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Original Research Article

Uterine smooth muscle tumours and the role of Ki67 expression in determining their biological behaviour

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ABSTRACT

Background: Uterine smooth muscle tumours include leiomyoma, intravenous leiomyoma, metastasizing leiomyoma, smooth muscle tumour of uncertain malignant potential (STUMP) and leiomyosarcoma (LMS). Diagnosis primarily relies on histopathological features. In cases with overlapping morphological findings, immunohistochemical markers can help in differentiating benign and malignant lesions. This study evaluates the role of Ki67 immunohistochemical expression in determining the biological behaviour of uterine smooth muscle tumours.
Methods: In this study a total of 58 cases of uterine smooth muscle tumours were analysed. Gross findings were observed. The microscopic evaluation was done on formalin fixed paraffin embedded sections and haematoxylin and eosin-stained slides. This was followed by Ki67 staining by immunohistochemistry and was graded depending on percentage of nuclear expression. Association of Ki67 grade with gross and microscopic findings were determined.
Results: Among 58 tumours, 52 (90%) were leiomyomas, 3 (5%) STUMPs, and 3 (5%) LMS. Most patients were between 41 and 50 years of age, with menorrhagia being the most common presenting complaint. Ki67 expression was low (86.2%) in all leiomyomas, consistently high in LMS and showed variable levels in STUMP. Ki67 levels demonstrated a significant association with mitotic activity, necrosis and cytologic atypia ($p<0.05$), but did not correlate with tumour size and secondary changes.
Conclusions: Combining histopathology with Ki67 immunostaining improves diagnostic efficacy in uterine smooth muscle tumours and thus contributes to a more effective clinical management.

Keywords: Uterus, Leiomyoma, Variants, STUMP, Leiomyosarcoma, Ki67

INTRODUCTION

The recent 7th edition of world health organization (WHO) 2022, classifies uterine smooth muscle tumours as leiomyoma (including the variants), intravenous leiomyoma, metastasizing leiomyoma, STUMP and LMS.[1]Among these, leiomyomas are the most common benign neoplasms occurring in women of reproductive age, influenced by hormonal, genetic and growth factor-related mechanisms.[1-3]

While most leiomyomas are asymptomatic, about one-third presents with abnormal bleeding, pain or infertility. Typical leiomyomas are easily recognized on imaging, but diagnostic difficulties may arise in large tumours, those with secondary changes or in cases with variations in microscopic findings.[4]

Histopathological diagnosis of STUMP and LMS is based on mitotic count, cytologic atypia and coagulative tumour cell necrosis.[1,5] Cellular and mitotically active leiomyomas lack atypia or necrosis, whereas STUMPs show intermediate features and LMS exhibits marked atypia, high mitotic activity and necrosis.[1,5] However, differentiating STUMP from LMS can be challenging, particularly when mitoses are difficult to identify.[6,7]

Ki67, a nuclear protein expressed during active phases of the cell cycle, serves as a marker of cellular proliferation and tumour aggressiveness.[6,7] Elevated Ki67 expression has been reported in LMS and in few leiomyoma variants.[6]

This study aims to evaluate the diagnostic utility of Ki67 immunohistochemistry in differentiating benign, borderline and the malignant uterine smooth muscle tumours.

METHODS

This was a cross-sectional study undertaken in the Department of Pathology, Yenepoya medical college, Deralakatte, Mangalore that included uterine smooth muscle tumours reported in the Department of Pathology from September 2023 to January 2025. The study was conducted after obtaining due clearance from institutional ethics committee.

The myomectomy specimens and hysterectomy specimens with uterine smooth muscle tumours received in the department of pathology at Yenepoya Medical College collected during the study period was included.

The specimens were received fixed in 10% formalin. Gross examination and findings like tumour size, number and appearance was noted. Grossing was done according to the standard grossing protocol of 1 section per 1cm size and if multiple leiomyomas, sections from each were taken accordingly. Tissue was processed and paraffin embedded. Sections were cut serially at a thickness of 4-5 microns and stained with haematoxylin and eosin. Microscopic features like architecture of tumour cells, degenerative changes, atypia, necrosis and mitotic figures were noted.

Immunohistochemistry of Ki67

Immunohistochemistry (IHC) was performed after selecting representative tumour blocks. Staining was done using primary antibody to Ki67 (Rabbit monoclonal antibody, Clone-SP6) following the manufacturer's (Biocare Medical) instructions. The PolyExcel HRP (Non-biotin, micro-polymer based) DAB detection system was used with adequate positive and negative controls.

Grading of Ki67

According to study conducted by Filho et al in order to estimate the expected prevalence of immunohistochemical evaluation of Ki67 expression in uterine smooth muscle tumours with 95% level of confidence, 6% margin of error, 58 cases were included in the study.[2]

Nuclear staining was considered positive for Ki67. Results were interpreted by 2 observers with a consensus reached regarding any discrepancy. Ki67 expression was evaluated by calculating the percentage of positive cells. Each slide was given a value composed of percentage of stained cells of the total number of cells.[2]

Percentage of stained cells=Cells with coloured nuclei×100/Total number of cells

The proportion of stained cells were graded as: grade 0: Staining in 0-10% of tumour cells, grade 1: Staining in 11-25% of cells, grade 2: Staining in 26-50% of cells and grade 3: staining in >50% of cells.[6]

Descriptive statistical analysis was carried out in the present study. Results on categorical data were shown as number and percentage. The entire data was statistically analysed using statistical package for social sciences (version 27) for MS Windows.

RESULTS

The present study analysed 58 cases of uterine smooth muscle tumours over a period of 1 year and 4 months which comprised of 52 leiomyomas (including the variants), 3 STUMP cases and 3 LMS cases. Among 58 cases of uterine smooth muscle tumours, the most common complaint was menorrhagia, followed by abdominal pain and infertility as shown in Figure 1. In STUMP and LMS, most common presenting complaint was menorrhagia.

Patient ages ranged from 30-75 years, with a peak incidence in the 41-50 years group, depicted in Figure 2. The mean age at presentation was 44 years in LMS and STUMP.

Cut surface of most tumours exhibited the typical pale white, whorled pattern (87.9%). Haemorrhagic infarction (red degeneration) and necrosis were observed in a few cases (5.2%) (Figure 3).

Leiomyoma accounted for 90% of tumours, with most showing usual histology. Variants included 10 cellular, 1 mitotically active and 2 epithelioid leiomyomas as shown in Table 1. 3 cases of STUMP was diagnosed, shown in Figure 4.

Secondary degenerative changes were present in 48.2%, with hyaline degeneration being most common.

A low mitotic index (0-3/10 HPF) was seen in 90% of cases. High mitotic rates (>10/10 HPF) were associated with LMS. Mild to moderate atypia occurred in 28% of cases; marked atypia was exclusive to LMS as shown in Figure 5. Necrosis was found in 9% of cases and strongly correlated with malignancy.

Ki67 expression

Leiomyoma

Most of these cases showing no/minimal staining (Grade 0-1) belonged to usual leiomyomas with a minor category comprising of variants of leiomyomas especially cellular leiomyoma (4%) and with a small proportion from the STUMP.

STUMP

STUMP showed variable expression from grade 0-2 with grade 2 comprising only of STUMP. STUMP tumours have Ki67% values in between leiomyoma and LMS, but their values are significantly different from LMS.

LMS

Three cases which showed grade 3 positivity was diagnosed as LMS. LMS cases tend to have higher Ki67% compared to leiomyomas, and this difference almost reaches statistical significance (p=0.054).

Most tumours (86.2%) had low Ki67 expression (Grade 0), indicating low proliferative activity. Grade 3 expression was observed only in LMS. Mean Ki67 indices were significantly higher in LMS compared to STUMP (Figure 4) and leiomyomas. Ki67 expression showed significant correlation with mitotic rate (p=0.00496), necrosis (p=0.004), and cytological atypia (p=0.0135). No significant association was found between Ki67 and tumour size or secondary changes.

Association between usual leiomyoma, variants of leiomyoma and STUMP was tested. A significant p=0.0025 confirms a strong association between Ki67 expression and tumour type. Low Ki67 expression is dominant in leiomyoma and leiomyoma variants as shown in Table 2.

In this study, Ki67 positive staining was seen significantly more in LMS as shown in Figure 6 when compared to STUMP and leiomyoma variants.

In the current study, 3 cases (5%) were diagnosed as LMS in which 2 were conventional type and 1 was myxoid type.

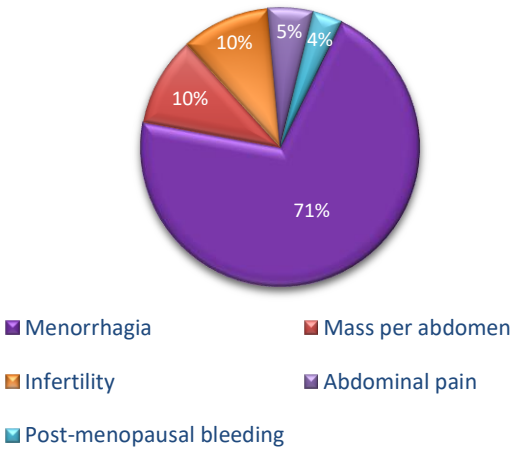

Figure 1: Distribution of presenting complaints, (n=58).

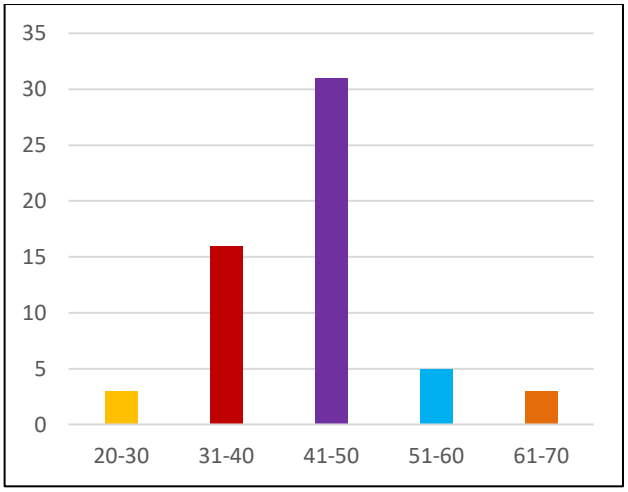

Figure 2: Age group distribution of uterine smooth muscle tumours, (n=58).

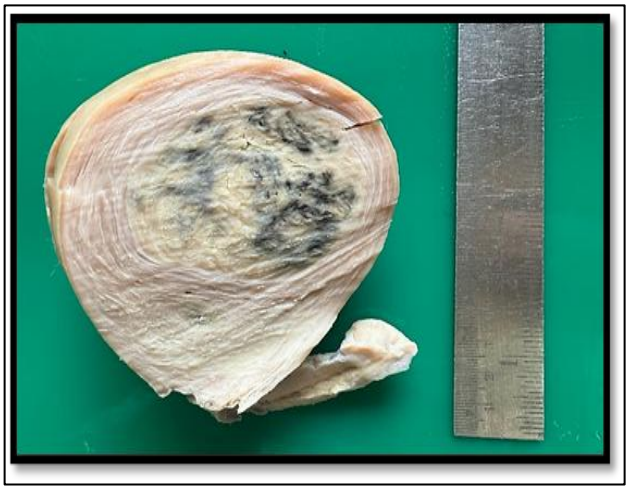

Figure 3: TAH specimen showing heterogenous appearance with haemorrhagic areas and pale-yellow areas diagnosed as STUMP.

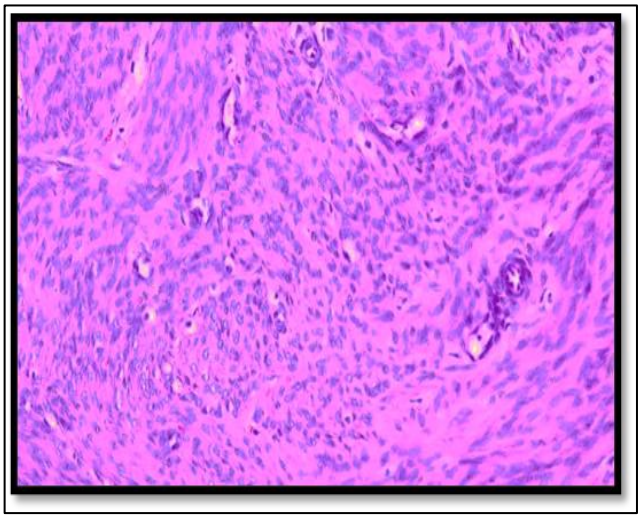

Figure 4: STUMP with mild atypia and 4-5 mitosis/10 HPF (H and E, 20×).

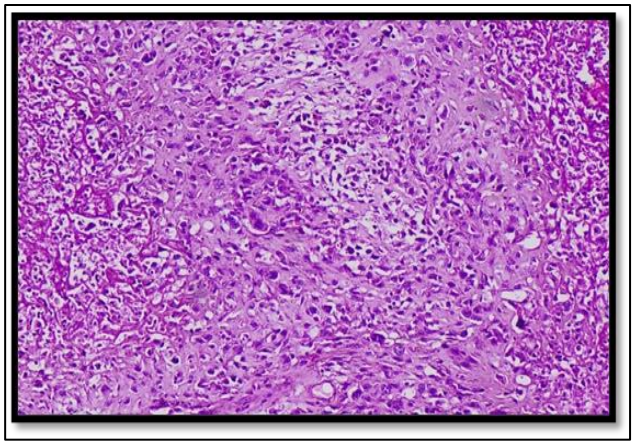

Figure 5: Conventional LMS in intersecting pattern, (H and E, 20×).

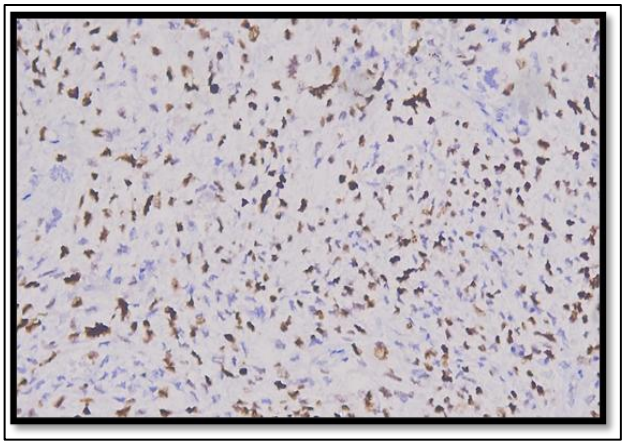

Figure 6: Ki67 grade 3 (76%) staining in LMS (IHC: Ki67, 20×).

Table 1: Leiomyoma and its variants, (n=52).

Category	N
Usual leiomyoma	39
Cellular leiomyoma	10
Epithelioid leiomyoma	2
Mitotically active leiomyoma	1

Table 2: Ki67 expression in uterine smooth muscle tumours.

Ki67 staining percentage	Grade 0 (0-10%)	Grade 1 (11-25%)	Grade 2 (26-50%)	Grade 3 (>50%)	Total	Mean Ki67 (%)	Positivity >10%
Leiomyoma	39	0	0	0	39	2.99	0 (0%)
Leiomyoma variants	10	3	0	0	13	4.3	3 (23%)
STUMP	1	1	1	0	3	20	2 (66.6%)
LMS	0	0	0	3	3	79	3 (100%)
Total	50	4	1	3	58		

DISCUSSION

Uterine smooth muscle tumours represent a spectrum ranging from benign leiomyoma to malignant LMS, with STUMP forming an intermediate group. Their overlapping histomorphological features often pose diagnostic challenges, emphasizing the role of immunohistochemical (IHC) markers in differentiating these entities. Among various markers studied, Ki67, a nuclear proliferation antigen, has been shown to correlate with tumour aggressiveness and remains one of the most valuable adjuncts in evaluating these tumours.

In the present study, 90% of cases were leiomyomas, 5% STUMP, and 5% were LMS, indicating that these benign tumours are the most commonly encountered uterine smooth muscle neoplasms.

Similar findings were observed in Delgado et al (59%), but with a lower percentage of leiomyomas, potentially due to differences in patient selection criteria or sample size.[8] The peak incidence occurred in the 41-50 year age group, reflecting hormonal dependence, similar to findings by Kale et al and Kaur et al.[9,10] Menorrhagia was the most frequent symptom, followed by abdominal pain, in agreement with previous reports. Intramural location was the most frequent (37.9%) and hyalinization was the predominant secondary change (34%), corroborating earlier findings by Dayal et al.[11]

In the present study, Ki67 staining revealed that 8 cases showing grade 1-2 positivity were primarily composed of cellular leiomyoma, STUMP and LMS.

Ki67 expression was minimal or absent in most leiomyomas (≤10%), consistent with their low proliferative activity. STUMP cases showed variable expression, reflecting their heterogeneous biological behaviour. All LMS demonstrated high Ki67 expression (>10%), confirming its association with malignancy and aggressive histologic features. Mittal and Demopoulos also observed that Ki67 levels exceeded 15% in 11 out of 12 LMS cases, while 5-10% expression was noted in 6 out of 7 STUMP cases.[12] Their findings suggest that Ki67 may

serve as a valuable marker in differentiating STUMP from leiomyoma. Petrović et al reported no Ki67 expression in LM (p=0.0001 for LMS vs. LM and STUMP vs. LM), supporting its diagnostic value.[13]

These studies, in conjunction with the current findings, indicate that Ki67 expression correlates with tumour aggressiveness and may assist in distinguishing between leiomyoma, STUMP and LMS.

While morphology remains the cornerstone of diagnosis, Ki67 can aid in distinguishing STUMP and LMS from benign leiomyomas, particularly in histologically ambiguous cases.

In the present study however sample size was relatively small, therefore further studies with larger cohort and also incorporation of additional markers could enhance diagnostic precision and improve prognostic assessment in cases with diagnostic dilemma.

CONCLUSION

This study emphasizes the significance of Ki67 as a crucial biomarker for differentiating between benign and malignant uterine smooth muscle tumours. The findings emphasize that histopathology combined with Ki67 immunostaining enhances diagnostic accuracy. The results also align with previous studies, reinforcing the significance of proliferative markers in tumour classification.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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