

Serum Vitamin D, Superoxide Dismutase and Catalase Levels in Females with Uterine Fibroid

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ABSTRACT

Objective: To determine the serum vitamin D, superoxide dismutase and catalase levels in females with uterine fibroids.

Methodology: This study was performed in the Obstetrics & Gynaecology Department of Sharif Medical City Hospital, Lahore after taking approval from the ethical committee of the institution. It was a case-control study that included 25 females of reproductive age (25-45 years) having uterine fibroid diagnosed on ultrasonography & 25 females were enrolled as age and gender matched controls. Informed written consent was obtained from all patients before taking the blood sample. Serum vitamin D, SOD and CAT levels were measured by ELISA kit.

Results: This study analyzed serum vitamin D and antioxidant enzymes SOD and CAT levels in uterine fibroid women which were compared with healthy women (non-fibroid females) of the same age group. The mean levels of vitamin D and antioxidant enzymes SOD & CAT in patients with uterine fibroid were 11.29 ± 1.08 pmol/L, 0.13 ± 0.0019 U/ml and 3.30 ± 0.0264 U/L respectively. In the control group the levels of vitamin D, SOD & CAT were 17.88 ± 2.16 pmol/L, 0.49 ± 0.0034 U/ml and 5.26 ± 0.376 U/L respectively. The levels of vitamin D and antioxidant enzymes SOD and CAT were found significantly decreased in females with uterine fibroid as compared to the controls.

Conclusion: The levels of vitamin D and antioxidant enzymes SOD and CAT are decreased in uterine fibroid females in comparison to the healthy females. It indicates a positive relationship between reduced levels of vitamin D, SOD and CAT to the risk of development of uterine fibroid. So, vitamin D and antioxidants supplementation might reduce the risk factor of uterine fibroid and can be helpful in the non-surgical treatment of uterine fibroid.

Keywords: Uterine fibroid. Serum vitamin D levels. Superoxide dismutase (SOD). Catalase (CAT). ELISA.

INTRODUCTION

Uterine fibroids also called leiomyomas are benign monoclonal tumors of smooth muscle cells originating from myometrium.¹ Uterine fibroid is the most common benign gynecological tumors appearing in 60-80% of women in the reproductive age group.² At 50 years of age the incidence rate of uterine fibroid may reach approximately 70-80% in the western population.³ According to the literature majority of the patients present with uterine fibroid usually between ages of 28-52 years.⁴ Majority cases of fibroids are asymptomatic and remain undiagnosed while other present with multiple symptoms such as heavy menstrual bleeding, dysmenorrhea, chronic abdomino-pelvic pain. Some women feel only pressure effects such as bloating and bowel disturbances.⁵ Most of the fibroids located beneath the endometrium affect the endometrial blood supply to the embryo which further ceases the growth and early development of embryo causing subfertility abortions and late pregnancy complications.^{5,6} Normal vitamin D levels play an important role to decline the growth of the fibroid. Normal levels of vitamin D in

both children and adults is >30 ng/ml (67.41 pmol/L) and vitamin D levels <20 ng/ml (44.94 pmol/L) are considered as vitamin D deficiency.⁷ Decreased levels of serum vitamin D is considered as a risk factor for the development of uterine fibroid. Vitamin D is known to act as an antifibrotic agent causing fibrosis of human uterine fibroid by acting on transforming growth factor beta-3 (TGF β 3). Vitamin D inhibits the growth of uterine fibroid by different mechanisms. It declines the growth of cells and induces apoptosis through the suppression of catechol-O-methyltransferase (COMT) and downregulation of cyclin-dependent kinase (CDK1).⁸ Vitamin D is also a membrane antioxidant causing a reduction in the oxidative stress which leads to shrinkage of uterine fibroids.⁹ Another mechanism of action of vitamin D is that it acts as a profibrotic factor and inhibits the growth of the tumor by acting on vitamin D receptor (VDR). Vitamin D receptor is a nuclear transcription factor that induces growth arrest and enhances the process of apoptosis. Vitamin D also inhibits the TGF β 3 dependent key profibrotic factors which cause a paradoxical increase in matrix metalloproteinases (MMPs) in uterine fibroids. So, serum vitamin D inhibits the expression of MMPs which cause shrinkage of fibroid.¹⁰ Vitamin D shows antiproliferative action and regulates different regulatory genes and controls the activity of cyclin-dependent kinases (CDKs), which further arrest the G0-G1 phase by interrupting the S phase and decreasing the number of cells in the uterine fibroid. Another important factor involved in the development of uterine fibroid is oxidative stress which leads to the

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deposition of newly formed extracellular matrix (ECM) in the uterine fibroid.¹¹ The main cause of oxidative stress is generation of reactive oxygen species (ROS) such as NO, H₂O₂ and hydroxyl radical all of which are involved in an imbalance between connective tissue production and degradation resulting in deposition of extracellular matrix in uterus and also an increase in the production of cytokines and growth factors, angiogenesis, proliferation and inhibition of apoptosis. All these processes simultaneously promote the development of uterine fibroid.¹² Several antioxidant enzymes play a major protective role against oxidative stress such as SOD and CAT. Superoxide dismutase catalyzes superoxide ion to hydrogen peroxide and CAT further catalyzes H₂O₂ into O₂ and H₂O which protect the cell and maintain cellular redox balance.¹³

The objective of this study was to find out the levels of serum vitamin D and serum antioxidant enzymes SOD and CAT in females of reproductive age group with uterine fibroid in comparison to the healthy controls (non-fibroid females). The study focused on the probable therapeutic role of vitamin D supplementation and antioxidants in the uterine fibroid.

METHODOLOGY

This study was conducted at the Obstetrics & Gynaecology Department of Sharif Medical City Hospital, Lahore. In this study, 50 participants were enrolled who underwent a gynaecological ultrasound scan at our institution. Out of 50, 25 women were diagnosed with the uterine leiomyomas on ultrasound and 25 served as control subjects (non-fibroid females). The patients with history of total thyroidectomy, total hysterectomy, osteoarthritis and parathyroid disorder were excluded from the study. All patients variables including age, height, weight, age of menarche, menopausal status, alcohol drinking, smoking status, any past medical history, any past surgical history, present medication and also the purpose of the study were recorded in the form of a questionnaire. Using aseptic techniques, 5 ml of the venous blood sample was collected from the median cubital vein after taking informed written consent. Serum was separated from the blood after centrifugation and serum vitamin D, SOD and CAT levels were measured using a standard ELISA Kit.

STATISTICAL ANALYSIS

The data was analyzed using Statistical Package for the Social Sciences (SPSS) version 23 and expressed as the mean±standard deviation. The results of the two groups were compared by applying independent t-test. A p-value ≤0.05 was taken as statistically significant.

RESULTS

The study showed a significant decline in serum vitamin D levels in uterine fibroid women (11.29±1.08 pmol/L) as compared to the healthy females (17.88±2.16 pmol/L) (Figure 1). Table 1 showed that the results were statistically significant with a p-value of 0.027.

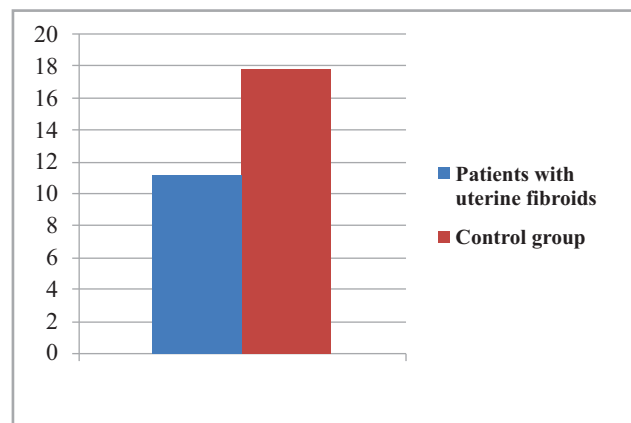


Figure 1: Levels of Vitamin D (pmol/L) in Study Subjects

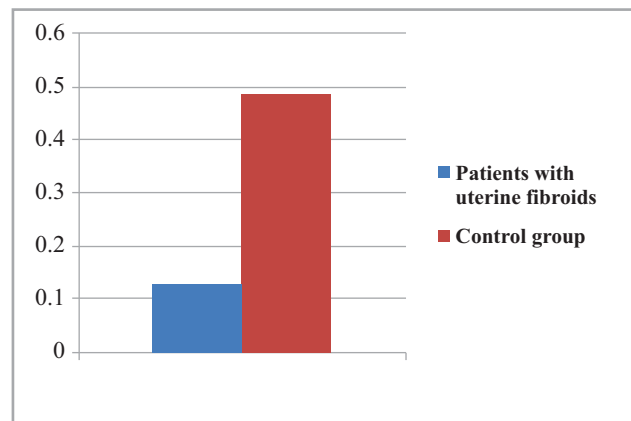


Figure 2: Levels of SOD (U/ml) in Study Subjects

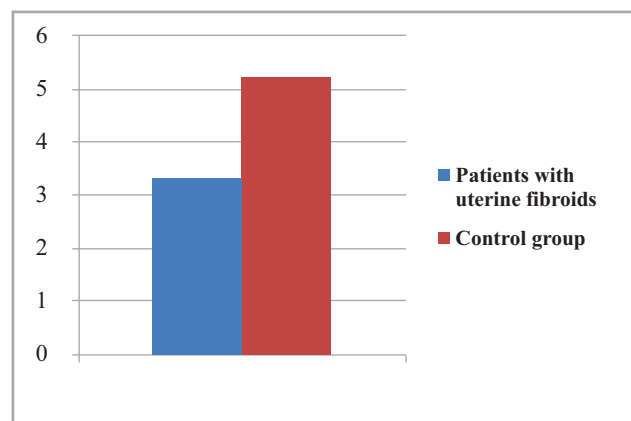


Figure 3: Serum CAT (U/L) Levels in Study Subjects

Table 1: Comparison of Vitamin D, SOD & CAT Levels in Patients with Uterine Fibroid and Control Group

Study Variables	Patients with Uterine Fibroids	Control Group	p-value
Vitamin D	11.29±1.08 pmol/L	17.88±2.16 pmol/L	0.027
SOD	0.13±0.0019 U/ml	0.49±0.0034 U/ml	0.011
CAT	3.30±0.0264 U/L	5.26±0.376 U/L	0.016

The levels of antioxidant enzyme SOD and CAT in uterine fibroid women were 0.13±0.0019 U/ml and 3.30±0.0264 U/L respectively. In the healthy females, the SOD & CAT levels were 0.49±0.0034 U/ml and 5.26±0.376 U/L respectively. The levels of SOD and CAT obtained were statistically significant in uterine fibroid females with p-values of 0.011 and 0.016 respectively (Figure 2, 3 & Table 1).

DISCUSSION

Oxidative stress plays a major role in different profibrotic gynecological disorders such as fibroids, endometriosis and postoperative adhesions. Several powerful antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) protect cells against the oxidative damage and maintain the cellular redox balance.¹⁴ Vitamin D is a strong antioxidant and the main regulator of calcium homeostasis. It has the property to decrease the whole process of lipid peroxidation that is why vitamin D is comparable to other anticancer drugs like tamoxifen and its 4 hydroxy metabolites.¹⁵ The current study shows a significant decrease in the levels of vitamin D, SOD and CAT in a patient with fibroid uterus compared to healthy controls (non-fibroid females). The results obtained were statistically significant and we found that vitamin D, SOD and CAT are inversely related with the occurrence of uterine fibroid.

In another study by Sabry et al., the serum vitamin D levels were measured in females with uterine leiomyomas. A statistically significant relationship of low serum vitamin D and the incidence of uterine leiomyoma was found (p-value= 0.01) coinciding with the results of the present study. According to them, normal levels of vitamin D act as a protective factor in uterine fibroid as it has an ability to cause apoptosis and inhibit the proliferation of leiomyoma cells which leads to shrinkage of uterine fibroid.¹⁶ In another study by Baird et al., it was indicated that sufficient vitamin D is associated with reduced risk of uterine fibroid. He randomly selected 35-49 year old females and their fibroid status was determined by ultrasound screening. Vitamin D status was assessed in stored plasma by radioimmunoassay. After exposure to sun rays, it was seen that each 10 ng/ml increase in 25(OH) D was associated with a 20% lower risk of fibroid, strengthening the importance of normal vitamin D levels indicated in the present study.¹⁷ Another study by

Abdelraheem et al., identified an inverse relation between serum vitamin D level and uterine fibroid volume and also pointed out an inverse relationship between serum vitamin D level and mean number of uterine fibroid. The results were similar to the present study.¹⁸ Brakta et al. also demonstrated that decreased serum vitamin D levels are associated with an increased risk of uterine fibroid.¹⁹ Halder et al. determined whether vitamin D₃ inhibits TGF β₃ induced fibrosis related gene expression in fibroid cells. He concluded that vitamin D₃ acts as an antifibrotic factor that reduces TGF β₃ which automatically inhibits phosphorylation of SMAD2 and limits the fibrosis of uterine fibroid, so vitamin D₃ is potentially useful therapeutically in the uterine fibroid.²⁰ According to Halder et al., vitamin D is a potent anti-estrogenic receptor and can reduce estrogenic action in uterine leiomyomas that's why vitamin D might be used as a treatment option in the uterine fibroid.²¹ Another research conducted by Halder et al., pointed out that vitamin D₃ acts as an antitumor agent and treatment with vitamin D shrinks uterine fibroid cells in an Eker rat model.²² These results potentiate the therapeutic significance of vitamin D pointed out in the present study. According to Blauer et al., vitamin D affects the cell growth of the uterine fibroid. He found that normal vitamin D levels inhibited growth in uterine leiomyomas 12% in comparison with the controls. This study strengthens the results of the current study according to which low vitamin D levels were seen in uterine fibroid females thereby further potentiating the need for vitamin D supplementation in females with fibroids.²³

According to Ciavattini et al. and Paffoni et al., decreased serum levels of vitamin D are strongly associated with the prevalence of uterine leiomyomas similar to our results.^{24,25} According to Mitro et al., there is no indicated relationship between decreased serum vitamin D levels and the prevalence of uterine fibroid. In his analysis of 3,590 from the National Health and Nutrition Examination Survey (NHANES) 2001-2006 no significant relationship was observed between uterine leiomyoma and serum vitamin D contrary to the results of the current study.²⁶

Superoxide dismutase is a main key antioxidant enzyme that plays a protective role in lipid peroxidation and is responsible for the elimination of superoxide radicals. In our study SOD levels were decreased in patients with uterine leiomyomas as compared to the

healthy females. Our results are consistent with another study by Pejic et al. According to them, serum levels of SOD in the blood are decreased 20% in patients with uterine myomas in comparison to the controls. Activated oxygen metabolites are responsible for cell damage and reduced antioxidant SOD might be responsible for uterine malignancy.²⁷ This is in accordance with the current study. In some other studies, contrary to the result of our study, SOD levels in different tissue with neoplastic changes appeared to be increased and remained unchanged in comparison to the healthy females.²⁸ Pejic et al. demonstrated that serum levels of SOD in leiomyoma and the different gynecological patient is decreased comparatively to the healthy subjects but serum levels of CAT remain unchanged in myoma patients,²⁹ which is contrary to the present study. Manoharan et al. concluded that there is an increased serum CAT level in cervicitis patients and decreased levels of CAT in myoma patients,³⁰ similar to the current study.

In our study, it has been observed that there is a decreased serum level of vitamin D and antioxidants SOD and CAT in uterine fibroid female as compared to the non-fibroid uterus females (controls). Our study results support that decreased levels of serum vitamin D and antioxidants are strongly associated with the occurrence of uterine leiomyomas.

CONCLUSION

The levels of serum vitamin D, SOD and CAT are decreased in uterine fibroid females in comparison to the non-fibroid uterus females. Vitamin D and antioxidant supplementation on a regular basis in daily routine might reduce the risk of occurrence of uterine fibroid. Hence, vitamin D and antioxidants can be useful as a non-surgical tool in treating uterine fibroids.

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