Section: Pathology

Original Research Article

COMPARATIVE IMMUNOHISTOCHEMICAL EXPRESSION OF BCL-2 IN NORMAL MYOMETRIUM AND UTERINE LEIOMYOMA

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ABSTRACT

Background: Altered regulation of apoptosis may contribute to the development and persistence of uterine leiomyoma. B-cell lymphoma-2 (Bcl-2), an anti-apoptotic protein, provides a potential marker for investigating differences in cellular survival pathways between leiomyoma and normal myometrium. **Aim:** To compare Bcl-2 immunohistochemical expression in uterine leiomyoma and paired normal myometrium and assess its association with selected clinicopathological characteristics.

Materials and Methods: A hospital-based comparative cross-sectional study was conducted in the Department of Pathology, Mandya Institute of Medical Sciences, Mandya, from November 2023 to October 2024. Forty-two hysterectomy specimens provided 42 leiomyoma and 42 paired normal myometrial samples. Following histopathological examination, Bcl-2 immunoreactivity was graded from 0 to 3+. Expression was assessed in relation to age, menstrual-cycle phase and secondary degenerative changes. Paired comparisons were evaluated using exact McNemar, Wilcoxon signed-rank and exact sign tests. Subgroup grade distributions were assessed using exact conditional tests. Statistical significance was defined as $p<0.05$.

Results: Bcl-2 positivity occurred in 38 leiomyomas (90.5%) and 32 normal myometrial samples (76.2%), without a statistically significant paired difference ($p=0.180$). Higher-grade expression (2+-3+) occurred in 26 leiomyomas (61.9%) and 13 normal myometrial samples (31.0%), yielding a difference of 31.0 percentage points (95% CI: 11.7-50.2; $p=0.007$). Overall expression grades were significantly higher in leiomyoma (Wilcoxon $W=122.0$; $p<0.001$). Leiomyoma exhibited a higher grade in 30 pairs (71.4%), an equal grade in six (14.3%) and a lower grade in six (14.3%). Among non-tied pairs, 83.3% showed higher expression in leiomyoma (95% CI: 67.2-93.6; exact sign test $p<0.001$). Expression-grade distribution was associated with menstrual phase ($p=0.016$) and secondary degenerative changes ($p<0.001$), but not age ($p=0.775$).

Conclusion: Leiomyomas exhibited higher Bcl-2 expression grades than paired normal myometrium. Higher grades were more frequent during the secretory phase and in lesions without secondary degeneration. These findings support an association between Bcl-2 expression and leiomyoma biology, warranting further functional and longitudinal investigation.

Keywords: Bcl-2; Uterine leiomyoma; Immunohistochemistry.

INTRODUCTION

Uterine leiomyomas are benign smooth-muscle tumours characterised by variable amounts of extracellular matrix and considerable heterogeneity in their size, location and clinical presentation. Stewart et al. (2016),[1] described leiomyomas as clonal uterine neoplasms containing smooth-muscle cells, fibroblasts and abundant fibrous extracellular matrix, highlighting the contribution of these components to tumour development. Although benign, their clinical consequences make them an

important subject of gynaecological and pathological research.

Giuliani et al. (2020),[2] emphasised that symptomatic leiomyomas can cause abnormal uterine bleeding, anaemia, pelvic pain, pressure symptoms and reproductive difficulties, substantially affecting women's health and quality of life. Stewart et al. (2017),[3] in their systematic review, demonstrated considerable variation in the reported epidemiology of uterine fibroids and examined the contribution of demographic and reproductive risk factors, supporting the need to consider patient characteristics when investigating their biology.

The development and persistence of leiomyomas involve complex interactions between hormonal stimulation, genetic alterations, extracellular matrix accumulation and cellular survival pathways. Yang et al. (2022),[4] highlighted the involvement of sex hormones, growth factors, epigenetic mechanisms and several intracellular signalling pathways in leiomyoma development, illustrating why their biological behaviour cannot be explained by increased cellular proliferation alone.

Reduced programmed cell death may also contribute to the persistence of tumour cells. B-cell lymphoma-2 (Bcl-2) is an anti-apoptotic protein, making its expression relevant to the investigation of cellular survival in leiomyomas. Elnaghy et al. (2018),[5] investigated Bcl-2 in leiomyomatous tissue and normal myometrium using immunohistochemistry and biochemical methods, providing a basis for comparative assessment of this marker in uterine smooth-muscle tissues.

Aim

To compare the immunohistochemical expression of Bcl-2 in normal myometrium and uterine leiomyoma.

Objectives

1. To assess the distribution and grade of Bcl-2 immunohistochemical expression in normal myometrium and uterine leiomyoma.
2. To compare Bcl-2 immunohistochemical expression between paired leiomyoma and normal myometrial tissue samples.
3. To evaluate the association of Bcl-2 expression with age, menstrual-cycle phase and secondary degenerative changes in leiomyoma.

MATERIALS AND METHODS

Source of Data

The study material comprised hysterectomy specimens received in the Histopathology Laboratory, Department of Pathology, Mandya Institute of Medical Sciences, Mandya. Clinical information was obtained from histopathology requisition forms and the corresponding inpatient and outpatient records. Leiomyoma tissue and accompanying histologically normal myometrium were evaluated from the selected hysterectomy specimens.

Study Design

The study was a hospital-based comparative cross-sectional study. Bcl-2 immunohistochemical expression was assessed in two tissue groups: uterine leiomyoma and normal myometrium. Because both tissue types were obtained from the same women, the comparison represented a paired, within-patient comparison. The dissertation reported simple random sampling as the sampling method; the specific random-selection procedure was not described.

Study Location

The study was conducted in the Department of Pathology, Mandya Institute of Medical Sciences, Mandya, Karnataka. Histopathological examination and immunohistochemical assessment were performed in the departmental laboratory.

Study Duration

The study was conducted over 12 months, from November 2023 to October 2024.

Sample Size

The final study comprised 42 samples in each tissue group, providing 42 matched pairs and 84 tissue samples.

Thus, the corrected calculation yielded a minimum of 39 samples per group, after rounding upward. The actual study included 42 samples per group, exceeding this minimum.

Calculation clarification: The dissertation's stated combined SD of 50.62 was inconsistent with the two supplied SDs; their pooled SD was approximately 47.96. The above calculation corrects that arithmetic without inventing an additional recruitment allowance. It is an independent-means approximation, rather than a formal paired-design calculation. A paired calculation would require the SD of within-patient differences, or an assumed correlation between measurements, which was not supplied. Furthermore, biochemical Bcl-2 measurements do not directly establish power for an ordinal immunohistochemical grade.

Inclusion Criteria

The study included hysterectomy specimens received in the Histopathology Laboratory during the study period. For the comparative evaluation, selected specimens provided histologically confirmed uterine leiomyoma and accompanying normal myometrial tissue suitable for histopathological and immunohistochemical examination.

Exclusion Criteria

1. Hysterectomy specimens from women with a previous history of myomectomy were excluded.
2. Uterine leiomyomas associated with pregnancy were excluded.

These were the exclusions stated in the dissertation. Additional exclusions should only be incorporated if supported by the original study records.

Procedure and Methodology

Hysterectomy specimens were evaluated along with the accompanying clinical information. Relevant details included age, presenting complaints, duration of symptoms and menstrual history. Gross examination documented the appearance and

dimensions of the leiomyoma and associated uterine findings. Representative tissue was examined to confirm the diagnosis and identify secondary changes.

Haematoxylin and eosin-stained sections were reviewed to assess leiomyoma morphology and the accompanying normal myometrium. Endometrial sections were examined to determine the menstrual-cycle phase using histological features and the Noyes criteria described in the dissertation. Cases were classified into proliferative or secretory phases where these features were identifiable.

Bcl-2 immunohistochemistry was performed on sections from the leiomyoma and normal myometrium. Brown cytoplasmic or perinuclear staining was recorded as immunoreactivity. Expression was assessed in relation to tissue type, age, menstrual-cycle phase and secondary degenerative changes.

The dissertation recorded expression using the following categories:

Grade	Description reported in the dissertation
0	No positive cells
1+	Weak or focal positivity
2+	Strong positivity involving 40-70% of cells
3+	Very strong positivity involving 80-100% of cells

Scoring clarification: This scheme mixes staining intensity and extent and leaves some percentage ranges undefined. The original scoring worksheet should be checked before publication. These categories describe **immunoreactivity**, not histological tumour grade, and should not be silently replaced with a different scoring system.

Sample Processing

The specimens were fixed in 10% formalin and processed for paraffin embedding. Sections approximately 5 µm thick were prepared for routine haematoxylin and eosin staining. The sections were deparaffinised in xylene, rehydrated through alcohol, stained with haematoxylin, differentiated and counterstained with eosin. Following dehydration and clearing, the slides were mounted for microscopic examination.

For immunohistochemistry, approximately 3 µm sections were mounted on poly-L-lysine-coated slides. The sections were deparaffinised and rehydrated before heat-induced antigen retrieval using an EDTA-based buffer at pH 9. Endogenous peroxidase activity was blocked with hydrogen peroxide, and a protein-blocking step was used to reduce nonspecific staining.

The sections were incubated with the primary antibody against Bcl-2 for approximately one hour, followed by washing and application of the secondary detection reagents and horseradish peroxidase system. Diaminobenzidine was used as the chromogen to produce a brown reaction product. Sections were counterstained with haematoxylin, dehydrated, cleared and mounted.

Laboratory details requiring verification: The antibody clone, manufacturer, dilution, control tissues and validated antigen-retrieval conditions should be added from laboratory records. The dissertation's stated microwave temperature of 300°C was not reproduced because it appears to be a reporting error.

Statistical Methods

Data were entered into Microsoft Excel and analysed using IBM SPSS Statistics version 26. Categorical variables were summarised as frequencies and percentages. Continuous variables were summarised as mean and standard deviation, and their distribution was assessed using the Shapiro-Wilk and Kolmogorov-Smirnov tests. The dissertation reported the use of chi-square or Fisher's exact tests for categorical comparisons and independent-samples t-tests or Mann-Whitney U tests for quantitative comparisons. A p value below 0.05 was considered statistically significant.

Required correction for the paired tissue comparison: The tests above describe the reported analysis; they do not adequately account for pairing when comparing leiomyoma with myometrium from the same woman. For reanalysis, ordinal Bcl-2 grades should be compared using the Wilcoxon signed-rank test, with attention to ties. Binary positivity should be compared using McNemar's test. If continuous percentage-positive measurements are available, a paired t-test may be used when within-patient differences are approximately normally distributed; otherwise, a suitable paired nonparametric test should be used. Effect estimates and 95% confidence intervals should accompany the relevant comparisons. These revised tests should only be described as completed in the final manuscript after reanalysis.

Data Collection

Clinical, gross pathological, histological and immunohistochemical findings were compiled in a structured data sheet. Recorded variables included age, presenting symptoms, symptom duration, menstrual history, leiomyoma dimensions, histological diagnosis, secondary changes, endometrial phase and Bcl-2 expression in both tissue types. The leiomyoma and normal myometrial observations were linked to the same case to preserve their paired relationship. Clinical information was obtained from requisition forms and hospital records, while pathological and immunohistochemical findings were obtained from specimen and slide examination.

RESULTS

Table 1: Comparison of Bcl-2 immunohistochemical expression between normal myometrium and uterine leiomyoma

Parameter	Normal myometrium (n=42)	Uterine leiomyoma (n=42)	Difference: leiomyoma − myometrium (95% CI)	Test of significance	p value
Any positive expression, grades 1+-3+, n (%)	32 (76.2)	38 (90.5)	14.3 percentage points (−2.6 to 31.2)	Exact McNemar; discordant pairs=10 versus 4	0.180
Higher-grade expression, grades 2+-3+, n (%)	13 (31.0)	26 (61.9)	31.0 percentage points (11.7 to 50.2)	Exact McNemar; discordant pairs=17 versus 4	**0.007**
Numerically coded expression grade, mean (SD)	1.07 (0.75)	1.76 (0.93)	0.69 grade units (0.36 to 1.02)	Wilcoxon signed-rank W=122.0	**<0.001**

Among the 42 paired tissue samples, positive Bcl-2 expression was observed in 38 leiomyomas (90.5%) and 32 normal myometrial samples (76.2%). The difference of 14.3 percentage points was not statistically significant (95% CI: −2.6 to 31.2; exact McNemar p=0.180). However, higher-grade expression, comprising grades 2+ and 3+, was significantly more frequent in leiomyoma than in normal myometrium, occurring in 26 (61.9%) and 13 (31.0%) samples, respectively. The paired difference was 31.0 percentage points (95% CI: 11.7-50.2; p=0.007). The mean numerically coded expression grade was also higher in leiomyoma, at 1.76 (SD 0.93), compared with 1.07 (SD 0.75) in normal myometrium, with a descriptive mean difference of 0.69 grade units (95% CI: 0.36-1.02). The Wilcoxon signed-rank test demonstrated a statistically significant difference in paired expression grades (W=122.0; p<0.001).

Table 2: Distribution and grade of Bcl-2 immunohistochemical expression in normal myometrium and uterine leiomyoma

Bcl-2 grade	Normal myometrium, n (%)	95% CI for percentage	Uterine leiomyoma, n (%)	95% CI for percentage	Discordant pairs: leiomyoma only / myometrium only*	Exact McNemar p value
0: Negative	10 (23.8)	13.5-38.5	4 (9.5)	3.8-22.1	4 / 10	0.180
1+: Weak/focal positivity	19 (45.2)	31.2-60.0	12 (28.6)	17.2-43.6	10 / 17	0.248
2+: Strong positivity	13 (31.0)	19.1-46.0	16 (38.1)	25.0-53.2	12 / 9	0.664
3+: Very strong positivity	0 (0.0)	0.0-8.4	10 (23.8)	13.5-38.5	10 / 0	**0.002**
Total	**42 (100.0)**		**42 (100.0)**			

Normal myometrium most frequently exhibited grade 1+ Bcl-2 expression, observed in 19 samples (45.2%), followed by grade 2+ in 13 (31.0%) and absent expression in 10 (23.8%). No normal myometrial sample exhibited grade 3+ expression. In contrast, leiomyoma most frequently showed grade 2+ expression, observed in 16 samples (38.1%), followed by grade 1+ in 12 (28.6%), grade 3+ in 10 (23.8%) and absent expression in four (9.5%). Grade-specific paired comparisons showed no statistically significant differences for grade 0 (p=0.180), grade 1+ (p=0.248) or grade 2+ (p=0.664). However, grade 3+ expression was significantly more frequent in leiomyoma (23.8%; 95% CI: 13.5-38.5) than in normal myometrium (0.0%; 95% CI: 0.0-8.4), with an exact McNemar p value of 0.002.

Table 3: Paired comparison of Bcl-2 expression in leiomyoma and normal myometrial tissue

A. Cross-tabulation of paired expression grades

Normal myometrial grade	Leiomyoma grade 0	Leiomyoma grade 1+	Leiomyoma grade 2+	Leiomyoma grade 3+	Total
0	0 (0.0)	8 (19.0)	1 (2.4)	1 (2.4)	10 (23.8)
1+	2 (4.8)	2 (4.8)	11 (26.2)	4 (9.5)	19 (45.2)
2+	2 (4.8)	2 (4.8)	4 (9.5)	5 (11.9)	13 (31.0)
3+	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	**4 (9.5)**	**12 (28.6)**	**16 (38.1)**	**10 (23.8)**	**42 (100.0)**

B. Direction of the paired difference

Paired outcome	n (%)	95% CI for percentage	Test statistic / test	p value
Higher grade in leiomyoma	30 (71.4)	56.4-82.8		

Same grade in both tissues	6 (14.3)	6.7-27.8		
Lower grade in leiomyoma	6 (14.3)	6.7-27.8		
Higher grade in leiomyoma among non-tied pairs	30/36 (83.3)	67.2-93.6	Two-sided exact sign test: 30 higher versus 6 lower	**<0.001**

Paired assessment demonstrated a higher Bcl-2 expression grade in leiomyoma than in the corresponding normal myometrium in 30 women (71.4%; 95% CI: 56.4-82.8). Equal expression grades were observed in six women (14.3%; 95% CI: 6.7-27.8), while a lower grade in leiomyoma was observed in six (14.3%; 95% CI: 6.7-27.8). The most frequent paired combination was grade 1+ in normal myometrium and grade 2+ in leiomyoma, occurring in 11 women (26.2% of all pairs), followed by grade 0 in normal myometrium and grade 1+ in leiomyoma in eight women (19.0%). After excluding the six tied pairs, 30 of 36 women (83.3%; 95% CI: 67.2-93.6) had higher expression grades in leiomyoma. The two-sided exact sign test demonstrated a statistically significant predominance of higher grades in leiomyoma (30 higher versus six lower; p<0.001).

Table 4: Association of leiomyoma Bcl-2 expression with age, menstrual-cycle phase and secondary degenerative changes

Characteristic	n	Grade 0, n (%)	Grade 1+, n (%)	Grade 2+, n (%)	Grade 3+, n (%)	Grades 2+-3+, n (%)	95% CI for grades 2+-3+ (%)	Pearson χ^2 statistic†	Exact p value†
Age									
≤45 years	30	3 (10.0)	7 (23.3)	12 (40.0)	8 (26.7)	20 (66.7)	48.8-80.8	1.493	0.775
>45 years	12	1 (8.3)	5 (41.7)	4 (33.3)	2 (16.7)	6 (50.0)	25.4-74.6		
Menstrual-cycle phase									
Proliferative	21	1 (4.8)	10 (47.6)	8 (38.1)	2 (9.5)	10 (47.6)	28.3-67.6	9.933	**0.016**
Secretory	21	3 (14.3)	2 (9.5)	8 (38.1)	8 (38.1)	16 (76.2)	54.9-89.4		
Secondary degenerative changes									
Absent	32	0 (0.0)	6 (18.8)	16 (50.0)	10 (31.3)	26 (81.3)	64.7-91.1	25.463	**<0.001**
Present	10	4 (40.0)	6 (60.0)	0 (0.0)	0 (0.0)	0 (			

The distribution of leiomyoma Bcl-2 expression grades did not differ significantly between women aged ≤45 years and those aged >45 years (Pearson χ^2=1.493; exact p=0.775). Higher-grade expression was observed in 20 of 30 women aged ≤45 years (66.7%; 95% CI: 48.8-80.8), compared with six of 12 women aged >45 years (50.0%; 95% CI: 25.4-74.6). In contrast, expression-grade distribution was significantly associated with menstrual-cycle phase (Pearson χ^2=9.933; exact p=0.016). Higher-grade expression occurred in 16 of 21 secretory-phase cases (76.2%; 95% CI: 54.9-89.4), compared with 10 of 21 proliferative-phase cases (47.6%; 95% CI: 28.3-67.6); grade 3+ expression was also more frequent during the secretory phase (38.1% versus 9.5%). A significant association was observed between expression-grade distribution and secondary degenerative changes (Pearson χ^2=25.463; exact p<0.001). Among leiomyomas without secondary changes, 26 of 32 (81.3%; 95% CI: 64.7-91.1) exhibited grades 2+-3+. None of the 10 leiomyomas with secondary changes exhibited higher-grade expression; four (40.0%) were negative and six (60.0%) showed grade 1+ expression.

DISCUSSION

The present study compared Bcl-2 immunohistochemical expression in 42 paired samples of uterine leiomyoma and normal myometrium. Its principal finding was a shift towards higher expression grades in leiomyoma, rather than a statistically significant difference in positivity alone. Higher-grade expression was associated with menstrual-cycle phase and the absence of secondary degenerative changes, whereas the association with age was not statistically significant. These findings support an association between leiomyoma and increased expression of an anti-apoptotic protein, although immunohistochemistry alone cannot establish the functional rate of apoptosis or demonstrate causation.

Comparison of Bcl-2 expression between leiomyoma and normal myometrium

Csatlós et al. (2015),[1] examined Bcl-2 and Bax gene expression in samples obtained from 101 women undergoing surgery for uterine leiomyoma. They demonstrated significant Bcl-2 overexpression in leiomyoma compared with controls, without a corresponding significant difference in Bax expression. Their findings are directionally consistent with the present study, in which higher-grade Bcl-2 expression occurred in 61.9% of leiomyomas compared with 31.0% of normal myometrial samples, representing a paired difference of 31.0 percentage points (95% CI: 11.7-50.2; p=0.007). However, their assessment used RT-PCR, whereas the present study evaluated protein immunoreactivity; consequently, the magnitude of expression cannot be compared directly between the studies.

Desouky et al. (2015),[2] provided a direct immunohistochemical comparison involving 30 fibroid specimens and 20 adjacent normal myometrial specimens. They reported a significantly greater area percentage of Bcl-2 expression in leiomyoma than in normal myometrium, with reported values of 1.37±0.016 and 0.31±0.007, respectively (p=0.045). This finding agrees with the higher expression grades observed in the present study. Nevertheless, their image-based area measurement differs from the ordinal grading used here. Their additional finding of increased CD34-assessed microvessel density suggests that altered cellular survival and vascular characteristics may coexist in leiomyoma, although neither study establishes a causal relationship between these processes.

Elnaghy et al. (2018),[3] reported intense grade 3+ Bcl-2 staining in all 24 leiomyomas, while the corresponding myometrial samples showed mild or moderate staining. Their biochemical assessment also demonstrated higher Bcl-2 content in leiomyoma than in accompanying myometrium. The direction of this difference agrees with the present findings, but the distribution was less uniform in the present study: only 23.8% of leiomyomas showed grade 3+ expression, and 9.5% were negative. Differences in scoring definitions, tissue selection, laboratory procedures and the proportion of degenerative lesions could contribute to this variation. These remain possible explanations rather than demonstrated causes of the between-study difference.

Banas et al. (2017),[4] compared leiomyosarcomas, leiomyomas and normal myometrium, including 53 cases in each principal group. They reported the highest Bcl-2 expression in leiomyoma and the lowest in leiomyosarcoma. This supports the observation that strong Bcl-2 expression can occur in benign uterine smooth-muscle tumours. It also cautions against interpreting increased Bcl-2 staining as evidence of malignancy or malignant transformation. The present study assessed expression differences between benign leiomyoma and normal myometrium and did not evaluate diagnostic discrimination between benign and malignant tumours.

Stewart et al. (2016),[5] described leiomyomas as heterogeneous tumours containing smooth-muscle cells, fibroblasts and extracellular matrix, while Yang et al. (2022)[6] outlined the interaction of hormonal, genetic, extracellular-matrix and cell-survival pathways in their development. This broader biological framework helps interpret Table 1: Bcl-2 positivity was common in both tissues, at 90.5% in leiomyoma and 76.2% in normal myometrium, and the difference was not statistically significant (p=0.180). The more informative finding was the greater frequency of higher grades in leiomyoma. Thus, the present results support altered expression within an existing survival pathway rather than expression of a protein entirely absent from normal tissue.

Distribution and grading of Bcl-2 expression

Adekunle et al. (2024),[7] evaluated 151 leiomyomas and reported Bcl-2 positivity in 81 cases (53.6%), considerably below the 90.5% observed in the present study. This difference demonstrates that Bcl-2 positivity rates are not uniform across published series. The present study predominantly showed grade 2+ expression in leiomyoma and grade 1+ expression in normal myometrium. Grade 3+ staining occurred in 10 leiomyomas but in no normal myometrial sample, producing a significant paired difference (p=0.002). Although this finding supports stronger staining in a subset of leiomyomas, it should not be interpreted as establishing grade 3+ staining as a diagnostic criterion without independent validation.

Adekunle et al. (2025),[8] similarly reported Bcl-2 positivity in 74 of 141 leiomyomas (52.5%). Their study used an Allred-based assessment and reported low, moderate and high immunoreactivity in 13.5%, 24.1% and 14.9% of cases, respectively. These categories are not directly interchangeable with the present grading scheme. The two Adekunle reports also involved the same institution and specimen-collection period, so their samples may overlap and should not be treated as fully independent replication. Together, they illustrate the importance of standardising staining protocols, positivity thresholds and scoring methods before comparing prevalence estimates across centres.

The grade-specific comparisons in Table 2 showed no significant differences for grades 0, 1+ or 2+ considered separately. This does not contradict the significant overall ordinal comparison. A shift distributed across several categories can produce an overall difference even when individual category comparisons lack precision. Furthermore, these category-specific tests were exploratory. The mean coded grades of 1.76 and 1.07 should therefore be regarded as supplementary summaries of an ordinal scale, rather than quantitative measurements of Bcl-2 protein concentration.

Paired comparison of expression grades

Mehine et al. (2016),[9] identified molecular subtypes of uterine leiomyoma with distinct driver pathways and biomarkers, demonstrating substantial biological heterogeneity within this tumour category. This provides a plausible context for the variation seen in Table 3: leiomyoma showed higher expression in 30 women, equal expression in six and lower expression in six. The present study did not assess molecular subtypes, so the discordant expression patterns cannot be attributed to particular mutations. Nevertheless, the findings show that increased Bcl-2 expression was frequent rather than universal.

Bulun et al. (2015),[10] discussed the relationship between progesterone signalling, leiomyoma stem cells and tumour growth. In the present study, paired sampling allowed comparison of tissues exposed to the same patient-level hormonal and demographic environment. Among the 36 non-tied pairs, 30 (83.3%; 95% CI: 67.2-93.6) showed higher expression in leiomyoma, with a significant exact

sign test ($p<0.001$). This strengthens the evidence for a tissue-associated difference. However, adjacent histologically normal myometrium is not necessarily equivalent to myometrium from a uterus without leiomyoma, and pairing does not eliminate differences in cellular composition, local signalling or tissue processing.

Association with age

Stewart et al. (2017),[11] and Pavone et al. (2018),[12] identified age among the factors associated with fibroid occurrence. These epidemiological findings address the probability of developing fibroids, whereas the present analysis examined expression grades among women who already had leiomyoma. Higher-grade expression occurred in 66.7% of women aged ≤45 years and 50.0% of those aged >45 years, but the complete grade distribution did not differ significantly (exact $p=0.775$). Therefore, the absence of a significant expression association does not conflict directly with the recognised relationship between age and fibroid prevalence.

Giuliani et al. (2020),[13] emphasised that leiomyomas mainly affect women during their reproductive years. Chronological age alone may therefore be an incomplete indicator of the hormonal environment relevant to tumour biology. In contrast to the present result, Adekunle et al. (2024),[7] reported a significant association between age and Bcl-2 immunoreactivity. Differences in age distribution, scoring methods and sample size may explain the discrepancy. With only 12 women in the older category and wide confidence intervals, the present findings should be interpreted as insufficient evidence of an age association, rather than proof that age has no relationship with expression.

Association with menstrual-cycle phase

Ali et al. (2023),[14] reviewed progesterone-mediated pathways in fibroid development, including the regulation of Bcl-2 and suppression of apoptosis. This provides biological context for the present association between menstrual phase and expression-grade distribution (exact $p=0.016$). Higher-grade expression occurred in 76.2% of secretory-phase cases compared with 47.6% of proliferative-phase cases, while grade 3+ expression occurred in 38.1% and 9.5%, respectively. These findings are compatible with hormonal regulation of Bcl-2, but the study did not measure circulating progesterone, receptor expression or functional apoptosis. Consequently, progesterone-mediated causation cannot be established from these observations.

Szucio et al. (2024),[15] described progesterone signalling through nuclear and membrane-associated mechanisms and its relationship with extracellular matrix and angiogenesis. More directly, Adekunle et al. (2025),[8] found a positive correlation between progesterone-receptor and Bcl-2 immunoexpression ($r=0.563$; $p<0.001$). These findings support the relevance of studying hormonal receptors alongside Bcl-2. Nevertheless, receptor correlation and differences between menstrual phases represent different observations; neither alone demonstrates that progesterone is responsible for the expression pattern in the present sample.

Yun et al. (2015),[16] provide a useful qualification to a simple progesterone-Bcl-2 explanation. In their study of 38 women, treatment with a selective progesterone-receptor modulator was associated with increased caspase-3 protein expression, but no significant between-group difference in Bcl-2 mRNA was found. Their results indicate that treatment-related changes in apoptosis may involve pathways other than Bcl-2. Similarly, Elnaghy et al,[3] reported no significant phase-related difference in their endometrial measurements; because these concerned a different tissue assessment, they are not a direct replication of the present leiomyoma-grade analysis.

Association with secondary degenerative changes

Islam et al. (2018),[17] described extracellular-matrix accumulation and remodelling as central features of leiomyoma biology, while Jamaluddin et al. (2018)[18] investigated the extracellular-matrix proteome of human fibroids. These studies provide tissue-biological context for the strong association observed in Table 4. Higher-grade Bcl-2 expression occurred in 26 of 32 leiomyomas without secondary changes (81.3%) but in none of the 10 with secondary changes; the overall grade distribution differed significantly (exact $p<0.001$). However, neither cited study directly confirms this specific association between degeneration and Bcl-2 grade.

Lower expression in degenerative lesions could reflect altered cellular survival, reduced viable smooth-muscle content, sampling differences or changes in antigen preservation. These are alternative interpretations of the present finding, not mechanisms demonstrated by the data. The cross-sectional design cannot determine whether reduced Bcl-2 expression preceded degeneration or developed alongside it. Moreover, zero higher-grade cases among 10 degenerative lesions does not establish that higher-grade expression never occurs in such lesions; the corresponding upper 95% confidence limit was approximately 27.8%.

Biological and clinical implications

Machado-Lopez et al. (2021),[19] described fibroid development as involving interacting genetic, epigenetic, hormonal and cellular mechanisms. Their integrated account supports interpreting Bcl-2 as one component of leiomyoma biology. The present findings would be strengthened by simultaneous evaluation of pro-apoptotic markers, proliferation indices, hormone receptors and relevant molecular subtypes. Such measurements could help distinguish increased anti-apoptotic protein expression from demonstrably reduced cell death.

Donnez and Dolmans (2016),[20] reviewed the importance of symptom burden, fertility preferences and tumour characteristics in fibroid management. Subsequently, Al-Hendy et al. (2021),[21] and Schlaff et al. (2020)[22] demonstrated clinical benefits of hormonal suppression strategies for fibroid-associated symptoms. These treatment studies support the clinical relevance of hormone-responsive

fibroid biology, but they did not validate the present Bcl-2 grading system as a predictor of treatment response. Accordingly, the present findings support further biomarker research but do not justify selecting treatment on the basis of Bcl-2 staining alone.

The strongest inference from these tables is that leiomyomas frequently exhibit higher Bcl-2 expression grades than paired normal myometrium. Associations with menstrual phase and degenerative changes merit further investigation using standardised scoring, larger samples and adjustment for potential confounding. The substantial expression overlap between tissues and variation across published studies limit claims about diagnostic specificity, prognosis or therapeutic prediction.

CONCLUSION

Uterine leiomyomas demonstrated significantly higher grades of Bcl-2 immunohistochemical expression than paired normal myometrium. Higher-grade expression (2+-3+) was observed in 61.9% of leiomyomas compared with 31.0% of normal myometrial samples (p=0.007), although the difference in overall positivity was not statistically significant. Leiomyoma showed a higher expression grade in 71.4% of paired comparisons. Expression-grade distribution was significantly associated with menstrual-cycle phase and secondary degenerative changes, with higher grades more frequent during the secretory phase and in leiomyomas without degeneration. No statistically significant association with age was identified. These findings support the involvement of Bcl-2-associated cell-survival pathways in leiomyoma biology; however, they do not establish reduced functional apoptosis or validate Bcl-2 as a diagnostic, prognostic or treatment-selection marker.

Limitations of the Study

1. **Small sample size:** The study included 42 women, limiting the precision of estimates and the ability to detect modest associations, particularly within smaller age and degenerative-change subgroups.
2. **Single-centre, surgically selected population:** Specimens were obtained from women undergoing hysterectomy at one tertiary care institution. The findings may not represent asymptomatic women or those managed conservatively.
3. **Cross-sectional design:** Expression was assessed at a single time point, preventing determination of whether altered Bcl-2 expression preceded leiomyoma development or secondary degeneration.
4. **Limitations of the comparison tissue:** Adjacent histologically normal myometrium shared the local uterine environment with leiomyoma and might not represent myometrium from women without fibroids.
5. **Semiquantitative assessment:** Immunohistochemical grading was subject to observer interpretation. The scoring scheme combined staining intensity and extent, and interobserver reproducibility was not reported.
6. **Incomplete assessment of hormonal influences:** Menstrual phase was determined histologically, without simultaneous measurement of circulating hormones or oestrogen and progesterone receptors.
7. **Absence of functional and molecular validation:** Bcl-2 staining was not supplemented by direct apoptosis assays or assessment of complementary markers such as Bax, cleaved caspase-3 and Ki-67.
8. **Potential confounding and exploratory analyses:** Subgroup associations were not adjusted for potential confounders. Multiple exploratory comparisons also increased the possibility of chance findings.
9. **No longitudinal follow-up:** The study did not determine whether Bcl-2 expression predicted tumour growth, recurrence, symptom severity or response to treatment.

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