

Title: Endometrial BCL6 Overexpression in Unidentified Recurrent Pregnancy Loss (uRPL) and Unexplained Infertility (UI): A Case-Control Study Establishing Its Role as a biomarker of Subclinical Endometriosis and Progesterone Resistance



INTRODUCTION

Compromised fertility affects millions globally, with 48.5 million couples unable to conceive after 5 years (1). Unexplained infertility (UI, 15–30%) and unexplained recurrent pregnancy loss (uRPL, 1–3%) are significant subsets of sub-fertility (2). Despite known causes like genetic, endocrine, or structural abnormalities, ~50% of RPL cases remain unexplained.

Endometriosis, an immune related inflammatory condition altering endometrial function, is linked to uRPL and UI (3,4). Endometriosis has been linked to the genesis of endometrial progesterone resistance and to specific nuclear proteins, including overexpression of endometrial B-cell CLL/lymphoma 6 (BCL6). BCL6 overexpression (HSCORE >1.4), detectable via immunohistochemistry, is a biomarker for endometriosis and progesterone resistance, correlating with poor reproductive outcomes (5). It is elevated in UI patients and the decidua of women with uRPL.

MATERIAL AND METHODS

Study design and settings

This is a case-control study performed at a tertiary care hospital in rural Maharashtra from August 2023 to July 2024 with total 45 sample size with 15 patients in each group.

Study groups comprised of:

- (1) Unidentified recurrent pregnancy loss (uRPL): ≥2 consecutive pregnancy losses with unknown causes (2) Unexplained infertility (UI): Infertility ≥12 months with normal semen analysis, ovulatory function, tubal patency with no existing uterine structural anomalies or pathologies. (3) Control group: consisted of fertile age-matched women with no history of infertility or pregnancy loss and atleast 1 successful pregnancy.

Participant Selection:

Inclusion Criteria: Women aged 20–40 years with regular menstrual cycles (21–35 days).

Exclusion Criteria: Uterine pathologies or structural anomalies, previous pelvic surgery unrelated to infertility, autoimmune/coagulation disorders, endometriosis, BMI >30 kg/m², hormonal treatment in the last 3 months, PCOS, or positive APLA.

Sample Collection: Endometrial Biopsy was performed 7–10 days post-LH surge using a pipelle device. Samples were processed for immunohistochemistry (IHC). Staining for BCL6 using an automated system. **BCL6 expression was assigned a histological score (HSCORE). Negative BCL6 expression was based on HSCORE of ≤1.4, Medium overexpression was >1.4 to 2.7 and high overexpression was BCL 6 score > 2.7**

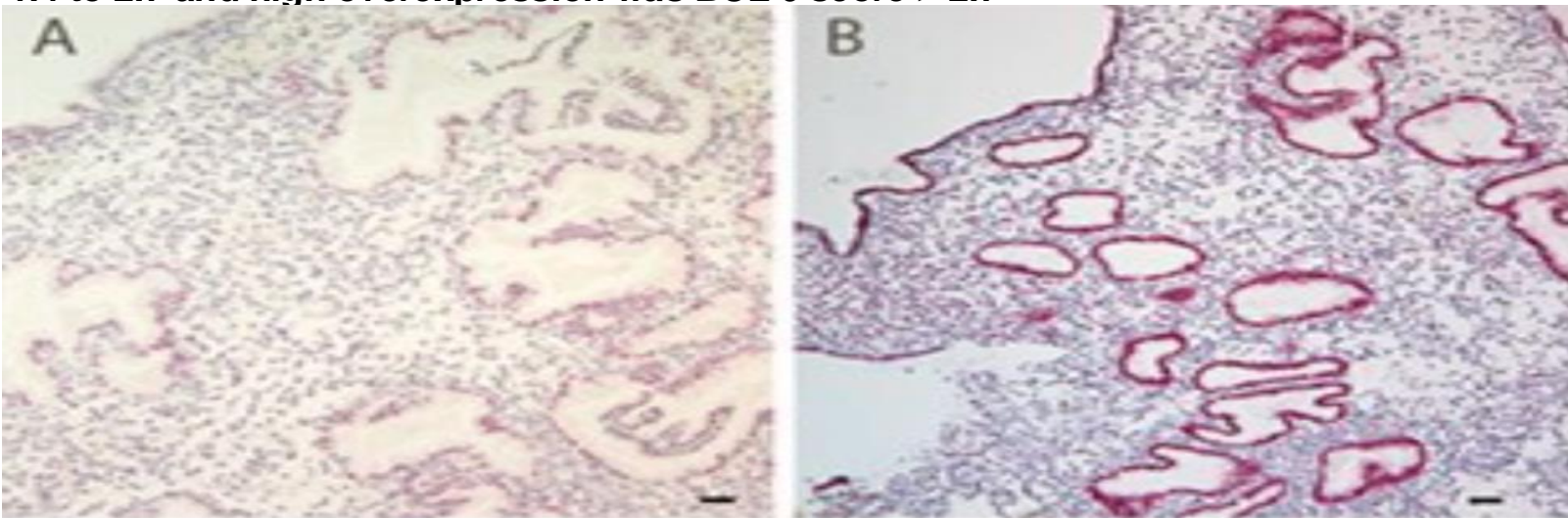


Image 1: A- Immunostaining in eutopic endometrium from women with low (A) and high (B) B-cell CLL/lymphoma (BCL6).

Laparoscopy (Optional) was performed in subset of uRPL/UI population with elevated BCL6 expression to confirm the diagnosis of endometriosis.

Luteal phase serum estradiol and progesterone was measured. Suboptimal levels (<10 ng/mL) of progesterone, E2/P ratio of > 40 pg/ng and menstrual irregularities were considered markers of progesterone resistance.

OBSERVATIONS AND RESULTS

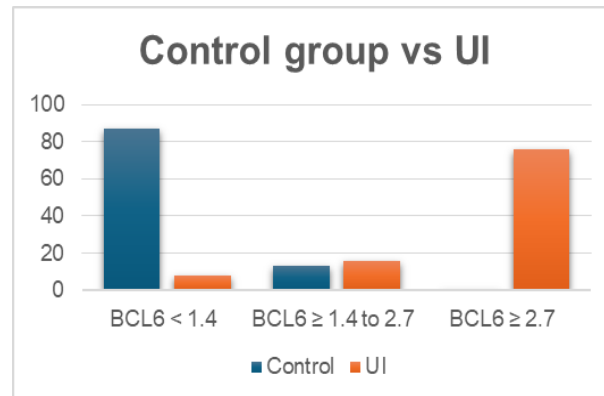
Table 1: Showing descriptive data indicating general characteristics of study population of groups Control, uRPL and UI:

Characteristics	Control (n=15)	uRPL (n=15)	UI (n=15)	P-value
Age (years)	31.8 ± 1.9	33.26 ± 1.4	32.2 ± 2.3	0.7
BMI (kg/m ²)	23.20 ± 3.01	22.84 ± 2.6	21.36 ± 2.1	0.8
Gravidity	1.6 ± 0.63	2.8 ± 0.7	0.53 ± 0.51	<0.001
Parity	1.4 ± 0.5	0.33 ± 0.4	0.2 ± 0.41	<0.001

Table 2: Comparison of BCL6 levels amongst various groups:

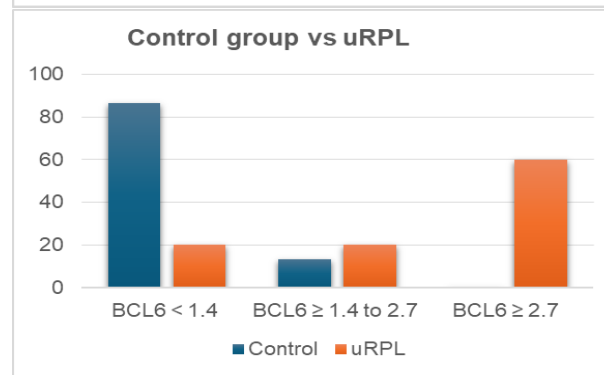
A: Control group vs UI:

Characteristics	Control (n=15)	UI (n=15)	P-value
BCL6 ≤1.4 (negative)	13 (86.67%)	2 (8%)	<0.001
BCL6 > 1.4 to 2.7 (medium)	2 (13.33%)	4 (16%)	
BCL6 > 2.7 (high)	0 (0%)	19 (76%)	



B: Control group vs uRPL:

Characteristics	Control (n=15)	uRPL (n=15)	P-value
BCL6 < 1.4 (negative)	13 (86.67%)	3 (20%)	<0.006
BCL6 ≥ 1.4 to 2.7 (medium)	2 (13.33%)	3 (20%)	
BCL6 ≥ 2.7 (high)	0 (0%)	9 (60%)	



C: uRPL vs UI:

Characteristics	uRPL (n=15)	UI (n=15)	P-value
BCL6 < 1.4 (negative)	3 (20%)	2 (8%)	0.345
BCL6 ≥ 1.4 to 2.7 (medium)	3 (20%)	4 (16%)	
BCL6 ≥ 2.7 (high)	9 (60%)	19 (76%)	

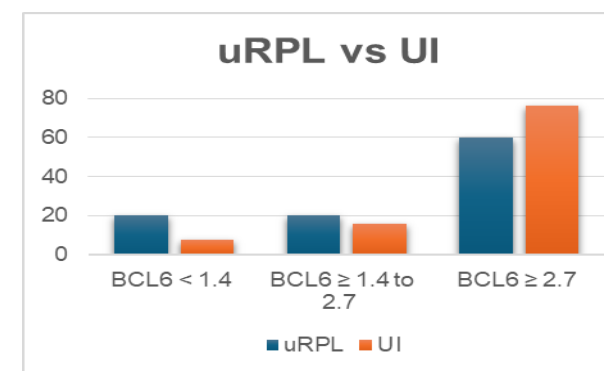


Table 3: Percentage of subset of population positive for endometriosis during laparoscopy:

Subset of population underwent laparoscopy	uRPL (n=8)	UI (n=9)	Total (n=17)
Positive for endometriosis	6 (75%)	8 (88.89%)	14 (82.35%)
Negative for endometriosis	2 (25%)	1 (11.11%)	3 (17.64%)
Total	8 (100%)	9 (100%)	17 (100%)

Table 4: Markers of Progesterone Resistance in each group:

A: Control group:

Markers of progesterone resistance	<1.4 (n=13)	>1.4 (n=2)	p-value
Menstrual irregularities (%)	2 (15.4%)	2 (100%)	0.097
Estradiol (E2) to Progesterone ratio (E2/P ratio) (pg/ng)	25 ± 5.11	21 ± 2.4	0.229
Luteal phase Se. Progesterone levels (ng/ml)	14.4 ± 2.3	15.75 ± 1.06	0.571

B: uRPL group:

Markers of progesterone resistance	<1.4 (n=3)	>1.4 (n=12)	P-value
Menstrual irregularities	1 (33.33%)	10 (83.33%)	0.307
Estradiol (E2) to Progesterone ratio (E2/P ratio) (pg/ng)	43 ± 7.15	69.1 ± 13.4	0.001
Luteal phase Se. Progesterone levels (ng/ml)	7.5 ± 0.7	5.4 ± 0.8	0.008

C: UI group:

Markers of progesterone resistance	<1.4 (n=2)	>1.4 (n=13)	P-value
Menstrual irregularities	1 (50%)	9 (69.2%)	1.0
Estradiol (E2) to Progesterone ratio (E2/P ratio) (pg/ng)	42.11 ± 0.07	59.2 ± 16.1	0.019
Luteal phase Se. Progesterone levels (ng/ml)	7.1 ± 0.4	5.4 ± 0.9	0.114

CONCLUSION

- BCL6 as a Biomarker:** Endometrial BCL6 overexpression is a strong potential biomarker for diagnosing subclinical endometriosis and progesterone resistance, particularly in patients with uRPL and UI.
- Endometriosis and Progesterone Resistance:** Elevated BCL6 levels are linked to higher rates of endometriosis and markers of progesterone resistance, including menstrual irregularities, elevated E2/P ratios, and reduced luteal phase progesterone levels.
- Clinical Implications:** BCL6 expression analysis could be integrated into routine diagnostic protocols for identifying endometrial defects in women with uRPL or UI, potentially guiding tailored interventions to improve reproductive outcomes.
- Future Research:** Larger studies are needed to confirm these findings and explore the therapeutic implications of targeting BCL6 in improving endometrial receptivity.

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