



Evaluation of serum oxidative stress markers in women with leiomyoma

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Abstract

Background & Aims: Background: One of the most common benign tumors that arise from the smooth muscles of the uterine wall is leiomyoma. In this study, the levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) were assessed in leiomyoma patients and compared with healthy individuals.

Materials & Methods: In this case-control study, 50 women with leiomyoma and 50 healthy women were selected as case and control groups, respectively. ELISA method was used to measure MDA and TAC.

Results: The results showed that the serum level of MDA in leiomyoma patients increased significantly, as compared to healthy individuals (0.52 ± 1.48 vs. 0.65 ± 1.3), while that of TAC had a significant reduction in patient group compared to healthy individuals (151 ± 151 vs. 466 ± 212).

Conclusion: Our findings show a relationship between leiomyoma and oxidative stress indicators.

Keywords: Leiomyoma, Malondialdehyde, Total antioxidant capacity

Received 15 July 2022; accepted for publication 22 February 2023

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Introduction

Leiomyoma, also called myomas or fibroids, is one of the most common benign tumors in women occurs in their 50s (1, 2). Evidence has revealed that most of these benign tumors are asymptomatic. Pathogenesis of the disease is unknown, but abnormal uterine bleeding or abdominal pain has been reported by 25% of women. Identifying the risk factors of a disease can have beneficial effects on the treatment of illnesses and surgical procedures (3, 4). For instance, leiomyoma in acute stages requires uterine surgery and removal of

the uterus. Leiomyoma is caused by the smooth muscles of the uterine wall and is dependent on the hormones estrogen and progesterone. The causes of this disease are not yet well known, but it seems that factors such as age, premature menarche, blood pressure, obesity, and uterine infections, vascular abnormalities, smoking, and contraceptives can affect the incidence of the disease (5, 6). Studies have shown that most of these benign tumors reach their maximum growth during female reproduction and are easily detectable.

Oxidative stress indices are highly effective in the early detection of diseases, and reactive oxygen species have several physiological functions (7, 8). In mammals, in addition to the overproduction of reactive oxygen species, there is a good antioxidant system that is composed of the malondialdehyde (MDA), total antioxidant capacity (TAC), and glutathione (GSH) (9, 10). The natural product of the peroxidation of unsaturated fatty acids is malondialdehyde. In leiomyoma, free radicals are formed by increasing oxygen consumption and increasing metabolism (11, 12). Studies have demonstrated different results between increasing or decreasing the oxidative and antioxidant activity in leiomyoma (13). It has also been exhibited that there is a link between the antioxidant system and sex hormones during endometrial cycles, in which superoxide dismutase plays an important role in maintaining the integrity of luteal cells. Estrogen appears to reduce the production of free radicals, and estrogen synthesis in postmenopausal women is responsible for the production of oxidative stress indicators (14). Numerous investigations have suggested that natural molecules such as vitamin D and vitamin B6 are effective for benign tumors such as uterine fibroids. In a recent study, Cibiera et al. have indicated that natural compounds are useful for treating fibroids and using vitamins D and A could improve the symptoms of uterine fibroids in women (15). In this study, we investigated the level of MDA and TAC in leiomyoma patients as a case group and compared these patients with healthy individuals as a control group.

Materials & Methods

Patients:

As endometriotic cells display an increase in endogenous oxidative stress and alterations in ROS detoxification pathways, women with macroscopic endometriosis during abdomino-pelvic surgical exploration, as well as past history of hormonal and/or surgical treatment for endometriosis, were excluded from the study. The inclusion criteria were women who referred to a gynecologist, and their disease was

diagnosed by ultrasound by a specialist. Overall, 50 women with leiomyoma and 50 healthy women, as case and control group, respectively, were included in the study. The serum level of MDA was measured as a biological indicator of lipid peroxidation, and that of TAC was measured by ELISA. To perform measurements, we collected 5 ml of venous blood from the patients, and after centrifugation and separation of the serum, the samples were kept at -20 °C for analysis.

MDA assay:

ELISA method was used to measure the serum level of MDA using Human Malondealdehyde ELISA Kit (Bioassay, China). For this purpose, 20 standard solutions were first mixed and diluted with 120 µl of diluent, and then 50 µl of standard and horseradish peroxidase solution (40 µl of serum and 10 µl of MDA antibodies) were added to each microplate. After washing and adding dyes A and B to the wells, a reaction stopping solution was added to microplates. Finally, the light absorption of the samples was read by an ELISA reader at a wavelength of 450 nm, and the unit conversion was performed. The sensitivity of ELISA kit was 0.14 nmol/ml, and the diagnostic range was 0.2-60 nmol/ml.

TAC assay:

To measure the TAC, we used the ELISA method and kit (Zellbio, Germany). The kit contained one ready-to-use reagent, ×100buffer, dye powder, reaction-stopping solution, and a 96-well microplate. In this assay, the amount of the TAC, equivalent to an amount of antioxidants in the sample, was compared with ascorbic acid as a standard. The sensitivity of kit was equal to 0.1 mM, and the diagnostic range was 0.125-2 mM. The final absorbance was read at 490 nm, and unit conversion was performed.

Statistical analysis:

The collected data were analyzed using SPSS software (version 21). The student's t-test was used to compare the groups. A p-value of < 0.05 was considered statistically significant.

Results

The characteristics of patients and fibroids, as well as laboratory findings of the two study groups are presented in Table 1. Among the 50 histologically proven leiomyoma patients, the mean number of leiomyoma was 5.5 ± 7.4 for each woman. Also, the mean total weight of uterine leiomyoma and the mean total size were 193.6 ± 364.7 g and 15.8 ± 10.4 cm, respectively. There were no differences in age and parity between case and control groups. Blood serum

samples from 50 leiomyoma patients and healthy subjects were analyzed, and TAC level and MDA activity were measured. Patients with leiomyoma showed significantly lower TAC levels, as compared to the control group. There was also a statistically significant difference between the case and control groups so that MDA analysis indicated a significant increase in the MDA activity of leiomyoma patients relative to healthy group.

Table 1. Baseline characteristics of patients and fibroid and laboratory findings

Characteristics	Fibroids (n=50)	Controls (n=50)	P value
Patients' characteristics			
Age (years)	31.2 ± 2.7	23.7 ± 4.5	0.407
Height (cm)	153.8 ± 5.7	154.6 ± 4.1	0.327
Weight (kg)	55.7 ± 11.0	52.1 ± 11.3	0.050
Parity ^a	0.3 ± 0.5	0.3 ± 0.9	0.327
Dysmenorrhea	3.7 ± 4.3	3.7 ± 3.1	0.068
Lower urinary symptoms	0.1 ± 0.5	0.1 ± 0.5	0.932
Laboratory findings			
CRP (mg/l)	1.5 ± 0.7	2.2 ± 4.2	0.120
WBC (U/mL)	$4700,0 \pm 1128.5$	5416.7 ± 1621.8	0.101
Fibroid characteristics			
Total weight (g)	193.6 ± 364.7	NA	NA
Total size (cm)	15.8 ± 10.4	NA	NA
Total number	5.5 ± 7.4	NA	NA

Table 2. Comparison of TAC levels with MDA activity in study and control groups

Parameter	Control (n = 50)	Cases (n = 50)	P value
TAC	466 ± 212	321 ± 151	0.022
MDA	1.3 ± 0.65	1.48 ± 0.5	0.003

Discussion

In this study, the serum levels of MDA and TAC were evaluated in patients with leiomyoma and compared with healthy individuals. The results showed that the serum MDA level increased, while that of TAC decreased. The production of oxidative stress indicators occurs when there is no balance between the production and neutralization of free radicals due to normal aerobic metabolism or pathological functions

inside and outside the cell. As a result, body cannot counteract the harmful and destructive effects of overproduction of free radicals neutralized by producing antioxidants (16, 17).

Oxidative stress markers play an important role in the early detection of diseases, including leiomyoma (18). MDA and TAC have an effective role in a wide range of diseases such as endometriosis (19). The results of studies by Hilali et al. have shown that the

serum level of MDA in female patients with leiomyoma increased compared to healthy women (20, 21). Similarly, Zhu et al. have reported that the serum levels of MDA, SOD, and GSH increase in women with leiomyoma compared to healthy women (22). Zhang et al.'s research has also indicated that the MDA level in patients with endometriosis enhances compared to the control group (23). The results of Turan et al.'s study showed that serum levels of MDA and GSH in patients with endometriosis increased compared to healthy individuals (24).

Although there is limited information on the causes of leiomyoma, it seems that the production of ROS causes growth and endometrial cells adhere to the uterine cavity (25). Studies have shown an increase in the level of ROS markers and a decrease in the overall level of antioxidants in the serum of women with leiomyoma. Turkilmaz et al. displayed an increase in the serum MDA levels and TAC in patients with leiomyoma (26) and concluded that antioxidants could be used as a valuable treatment for leiomyoma in future. However, conflicting results have been reported in some studies with more patient samples that failed to show oxidative or antioxidant balance. Pejić et al. exhibited that the serum level of MDA in patients with leiomyoma increased and the level of TAC decreased and deduced that the higher levels of MDA may indicate oxidative and antioxidant imbalance in patients with leiomyoma (27).

Conclusion

The findings of this study show that serum levels in patients with leiomyoma are different from those in healthy individuals, which can be effective in the rapid diagnosis of the disease and the diagnosis and identification of oxidative stress markers predisposing to the disease. Altogether, test on more samples, homogeneous patients, and different measurement methods is necessary to achieve definitive results.

Acknowledgments

We thank the management and staff of Ali Ibn Abi Taleb Hospital Research Centre, Zahedan, Sistan and

Baluchestan Province for providing the clinical samples of the subjects enrolled in this study.

Conflict of interest

The authors have no conflict of interest in this study.

Funding/support

The authors would like to acknowledge Dr. Mohammad Reza Rezaei, Dr. Hosnie Hoseini, and Dr. Fatemeh Rezaei for their financial support.

Data availability

The raw data supporting the conclusions of this article are available from the authors upon reasonable request.

Ethical Statement

This study was conducted according to the guideline for case series and approved by the Ethics Committee of Ali Ibn Abi Talib Hospital in the Zahedan Medical University, Zahedan, Iran (ethical code: IR.IAU.ZAH.REC.1400.010). Before the initiation of the study and after a detailed explanation of the therapeutic procedure, informed consent forms were signed by all the patients. A written consent was also obtained from each participant.

Authors' Contributions

All authors have contributed to data collection and wrote the manuscript. MR and HH conceived the structure of the manuscript and revised the manuscript. HH and FR made the figures and tables. HH, MR, and FR drafted the initial manuscript. All authors have read the manuscript, approved the current manuscript for publication, attested that they substantially contributed to the current work, and disclosed that there was no writing assistance. All authors have read and approved the final version of manuscript.

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