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# DYSREGULATION OF EZRIN EXPRESSION AND PHOSPHORYLATION IN UTERINE LEIOMYOMA

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*Uterine Leiomyoma (UL) is a major source of gynaecologic and reproductive dysfunction in females of reproductive age. They are highly fibrotic tumors with evident disturbance of the mechano-transduction molecules. Ezrin acts as a dynamic linkage between the cytoskeleton and the membrane-associated proteins known to be dysregulated in tumorigenesis.* ***Aim of the study:*** *The current study aimed to profile the expression of ezrin, phospho-ezrin, and their upstream effector molecules; RhoA and ROCK1 in UL, their adjacent tissues, and control myometrium.* ***Materials and Methods:*** *Ezrin, phospho-ezrin, RhoA and ROCK1 mRNA and proteins were assayed in the tissue lysate of fibroid, and adjacent myometrium of 43 pre-menopausal women with symptomatic UL and 18 non-fibroid myometrium as a control group.* ***Results:*** *Tissue levels of ezrin, phospho-ezrin, RhoA and ROCK1 in fibroids and adjacent tissues were higher compared to the control tissue. Moreover, the levels of phospho-ezrin and total RhoA proteins showed higher levels in fibroid compared to adjacent tissues. Phospho-ezrin and ROCK1 mRNA correlated positively with both uterine and fibroid size.* ***Conclusion:*** *Ezrin, phospho-ezrin, and RhoA-ROCK1 upregulation in leiomyoma and its adjacent myometrium signify the central role of ezrin and its upstream and downstream effector on the pathogenesis and progression of UL.*

***Keywords:*** *Ezrin; phospho-ezrin, RhoA, ROCK1, leiomyoma*

## INTRODUCTION

Uterine leiomyomas (UL) are benign tumours affecting the myometrium during the reproductive age. Yet, they are the primary indication for hysterectomy and a major source of gynaecologic and reproductive dysfunction including pelvic pain, heavy menses, infertility, and pregnancy complications [1]. These presenting manifestations depend on the number, size, and localization of the fibroids. The main known features of UL are uncontrolled cell proliferation and excessive extracellular matrix (ECM) production that leads to the transformation of fibroblasts into myofibroblasts, which eventually contributes to production of the excessive ECM microenvironment and stimulate leiomyoma cell proliferation[1, 2]. In Egypt, the prevalence of UL ranged from 9.8% to 17.8% in the 40-49 age group year and about 25% of them underwent hysterectomy due to symptomatic UL[3].

The cytoskeleton is the main determinant of cellular mechanics and structural stability. It also serves as a mediator of cell signalling by regulating the location, duration, and intensity of signalling through a diverse set of mechanisms[4]. The dynamic functions of the cytoskeleton are dependent on their interaction with cytoskeletal-associated proteins,

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Dysregulated cytoskeletal proteins may be involved in the development of the leiomyoma[5, 6]. Dysregulation of these interactions can lead to numerous diseases, such as tumorigenesis and fibrotic diseases[7, 8].

Ezrin is a membrane-cytoskeleton linker protein consisting of 585 amino acids. It belongs to the Ezrin-radixin-moesin (ERM) family of proteins that makes ezrin essential for signal transduction, determination of the cell shape, surface structure, cell adhesion, motility, cytokinesis, and phagocytosis, all of which are involved in tumor development/growth[9, 10].

Ezrin is a cytoskeletal protein that connects the ECM with the intracellular actin cytoskeleton through transmembrane integrins. Over phosphorylation of ezrin causes over crosslinking of actin bundles, integrins and ECM, increasing cell-cell and cell-matrix adhesions, which causes deformation of ECM, its stiffness and surface topography alteration which may further alter ECM properties[9, 11, 12].

Indeed, ezrin is an important protein in the maintenance of fibroblast size and its dysregulation promotes fibroblast proliferation and collagen deposition causing ECM stiffness and tissue fibrosis [13, 14], both of which are landmarks in the development of leiomyoma. Moreover, increased ezrin phosphorylation is reported to occur in many inflammatory and fibrotic diseases such pulmonary fibrosis[15-17]. The phosphorylation of Thr567 residue of ezrin occurs in a tissue- and cell-dependent manner and is significantly activated by oestrogen. Dynamic regulation of ezrin phosphorylation at amino acid Thr567 plays a pivotal role in tumorigenesis[18, 19].

Dynamic changes in the cytoskeleton and its associated proteins are tightly regulated by the Rho family of small GTPases as Ras homolog gene family member A (RhoA) in response to intra- or extracellular signaling [20, 21]. RhoA is a small GTPase protein, that serves as a molecular switch between RhoA (GTP-bound) and (GDP-bound) forms in intracellular signaling, crucial for controlling a range of cellular activities, such as cytoskeletal dynamics, cell shape, adhesion, growth, proliferation, and transcriptional activity in the cells[21, 22].Total RhoA protein includes both GTP- and GDP-bound forms[23].

Notably, RhoA activates Rho-associated coiled-coil-containing protein kinase (ROCK1) with an impact on cytoskeletal reorganization, via ezrin activation[24]. Furthermore, ROCK1 kinase promotes collagen deposition, fibronectin matrix assembly, elevated tissue stiffness, and cell adhesion[25, 26]. Also, fibroblasts transformation into myofibroblasts in response to either biochemical stimuli such as TGF-β or biomechanical stimuli such as increased stiffness of ECM, evident findings in leiomyoma was reported in response to ROCK1 activation [27].

Accordingly, the current study investigated expression levels of ezrin gene and its regulators (RhoA and ROCK1 kinase) and tissue levels of ezrin, phospho-ezrin and total RhoA proteins in UL, its adjacent tissues and myometrium from non-fibrotic uterine tissues. We also explored the influence of these molecules on the UL features including site (type), size, and number.

## PATIENTS AND METHODS

The current study included 43 pre-menopausal women, who have undergone hysterectomy or myomectomy for symptomatic UL at the Women Health Hospitals, Assiut University from July-2021 to March-2023. Also, the study included 18 pre-menopausal women who were undergoing hysterectomy for benign, non-fibroid indications including irregular bleeding, chronic pelvic pain, and genital prolapse. Participants received thorough medical examination and preoperative assessment. Women were excluded if they had malignancy, received hormonal treatment in last 3 months, or suffered chronic illnesses including hypertension, diabetes, liver or kidney dysfunction. UL patients were diagnosed preoperatively using clinical examination and ultrasound imaging.

All leiomyoma tissues were confirmed histologically as benign ordinary leiomyomas. The study protocol was approved by Assiut Medical School Institutional (Ethical Committee of The Faculty of Medicine, Assiut University) Review Board (IRB no: 17300349). Informed written consent was given by each participant.

### Human tissue collection

The site, size and number of fibroids and the size of the uterus were assessed. Volumes

of the uterus and fibroids were determined according to the formula (a × b × c × 0.523) where "a" is height, "b" is width, and "c" is depth . According to the surgical operation, the biopsies were acquired from the fibroid tumor (*Myo-F group*) and its adjacent tissue located ½ inch from the fibroid (*Myo-A group*). Normal myometrial tissue biopsies were taken as control group from women undergoing hysterectomy for non-fibroid indications (*Myo-N group*). Tissue samples were washed in PBS, split into 2 portions, both of which were snap frozen in liquid nitrogen and stored at -80 °C for further analysis.

**Real-time quantitative PCR**

Total mRNA was extracted using the Rneasy mini kit (QIAGEN, Cat. No. 217004) according to the manufacturer's instructions. RNA concentration and purity were examined using nanodrop and 500 ng of RNA were reverse transcribed into cDNA (Enzynomic, Cat. No. K1622) in 96-well thermal cycler (Veritti, Applied Biosystems, Germany). The cDNA was then amplified in SYBR-Green PCR master mix (Enzynomics, Cat. No. # P725) on Applied Biosystems 7500 Fast Real-time PCR machine (Applied Biosystems, Germany). The primer sets shown in **Table 1** (Invitrogen) were used to evaluate the gene expression levels of Ezrin, RhoA, and ROCK1. The 18s rRNA was used as an internal control gene which more valid in cell proliferation and hypoxia conditions[28]. The amplification reaction comprised of initial denaturation at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for one minute. Gene expression was calculated according to $2^{-\Delta\Delta CT}$ method and presented as fold-change relative to control myometrium.

**Protein extraction & Enzyme-Linked Immunosorbent Assay (ELISA)**

Tissue homogenates were prepared by adding 900 µl of ice-cold PBS buffer to 100 mg tissue sample. Then, the tissue was homogenized mechanically using Glass-Col Homogenizer, followed by centrifugation at 10000 g for 10 min. Fifty µl of the supernatant was drawn for colorimetric protein [31].

Commercial ELISA kits were used to measure ezrin protein (Elabscience, Cat. No. E-EL-H2079,) Phospho-ezrin (Threonine 567); (Cat. No. I7007-Glory Science Co.) and Total RhoA protein (Cat. No. 11592 - Glory Science Co.) according to the manufacturer's instructions. Levels of the study parameters are expressed relative to tissue protein concentration.

**Statistical analysis**

GraphPad Prism was used to analyse the data. Data are displayed as mean ± SE. Kruskal-Wallis's test and Mann Whitney's test were used for three and two group comparison respectively. Correlation analyses were performed using Spearman's co-efficient U tests. p-value of less than 0.05 was considered significant.

**Table 1:** Primer sequences of the studied genes.

| | Forward primer | Reverse primer |
|---|---|---|
| Ezrin[29] | 5'-GAATACACAGCCAAGATTGC-3' | 5'-CTCATGTTCTCGTTGTGGAT-3' |
| RhoA[30] | 5'-ACAGCCCTCCTCTGCACTCCC-3' | 5'CTGCCCACCCCAGAGCTATGCC-3' |
| ROCK1[30] | 5'-GCGCTGCCTTACACAAAATGACCTG-3' | 5'-TGCCCATCTGCATCCTGACGTT-3' |
| 18S rRNA[28] | 5'-GGCGCCCCCTCGATGCTCTTAG-3' | 5'-GCTCGGGCCTGCTTTGAACACTCT3' |

## RESULTS AND DISCUSSION

### Results

Demographic and clinical characteristics of the study groups are presented in **table 2**. Eighteen premenopausal women who underwent hysterectomy for non-fibroid indications aged (44.89 ± 0.92) years were considered as controls and 43 fibroid patients who underwent hysterectomy (n=22) and myomectomy (n=21) aged (40.47 ± 0.79). There were no statistically significant differences in age (year), BMI ($Kg/m^2$), Hb (g/dl) and hematocrit value (%) between controls, and fibroid patients. As expected, uterine volumes varied significantly between the study groups P<0.0001. Most of the fibroids were intramural n=26 (60.5%), and the least common type was submucous n=7 (16.3%). Single fibroids were detected in 18 patients (42%), while multiple fibroids were detected in 25 patients (58 %). Around 80 % of the patients were presented with fibroids ≥ 4 cm in size.

**Table 2:** Demographic and clinical characteristics of controls and fibroid group.

| | Controls (n=18) | Fibroid patients (n=43) | P value |
|---|---|---|---|
| Age (year) | 44.89 ± 0.92 | 40.47 ± 0.79 | NS |
| BMI ($Kg/m^2$) | 33.19 ± 1.16 | 30.84 ± 0.73 | NS |
| Hb (g/dl) | 11.88 ± 0.33 | 11.12 ± 0.20 | NS |
| Hematocrit % | 36.59 ± 0.92 | 34.28 ± 0.53 | NS |
| Uterine volume ($cm^3$) | 100.3 ± 7.129 | 378.6 ± 51.98 | P*<0.0001 |
| Menstrual cycle phase n (%)<br>Proliferative phase<br>Disordered<br>Lutel phase | <br>11 (61.1%)<br>6 (33.3%)<br>1 (5.6%) | <br>39 (90.7%)<br>2 (4.7 %)<br>2 (4.7 %) | |
| Main complication n (%)<br>Heavy menstrual bleeding<br>Abdominal pain<br>Dyspernia<br>Infertility<br>Abortion | <br>11(61.1%)<br>6 (33.3%)<br>1(5.6%)<br>0<br>0 | <br>22 (51.2 %)<br>16 (37.2%)<br>0<br>3 (7.0 %)<br>2 (4.7 %) | |
| Site of fibroids n (%)<br>Intramural<br>Submucous<br>Mixed type | | <br>26 (60.5%)<br>7 (16.3%)<br>10 (23.2%) | |
| Number of fibroids n (%)<br>Single fibroid<br>Multiple fibroids | | <br>18 (41.9%)<br>25 (58.1 %) | |
| Fibroid size n (%)<br>Fibroid ≥ 4 cm<br>Fibroid < 4 cm | | <br>34 (79.1%)<br>9 (20.9 %) | |
| Largest fibroid volume $cm^3$ | | 102.7 ± 15.33 | |

Data presented as mean ± SE. Statistical analyses were performed by Mann Whitney's test. Statistically significant differences at (P*< 0.05).

The relative mRNA expression of ezrin and ezrin protein were significantly upregulated in fibroids, and its adjacent tissues compared to the normal myometrium. Yet, no variations were reported between fibroid and adjacent tissues in that regard. However, phosphor-ezrin showed higher levels in fibroid tissues compared to both controls' (p=0.001) and adjacent myometrium (p=0.038) signifying the central role of ezrin activation not only in the development but also in progression of fibroid (as shown in **Fig.1**). Likewise, both RhoA and ROCK1 the upstream effectors and important modulator for ezrin activation and phosphorylation, were elevated in fibroid tissues compared to control and adjacent tissues (as shown in **Fig.2**). Interestingly, both RhoA and phospho-ezrin showed significant positive correlation with both uterine and fibroid volume (as shown in **Fig. 3**). With the exception of total RhoA that showed significant differences according to the type of fibroid, none of the other study parameters vary based on the site or number of fibroids (as indicated in **Table 3** and **4**).

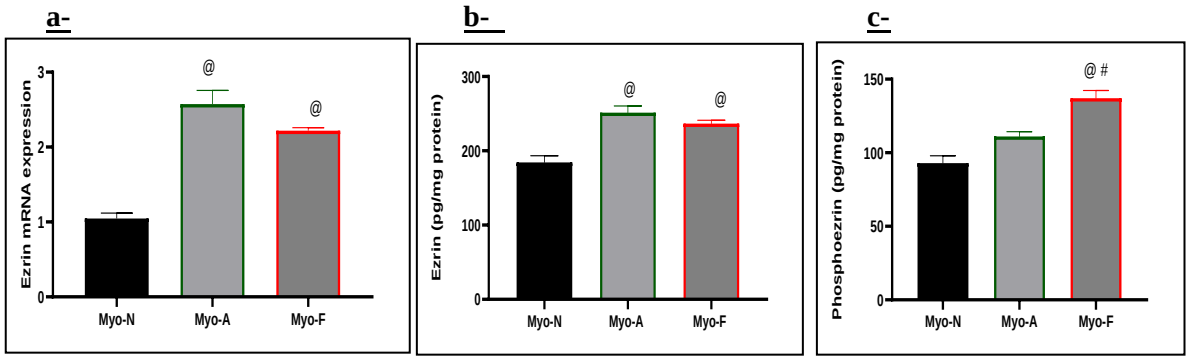


**Fig.1 :** Ezrin expression in fibroid tissues (Myo-F), adjacent myometrium (Myo-A) and myometrium from control group (Myo-N). (a)-Ezrin mRNA expression by qPCR, data are presented as mean fold change relative to controls. (b)-Ezrin protein levels. (c)- Phospho-ezrin protein levels expressed as pg ezrin /phospho-ezrin relative to mg protein. Data presented as mean ± SE. Statistical analyses were performed by ANOVA-Kruskal-Wallis's test. @ indicates post hoc test p <0.05 for Myo-N compared to Myo-A and Myo-F groups and # for Myo-F compared to Myo-A.

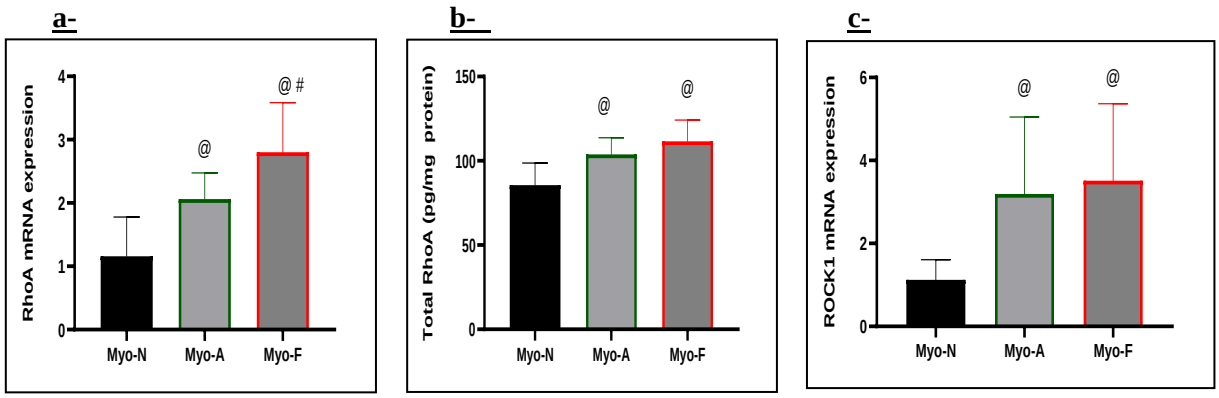


**Fig.2**: RhoA and ROCK1 expression in fibroid tissues (Myo-F), adjacent myometrium (Myo-A) and myometrium from control group (Myo-N). (a)- RhoA mRNA expression. (b)- Total RhoA protein. (c)- ROCK1 mRNA expression. mRNA expression by qPCR, data are presented as mean fold change relative to controls, protein levels expressed as pg per mg protein. Data are presented as mean ± SE. Statistical analyses were performed by ANOVA- Kruskal-Wallis's test. @ indicates post hoc test p <0.05 for Myo-N compared to Myo-A and Myo-F groups and # for Myo-F compared to Myo-A.

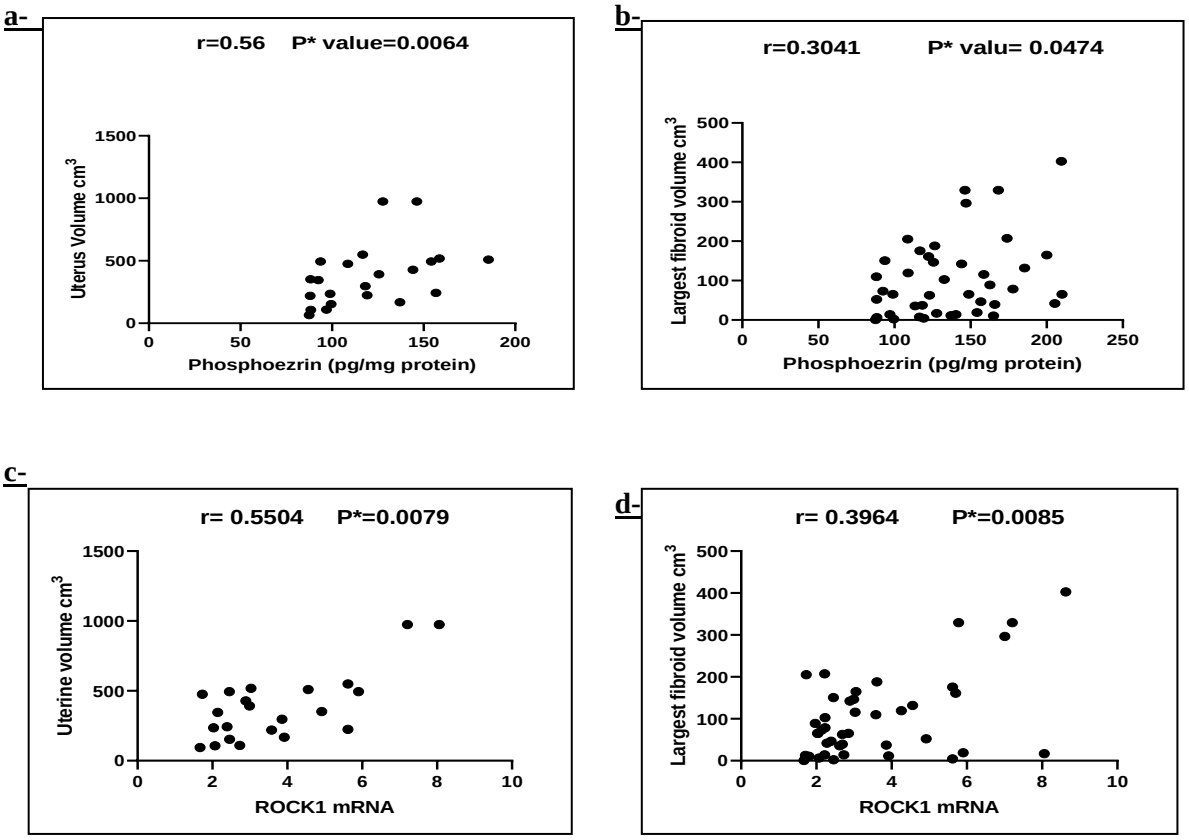


**Fig.3:** Correlations between the studied parameters and clinical data of fibroid patients. (a)- Spearman correlation between phospho-ezrin and uterine volume. (b)- Spearman correlation between phospho-ezrin and largest fibroid volume. (c)- Spearman correlation between ROCK1 mRNA and uterine volume. (d)- Spearman correlation between ROCK1 mRNA and largest fibroid volume. Statically significant differences at (P*< 0.05).

**Table 3:** Studied parameters according to the type of fibroids.

| | Intramural type n=26 | Mixed types n=10 | Submucous type n=7 | P value |
|---|---|---|---|---|
| Ezrin mRNA expression | 2.27 ±0.05 | 2.13 ± 0.07 | 2.15 ± 0.09 | NS |
| Ezrin (pg/mg protein) | 242 ± 30.7 | 220 ± 32.2 | 241 ± 13.2 | NS |
| Phospho-ezrin (pg/mg protein) | 136 ± 38.7 | 136 ± 30.3 | 141 ± 31.2 | NS |
| RhoA mRNA expression | 2.79 ± 0.17 | 2.94 ± 0.24 | 2.63 ± 0.13 | NS |
| Total RhoA (pg/mg protein) | 110 ± 2.36 | 119 ± 4.01 | 105 ± 4.11 | 0.0418* |
| ROCK1 mRNA expression | 3.39 ± 0.38 | 4.11 ± 0.69 | 3.14 ± 0.34 | NS |

Data presented as mean ± SE. Statistical analyses were performed by ANOVA Kruskal-Wallis's test. Statistically significant differences at (P*< 0.05).

**Table 4:** Studied parameters according to the number of fibroids.

| | Single fibroid n=18 | Multiple fibroids n=25 | P value |
|---|---|---|---|
| Ezrin mRNA expression | 2.27 ± 0.07 | 2.18 ± 0.05 | NS |
| Ezrin (pg/mg protein) | 242 ± 31.2 | 233 ± 28.8 | NS |
| Phospho-ezrin (pg/mg protein) | 132 ± 35.7 | 136 ± 33 | NS |
| RhoA mRNA expression | 2.81 ± 0.23 | 2.8 ± 0.13 | NS |
| Total RhoA (pg/mg protein) | 108 ± 2.88 | 114 ± 2.47 | NS |
| ROCK1 mRNA expression | 3.26 ± 0.34 | 3.7± 0.42 | NS |

Data presented as mean ± SE. Statistical analyses were performed by Mann Whitney test's test. Statistically significant differences at ($P< 0.05$).

**Discussion**

Uterine leiomyomas (UL) are the most prevalent type of non-malignant growth of the female reproductive system with tight cell-cell/cell-matrix adhesions [2, 32]. Fibrosis, stiffness, and disordered ECM are major characteristics of leiomyomas [33]. Myometrial and fibroid cells are found to convert mechanical stimuli from the disordered ECM into biochemical signals through a process named mechano-transduction [9, 33, 34]. This creates a highly complex response to mechanical force involving the coordinated assembly of many cytoskeletal proteins and plasma membrane [13] that in turn enhance the progression of leiomyoma. One of these proteins, ezrin, is a cytoskeletal organizer forming membrane protein-ezrin- cytoskeletal protein complex that plays a key role many cellular activities, angiogenesis, proliferation, apoptosis and cell adhesion to the ECM[9] Mechanically, ezrin could affect various signalling pathways and molecules involved in tumor progression [35].

Herein, the current study showed significant up-regulation of ezrin in fibroids and its adjacent tissues compared to its expression in the normal myometrium implying its role in the pathogenesis of UL. Nonetheless, the role of ezrin in the pathogenesis of leiomyoma remains poorly investigated, ezrin upregulation in other benign tumors and conditions characterized by fibrosis and aberrant ECM such as benign breast disease[36], endometrial tissues from women with endometriosis [37] and synovial tissue from patients with rheumatoid arthritis[38], as well as hypertrophic and keloid scars relative to normal skin [39].

Ezrin exists in an inactive. Multiple sites on ezrin can be phosphorylated by several kinases for the purpose of activation. Phosphorylation of the threonine residue (Thr567) is the key step to ezrin activation, which allows the actin binding domains to interact with other membrane proteins. The threonine phosphorylation is a Rho-dependent. Altered protein phosphorylation in cellular pathways is strongly associated with inhibition of apoptosis and activation of cell survival pathways in UL [40]. Furthermore, altered phosphorylation of Thr residues of ezrin is often linked to cell surface rigidity and cell shape change[4, 41, 42].

The results of the present study showed that phospho-ezrin levels were significantly higher in fibroids and adjacent tissues than normal myometrium, supporting its role in the development of UL. This enlightens us on the importance of ezrin activation in the progression from a disordered myometrium into actual fibroid, taking into consideration the suggestion that the myometrium from fibroid patients represent a transition state between the non-fibroid myometrium and the fibroid tumors [43]. Interestingly, both the uterine volume and largest fibroid size were in positive correlation with phospho-ezrin levels. In contrast to this, Davis et al. reported lowered ezrin gene expression in leiomyomas from older black women; expected to have larger fibroid compared to leiomyomas from older white women, expected to have smaller fibroid; but this could be explained by age difference and genetic context [44]. Supporting our findings, hyper-phosphorylation of Thr residue 567 of ezrin was determined in many benign diseases such as endometrial lesions [37], chronic

autoimmune disease of salivary glands Sjogren's Syndrome [45], and synovial tissue from patients with rheumatoid arthritis[46].

Ezrin overexpression disturbs many biological processes involved in leiomyoma pathogenesis. Many studies reported that high levels of ezrin expression and activation contribute to both ECM stiffness and fibroblast proliferation as in hypertrophic and keloid skin scars through the transcription factor, yes-associated protein (YAP1)[13, 39]. Furthermore, the dual role of ezrin protein as both a mechanical linkers and regulators of the intracellular signalling cascades renders it a potent modulator of the stem cell biomechanics that are involved in the conversion of stem cells to tumor-initiating stem cells, another pathogenic finding in UL [47, 48]. Moreover, ezrin, as a cross-linker between the plasma membrane and the actin cytoskeleton regulates the plasma membrane tension that is known to be high UL [49].

On the other-hand, multiple features and risk factors for UL are considered responsible for ezrin over expression including hypoxia, hormonal effects and of course the ECM stiffness. Hypoxia encountered in UL stimulates expression of hypoxia inducible factor (HIF)-1α that further induces ezrin phosphorylation[50, 51]. Of the utmost importance, UL is a hormone-dependent tumour. Two independent studies reported that ezrin expression is modified in leiomyomas treated by progesterone antagonists[52, 53].

Ezrin performs both upstream and downstream to RhoA/ROCK1. Ezrin is activated by phosphorylation at threonine 567 by Rho kinases, and subsequently regulates other Rho-related GTPase activities. RhoA plays an important role in regulating cytoskeleton, cell growth and ECM fibrosis. ROCK1 enzyme is a serine/threonine kinase, which regulates a variety of cellular processes, including the regulation of cell morphology, cytokinesis, cell migration, differentiation, proliferation, and apoptotic resistance [54]. Furthermore, ROCK1 kinase activates pro-fibrotic genes in fibroblasts and myofibroblasts through transcription factors as MRTF/SRF, YAP/TAZ/TEAD, and TGF-β signalling[55]. We reported a significant up-regulation of RhoA and ROCK1 in fibroids and its adjacent tissues, compared to its expression in the non-fibroid myometrium and a significantly higher expression levels of RhoA in fibroids than adjacent tissues. Upregulation of RhoA could be a direct result of the stiff matrix[20]. Previous study demonstrated higher RhoA levels in 3D leiomyoma cell culture compared to 2D culture[56]. The same study highlighted how Rho-kinase inhibitor lowered collagen1A, fibronectin, fibromodulin, and versican which constitute the majority of ECM. In addition**, Afrin et al.**[33] revealed reduced levels of mechanical signaling proteins including RhoA, and ROCK1 on simvastatin treatment of leiomyoma cell culture [33]. Intriguingly, UL cells cultured on stiff matrix plates showed augmented progesterone response mediated by RhoA/ ROCK1 and attenuated by the use of their antagonists [57].

## Conclusion

Upregulation of Ezrin/RhoA/ROCK1 pathway was observed in UL and adjacent tissues compared to normal myometrium, providing evidence that these proteins can be considered important players in the development and progression of UL. It is not obvious if ezrin and Phospho-ezrin augmented pathway are a cause or consequence of altered mechano-transduction and ECM stiffness. Although the use of progesterone inhibitors and simvastatin modified this pathway, specifically targeting this pathway by small molecule inhibitors against ezrin requires further investigation.

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## Limitations

This is a small-scale study that is limited to investigating ezrin and the direct effector molecules. None of the other cytoskeletal, ECM proteins were included in the study.

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**Ethical approval**

Written informed consent was obtained from each patient before surgery according to the approved protocol by Ethical Committee of The Faculty of Medicine, Assiut University, (Ethical approval number:17300349).

## REFERENCES


1. Q. Yang and A. Al-Hendy, "Update on the Role and Regulatory Mechanism of Extracellular Matrix in the Pathogenesis of Uterine Fibroids", ***Int J Mol Sci,*** 24(6), 5778 (2023).
2. W. Szucio, P. Bernaczyk, D. Ponikwicka-Tyszko, G. Milewska, A. Pawelczyk, S. Wolczynski, *et al.*, "Progesterone signaling in uterine leiomyoma biology: Implications for potential targeted therapy", ***Adv Med Sci,*** 69(1), 21-28 (2024).
3. A. El-Hakim, A.Abd El-Razik, M. F.Moustafa, A. El-Rahim, and A. Hassan, "Effect of Educational Program on Knowledge Regarding Leiomyomas among Women in Reproductive Age", ***Minia Scientific Nursing Journal,*** 11(1), 40-47 (2022).
4. O. Moujaber and U. Stochaj, "The Cytoskeleton as Regulator of Cell Signaling Pathways", ***Trends Biochem Sci,*** 45(2), 96-107 (2020).
5. M.S. Ong, S. Deng, C.E. Halim, W. Cai, T.Z. Tan, R.Y. Huang, *et al.*, "Cytoskeletal Proteins in Cancer and Intracellular Stress: A Therapeutic Perspective", ***Cancers (Basel),*** 12(1), 238 (2020).
6. A.A. Ayanlaja, X. Hong, B. Cheng, H. Zhou, K. Kanwore, P. Alphayo-Kambey, *et al.*, "Susceptibility of cytoskeletal-associated proteins for tumor progression", ***Cell Mol Life Sci,*** 79(1), 13 (2021).
7. A.I. Ivanov, H.T. Le, N.G. Naydenov, and F. Rieder, "Novel Functions of the Septin Cytoskeleton: Shaping Up Tissue Inflammation and Fibrosis", ***Am J Pathol,*** 191(1), 40-51 (2021).
8. M.T. Nguyen, Q.K. Ly, H.J. Kim, and W. Lee, "FLII Modulates the Myogenic Differentiation of Progenitor Cells via Actin Remodeling-Mediated YAP1 Regulation", ***Int J Mol Sci,*** 24(18), 14335 (2023).
9. R.G.M. Buenaventura, G. Merlino, and Y. Yu, "Ez-Metastasizing: The Crucial Roles of Ezrin in Metastasis", ***Cells,*** 12(12), 1620 (2023).
10. Y. Zhu, X. Zhang, Y. Chen, Q. Liu, J. Yang, X. Fan, *et al.*, "Ezrin's role in gastric cancer progression: Implications for immune microenvironment modulation and therapeutic potential", ***Heliyon,*** 10(5), e27155 (2024).
11. H. Hirata, M. Sokabe, and C.T. Lim, Chapter Six – "Molecular Mechanisms Underlying the Force-Dependent Regulation of Actin-to-ECM Linkage at the Focal Adhesions", ***Prog Mol Biol Transl Sci***, 126, 135-54 (2014).
12. P. Wang, J. Li, and Q. Wei, "Understanding the interplay between cell force and cell adhesion processes", ***Engineered Regeneration,*** 4(3), 277-288 (2023).
13. C. Quan, Y. Yan, Z. Qin, Z. Lin, and T. Quan, "Ezrin regulates skin fibroblast size/mechanical properties and YAP-dependent proliferation", ***J Cell Commun Signal,*** 12(3), 549-560 (2018).
14. S. Zhao, J. Luo, J. Hu, H. Wang, N. Zhao, M. Cao, *et al.*, "Role of Ezrin in Asthma-Related Airway Inflammation and Remodeling", ***Mediators Inflamm,*** 2022, 6255012 (2022).
15. M. Adada, D. Canals, Y.A. Hannun, and L.M. Obeid, "Sphingolipid regulation of ezrin, radixin, and moesin proteins family: implications for cell dynamics", ***Biochim Biophys Acta,*** 1841(5), 727-37 (2014).
16. A. Peloggia, M.P. Andres, and M.S. Abrao, "Expression of ezrin protein and phosphorylated ezrin in pelvic endometriotic lesions", ***Clinics (Sao Paulo),*** 77, 100074 (2022).
17. L.M. Yin and M. Schnoor, "Modulation of membrane-cytoskeleton interactions: ezrin as key player", ***Trends Cell Biol,*** 32(2), 94-97 (2022).
18. L.M. Lathika, J. Nair, V.N. Saritha, K. Sujathan, and S. Sreeja, "Role of phospho-ezrin in differentiating thyroid carcinoma", Sci Rep, 9(1), 6190 (2019).
19. S. Du, X. Song, Y. Li, Y. Cao, F. Chu, O.A. Durojaye, *et al.*, "Celastrol inhibits ezrin-mediated migration of hepatocellular

carcinoma cells", ***Sci Rep,*** 10(1), 11273 (2020).
20. K. Burridge, E. Monaghan-Benson, and D.M. Graham, "Mechanotransduction: from the cell surface to the nucleus via RhoA", ***Philos Trans R Soc Lond B Biol Sci,*** 374(1779), 20180229 (2019).
21. N. Mosaddeghzadeh and M.R. Ahmadian, "The RHO Family GTPases: Mechanisms of Regulation and Signaling", ***Cells,*** 10(7), 1831 (2021).
22. E.A. Pinoșanu, D. Pîrșcoveanu, C.V. Albu, E. Burada, A. Pîrvu, R. Surugiu, *et al.*, "Rhoa/ROCK, mTOR and Secretome-Based Treatments for Ischemic Stroke: New Perspectives", ***Curr Issues Mol Biol,*** 46(4), 3484-3501 (2024).
23. O. Al-Shboul, "The role of the RhoA/ROCK pathway in gender-dependent differences in gastric smooth muscle contraction", ***J Physiol Sci***, 66(1), 85-92 (2016).
24. N. Xie, C. Xiao, Q. Shu, B. Cheng, Z. Wang, R. Xue, *et al.*, "Cell response to mechanical microenvironment cues via Rho signaling: From mechanobiology to mechanomedicine", Acta Biomater, 159, 1-20 (2023).
25. L. Julian and M.F. Olson, "Rho-associated coiled-coil containing kinases (ROCK): structure, regulation, and functions", ***Small GTPases,*** 5, e29846 (2014).
26. L. Wei, M. Surma, S. Shi, N. Lambert-Cheatham, and J. Shi, "Novel Insights into the Roles of Rho Kinase in Cancer", Arch Immunol Ther Exp (Warsz), 64(4), 259-78 (2016).
27. R.S. Knipe, C.K. Probst, D. Lagares, A. Franklin, J.J. Spinney, P.L. Brazee, *et al.*, "The Rho Kinase Isoforms ROCK1 and ROCK2 Each Contribute to the Development of Experimental Pulmonary Fibrosis", ***Am J Respir Cell Mol Biol,*** 58(4), 471-481 (2018).
28. S. Dupasquier, A.S. Delmarcelle, E. Marbaix, J.P. Cosyns, P.J. Courtoy, and C.E. Pierreux, "Validation of housekeeping gene and impact on normalized gene expression in clear cell renal cell carcinoma: critical reassessment of YBX3/ZONAB/CSDA expression", ***BMC Mol Biol***, 15, 9 (2014).
29. H.J. Wang, J.S. Zhu, Q. Zhang, H. Guo, Y.H. Dai, and X.P. Xiong, "RNAi-mediated silencing of ezrin gene reverses malignant behavior of human gastric cancer cell line SGC-7901", J Dig Dis, 10(4), 258-64 (2009).
30. S. Wang, H. Duan, Y. Zhang, and F.Q. Sun, "Abnormal Activation of RhoA/ROCK-I Signaling in Junctional Zone Smooth Muscle Cells of Patients With Adenomyosis", ***Reprod Sci,*** 23(3), 333-41 (2016).
31. E. Subroto, E. Lembong, F. Filianty, R. Indiarto, G. Primalia, M.S.K.Z. Putri, *et al.*,"The Analysis Techniques Of Amino Acid And Protein In Food And Agricultural Products", ***International Journal of Scientific & Technology Research,*** 9, 29-36 (2020).
32. H.M. Chen, Y.H. Lin, Y.M. Cheng, L.Y. Wing, and S.J. Tsai, "Overexpression of integrin-beta1 in leiomyoma promotes cell spreading and proliferation", ***J Clin Endocrinol Metab,*** 98(5), E837-46 (2013).
33. S. Afrin, M.S. Islam, K. Patzkowsky, M. Malik, W.H. Catherino, J.H. Segars, et al.,"Simvastatin ameliorates altered mechanotransduction in uterine leiomyoma cells", ***Am J Obstet Gynecol,*** 223(5), 733 e1-733 e14 (2020).
34. S. Rafique, J.H. Segars, and P.C. Leppert, "Mechanical Signaling and Extracellular Matrix in Uterine Fibroids", Semin Reprod Med, 35(6), 487-493 (2017).
35. C. Quan, J. Sun, Z. Lin, T. Jin, B. Dong, Z. Meng, *et al.*, "Ezrin promotes pancreatic cancer cell proliferation and invasion through activating the Akt/mTOR pathway and inducing YAP translocation", ***Cancer Manag Res,*** 11, 6553-6566 (2019).
36. M.H. Jais, R.R. Md Zin, N.A. Muhd Hanapi, and S.A. Md Ali, "Ezrin is Significantly Overexpressed in Luminal A, Luminal B, and HER2 Subtype Breast Cancer", ***Appl Immunohistochem Mol Morphol,*** 25(1), 44-48 (2017).
37. T. Ornek, A. Fadiel, O. Tan, F. Naftolin, and A. Arici, "Regulation and activation of ezrin protein in endometriosis", ***Hum Reprod,*** 23(9), 2104-12 (2008).
38. Q. Chen, K. Fan, X. Chen, X. Xie, L. Huang, G. Song, *et al.*, "Ezrin regulates

synovial angiogenesis in rheumatoid arthritis through YAP and Akt signalling", ***J Cell Mol Med,*** 25(19), 9378-9389 (2021).
39. Y. K.T. Kim, E.Park, A. Fadiel and F. Naftolin, "Ezrin Expression and Activation in Hypertrophic and Keloid Scar", ***Gynecological and Reproductive Endocrinology & Metabolism,*** 1(1), 29-36 (2020).
40. B. Ura, L. Monasta, G. Arrigoni, I. Battisti, D. Licastro, G. Di Lorenzo, *et al.*, "Phosphoproteins Involved in the Inhibition of Apoptosis and in Cell Survival in the Leiomyoma", ***J Clin Med,*** 8(5) (2019).
41. K. Giehl, C. Keller, S. Muehlich, and M. Goppelt-Struebe, "Actin-mediated gene expression depends on RhoA and Rac1 signaling in proximal tubular epithelial cells", ***PLoS One,*** 10(3), e0121589 (2015).
42. K. Tachibana, S.M. Haghparast, and J. Miyake, "Inhibition of cell adhesion by phosphorylated Ezrin/Radixin/Moesin", ***Cell Adh Migr,*** 9(6), 502-12 (2015).
43. E.N. Paul, G.W. Burns, T.J. Carpenter, J.A. Grey, A.T. Fazleabas, and J.M. Teixeira, "Transcriptome Analyses of Myometrium from Fibroid Patients Reveals Phenotypic Differences Compared to Non-Diseased Myometrium", ***Int J Mol Sci,*** 22(7), (2021).
44. B.J. Davis, J.I. Risinger, G.V. Chandramouli, P.R. Bushel, D.D. Baird, and S.D. Peddada, "Gene expression in uterine leiomyoma from tumors likely to be growing (from black women over 35) and tumors likely to be non-growing (from white women over 35)", ***PLoS One,*** 8(6), e63909 (2013).
45. P. Perez, S. Aguilera, N. Olea, C. Alliende, C. Molina, M. Brito, *et al.*, "Aberrant localization of ezrin correlates with salivary acini disorganization in Sjogren's Syndrome", ***Rheumatology (Oxford),*** 49(5), 915-23 (2010).
46. Y. Xiao, M. Sun, Z. Zhan, Y. Ye, M. Huang, Y. Zou, *et al.*, "Increased phosphorylation of ezrin is associated with the migration and invasion of fibroblast-like synoviocytes from patients with rheumatoid arthritis", ***Rheumatology (Oxford),*** 53(7), 1291-300 (2014).
47. I. Titushkin, S. Sun, A. Paul, and M. Cho, "Control of adipogenesis by ezrin, radixin and moesin-dependent biomechanics remodeling", ***J Biomech,*** 46(3), 521-6 (2013).
48. M. El Sabeh, S. Afrin, B. Singh, M. Miyashita-Ishiwata, and M. Borahay, "Uterine Stem Cells and Benign Gynecological Disorders: Role in Pathobiology and Therapeutic Implications", ***Stem Cell Rev Rep,*** 17(3), 803-820 (2021).
49. R. Rogers, J. Norian, M. Malik, G. Christman, M. Abu-Asab, F. Chen, *et al.*, "Mechanical homeostasis is altered in uterine leiomyoma", ***Am J Obstet Gynecol,*** 198(4), 474 e1-11 (2008).
50. N.M. Fletcher, M.G. Saed, H.M. Abu-Soud, A. Al-Hendy, M.P. Diamond, and G.M. Saed, "Uterine fibroids are characterized by an impaired antioxidant cellular system: potential role of hypoxia in the pathophysiology of uterine fibroids", ***J Assist Reprod Genet,*** 30(7), 969-74 (2013).
51. M. Fedotova, E. Barysheva, and O. Bushueva, "Pathways of Hypoxia-Inducible Factor (HIF) in the Orchestration of Uterine Fibroids Development", ***Life (Basel),*** 13(8), (2023).
52. A. Tinelli, I.P. Kosmas, O.A. Mynbaev, A. Malvasi, R. Sparic, and D. Vergara, "The Biological Impact of Ulipristal Acetate on Cellular Networks Regulating Uterine Leiomyoma Growth", ***Curr Pharm Des,*** 26(3), 310-317 (2020).
53. I. Szydlowska, M. Grabowska, J. Nawrocka-Rutkowska, M. Piasecka, and A. Starczewski, "Markers of Cellular Proliferation, Apoptosis, Estrogen/Progesterone Receptor Expression and Fibrosis in Selective Progesterone Receptor Modulator (Ulipristal Acetate)-Treated Uterine Fibroids", ***J Clin Med,*** 10(4) (2021).
54. J. Barcelo, R. Samain, and V. Sanz-Moreno, "Preclinical to clinical utility of ROCK inhibitors in cancer", ***Trends Cancer,*** 9(3), 250-263 (2023).
55. B. Yu, N. Sladojevic, J.E. Blair, and J.K. Liao, "Targeting Rho-associated coiled-coil forming protein kinase (ROCK) in cardiovascular fibrosis and stiffening",

***Expert Opin Ther Targets,*** 24(1), 47-62 (2020).

56. M. Malik, J. Britten, J. Segars, and W.H. Catherino, *"*Leiomyoma cells in 3-dimensional cultures demonstrate an attenuated response to fasudil, a rho-kinase inhibitor, when compared to 2-dimensional cultures*"*, ***Reprod Sci,*** 21(9), 1126-38 (2014).
57. C.N. Cordeiro Mitchell, M.S. Islam, S. Afrin, J. Brennan, K.J. Psoter, and J.H. Segars, *"*Mechanical stiffness augments ligand-dependent progesterone receptor B activation via MEK 1/2 and Rho/ROCK-dependent signaling pathways in uterine fibroid cells*"*, ***Fertil Steril,*** 116(1), 255-265 (2021)

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# خلل تنظيم تعبير الإزرين والفسفرة في الورم العضلي الأملس الرحمي

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**الخلفية**: الورم العضلي الأملس الرحمي (UL) هو مصدر رئيسي للخلل الوظيفي الإنجابي وأمراض النساء لدى الإناث في سن الإنجاب. وهي أورام ليفية مع دليل اضطراب واضح في جزيئات النقل الميكانيكي. يعمل الإزرين بمثابة رابط ديناميكي بين الهيكل الخلوي والبروتينات المرتبطة بالغشاء المعروفة بأنها غير منتظمة في تكوين الأورام.

**هدف الدراسة**: هدفت الدراسة الحالية إلى التعرف على تعبير الإزرين و الفوسفو-إزرين وجزيئاتها المؤثرة RhoA و ROCK1 في UL ، والأنسجة المجاورة لهما، والتحكم في عضل الرحم.

**المواد والطرق**: تم تقييم إزرين وفوسفو-إزرين و RhoAو ROCK1 mRNA والبروتينات في المحللة النسيجية للورم الليفي وعضل الرحم المجاور لـ ٤٣ امرأة في مرحلة ما قبل انقطاع الطمث مع أعراض ULو١٨ عينة من عضل ليفي غير ورم كمجموعة مراقبة.

**النتائج**: كانت مستويات أنسجة الإزرين و الفوسفو-إزرين والـ RhoA وROCK1 في الأورام الليفية والأنسجة المجاورة أعلى مقارنة بالأنسجة الضابطة. علاوة على ذلك، أظهرت مستويات الفوسفو-إزرين وبروتينات RhoA الكلية مستويات أعلى في الورم الليفي مقارنة بالأنسجة المجاورة. كما وجد ارتباط الفوسفو-إزرين و ROCK1 mRNA بشكل إيجابي مع حجم الرحم والورم الليفي.

**الاستنتاج**: إن تنظيم الإزرين، والفوسفو-إزرين، و RhoA-ROCK1 في الورم العضلي الأملس وعضل الرحم المجاور له يدل على الدور المركزي للإزرين ومؤثره على التسبب وتطور الورم العضلي الأملس الرحمي.

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