



## STUDY OF RETICULIN FIBRE CHANGES IN DYSFUNCTIONAL UTERINE BLEEDING WITH SPECIAL STAIN

### Pathology

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### ABSTRACT

**Aim:** This study was carried out to evaluate the changes in reticulin fibre network in endometrium with special stain to aid classification of histomorphology of endometrium in Dysfunctional Uterine Bleeding.

**Material and Methods:** This case control study was carried out among 100 women who were diagnosed with Dysfunctional uterine bleeding in our tertiary care medical college hospital between 2012 and 2015 with 25 normal endometrium with cyclical changes corresponds to LMP. Slides were stained with conventional Haematoxylin & Eosin, and Gomeri's silver impregnation method for reticulin.

**Results:** Special stains are useful in emphasizing the architecture of normal endometrium and endometrial stromal abnormalities in DUB and also useful in demonstrating the spiral arteriole. In conventional H&E stain reticulin not easily detectable.

**Conclusion:** This study has highlighted the changes in reticulin network with the help of special stain in the endometrial biopsy for an in depth analysis and categorization of DUB

### KEYWORDS

DUB, histomorphology, special stain, reticulin fibre network

### INTRODUCTION:

Alteration in the hormone changes produce arrest in follicular maturation at different stages of its development causing morphological changes that corresponds to the stage of maturation. Aim of this study is to evaluate the changes in reticulin fibre network in endometrium with special stain to aid classification of histomorphology of endometrium.<sup>1,2</sup> This study was carried out at the Department of Pathology in our medical college hospital, Salem for a period of three years between July 2012 and June 2015 as a case control study in 100 DUB cases with 25 normal endometrium to demonstrate the normal cyclical changes corresponding to LMP.

### Study participants

All the patients who were clinically diagnosed with Dysfunctional Uterine Bleeding during the study period were included in the study.

### Inclusion criteria

Age between 21-55 years  
Willing to participate in the study

### Exclusion criteria

Adolescent girls between 10-20 years of age  
Pregnant women  
Women with established causes of bleeding

The endometrial samples received were checked for adequate fixation, fixed in 10% formalin of quantity 20 times the volume of endometrial issue fragment. Slides were stained with conventional Haematoxylin & Eosin, Periodic acid Schiff method and Gomeri's method for reticulin fibre.

### GOMERI'S METHOD FOR RETICULIN FIBER<sup>14,15</sup>

Sections were dewaxed and brought to water. They were treated with 1% potassium permanganate solution for 2 minutes. After rinsing in tap water, the sections were bleached in 2% potassium metabisulphite solution and again rinsed in tap water. They were then treated with 2% iron alum for 2 minutes and further washed in several charges of distilled water. The Sections were dewaxed and brought to water. They were treated with 1% potassium permanganate solution for 2 minutes. After rinsing in tap water. The sections were then placed in Coplin jar of silver solution for 1 minute and washed in several changes of distilled water. Further to this, they were reduced in 4% aqueous formalin solution for 3 minutes and rinsed in tap water and toned in 0.2% gold chloride solution for 10 minutes followed by rinsing in tap water. The sections were then placed in Coplin jar of silver solution for 1 minute and washed in several changes of distilled water. Further to this, they were reduced in 4% aqueous formalin solution for 3 minutes and rinsed in tap water and toned in 0.2% gold chloride solution for 10 minutes followed by rinsing in tap water. The slides were then treated

with 2% potassium metabisulphite solution for 1 minute. After this, the sections were rinsed in tap water and treated with 2% sodium thiosulphate solution for 1 minute and further rinsed in tap water and counterstained with eosin. This was then dehydrated in graded alcohol, cleared in xylene and mounted in resinous mountant. Staining was done also, with the control. After staining slides were analysed and the sample biopsies were categorized to the complete DUB classification by Gisela Dallenbach – Hellweg in 'Haines and Taylor' – obstetrical and Gynecological pathology (2003) the finding correlated with the classical histomorphological findings in DUB and analyzed.

### RESULTS

A total of 100 cases were analyzed in this study and majority of the cases consisted of anovulatory disturbances (62%) followed by ovulatory disturbances (34%). The histomorphological categorization of the cases is given in table. While majority of the anovulatory cases were irregular proliferation (27%), majority of the ovulatory cases were deficient secretory state with co-ordinated delay (11%).

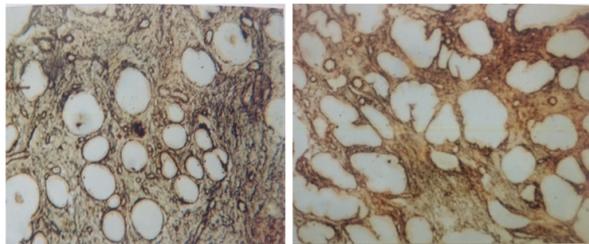
**Table: Data of Histomorphological Categories of DUB**

S. No	Categories	Frequency (n=100)	Percentage
1	ANOVLATORY// PROLIFERATIVE		
1.1	Early Proliferative	9	9
1.2	Mid Proliferative	10	10
1.3	Late Proliferative	5	5
1.4	Atrophy	1	1
1.5	Deficient Proliferation	10	10
1.6	Irregular proliferation	27	27
2	OVULATORY/- SECRETORY		
2.1	Late Secretory phase	6	6
2.2	Deficient Secretory state with co-ordinated delay	11	11
2.3	Deficient Secretory state with dissociated delay	9	9
2.4	Irregular shedding	7	7
2.5	Dysmenorrhea Membranacea	1	1
3	Simple Hyperplasia	4	4

### DISCUSSION

Reticulin stain is helpful in detecting early stromal dissolution, dissociation with glandular maturation, irregular shedding and areas of pseudodecidualisation. It also highlights the spiral arteriole.<sup>1,2,3,4</sup> In controls cases the endometrium exhibit normal pattern of reticulin network corresponding to the cyclical activity. In proliferative endometrium, reticulin network seen as thin periglandular basket and as discrete fibre in the stroma. In secretory phase reticulin fibre forms

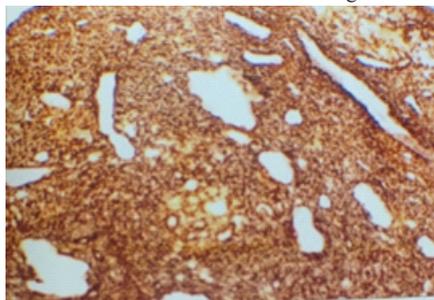
thick per glandular basket and around blood vessel it is thick and prominent. The inter glandular fibre form mosaic pattern around predecidual cells.<sup>1,2,3</sup> Histomorphological analysis significantly helps in categorization of DUB with the exception of receptor deficient and estrogen withdrawal bleeding.<sup>4,5</sup>



**Fig:1 Reticulin stain in Proliferative and secretory phase of endometrium**

In our study, all the DUB case showed significant morphological findings. Only six cases showed classical late secretory feature which corresponds to the day menstrual cycle on which, the endometrial biopsy was done.

Endometrial granulocytes thought to secrete, proteolytic hormone-relaxin and dissolves the reticulin network. In our study, the endometrial granulocyte was found 62% of cases with ovulatory disturbances.<sup>6,7,8,9</sup> The reticulin network shows evidence of hormonal influence as the maturation takes place under the control of progesterone. The fibers grow thicker and denser with increasing progesterone levels. As long as the corpus luteum continues to produce progesterone, the reticulin network remains intact. Its dissolution denotes decreased progesterone level. The disintegration starts with appearance of endometrial granulocyte which secretes hormone relaxin.<sup>1,2,3</sup> In our study, the dissolution of dense network corresponding to the hormonal status in different categories was found in 59.40% which, appeared to be statistically significant. Periodic acid-Schiff (PAS) reaction, which takes advantage of the high polysaccharide content of reticular fibers. Gomori and Wilder silver nitrate staining procedures, which make the fibres appear black (argyrophilic).<sup>14,15</sup> The PAS stain, highlight the glycogen granules in the glandular as well as stromal cells and also stain the ground substances.



**Fig:2: Reticulin stain for a case Dysmenorrhea membranacea**

In deficit proliferation, due to decreased progesterone level incomplete reticulin network seen. In irregular proliferation, irregular basket of reticulin with varying thickness and seen in patchy areas of stroma. Amount of reticulin varies from region to region of endometrial tissue. In deficit secretion with co-ordinate delay reticulin basket is weak in peri glandular space and around stromal cells. In Deficit secretion with dissociated delay stromal and glandular maturation not correlated. No reticulin basket around the gland and patch stromal dissolution seen. In irregular shedding reticulin fail to undergo dissolution and seen intact as tightly knit network. This is due to persistent corpus luteum. In dysmenorrhea membranacea no basket weave around gland and thick complete mosaic seen around stromal cells. This because of fall in progesterone level takes place very lately and the dissolution cannot take place. The entire endometrial layer discharged as a whole<sup>11,12</sup>

## CONCLUSION

Histomorphological analysis of the DUB cases on our study highlighted certain important diagnostic features in the interpretation of endometrial biopsy. Along with glandular epithelial lining, glandular epithelial mitosis, secretory activity of epithelial cells, stromal cell morphology, stromal cell mitosis and appearance, pattern,

and quality of reticulin network with the help of special stain were found to be, very useful in the classification of endometrium in DUB.

## Declaration

**Conflict of interest -nil**

**Funding -nil**

**No ethical issues**

## REFERENCES.

- 1) Functional Disturbances of Endometrium - text book- Gisela Dallenbach- Hellweg (author) (5th ed) 2003 Springer, Berlin
- 2) Haines and Taylor obstetric and Gynecologic pathology Text book, Harold Fox (ed) (5th ed) 2003 Churchill Livingstone, London
- 3) Linda D. Bradley, Tommaso Falcone (author) Text book Hysteroscopy: Office Evaluation and Management of the Uterine Cavity, (1st ed) 2009 Elsevier, U.S
- 4) Dating the endometrial biopsy – Fertility and Sterility Journal, Noyes, R.W., Hertig A.T., and Rock J (author) (1950) Fertil. Steril., 1, 3-25, Elsevier, U.S
- 5) Elizabeth A. Steward – Yen & Jaffe's Reproductive endocrinology, Jerome Strauss, Robert Barbieri (ed) (6th ed) Elsevier, U.S
- 6) Diagnostic Gynecology & obstetric pathology – An Atlas and Text – Roger A. Reichert (author) (1st ed) (2012) Lippincot Williams and Wilkins, London
- 7) Claude Gompel and Steven G Silverberg (ed) Text book - Pathology in Gynecology and obstetrics (4th ed) 1994 Lippincot Williams and Wilkins, London
- 8) Rosai and Ackerman – Surgical Pathology Text Book, Juan Rosai, Lauren V Ackerman (author) (10th ed) 2011 Elsevier, New York
- 9) Sternberg's Diagnostic surgical pathology Text Book, Stacey E Mills (ed) (5th ed) 2009 Lippincot Williams and Wilkins, London
- 10) Marisa Nucci, Esther Oliva (author) Gynecologic Pathology - A volume in the series : Foundations in Diagnostic Pathology, series editor John R Goldblum (1st ed) 2009 Churchill Livingstone, London.
- 11) Robert J Kurman (ed) Text book Blaustein's Pathology of Female genital Tract. 6th edition. 2010 springer: New York
- 12) Fox H., Buckley C. H. (Author) pathological factors in DUB – Text Book – Pathology of Female Genital Tract (5th ed) 1992, Springer, Netherlands
- 13) FIGO Classification of AUB- Malcolm G. Munro (author) International Journal of Gynecology & Obstetrics (After exclusion of FIGO PALM – COEIN – AUB Classified) Int. J. Gynecol. Obstet. Jan 2011, Page 3-13, Wiley-Blackwell, U.S
- 14) Staining technique PAS Reaction McManus Method – text book, Bancroft – Theory and Practice of Histological Technique, John D Bancroft, Marilyn Gamble (author) (6th ed) 2008, Churchill Livingstone, London.
- 15) Staining Technique Gomori's Method for reticulin fibre -text book Bancroft – Theory and Practice of Histological Technique, John D Bancroft, Marilyn Gamble (author) (6th ed) 2008 Churchill Livingstone, London.