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Tocilizumab's Potential Therapeutic Effect in the therapy of Endometriosis

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Abstract Introduction: Endometriosis is a chronic inflammatory syndrome that typically affects premenopausal women and is characterized by the growth of endometrial tissue and stromal cellbeyond the uterus.Many pro-inflammatory cytokines, particularly tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, IL8 and monocyte chemotactic protein-1 (MCP-1) play important roles in endometriosis pathogenesis.

Materials/ Methods:Endometriosis was studied using 18 of female Sprague rats. Animals were randomly separated into two sets: tocilizumab-treated animals and saline-treated animals.After endometrial tissues volumes were measured, immunohistochemical examination was evaluated.

Results:The treated and control groups had similar pretreatment spherical volumes (P>.05). There was a significant change between pretreatment and posttreatment implant volumes after treatment (P<.05).). Also immunohistochemistry technique show differences in immunoreaction intensity for IL6 in control and treated Group.

keywords: (Endometriosis. Cytokines. Interleukin-6.)

1.Introduction

Endometriosis is a chronic inflammatory syndrome that typically affects premenopausal women and is defined by the development of endometrial tissue and stromal cell beyond the uterus (Zondervan *et al*., 2018). Endometriosis affects not just the pelvic area, but also the ovaries and the rectovaginal septum. It can also occur in non-pelvic locations such as the gastrointestinal system, the abdomen, the pericardium, the skin, the lungs, the pleura, and the diaphragm (14); (19); (12). Endometrial tissue implants and proliferates in these locations, producing inflammation, fibrosis, and deformation of normal tissue while living in the ectopic sites and relying primarily on estrogen and immune response (4); (16). Clinically, the most frequent signs of endometriosis are discomfort and infertility. Pain occurs before menstruation and lasts for the remainder of the menstrual cycle. It is sometimes associated with dyspareunia, dysuria, dyschezia, and aperiodic pelvic discomfort (15); (5).

Immunological dysregulation and chronic inflammation play important roles in the development of endometriosis in the peritoneal fluid (1). Excessive levels of pro-inflammatory cytokines and chemokines found in peritoneal fluid of endometrial patients compared to non-endometrial patients suggest that their secretions are implicated in the endometriosis initiation and progression by promoting endometrial cell adhesion and proliferation (3) ; (5); (9).

Interleukin 6 (IL6) is an inflammatory cytokine that is involved in the genesis of tumours and inflammation. It belongs to the IL6 cytokine family and affects a variety of cellular metabolic and immunological processes.(8). In endometriosis, it is a pleiotropic cytokine produced by macrophages

that promotes endometrial cell proliferation and angiogenesis (17).It has been discovered to be increased in endometriosis women's PF and serum(10); (11). The purpose of this study was to investigate the efficacy of the anti-interleukin 6 (IL-6) in the treatment of endometriosis in a rat model before and after the biological treatment (Tocilizumab).

2. Materials and methods

Animal Model

In this experiment, 18 female Spraguerats weighing 200 to 250 g from the Mansoura Experimental Research Center (MERC) animal house were employed.

Rats were sedated with 50 mg/kg intramuscular ketamine, before being opened by a 5-cm vertical abdominal incision, A portion of the right uterine horn was implanted on the inner surface of the abdominal wall.

A month later, second surgery was done to assessendometrial implants. The implants were angiogenic. A digital camera was used to photograph those implants. implant volume was calculated and tissueswere then extracted from these endometrial implants for histological examination using hematoxylin and eosin stain (HE).

As a result of surgical difficulties, the residual 15 rats were separated into two groups: treatment (10 rats) and control (5 rats). The treated group received 8 mg/kg tocilizumab for 4 weeks, whereas the control group got a corresponding volume and frequency of 0.9 percent saline (Ahmed *et al.*,2020).

one month later. All rats were sedated, photos of the implants were taken and the volume of the implants was determined. After removing endometrial tissue, rats were sacrificed. Tissues were subsequently fixed in paraffin and stained with a 5 m thick H&E stain for histological examination.The presence of epithelial lining with underlying stroma verified the existence of endometrial lesions. The epithelium was categorized as either whole or attenuated..

Immunohistochemistry

The procedures were carried out in accordance with the manufacturer's instructions. 5-µm tissues were washed and antigen retrieval was accomplished by incubating for 20minutes in citrate buffer at room temperature for 20 minutes. After being treated with 3 percent H2O2 for 10 minutes to inactivate endogenous peroxidase activity, the sections were washed in phosphate-buffered saline (PBS). Primary antibodies were used to block regions.(IL-6; NB600-1131; Littleton, CO) were incubated overnight at room temperature. After washing, the sections were incubated with a goat anti-rabbit HRP conjugated secondary antibody for 1 hour at room temperature. Immunolabeling was visualised using DAB substrate, and slices were washed and dried overnight before being covered. The slices were examined using an Olympus CX31 bright field microscope.

Statistical Analysis

SPSS for Windows version 25 was used to analyse the data. The mean + SD was used to show numerical variables. To compare volume data before and after treatment, the Mann–Whitney U test was employed in each group.

3. Results and Discussion

Endometriosis has been experimentally diagnosed according to morphological examination of endometrial tissue from the implant. The implants morphologically became cysts and highly vascularized (Fig. 1).Pre- and posttreatment lesions volumes were evaluated in treated and control groups. Both treated and control groups showed similar pre-treatment spherical volumes. There was a significant difference between pretreatment and post-treatment implant volumes in the treated group.

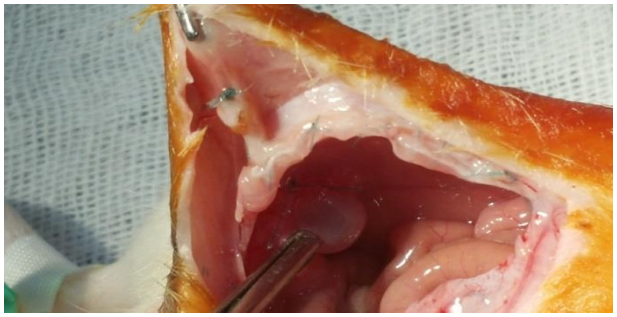

Fig 1. The morphology of uterine autografts on the abdominal peritoneum of rats

Immunohistochemistry for IL6

IL6 immunostaining was seen mostly in the epithelial cytoplasm and stroma (Fig. 2). Eutopic lesions was comparable in the control and treated groups show weak staining,while show sever stainingin the ectopic control

lesions. In ectopic treated lesions IL6 immunostaining was mild.

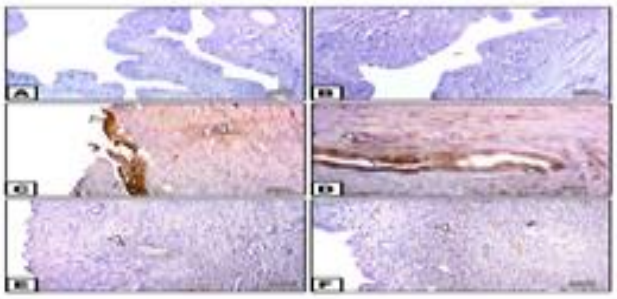

Fig 2. Immunohistochemistry for interleukin 6 (IL-6) immunostaining in the experimental groups. Immunostaining of endometrial eutopic lesions revealed low IL-6 immunoreaction in (A) the tocilizumab group eutopic and (B) the control group eutopic (C) and (D) the control group ectopic implant. Tocilizumab group ectopic implant showed minimal IL-6 immunoreaction (E) and (F). 100X magnification was used in the original.

Discussion

Endometriosis is a disease that affects menopausal females, characterized by symptoms ranging from pelvic discomfort to infertility (7). Endometriosis, while being a benign gynecological condition, is a burdensome disease for females owing to the symptoms that develop.The most prevalent symptoms are pelvic pain, vaginal injury, infertility, and malignant change. Accurate diagnosis of endometriosis might be delayed for years in the absence of particular symptoms and indicators. Complex interplay between genetic profile, hormonal activity, menstrual cycle, inflammatory state, and immunological factors form the endometriosis phenotype. Although the link between infertility and endometriosis is still contested, it is clinically recognised and well-documented in several studies (7).

Many anti-endometriosis medicines or prospective treatments, such as hormonal and immunomodulatory agents, impact the amount and profile of cytokines in endometriosis at the same time, as previously indicated. When opposed to reducing solely inflammatory cytokines, endometriosis therapy targets both inflammatory and anti-inflammatory cytokines, innate macrophages, and natural killer (NK) cells. Endometriosis therapy might be more successful (21).

In this study, experimental rat model was used to explain tocilizumab (TCZ) as an effective medicine for endometriosis treatment. Our current study explained that treatment with TCZ had histological and immunoreactive intensity differences in epithelial layer and also reduced implants volume.

Previous researches has shown that anti-IL-6 therapy can help cure endometriosis in the Wistar rat model. They used a surprisingly similar strategy to produce endometriosis in Wistar rats without using any exogenous estrogen. The test group received tocilizumab, whereas the control group received saline. The volume of the endometriotic lesions was found to be greatly reduced in the test group, a finding was identical to ours. The epithelium was observed to be considerably attenuated in the test group as compared to the control, whereas we discovered a propensity for attenuation in the test group without statistical significance.Furthermore, treatment with tocilizumab lowered VEGF levels in both ectopic and eutopic endometrium (18).

demonstrated that no statistically significant in IL-6 score immunohistochemistry between test and control groups. In contrast, we discovered that the immune-histochemical staining intensity of the epithelial lining of ectopic endometrium was strong in all control rats, but mild in the test group.

By inhibiting the death of ectopic implants in the peritoneum with tumour necrosis factor-alpha, IL-6 contributes to the genesis of endometriosis under a variety of inflammatory, environmental, and genetic effectors(6). IL-6 also promotes theemigration of endometrial implants, which can contribute to the development of extra-pelvic endometriosis (20). Researchers are looking for non-hormonal avenues to target in the therapy of the endometriosis microenvironment after the recent identification of a link between endometriosis and immunology(13).

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