International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

Evaluation Of KI-67 Expression in Uterine Leiomyoma and in Healthy Myometrium

Navdeep Kaur[1], Swati Singh[2], Richa Sharma[3], Adreena Mittal[4]

[1]Junior Resident, Department of Pathology- Santosh Medical College & Hospital, Santosh Deemed to be University, Ghaziabad, NCR Delhi- 201009, India

[2]Professor and Guide, Department of Pathology- Santosh Medical College & Hospital, Santosh Deemed to be University, Ghaziabad, NCR Delhi- 201009, India

[3]Assistant Professor and CoGuide, Department of Pathology- Santosh Medical College & Hospital, Santosh Deemed to be University, Ghaziabad, NCR Delhi- 201009, India

[4]Head of Department, Department of Pathology- Santosh Medical College & Hospital, Santosh Deemed to be University, Ghaziabad, NCR Delhi- 201009, India

OPEN ACCESS

Corresponding Author:

Dr. Swati Singh
Professor, Department of Pathology, Santosh Medical College & Hospital, Ghaziabad - 201009, India

Received: 10-05-2026
Accepted: 24-06-2026
Available online: 27-06-2026

ABSTRACT

Introduction: Uterine fibroids, also known as leiomyomas, are benign smooth muscle tumors of the uterus that affect up to 75% of women during reproductive years. Their growth is influenced by estrogen, genetic mutations such as MED12, and local growth factors. Clinically, they present with abnormal uterine bleeding, pelvic pressure, infertility, and adverse pregnancy outcomes. The Ki-67 antigen, a nuclear protein expressed during active phases of the cell cycle, is widely used as a proliferation marker. Its role in assessing tumor activity and severity in leiomyomas remains important for diagnostic and prognostic purposes.

Aim and Objective: The study aimed to evaluate Ki-67 expression in uterine leiomyomas and adjacent healthy myometrium and to assess its potential as a marker for tumor activity and severity across different morphological subtypes.

Methodology: This cross-sectional study was conducted at Santosh Medical College and Hospital, Ghaziabad, on 86 female patients aged 35–44 years with uterine leiomyomas scheduled for hysterectomy. Patients with prior hormonal therapy, other uterine pathologies, or previous uterine surgery were excluded. Tissue samples of leiomyoma and adjacent myometrium were collected and subjected to immunohistochemical staining for Ki-67. Expression was evaluated by two blinded observers, and statistical analysis was performed using Student's t-test and ANOVA with $p < 0.05$ considered significant.

Results: The majority of participants were in the 38–40 years age group (37.2%). Most had secondary education (38.4%) and belonged to the upper lower socioeconomic class (33.7%). Mean Ki-67 expression was significantly higher in leiomyomas (4.32 ± 1.67) than in myometrium (1.14 ± 0.61; $p < 0.001$). Among subtypes, atypical leiomyomas showed the highest expression (6.12 ± 2.05), followed by cellular (5.41 ± 1.83), ordinary (3.85 ± 1.42), and degenerated (2.67 ± 0.98). All subtypes had significantly higher expression than corresponding myometrium ($p < 0.001$).

Conclusion: Ki-67 expression was significantly elevated in leiomyomas compared to myometrium, with higher levels in atypical and cellular subtypes, suggesting its potential utility as a marker for tumor activity and severity in uterine leiomyomas.

Keywords: *Uterine leiomyoma; Fibroid; Ki-67 antigen; Immunohistochemistry; Myometrium; Tumor activity; Morphological subtypes; Proliferation marker.*

INTRODUCTION

Uterine fibroids, or leiomyomas, are benign tumors that originate from the smooth muscle of the uterus. They are highly prevalent, affecting up to 75% of women during reproductive years. Histologically, fibroids show abnormal proliferation of smooth muscle cells and excess extracellular matrix, which gives them their density and structure[1].

Their occurrence is closely linked with hormonal influence, particularly estrogen. Estrogen stimulates fibroid growth, while regression is often observed after menopause when estrogen levels decline. Apart from hormones, genetic mutations (such as in the MED12 gene) and local growth factors also contribute to their development[1].

Clinically, fibroids vary in size, number, and location, which determines symptom severity. About 30% of affected women experience abnormal uterine bleeding, most often heavy or prolonged menses[2]. Enlarged fibroids may cause pelvic heaviness or pressure, leading to constipation, urinary frequency, or incontinence due to compression of adjacent organs[2]
.
Fibroids also impact reproductive health. They are associated with infertility and pregnancy complications, including miscarriage, fetal malformations, preterm birth, and obstructed labor requiring cesarean section[2,3]. These risks highlight the importance of early diagnosis and management, especially in women of childbearing age.

From a biological perspective, research into cellular proliferation helps explain tumor behavior. The Ki-67 antigen, a nuclear protein, is a key marker of cell proliferation. It is expressed in all active phases of the cell cycle (G1, S, G2, M) but absent in resting cells, making it a reliable measure of proliferative activity.

The study aimed to assess whether Ki-67 expression correlates with clinical and demographic factors such as patient age, tumor size, location, and symptom profile and also aimed at evaluation of KI-67 expression in uterine leiomyoma and in healthy myometrium thereby assess the potential of KI-67 expression as a marker for tumor activity and severity in different morphological subtypes of uterine leiomyomas.

MATERIAL AND METHODS

This cross-sectional study was conducted at Santosh Medical College and Hospital, Ghaziabad, with a sample size of 86, calculated at a 95% confidence level, 5% margin of error, and an expected prevalence of 0.06% as reported by Rubisc. The study included female patients aged 35 to 44 years diagnosed with uterine leiomyomas and scheduled for hysterectomy, who provided written informed consent. Patients with a history of hormonal therapy within six months, other uterine pathologies such as adenomyosis or endometrial hyperplasia, or prior uterine surgery affecting tissue integrity were excluded. A consecutive sampling technique was used, where all eligible patients during the study period were approached, and those fulfilling the criteria were included. During hysterectomy, both leiomyoma and adjacent myometrial tissues were collected for analysis. The samples were processed for immunohistochemical staining with the Ki-67 antibody, and evaluation was performed by two independent blinded observers.

Immunohistochemistry (IHC) was the primary method used to detect Ki-67 expression. Tissue sections were mounted on slides, subjected to antigen retrieval, and incubated with the primary Ki-67 antibody, followed by a secondary antibody conjugated with a chromogenic enzyme. The reaction product produced distinct nuclear staining in Ki-67 positive cells, which was visualized under a high-resolution microscope. Digital imaging analysis systems were used to quantify the staining, ensuring accuracy and reproducibility.

The study procedure involved immediate fixation of the excised tissues in formalin, embedding them in paraffin blocks, sectioning, and staining for Ki-67. Data on Ki-67 expression were collected by quantifying the percentage of positively stained nuclei in multiple high-power fields of leiomyoma and myometrial tissues. Alongside histological evaluation, clinical data such as patient age, symptoms of anemia, bleeding, and pain were collected through interviews and review of medical records.

Statistical analysis was carried out using appropriate medical research software. The Student's t-test was applied to compare mean Ki-67 expression levels between leiomyoma and myometrial tissues, while Pearson's correlation coefficient was used to assess associations between Ki-67 expression and clinical parameters including severity of anemia, bleeding, and pain. A p-value of less than 0.05 was considered statistically significant. This analysis aimed to identify significant differences in Ki-67 expression between tumor and adjacent normal tissue and to evaluate its potential as a marker of tumor activity and severity in uterine leiomyomas.

RESULT

Table 1: Age Distribution (n=86)

Age Group (Years)	Frequency (n)	Percentage (%)
35–37	24	27.9
38–40	32	37.2

41–44	30	34.9
Total	**86**	**100.0**

The above table illustrates that out of the total study participants, 24 (27.9%) were in the age group of 35–37 years, 32 (37.2%) were in the age group of 38–40 years, and 30 (34.9%) were in the age group of 41–44 years, making a total of 86 participants (100%).

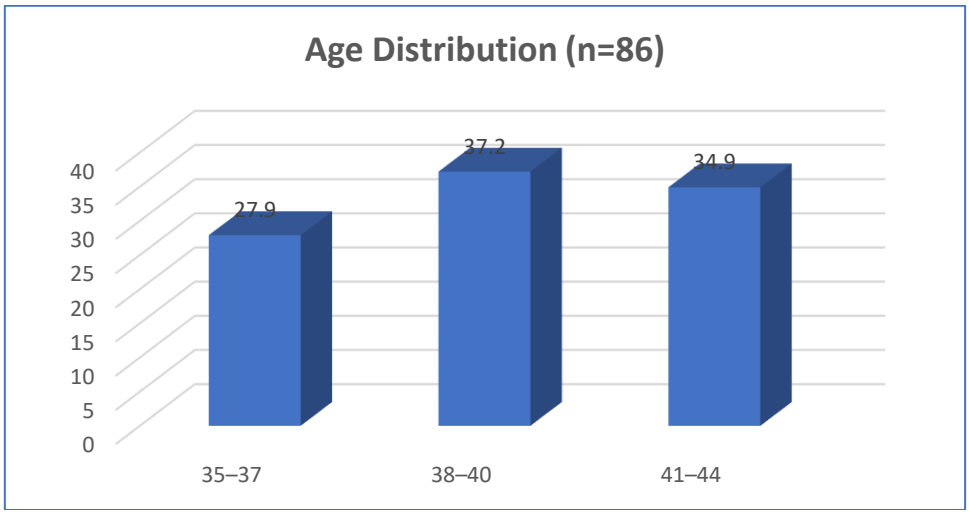


Table 2: Educational Status

Education Level	Frequency (n)	Percentage (%)
Illiterate	14	16.3
Primary (1–5 yrs)	19	22.1
Secondary (6–12 yrs)	33	38.4
Graduate	15	17.4
Postgraduate	5	5.8
Total	**86**	**100.0**

The above table illustrates that out of the total study participants, 14 (16.3%) were illiterate, 19 (22.1%) had primary education, 33 (38.4%) had secondary education, 15 (17.4%) were graduates, and 5 (5.8%) were postgraduates, making a total of 86 participants (100%).

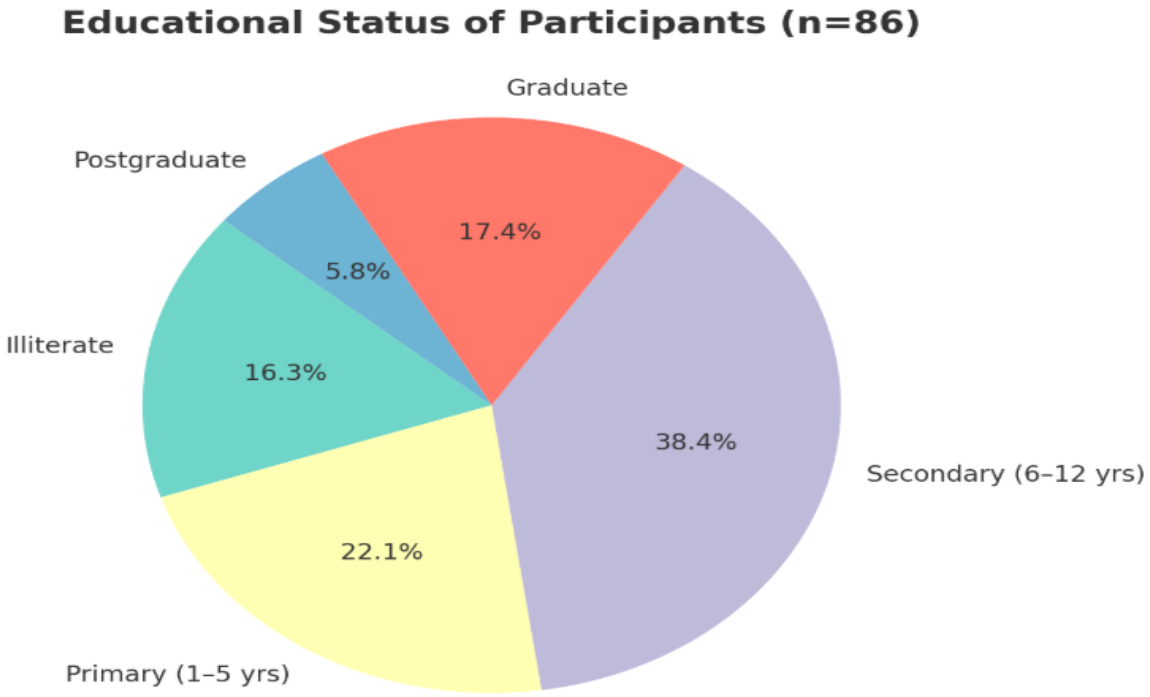


Table 3: Socioeconomic Status (Kuppuswamy Scale)

Socioeconomic Class	Frequency (n)	Percentage (%)
Upper (I)	7	8.1
Upper Middle (II)	12	14.0
Lower Middle (III)	26	30.2
Upper Lower (IV)	29	33.7
Lower (V)	12	14.0
Total	**86**	**100.0**

The Modified Kuppuswamy Scale is calculated based on three parameters: Education, Occupation, and Total Monthly Family Income.

We sum the scores from all three parameters (Range: 3 to 29) to classify the family into one of the following five strata:

Upper (Class I): 26 – 29
Upper Middle (Class II): 16 – 25
Lower Middle (Class III): 11 – 15
Upper Lower (Class IV): 5 – 10
Lower (Class V): 3 - 4

The above table illustrates that out of the total study participants, 7 (8.1%) belonged to the upper class, 12 (14.0%) were from the upper middle class, 26 (30.2%) were from the lower middle class, 29 (33.7%) were from the upper lower class, and 12 (14.0%) belonged to the lower class, making a total of 86 participants (100%).

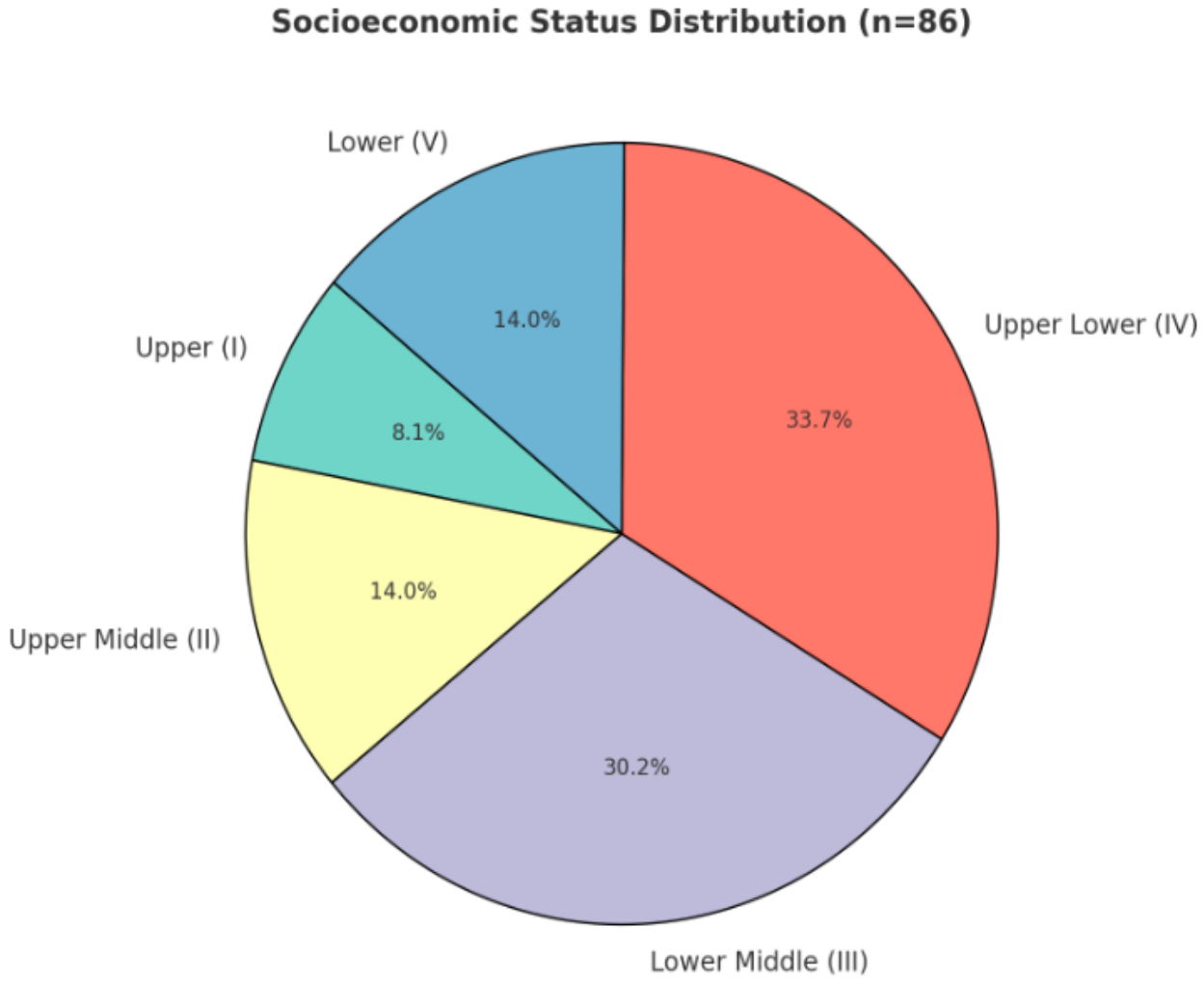


Table 4: Overall KI-67 Expression (n=86)

Tissue Type	KI-67 (% Positive Cells), Mean ± SD	p-value (t-test)
Leiomyoma	4.32 ± 1.67	**<0.001**
Myometrium	1.14 ± 0.61	

The above table illustrates that out of the total study participants, the mean Ki-67 expression in leiomyoma tissue was 4.32 ± 1.67, whereas in myometrium it was 1.14 ± 0.61, with the difference being statistically significant ($p < 0.001$).

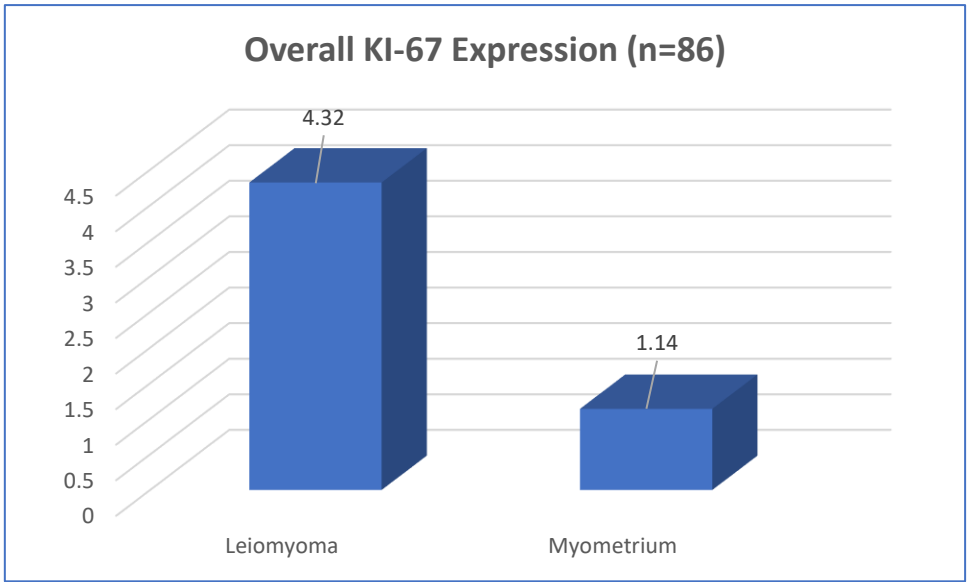


Table 5: KI-67 Across Subtypes

Subtype	KI-67 (% Positive Cells), Mean ± SD	p-value (ANOVA)
Ordinary	3.85 ± 1.42	**<0.001**
Cellular	5.41 ± 1.83	
Atypical	6.12 ± 2.05	
Degenerated	2.67 ± 0.98	

Cellular Leiomyoma has increased cellularity (more cellular than background myometrium), scant cytoplasm without increased mitotic activity and atypia.

Atypical Leiomyoma has bizarrely shaped, hyperchromatic, multilobulated nuclei with nuclear pseudoinclusions arranged in a multifocal to diffuse distribution in a background of a typical leiomyoma ,has low mitotic activity (< 5 mitoses/10 high power fields) and absence of tumor cell necrosis.

The above table illustrates that out of the total study participants, the mean Ki-67 expression in ordinary subtype was 3.85 ± 1.42, in cellular subtype it was 5.41 ± 1.83, in atypical subtype it was 6.12 ± 2.05, and in degenerated subtype it was 2.67 ± 0.98, with the difference found to be statistically significant ($p < 0.001$).

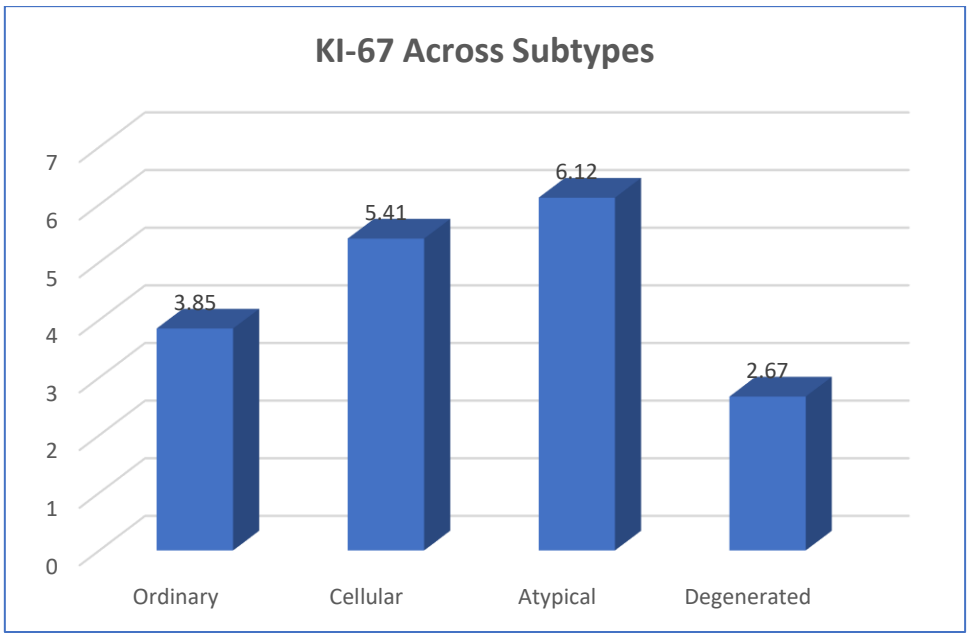
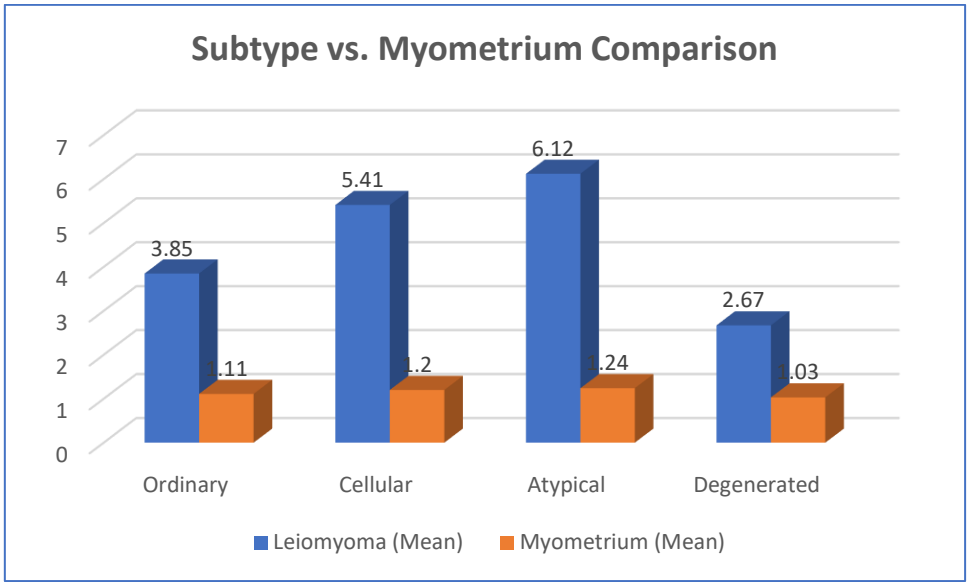


Table 6: Subtype vs. Myometrium Comparison

Subtype	Leiomyoma (Mean ± SD)	Myometrium (Mean ± SD)	p-value
Ordinary	3.85 ± 1.42	1.11 ± 0.59	<0.001
Cellular	5.41 ± 1.83	1.20 ± 0.64	<0.001
Atypical	6.12 ± 2.05	1.24 ± 0.69	<0.001
Degenerated	2.67 ± 0.98	1.03 ± 0.51	<0.001

The above table illustrates that out of total study participants, the mean Ki-67 expression in leiomyoma tissue was 3.85 ± 1.42 for the ordinary subtype, 5.41 ± 1.83 for the cellular subtype, 6.12 ± 2.05 for the atypical subtype, and 2.67 ± 0.98 for the degenerated subtype, while in the corresponding myometrium the values were 1.11 ± 0.59, 1.20 ± 0.64, 1.24 ± 0.69, and 1.03 ± 0.51 respectively, with all showing statistically significant p-values of <0.001.



DISCUSSION

The present study demonstrated that the mean Ki-67 expression was significantly higher in leiomyoma tissue (4.32 ± 1.67) compared to healthy myometrium (1.14 ± 0.61), with a statistically significant difference ($p < 0.001$). When evaluated across morphological subtypes, the highest Ki-67 expression was observed in atypical leiomyomas (6.12 ± 2.05), followed by cellular (5.41 ± 1.83), ordinary (3.85 ± 1.42), and degenerated subtypes (2.67 ± 0.98), with all differences reaching statistical significance. These findings suggest that Ki-67 expression correlates with tumor activity and may vary according to histological subtype.

The results of the present study are comparable with the observations of Watanabe et al. (2021)[4], who reported that Ki-67, along with cyclin E, was a critical biomarker in confirming the progression from leiomyoma to leiomyosarcoma in a postpartum patient. Their findings emphasized that increased Ki-67 activity can indicate malignant potential, which is in line with our study where higher Ki-67 expression was noted in atypical and cellular variants. Similarly, Guo et al. (2022)[5] highlighted the diagnostic complexity of leiomyoma with bizarre nuclei (LBN), noting its overlap with smooth muscle tumors of uncertain malignant potential (STUMP) and leiomyosarcoma. They suggested that although Ki-67 alone is not definitive, it may contribute as an adjunct marker in distinguishing these tumors. Our results support this by demonstrating variable Ki-67 expression across morphological subtypes, reflecting differences in tumor activity.

The findings are also consistent with those reported by Sparić et al. (2022)[6], who in their systematic review underscored the importance of integrating molecular and protein markers, including Ki-67, to aid in differential diagnosis between leiomyomas and leiomyosarcomas. The higher proliferation indices observed in our atypical and cellular subtypes align with their assertion that molecular markers provide added diagnostic value. Nishikawa et al. (2023)[7] also emphasized the role of molecular markers and genetic profiling in differentiating benign from malignant mesenchymal tumors, suggesting that Ki-67 expression may serve as one such marker for stratifying tumor aggressiveness. This is supported by our study where significant differences in Ki-67 expression were observed between leiomyoma subtypes and healthy myometrium. Patkar et al. (2023)[8] reported that leiomyomas with bizarre nuclei exhibited low mitotic activity and a very low Ki-67 proliferation index (0.5–1.0%), thereby confirming their benign nature despite atypical histology. This observation differs from our study, where the atypical subtype demonstrated the highest Ki-67 expression. The discrepancy may be attributed to sample size differences and histological heterogeneity within the atypical category. Nonetheless, both studies reinforce the utility of Ki-67 as a valuable adjunct marker in assessing tumor biology.

CONCLUSION

The present study concludes that Ki67expression was significantly elevated in leiomyoma tissue compared with normal myometrium, underscoring the heightened proliferative activity characteristic of these tumors. Notably, Ki-67 indices varied across histomorphological subtypes, with atypical leiomyomas demonstrating the highest proliferative fraction, followed by cellular and non-degenerated variants, while degenerated subtypes exhibited comparatively lower expression. These differences were statistically robust ($p < 0.001$).

Collectively, these findings reinforce the role of Ki-67 as a sensitive and reproducible marker of cellular proliferation in uterine smooth muscle tumors. The observed heterogeneity in proliferative indices among leiomyoma subtypes may have important implications for understanding tumor biology and could contribute to refined histopathological stratification in routine diagnostic practice.

REFERENCES

1. Commandeur AE, Styer AK, Teixeira JM. Epidemiological and genetic clues for molecular mechanisms involved in uterine leiomyoma development and growth. Hum Reprod Update. 2015;21(5):593–615.
2. Vargas-Hernández VM, Vargas-Aguilar VM, Tovar-Rodríguez JM, Flores-Barrios K, Acosta-Altamirano G, Moreno-Eutimio MA. Leiomiomatosis uterina. Aspectos epidemiológicos, fisiopatogénicos, reproductivos, clínicos y terapêuticos. Rev Hosp Jua Mex. 2013;80(3):173–82.
3. Boclin KLS, Faerstein E. Prevalência de diagnóstico médico auto-relatado de miomas uterinos em população brasileira: padrões demográficos e socioeconômicos no Estudo Pró-Saúde. Rev Bras Epidemiol. 2013;16(2):301–13.
4. Watanabe K, Hayashi T, Katsumata M, Sano K, Abiko K, Konishi I. Development of uterine leiomyosarcoma during follow-up after caesarean section in a woman with uterine leiomyoma. Anticancer Res. 2021;41(6):3001–3010. doi:10.21873/anticanres.15082
5. Guo E, Li C, Hu Y, Zhao K, Zheng Q, Wang L. Leiomyoma with bizarre nuclei: a current update. Int J Womens Health. 2022;14:1641–1656. doi:10.2147/IJWH.S388278
6. Sparić R, Andjić M, Babović I, Nejković L, Mitrović M, Štulić J, Pupovac M, Tinelli A. Molecular insights in uterine leiomyosarcoma: a systematic review. Int J Mol Sci. 2022;23(17):9728. doi:10.3390/ijms23179728
7. Nishikawa S, Yamamoto T, Kuno R, et al. Molecular classification and diagnostic markers of uterine mesenchymal tumors: a clinical approach. Int J Gynecol Pathol. 2023;42(1):45–53. doi:10.1097/PGP.0000000000000821
8. Patkar RR, Shukla DP, Kamat AM, Gandhi JS. Uterine leiomyoma with bizarre nuclei: a histopathological challenge. J Obstet Gynaecol India. 2023;73(Suppl 1):34–38. doi:10.1007/s13224-022-01737-6.