Original Article

Serum versican as a potential biomarker in patients with uterine fibroids: A study from Eastern India

Pratima Jasti[1], Suchitra Kumari[1], Sweta Singh[2], Anudeep PP[1]

[1]Department of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India, [2]Department of Obstetrics and Gynaecology, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India

Abstract

Objective: Versican is a chondroitin sulphate proteoglycan with raised expression at site of inflammation, and uterine fibroids are associated with local inflammation. Hence, this study aimed to estimate serum Versican levels in pre-menopausal women with uterine fibroids to evaluate its diagnostic efficiency. **Materials and Methods:** This case–control study included forty uterine fibroid cases and 40 healthy controls. Cases clinically evaluated with USG findings, that is number, location of fibroid and volume calculated by prolate ellipse formula a × b × c × 0.523 (a – height, b – width, c – depth). Biochemical investigations, that is serum Versican levels, were estimated by ELISA with total cholesterol, HDLc and LDLc. Triglycerides by fully automated chemistry analysers. Serum biochemical parameters were compared and correlated with volume of fibroid. Area under receiver operating characteristic curve was calculated along with cut-off value to determine diagnostic potential of Versican, differentiating women with fibroids. **Results:** In the present study, patients with fibroids had decreased levels of serum Versican (79.43 ± 18.60) as compared to healthy controls (101.81 ± 28.24, $P < 0.001$). There was a statistically significant negative correlation ($r = -0.307$, $P = 0.04$) between serum Versican level and volume of fibroid. Area under ROC was 0.726 (95% CI: 0.616-0.836; $P = 0.001$). The best cut-off value for serum Versican level was 96.90 ng/ml with 90% sensitivity and 48% specificity. **Conclusion:** Serum Versican levels were found significantly lower in women with fibroid with a negative correlation with volume of fibroid uterus. Furthermore, extensive study would help in substantiating diagnostic potential of serum Versican in fibroid uterus patients.

Keywords: Prolate ellipse formula, serum Versican, uterine fibroid

Introduction

Uterine fibroids (leiomyoma) are the most common benign mass in the female reproductive tract composed of smooth muscle and extracellular matrix. Its increasing incidence adversely affects the quality of reproductive life, that is abnormal uterine bleeding, infertility and abdominal pain in premenopausal women.

Uterine leiomyomas are composed of modified smooth muscle cells and a large amount of extracellular matrix (ECM).[1] Smooth muscle cells and a substantial amount of extracellular matrix (ECM) make up uterine fibroids.[2] One of the hallmarks of uterine fibroid is excessive ECM deposition, which is mainly responsible for the large sizes of fibroid despite having slow rate of smooth muscle proliferation Compared to normal myometrium, ECM in uterine fibroids shows increased collagens, laminins, fibulin-3, proteoglycans like chondroitin sulphate, dermatan sulphate, matrix metalloproteinase (MMPs) and tissue inhibitor of metalloproteinase (TIMPs), which are mediated by growth factors like TGF-β, Activin-A, PDGF, TNF-α and cytokines IL-11 and IL-13.[3] An inappropriate inflammatory response is proposed as one of the causes of uterine leiomyoma.[4] In the inflammatory milieu, myofibroblast activated to produce ECM components. But chronic inflammatory states lead

Address for correspondence: Dr. Suchitra Kumari, Department of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar - 751 019, Odisha, India.
E-mail: suchitrakumari76@gmail.com

Received: 18-02-2023 **Revised:** 23-06-2023
Accepted: 26-06-2023 **Published:** 29-08-2023

Access this article online

Quick Response Code:

Website: http://journals.lww.com/JFMPC

DOI: 10.4103/jfmpc.jfmpc_320_23

How to cite this article: Jasti P, Kumari S, Singh S, Anudeep PP. Serum versican as a potential biomarker in patients with uterine fibroids: A study from Eastern India. J Family Med Prim Care 2023;12:1704-9.

to increased and continuous ECM production leading to fibrosis.[5] Versican is a chondroitin sulphate proteoglycan and is one of the proteoglycans in ECM present widespread in various tissues. It is involved in cell adhesion, proliferation, migration and extracellular matrix assembly.[6] Previous studies have demonstrated role of versican as anti-adhesive molecule.[7-10] But its pro-adhesive properties are also reported recently.[11-13] Hence, versican plays a complex role in regulating the cell adhesion in extracellular environment. Elevated versican levels in fast-growing tissues point out role of versican in cellular proliferation. Pro- and anti-apoptotic effect of versican also have been studied. Earlier studies showed increased versican at the site of inflammation in ECM and uterine fibroids is developed by inflammation due to chronic stress like hypoxia, oxidative stress, implantation and mechanical stimuli.[14] Increase of versican is mediated by growth factors like PDGF and TGF-β, and also, it is known that in uterine fibroids, TGF-β is increased.[15] A few studies showed that there is increased expression of versican in uterine leiomyomas compared to myometrium.[8,12,15] However, we have less information about serum versican level in uterine fibroids patients and its association with the disease progression.

The middle and low-middle sociodemographic index population has the major burden of uterine fibroids.[16] In India, with increasing prevalence of asymptomatic uterine fibroids, it serves as a great challenge to primary care providers and family physicians dealing these patients routinely. Raising awareness of uterine fibroids, prompt diagnosis and improving levels of medical care are necessary to reduce future burden. As per earlier studies, there is an increased expression of versican in uterine leiomyomas; hence, it could be a surrogate marker for early diagnosis. This study was conducted with an objective to evaluate the serum versican levels in fibroid uterus cases as compared to healthy women and to correlate it with the volume of the fibroid mass, to explore the diagnostic potential of serum versican.

Materials and Methods

Subjects

A case–control study was conducted in the Department of Biochemistry, All India Institute of Medical Sciences, Bhubaneswar. Forty premenopausal women with ultrasonographically identified uterine fibroids (at least one fibroid) within age group of 25-45 yrs were enrolled as cases and 40 women without ultrasonographically identified UFs, apparently healthy, of age group 25-45 yrs, irrespective of their parity, attending the OPD of Obstetrics and Gynaecology Department, AIIMS Bhubaneswar, from July 2020 to May 2021, were enrolled as control in the study. Patients with idiopathic interstitial pneumonia, cardiovascular disorders, lung diseases, cancer, inflammatory bowel disease, PCOS, pregnancy, current use of oral contraceptives and known cases of diabetes and hypertension were excluded from the study.

Sample size

Sample size was calculated based on the Z_b value of 1.65 for 95% power and Z_a value of 1.96 for 0.05 significance level and difference as half of standard deviation.

Methods

This study was approved by Institutional Ethical Committee of AIIMS Bhubaneswar (IEC/AIIMS BBSR/STS/2020-21/2). All the study participants, that is, 40 cases and 40 controls, were evaluated on the basis of: questionnaire developed, biochemical investigations and clinical evaluation with ultrasonography (size and number of fibroids).

Clinical evaluation

Questionnaire was prepared pertaining to the study, that is parity (no of childbirth), age of menarche, last menstrual period, history of intake of oral contraceptive pills, miscarriage and any known case of diseases like hypertension, CVD and PCOS. The blood pressure was recorded. Height and weight were measured to calculate body mass index (BMI). Volume of all uterine fibroids was measured using prolate ellipse formula- $a \times b \times c \times 0.523$, where a is height, b is width, and c is depth of fibroid obtained from ultrasound.

Biochemical analysis

After having informed written consent, 3 ml of venous blood was collected by venepuncture from all participants for biochemical evaluation; then, it was processed to separate serum within 1 hour after withdrawal and was stored at -80°C until needed for assays. Serum versican level was evaluated by enzyme-linked immunosorbent assay (ELISA) using a commercial human VCAN (versican) ELISA kit (Elabscience, TX, USA) as per the manufacturer's recommendations. In brief, sample and standard were added to the respective wells in the ELISA plate and incubated, followed by a wash step, then by addition of biotinylated detection antibody working solution to each well and incubation. After the wash, HRP conjugate working solution was added and incubated. Subsequently, substrate reagent was added followed by addition of stop solution to stop the reaction. Plate results were measured at 450 nm using the ELISA reader (Biotek ELx50, VT, USA). Fasting plasma glucose and lipid profile were evaluated by fully automated chemistry analyzer (Beckman Coulter 5800, AU platform). Biochemical test results, clinical findings and anthropometric measurements were recorded for all participants in their case study form.

Statistical analysis

Data obtained from questionnaire, biochemical and clinical evaluation were analysed statistically using SPSS software. Various parameters like true-positive (TP), false-positive (FP), false-negative (FN) and true-negative (TN) were calculated. In addition, basic test accuracy measures were investigated: sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), false-positive rate (FPR) and false-negative rate (FNR).

Predictive values for differentiation between UFs and control were calculated. Changes in sensitivity and specificity were presented in receiver operating characteristic (ROC) curve. Then area under curve (AUC) of ROC curve was calculated to determine its usefulness for diagnosing uterine fibroids. Cut-off value was also evaluated to differentiate between patients with and without uterine fibroids. After categorising them into uterine fibroid positive, serum versican levels were correlated with kind of fibroid and also with volume of uterine fibroids using prolate ellipse formula.

Results

Clinicodemographic profile of all the study participants was analysed and presented in Table 1. Age at menarche was significantly lower in cases as compared to healthy control ($P < 0.05$). Most of the uterine fibroid cases were multiparous without any history of miscarriage.

Among the biochemical parameters, the serum versican level was found significantly low ($P < 0.001$) in fibroid uterus cases as compared to controls [Table 2, Figure 1] and the LDLc level was found higher in cases ($P < 0.05$).

Serum versican levels when analysed as per the fibroid types, and compared with Kruskal–Wallis test, the difference was not found significant, with the values of intramural type 82.83 ± 33.74, subserosal 63.50 ± 18.16, submucosal 80.96 ± 5.25 and cervical 78.02 ± 7.34 (*P* value: 0.120).

The volume of uterine fibroids as measured using prolate ellipse formula a × b × c × 0.523, (a is height, b is width, and c is depth) obtained from ultrasound was correlated with the serum versican levels in fibroid uterus cases and presented in Figure 2. Pearson's analysis was performed to evaluate correlation, and volume of uterine fibroids showed a negative correlation with the serum versican level ($r = -0.307$, *P* value: 0.054).

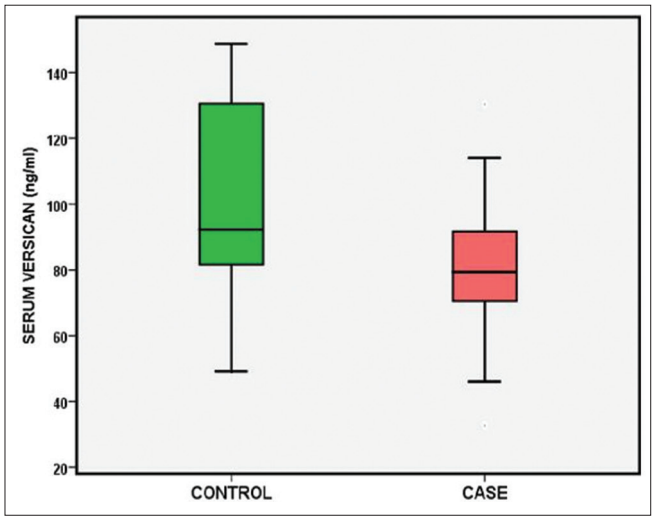

Figure 1: Comparison of serum versican levels between fibroid uterus cases and control

To evaluate the diagnostic potential of serum versican, ROC analysis was performed. The area was 0.726 (95% CI: 0.616-0.836; $P = 0.001$) as depicted in Figure 3. The best cut-off value for serum versican level was 96.90 ng/ml with 90% sensitivity and 48% specificity.

Usefulness of the diagnostic test was performed by analysing the true positive, false positive [Table 3] with a positive predictive value (PPV) of 63% and a negative predictive value (NPV) of 83%.

Discussion

Versican has a regulatory role in cellular proliferation, cell migration, extracellular matrix (ECM) assembly and inflammation. Versican can interact with various ECM substrates

Table 1: Clinicodemographic profile of study participants

Characteristics	Case (*n*=40)	Control (*n*=40)	*P*
Age (years)	42.55±6.71	39.87±8.49	0.060
Height (cm)	152.55±9.96	157.52±5.98	0.11
Weight (kg)	58.60±9.27	59.97±10.71	0.260
BMI (kg/m^2)	25.43±5.03	24.37±5.14	0.242
Age at menarche (years)	12.32±0.68	13.22±1.06	<0.001**
Parity			
Nulliparous	3 (7.5%)	5 (12.5%)	0.749
Uniparous	10 (25%)	10 (25%)	
Multiparous	27 (67.5%)	25 (62.5%)	
History of Miscarriage			
No	29 (72.5%)	37 (92.5%)	0.019*
Yes	11 (27.5%)	3 (7.5%)	
History of Oral Contraceptive Pill consumption			
No	26 (65%)	32 (80%)	0.133
Yes	14 (35%)	8 (20%)	
Blood Pressure			
Systolic	122.92±11.52	124.95±15.03	0.246
Diastolic	78.25±8.75	79.57±7.21	0.298
H/O Hypertension			
No	33 (82.5%)	35 (87.5%)	0.330
Yes	7 (17.5%)	5 (12.5%)	
H/O DM			
No	36 (90%)	32 (80%)	0.499
Yes	4 (10%)	8 (20%)	
H/O Hypothyroidism			
No	35 (87.5%)	40 (100%)	0.055
Yes	5 (12.5%)	0 (0%)	

Note: *=significant (*P*<0.05); **=highly significant (*P*<0.001)

Table 2: Biochemical parameters of the study population

Parameters	Cases	Control	*P*
Versican (ng/ml)	79.43±18.60	101.81±28.24	0.001**
FBS (mg/dl)	106.95±20. 73	102.05±43.36	0.312
Total Cholesterol (mg/dl)	156.95±35.70	151.22±33.89	0.620
LDLc (mg/dl)	113.12±19.67	90.32±14.63	0.043*
Triglycerides (mg/dl)	132.52±79.13	152.97±93.60	0.934
HDLc (mg/dl)	35.72±11.18	43.37±8.38	0.085

Note: *=significant (*P*<0.05); **=highly significant (*P*<0.001)

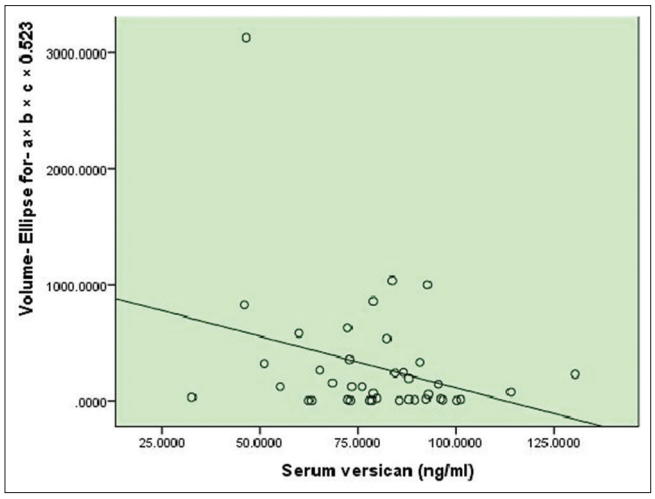

Figure 2: Correlation between the serum versican levels and volume of fibroid uterus

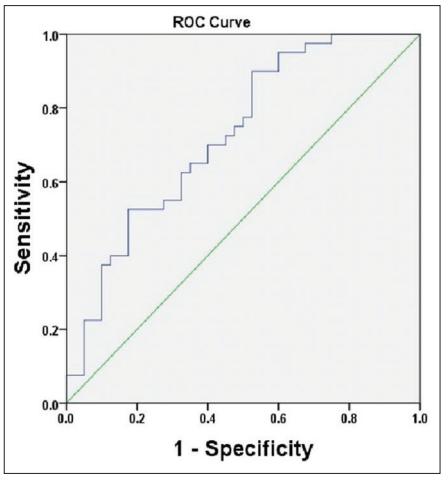

Figure 3: Receiver operating characteristic curve of serum versican levels for diagnosis of fibroid uterus

Table 3: Diagnostic performance of serum Versican levels in the study participants

Versican	UF positive (*n*, %)	UF negative (*n*, %)	Total (*n*, %)
Positive result	36 (TP), 90%	21 (FP), 53%	57,71%
Negative result	4 (FN), 10%	19 (TN), 47%	23,29%
Total	40	40	80

UF=Uterine fibroid, TP=True positive, FP=False positive, FN=False negative, TN=True negative

like hyaluronan and mediate ECM assembly.[17] Previous studies have demonstrated role of versican as anti-adhesive molecule. Versican is involved in the inflammatory response, and the macrophage-derived versican expression has been found to increase in response to acute inflammation. Uterine fibroid is a chronic inflammation state leading to altered expression of different gens. One of the hallmarks of uterine fibroid is excessive ECM deposition, which is mainly responsible for the large sizes of fibroid despite having slow rate of smooth muscle proliferation. Proteomic profiling study by Jamaluddin *et al.* showed a significant upregulated versican expression in small fibroids comparing to normal adjacent myometrial tissue.[18] Barker *et al.* demonstrated an increased versican mRNA level in leiomyoma comparing to normal myometrium.[19] In our present study, we found out that patients with uterine fibroids had decreased levels of serum versican as compared to healthy controls. Additionally, there is a statistically significant negative correlation between serum versican level and size of uterine fibroid, that is, the larger the volume of fibroid, the lesser is the serum versican levels, suggesting its possible involvement in pathogenesis of fibroid uterus. Among the various isoforms of versican, a study by Norian *et al.* showed similar results, in which V0 forms was found to be drastically increased in uterine leiomyoma tissue.[20] Elevated versican in leiomyoma and keloid, two common conditions resulting from fibrosis also reported previously[21] Gueye *et al.* demonstrated significantly high V0 and V1 variants in leiomyomal tissue comparing to normal myometrium.[22] In their study, they also reported an elevated (a disintegrin and metalloprotease with thrombospondin repeats) ADAMTS4 activity and increased cleaved versican forms in uterine fibroids. Increased versican expression in uterine tissue also demonstrated in leiomyomal sarcoma. Thus, elevated expression of versican in fibroid uterus is evaluated previously.[23] But alteration in serum versican level in uterine fibroid cases largely remains unexplored. In the present study, regression analysis was performed to evaluate the diagnostic potential of serum versican and the best cut-off value for serum versican level was found to be 96.90 ng/ml in fibroid uterus cases, with 90% sensitivity and 48% specificity. This serum values would be useful in detecting fibroid uterus in women presenting with suggestive clinical features. In situations with problematic differentiation, lower serum versican can be used as a marker for diagnosing uterine fibroids. The expression of several versican subtypes was reported to be higher in leiomyoma tissue and primary cells compared to their healthy tissue counterparts by microarray analysis.[24] In addition, Carrino *et al.* reported higher amounts of versican in uterine leiomyoma and keloid scars compared to corresponding healthy tissues (Carrino *et al.*, 2012), suggesting a molecular link in the ECM composition between these two fibrotic diseases.[21] One could speculate that an increased rate of degradation during stable disease is an attempt to normalize the increased versican levels found in ECM.[25] As per the findings of the present study, there is correlation between the serum versican levels and size of uterine fibroid (measured using prolate ellipse formula obtained from ultrasound). In primary healthcare set-ups, with nonavailability of the ultrasound facility, serum versican estimation could be a noninvasive tool for early diagnosis of uterine fibroid, thereby reducing the disease burden at grassroot level.[26] These preliminary findings pave the way for further functional studies related to tissue expression, local niche, activation of versican and its release into circulation, would provide an insight into the biochemical basis of serum expression in fibroid uterus.

Conclusion

The serum versican levels were found significantly lower in women with fibroid uterus as compared to healthy control. A significant negative correlation existed between serum versican levels and size of uterine fibroid suggesting the potential diagnostic and prognostic role of serum versican levels in fibroid uterus. Furthermore, extensive study in a different set of population would help in substantiating the efficacy of serum versican as a biomarker in fibroid uterus patients.

Key points

- Versican is a chondroitin sulphate proteoglycan and is one of the major proteoglycans in extracellular matrix.
- Uterine leiomyomas are composed of modified smooth muscle cells and a large amount of extracellular matrix.
- The serum versican level was found significantly low in fibroid uterus cases as compared to controls.
- The volume of uterine fibroids as measured using prolate ellipse formula- a × b × c × 0.523, (a is height, b is width, and c is depth) obtained from ultrasound was found to be correlated with the serum versican levels in fibroid uterus cases.
- The best cut-off value for serum versican level was 96.90 ng/ml with 90% sensitivity and 48% specificity.
- Serum versican would be useful in detecting fibroid uterus in women presenting with suggestive clinical features in primary healthcare set-ups.

Acknowledgement

This is an ICMR STS Awarded project work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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