gynecology. 1998; 91:25-9.
21. Kim SK, Park JY, Jee BC, Suh CS, Kim SH. Association of the neutrophil-to-lymphocyte ratio and CA 125 with the endometriosis score. Clinical and Experimental Reproductive Medicine. 2014; 41(4):151-7.
22. Defrere S, Gonzalez-Ramos R, Lousse JC, Colette S, Donnez O, Donnez J, et al. Insights into iron and nuclear factor-kappa B (NFkappaB) involvement in chronic inflammatory processes in peritoneal endometriosis. Histology & Histopathology. 2011; 26:1083-92.
23. Gonzalez-Ramos R, Defrere S, Devoto L. Nuclear factor-kappaB: a main regulator of inflammation and cell survival in endometriosis pathophysiology. Fertility and Sterility. 2012;98:520-8.
24. Verit FF, Erel O, Celik H. Serum paraoxonase-1 activity in women with endometriosis and its relationship with the stage of the disease. Human Reproduction. 2008;23:100-4.
25. Jackson LW, Schisterman EF, Dey-Rao R, Browne R, Armstrong D. Oxidative stress and endometriosis. Human Reproduction. 2005;20:2014-20.
26. Mackness MI, Arrol S, Abbott C, Durrington PN. Protection of low-density lipoprotein against oxidative modification by high-density lipoprotein associated paraoxonase. Atherosclerosis. 1993;104:129-35.
27. Shih DM, Xia YR, Wang XP, Miller E, Castellani LW, Subbanagounder G, et al. Combined serum paraoxonase knockout/ apolipoprotein E knockout mice exhibit increased lipoprotein oxidation and atherosclerosis. Journal of Biological Chemistry. 2000;275:17527-35.
28. Rozenberg O, Shih DM, Aviram M. Human serum paraoxonase 1 decreases macrophage cholesterol biosynthesis: possible role for its phospholipase-A2-like activity and lysophosphatidylcholine formation. Arteriosclerosis, Thrombosis, and Vascular Biology. 2003;23:461-7.
29. Rozenberg O, Rosenblat M, Coleman R, Shih DM, Aviram M. Paraoxonase (PON-1) deficiency is associated with increased macrophage oxidative stress: studies in PON-1 knockout mice. Free Radical Biology and Medicine. 2003;34:774-84.
30. Aviram M, Rosenblat M, Bisgaier CL, Newton RS, Primo-Parmo SL, La Du BN. Paraoxonase inhibits high density lipoprotein (HDL) oxidation and preserves its functions: a possible peroxidative role for paraoxonase. Journal of Clinical Investigation. 1998;101:1581-90.
31. Aviram M, Rosenblat M. Paraoxonases 1, 2, and 3, oxidative stress, and macrophage foam cell formation during atherosclerosis development. Free Radical Biology and Medicine. 2004; 37:1304-16.
32. Chen P, Zhang Z, Chen Q, Ren F, Li T, Zhang C, et al. Expression of Th1 and Th2 cytokine-associated transcription factors, T-bet and GATA-3, in the eutopic endometrium of women with endometriosis. Acta Histochemica. 2012; 114:779-84.
33. Chen P, Wang DB, Liang YM. Evaluation of estrogen in endometriosis



[ DOI: 10.32598/tbsrj.v4i4.10517 ]

patients: Regulation of GATA-3 in endometrial cells and effects on Th2 cytokines. Journal of Obstetrics and Gynaecology Research. 2016;42(6):669-77.
34. Tsudo T, Harada T, Iwabe T, Tanikawa M, Nagano Y, Ito M, et al. Altered gene expression and secretion of interleukin-6 in stromal cells derived from endometriotic tissues. Fertility and Sterility. 2000;73:205-11.
35. Garcia-Velasco JA, Arici A. Interleukin-8 expression in endometrial stromal cells is regulated by integrin-dependent cell adhesion. Molecular Human Reproduction. 1999;5:1135-40.
36. Arici A. Local cytokines in endometrial tissue: The role of interleukin-8 in the pathogenesis of endometriosis. Annals of the New York Academy of Sciences. 2002; 955:101-9.
37. Ho HN, Wu MY, Yang YS. Peritoneal cellular immunity and endometriosis. American Journal of Reproductive Immunology. 1997; 38:400-12.
38. Saia RS, Bertozi G, Cunha FQ, Cárnio EC. Estradiol and thermoregulation in adult endotoxemic rats exposed to lipopolysaccharide in neonatal life. Acta Physiologica. 2011;203:429-39.
39. Adolph L, Lorenz R. Enzyme diagnosis in diseases of the heart, liver, and pancreas. New York: S Karger. 1982:9-27 p.
40. Nalpas B, Vassault A, Le Guillou A, Lesgourgues B, Ferry N, Lacour B, et al. Serum activity of mitochondrial aspartate aminotransferase: a sensitive marker of alcoholism with or without alcoholic hepatitis. Hepatology .1984; 4:893-6.
41. Pol S, Nalpas B, Vassault A, Bousquet-Lemercier B, Franco D, Lacour B, et al. Hepatic activity and mRNA expression of aspartate aminotransferase isoenzymes in alcoholic and nonalcoholic liver disease. Hepatology. 1991; 14:620-5.
42. Ludwig S, Kaplowitz N. Effect of serum pyridoxine deficiency on serum and liver transaminases in experimental liver injury in the rat. Gastroenterology. 1980; 79:545-9.
43. Zhou SL, Gordon RE, Bradbury M, Stump D, Kiang C-L, Berk PD. Ethanol up-regulates fatty acid uptake and plasma membrane expression and export of mitochondrial aspartate aminotransferase in Hep G-2 cells. Hepatology. 1998; 27:1064-74.
44. Bergmeyer HU, Scheibe P, Wahlefeld AW. Optimization of methods for aspartate aminotransferase and alanine aminotransferase. Clinical Chemistry. 1978;24:58-73.
45. Vanderlinde RE. Review of pyridoxal phosphate and the transaminases in liver disease. Annals of Clinical & Laboratory Science. 1986; 16:79-93.
46. Allman MA, Pang E, Yau DF, Stewart PM, Tiller DJ, Truswell AS. Elevated plasma vitamers of vitamin B6 in patients with chronic renal failure on regular hemodialysis. European Journal of Clinical Nutrition. 1992; 46:679-83.
47. Crofton PM. Biochemistry of alkaline phosphatase isoenzymes. Critical Reviews in Clinical Laboratory Sciences. 1982; 16:161-94.
48. Fishman WH. Alkaline phosphatase isoenzymes: recent progress. Clinical Biochemistry. 1990; 23:99-104.
49. Weiss MJ, Ray K, Henthorn PS, Lamb B, Kadesch T, Harris H.
Structure of the human liver/bone/kidney alkaline phosphatase gene. Journal of Biological Chemistry. 1988; 263:12002-10.
50. Posen S, Grundstein HS. Turnover of skeletal alkaline phosphatase in humans. Clinica Chimica Acta. 1982; 28:153-4.
51. Moss DW. Physicochemical and pathophysiological factors in the release of membrane-bound alkaline phosphatase from cells. Clinica Chimica Acta. 1997; 257:133-40.
52. De Flamingh JP, van der Merwe JV. A serum biochemical profile of normal pregnancy. South African Medical Journal. 1984; 65:552-5.
53. Young DS. Effects of preanalytical variables on clinical laboratory tests, 2nd ed. Washington, DC: AACC Press; 1997:1285 p.
54. Anciaux ML, Pelletier AG, Attali P, Meduri B, Liguory C, Etienne JP. Prospective



[ DOI: 10.32598/tbsrj.v4i4.10517 ]

study of clinical and biochemical features of symptomatic choledocholithiasis. Digestive Diseases and Sciences. 1986; 31:449-53.
55. Doumas BT, Peters T. Serum and urine albumin: a progress report on their measurement and clinical significance. Clinica Chimica Acta. 1997; 258:3-20.
56. Bedogni G, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. Hepatology. 2005; 42:44-52.
57. Ioannou GN, Morrow OB, Connole ML, Lee SP. Association between dietary nutrient composition and the incidence of cirrhosis or liver cancer in the United States population. Hepatology. 2009; 50:175-84.
58. McCullough AJ. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. Clinical Liver Disease. 2004; 8:521-33.
59. Teli MR, Day CP, Burt AD, Bennett MK, James OF. Determinants of progression to cirrhosis or fibrosis in pure alcoholic fatty liver. Lancet. 1995; 346:987-90.
60. Mann RE, Smart RG, Govoni R. The epidemiology of alcoholic liver disease. Alcohol Research & Health. 2003; 27:209-19.
61. Wieckowska A, Feldstein AE. Diagnosis of nonalcoholic fatty liver disease: invasive versus noninvasive. Seminars in Liver Disease. 2008; 28:386-95.
62. Delbandi, AA, Mahmoudi M, Shervin A, Akbari E, Jeddi-Tehrani, M, Sankian M, et al. Eutopic and ectopic stromal cells from patients with endometriosis exhibit differential invasive, adhesive, and proliferative behavior. Fertility and Sterility. 2013; 100:761-9.
63. Howard FM. Endometriosis and mechanisms of pelvic pain. Journal of Minimally Invasive Gynecology. 2009; 16:540-50.
64. Bokor A, Kyama CM, Vercruysse L, Fassbender A, Gevaert O, Vodolazkaia A, et al. Density of small diameter sensory nerve fibres in endometrium: a semi-invasive diagnostic test for minimal to mild endometriosis. Human Reproduction. 2009; 24:3025-32.
65. Groothuis PG, Nap AW, Winterhager E, Grummer R. Vascular development in endometriosis. Angiogenesis. 2005; 8:147-56.
66. Berkes E, Oehmke F, Tinneberg HR, Preissner KT, Saffarzadeh M. Association of neutrophil extracellular traps with endometriosis-related chronic inflammation. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2014; 183:193-200.



[ DOI: 10.32598/tbsrj.v4i4.10517 ]