"DISTRIBUTION OF MAST CELLS IN UTERINE LEIOMYOMA – A HOSPITAL BASED OBSERVATIONAL STUDY "

Submitted by

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JUNE/JULY -2023

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LIST OF ABBREVIATIONS USED

S.No	Abbreviation	Expansion
1	MC	Mast cell
2	**AVG**	Average
3	**hpf**	High power fields
4	**MCD**	Mast cell density
6	**SD**	Standard Deviation
7	**WHO**	World Health Organization
8	**IQ**	Interquartile Range
9	**Eg**	Example
10	**N**	Total number
11	**H & E**	Hematoxylin and Eosin
12	**MMSC**	Myometrial mast cells
13	**TICs**	Tumor infiltrating stem cells
14	**UFs**	Uterine fibroids
15	**MED12**	Mediator of RNA Polymerase II transcription subunit 12
16	**HMGA2**	High mobility group AT- hook 2
17	**FH**	Fumarate hydratase
18	**DNA**	Deoxyribonucleic acid
19	**RNA**	Ribonucleic acid
20	**ER/PR**	Estrogen receptor/Progesterone receptor

21	**bFGF**	basic Fibroblast growth factor
22	**TGF beta**	Transforming growth factor beta
23	**EGF**	Epidermal growth factor
24	**IGF-1**	Insulin like growth factor
25	**FDA**	Food and Drug Administration
26	**SMA**	Smooth muscle actin
27	**CTMC**	Connective tissue mast cell
28	**MMC**	Mucosal mast cell
29	**TLRs**	Toll like receptors
30	**MHC I**	Major Histocompatibility complex I
31	**MMPs**	Matrix metalloproteases
32	**TNF Alpha**	Tumor necrosis factor Alpha
33	**NK cells**	Natural killer cells
34	**GnRH**	Gonadotropin releasing hormone

ABSTRACT

TITLE: DISTRIBUTION OF MAST CELLS IN UTERINE LEIOMYOMA -A HOSPITAL BASED OBSERVATIONAL STUDY

Introduction

Mast cells are immune cells of myeloid lineage present in connective tissues which regulate various physiological processes. Human uterus is relatively rich in mast cells. Uterine leiomyomas are the most common benign tumors originating from myometrium. However there is no consensus regarding the prognostic role of mast cells in the uterus. Hence further study is necessary for clarifying the relationship between the distribution of mast cells and various conditions of the myometrium.

Materials And Methods

The present study was a 3 year study from January 2019 to December 2021 conducted in the Department of Pathology, Jawaharlal Nehru Medical college, KAHER,Belagavi.

Retrospective tissue blocks from the archives of the department and Hysterectomy specimens of patients with uterine leiomyoma admitted at KLE'S Dr.Prabhakar Kore Charitable Hospital , a total of 100 cases, formed the source of data.

Two sections each were taken from the leiomyoma and its adjacent myometrium , processed routinely & stained with H & E and 1% Toluidine blue stain. The mast cells were counted under 40x magnification for 10 consecutive fields in each slide.

Results

Leiomyomas showed a perimenopausal age preponderance and 59% were Intramural in origin. An inverse correlation was found between the mast cell density and increasing age. The distribution of mast cells in uterine leiomyoma in comparison to the adjacent myometrium showed a clear twofold increase. The mast cell density of leiomyomas with degeneration were significantly lower than those without degeneration.

Conclusions

Based on the observations of this study, progressively decreasing mast cell counts were observed as we move from the uterine leiomyoma to the adjacent normal myometrium. This study also found a decline in the number of mast cells when a leiomyoma undergoes degeneration.

Keywords- Uterus, leiomyoma, mast cells, Endometrium, leiomyoma with degeneration

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INTRODUCTION

Mast cells also called Mastocyte or Labrocytes are immune cells of myeloid lineage, present in connective tissues. They contain many granules rich in histamine and heparin. These cells mature under the influence of the c-kit ligand and stem cell factor in the presence of other growth factors.

Under normal conditions, mature mast cells don't circulate in the blood stream. However the progenitors migrate into the tissue and differentiate under the influence of various cytokines.

Mast cells are known to regulate various physiological processes including vascular homeostasis, innate and adaptive immune response, vasodilation, and angiogenesis and have also been implicated in the pathophysiology of allergy, asthma, anaphylaxis, gastrointestinal disorders, many malignancies and cardiovascular diseases. [(1)]

Notwithstanding mounting evidence of mast cell accumulation in tumors, their exact role in tumorigenesis and progression is poorly understood.[(2)]

The human uterus is considered to be relatively rich in mast cells compared to other tissues of the body. The greatest number was found in the inner (luminal) surface of the myometrium while the endometrium contained significantly fewer mast cells. No change was noticed in the number, with different phases of the menstrual cycle, however there was a significant fall in all regions after menopause.[(3)]

Mast cells can be stained by Haematoxylin and eosin, Toluidine blue, Giemsa, Mast cell Tryptase, Chymase and CD117 although studies have revealed best results were obtained with CD117 and Toluidine blue. [(4)] However there exists no standardization for counting them.

Uterine fibroids are the most common benign tumours affecting women and they originate from the uterine smooth muscle cells (myometrium) whose growth is primarily dependent on the levels of circulating estrogen. [(5)]

Various studies- Maluf & Gersall[(101)], D'Souza et al[(102)], Orii et al[(116)], Zhou et al[(104)] have concluded that the abundance of mast cells in tumors indicate a good prognosis. However, according to studies done by Jiang et al [(107)] and others mast cells promote cancer growth.

Hence further study is necessary for clarifying the relationship between the distribution of mast cells and various conditions of the myometrium.

AIMS & OBJECTIVES

1. To study the distribution of mast cells in uterine leiomyoma
2. To study the distribution of mast cells in uterine leiomyomas according to their location.
3. To compare the distribution of mast cells in uterine leiomyoma with the normal adjacent myometrium
4. To study the distribution of mast cells in leiomyoma without degeneration and with degeneration and compare with normal myometrium

REVIEW OF LITERATURE

EMBRYOLOGY

Sex differentiation is a complex process which involves many genes including anatomical genes. Understanding the complex process is the key to many developmental disorders encountered in females. The female genital system involves uterus with cervix, bilateral fallopian tube along with ovary and vagina. The genital tract is lined by mesoderm mostly except the germ cells which are endodermal in origin. The epithelial lining of vagina and vulva are ectodermal in origin. [6]

The paramesonephric duct (Mullerian duct) develops into genital duct of female under the influence of estrogen and absence of testosterone and mullerian hormone. At 5- 6weeks of gestation the duct arises as coelomic invaginations that appear on the lateral surface of the paired urogenital ridges. This duct consists of three parts: 1. A cranial portion that opens into abdominal cavity, 2. A horizontal portion which crosses the mesonephric duct, 3. A caudal portion which fuses with the counter part from opposite side. In males this paramesonephric duct is suppressed leaving a small portion giving rise to appendix of testis. [6]

At 8weeks of gestation the paramesonephric ducts fuse vertically. The fused cranial and horizontal ends gives rise to what will ultimately become the fallopian tube, while the caudal component will fuse to form the uterus, cervix and upper third of the vagina. The uterine corpus remains under developed at birth and reaches functional and anatomical maturity at the time of Menarche. [7-9]

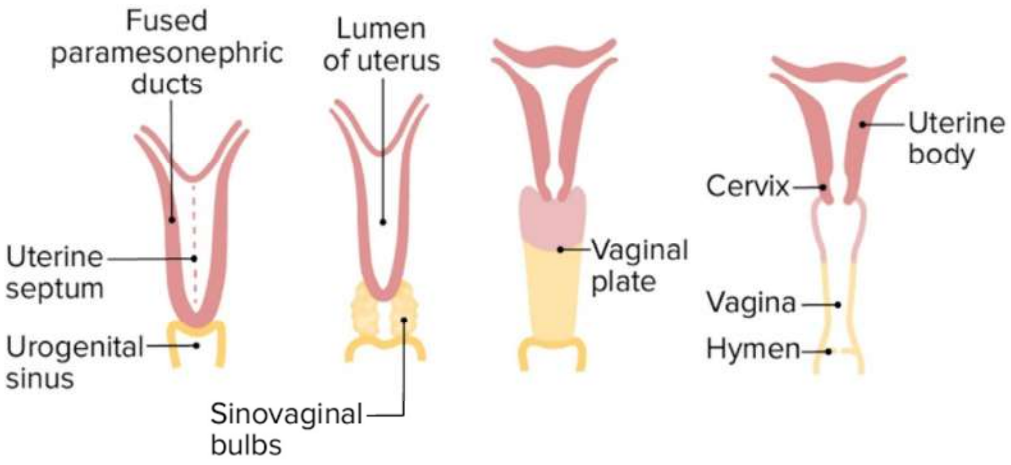


Fig 1: Development of uterus [7-9]

ANATOMY OF UTERUS

The main organ of female reproduction is the uterus with cervix. Its adnexal structures include uterine tubes, vagina and its ligaments. Uterus is pear shaped muscular organ located in the pelvis between rectum and urinary bladder.

The uterus lies in the midline of the abdominal pelvic cavity. It contains three layers: the endometrium (innermost layer), myometrium, and the perimetrium (outermost layer). The endometrium's thickness and structure vary based on hormonal stimulation. The Myometrium is a structural wall of uterus composed primarily of smooth muscle

Serosa (Perimetrium) is a thin, outermost layer of uterus consisting of loose connective tissue and mesothelium. [10]

The uterus has four parts: the fundus, corpus, isthmus, and cervix. The corpus is the largest segment and connects to the cervix via the isthmus.The fibromuscular

junction which is named internal cervical os points out the junction made by corpus and cervix. The uterus sits posterior to the bladder and anterior to the rectum. [11]

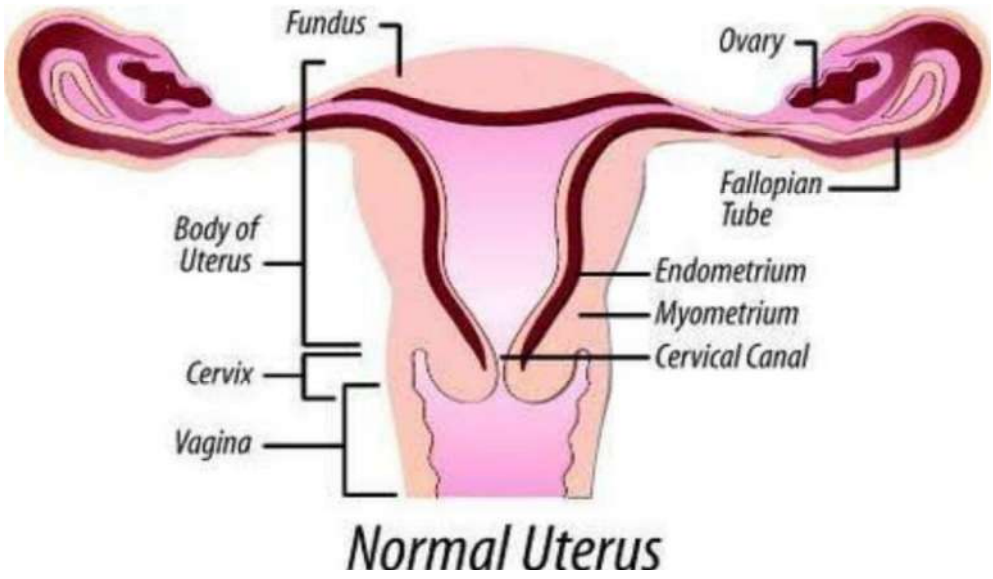
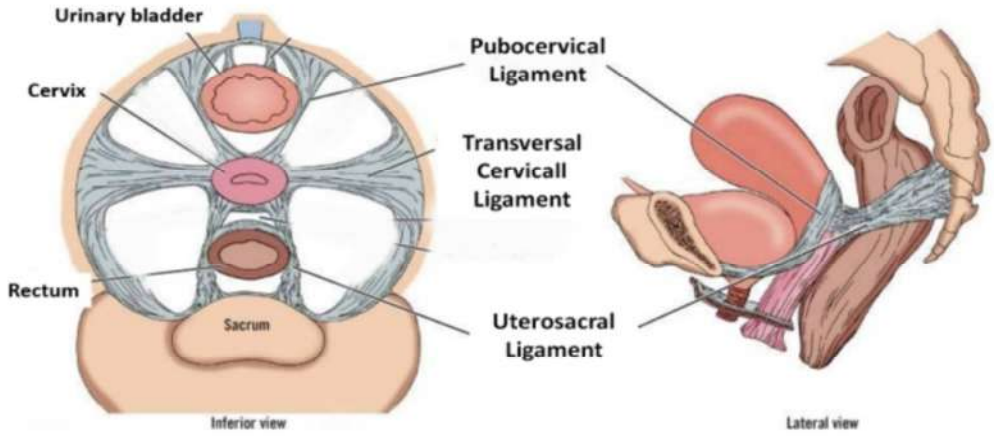


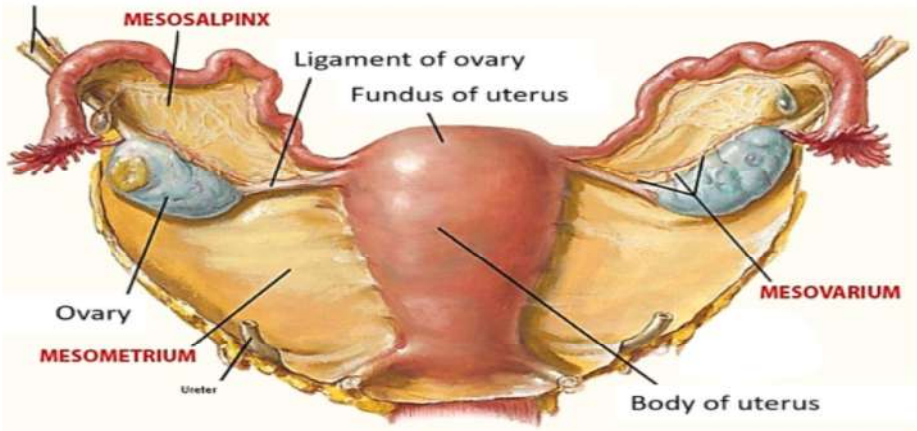
Fig 2 : Structure of Human uterus [11]

SUPPORTS OF THE UTERUS: The uterus is placed in position by various ligaments which include true and false ligaments. True/primary supports include: pelvic diaphragm, perineal body and urogenital diaphragm. Ligamentous true supports include transverse cervical ligaments of Mackendrodt, uterosacral ligaments, round ligament of uterus, pubocervical ligament and uterine axis. False supports include broad ligament, uterovesical ligament and rectovaginal fold of peritoneum. [10]

The round ligament connects the uterus to the abdominal wall and includes the artery of Sampson. The broad ligament connects the lateral part of the uterus with the fallopian tubes and ovary. The uterine artery, cardinal arteries and ureter travel within the broad ligament. The ovarian ligament connects the ovary to the lateral surface of the uterus. The Infundibulopelvic ligament connects the ovary to the abdominal wall. Within this are the ovarian artery and vein. [10]



(a)



(b)

Fig 3 (a,b): Supports of the uterus [(10)]

UTERINE VASCULATURE: The uterus is supplied by uterine artery,a branch of the anterior division of the Internal Iliac artery. In the broad ligament the uterine artery crosses the ureter anteriorly at the cervico-uterine junction. A major branch of it ascends along the uterus tortuously within the substance of the broad ligament. It anastomoses with branches of ovarian artery. The second branch descends to supply the cervix.

The venous drainage of the uterus is contributed by Internal Iliac veins.These venous plexus anastomose with ovarian and vaginal plexuses. [10]

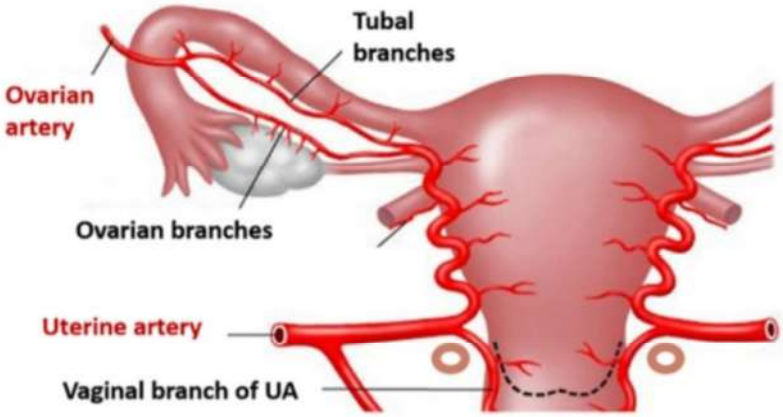


Fig 4 : Blood supply of the Uterus [10]

LYMPHATIC DRAINAGE: There are three groups of lymph nodes- External, Obturator and Internal Iliac nodes. These lymphatics are present in the superficial and deep regions of the uterine wall. The lymphatic drainage from uterine fundus and fallopian tube accompanies the lymphatic drainage of ovary into the para-aortic lymph nodes. [10]

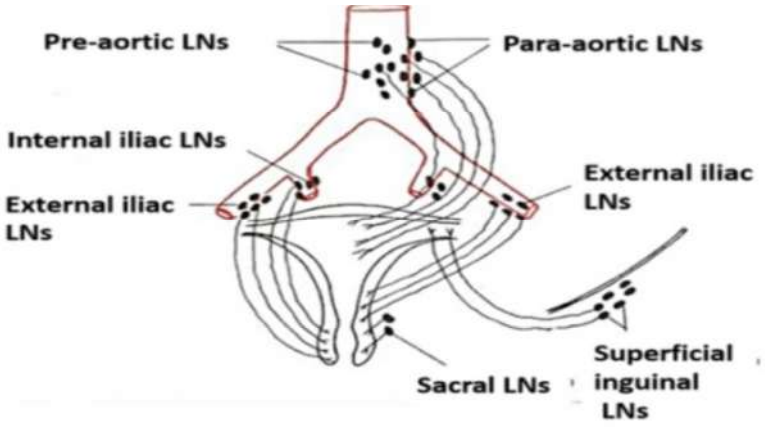


Fig 5: Lymphatic drainage of the uterus [10]

NERVE SUPPLY: The uterus is predominantly supplied by inferior hypogastric plexus. They supply uterine body and fallopian tube. This uterine nerve ends in myometrium and endometrium. Cervix is innervated by plexus containing paracervial ganglia. Preganglionic efferent sympathetic fibres are derived from T12 to L4 segments. Parasympathetic preganglionic fibres are derived from S2 to S4 spinal regions and end in paracervical ganglia.

Sympathetic innervation produces contraction of uterus along with vasoconstriction, while uterine inhibition and vasodilation are produced by presympathetic innervation. (10)

PHYSIOLOGY OF UTERUS

In the female reproductive cycle, there are two concurrent cycles, the ovarian cycle, and the uterine or the menstrual cycle. The Ovarian cycle consists of a series of events that occur during and following oocyte maturation. The Uterine cycle consists of a series of changes within the endometrium in preparation for the arrival of a fertilized ovum that will develop within the endometrium until birth.(12)

The reproductive cycle can be subdivided into the menstrual phase, preovulatory phase, ovulation, and postovulatory phase.

In the **menstrual phase**, a decline in estrogen and progesterone levels stimulates the release of prostaglandins, which leads to vasoconstriction of arterioles within the uterus. The vasoconstriction eventually leads to hypoperfusion of these cells, which results in cell death. This process initiates the sloughing off of blood, fluid, and epithelial cells from the endometrial walls into the cervix and out through the vagina. (12)

In the **preovulatory phase**, estrogen is released into the blood, which repairs the endometrium. The endometrium undergoes other changes and doubles in thickness.

During **ovulation**, the follicle ruptures and releases an oocyte that enters the uterine tube.

In the **postovulatory phase**, progesterone and estrogen both stimulate further growth of endometrial glands and thickening of the endometrium in preparation for implantation. If fertilization does not occur, progesterone and estrogen levels decline, and the menstruation stage occurs.

If the egg becomes fertilized, the zygote is propelled down the fallopian tubes into the uterus. The zygote cells divide rapidly during this descent. The cells of the zygote continue to divide until it becomes a blastocyst. This blastocyst implants by invading the wall of the endometrium. [(12)]

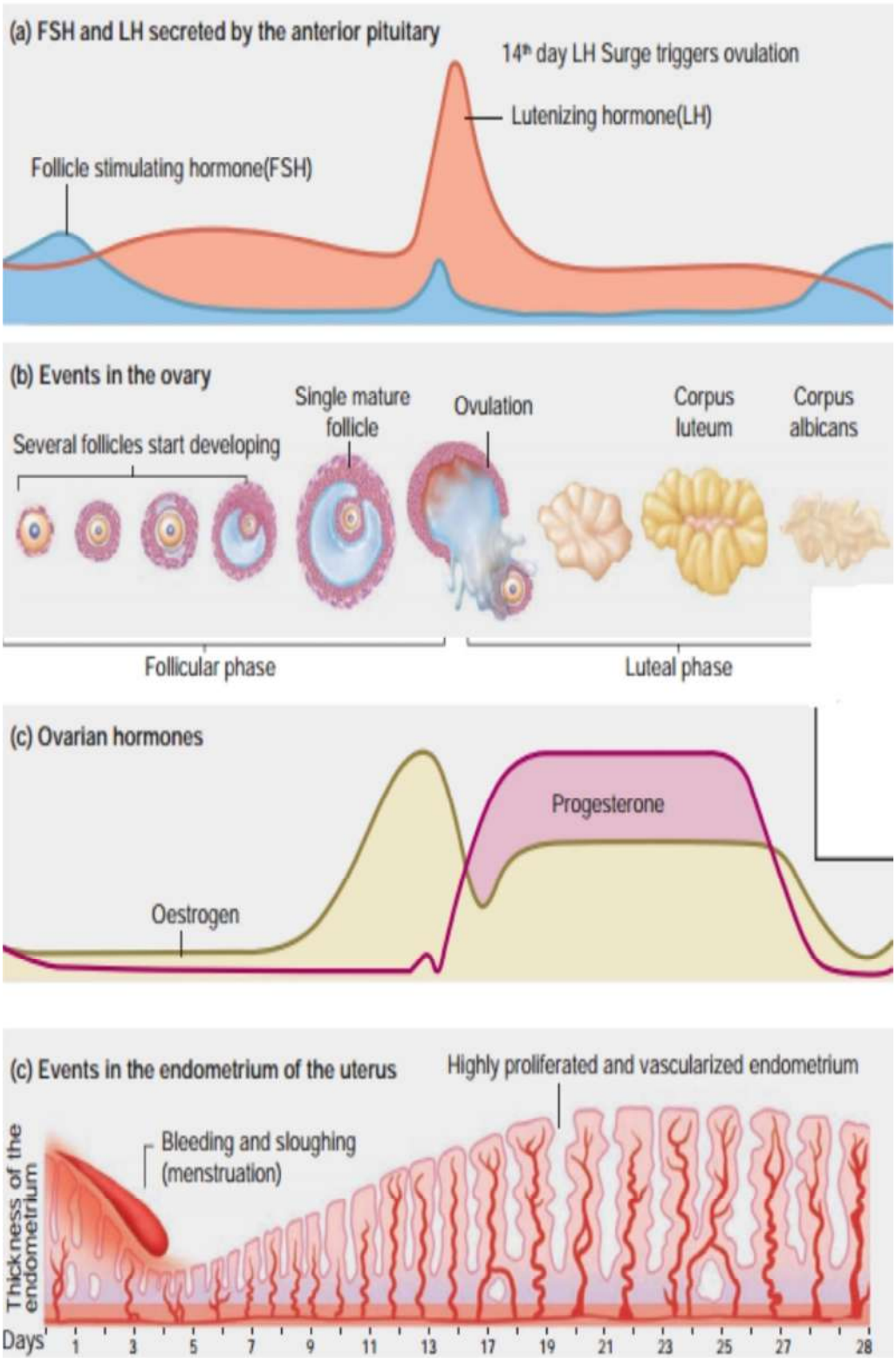


Fig 6: Menstrual and Ovarian cycle [(12)]

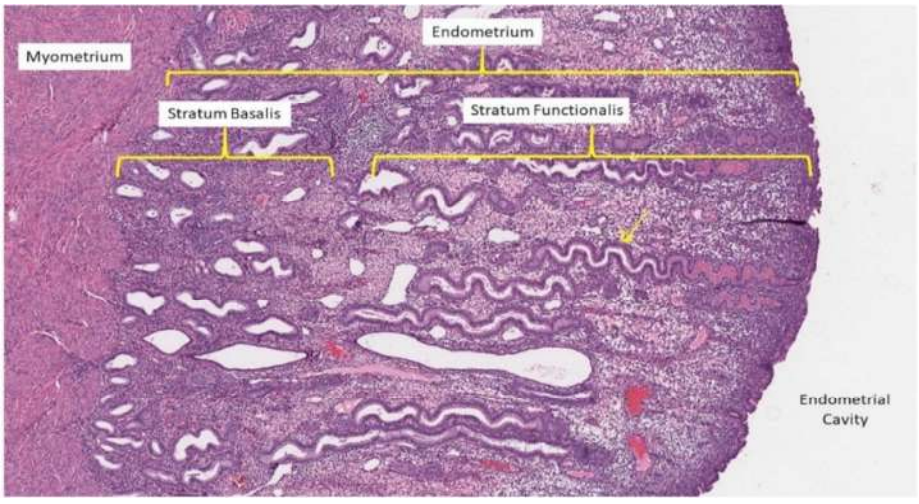
HISTOLOGY

The uterus is made up of an external layer of smooth muscle called the Myometrium and an internal layer called the Endometrium. The endometrium has three layers: Stratum compactum, Stratum spongiosum and Stratum basalis. The Stratum compactum and Stratum spongiosum develop into the Stratum functionalis during the first half of the menstrual cycle (during the Proliferative phase).

The endometrium is a mucosal layer lining the uterine cavity composed of endometrial glands and specialized stroma .

The Stratum basalis is a deep layer of endometrium which is minimally hormone responsive and serves to replenish the Stratum functionalis following menses.

Stratum functionalis is a hormone responsive superficial layer of endometrium which undergoes functional and morphologic changes throughout menstrual cycle.This layer is shed during menses. [13]

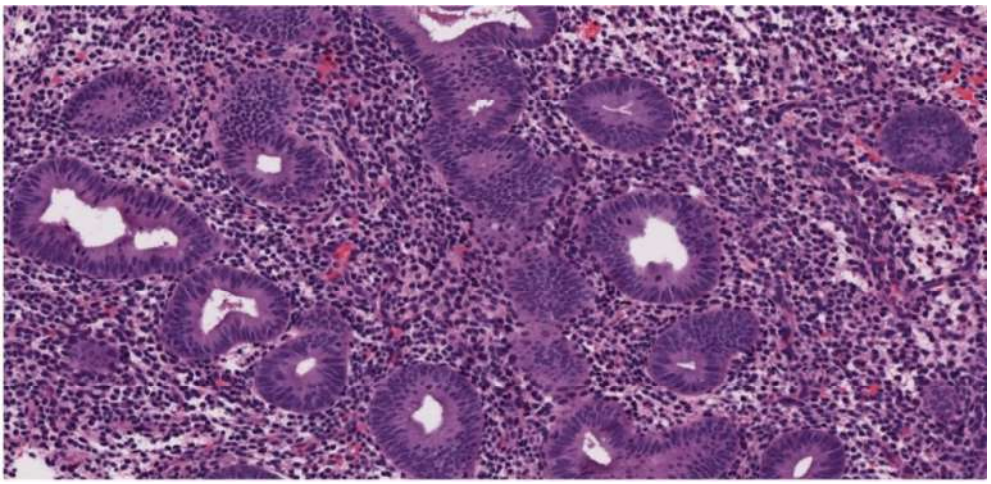


Photomicrograph 1: Histology of human uterus [13]

ENDOMETRIUM

Proliferative phase

A cellular blue appearance is seen at low power.The endometrium is mainly made up of round to tubular glands with even, regular spacing between glands. The glands are lined by pseudostratified columnar epithelium. Numerous mitotic figures are seen in glands and stroma. [14]



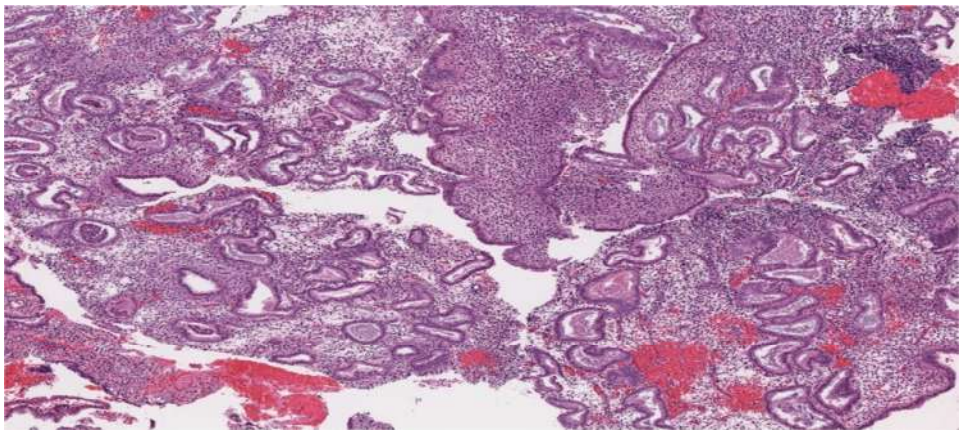
Photomicrograph 2: Low power image of Proliferative phase endometrium [13]

Interval phase (Day 16)

Partially developed subnuclear vacuoles are seen in the epithelium lining the glands. Mitoses is seen, but not as numerous as in proliferative phase. Less than 50% of the cells in a gland have continuous and well developed subnuclear vacuoles.[14]

Secretory phase

At low power, it appears relatively pink. The glands are convoluted and irregularly shaped lined by a single layer of columnar or cuboidal cells.[14]



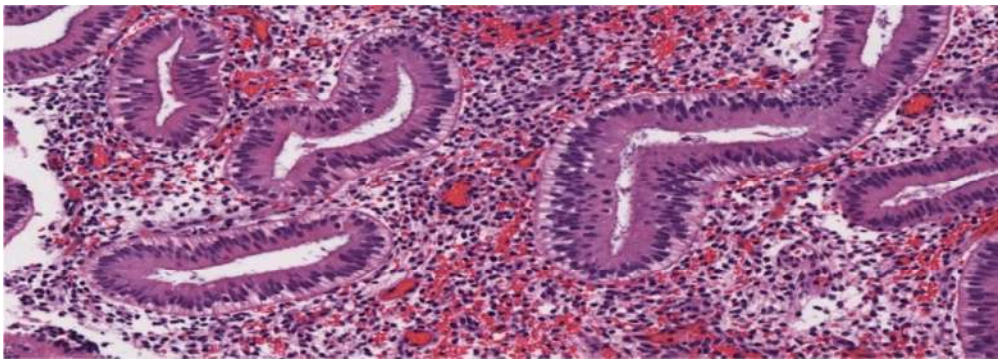
Photomicrograph 3: Low power image of endometrium in secretory phase [(14)]

Early secretory phase (Day 17 - 19)

Day 17: Continuous and well developed subnuclear vacuoles are seen in >50% of a gland with rare mitoses.

Day 18: Sub and supranuclear vacuoles (piano keys) with nuclei are seen in the center of cell.

Day 19: Presence of nuclei at base of cell, with supranuclear vacuoles are noted. Some amount of luminal secretions are also seen.



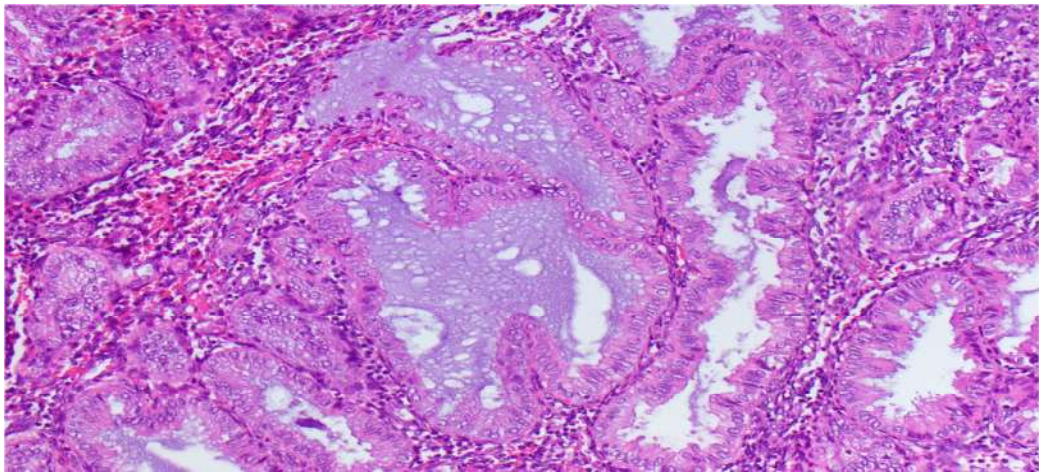
Photomicrograph 4: Early secretory endometrium with subnuclear vacuoles [(13)]

Mid secretory phase (Day 20 - 22)

Day 20: Maximal amount of intraluminal secretions are seen. Stromal cells with hyperchromatic nuclei and high N:C ratio are also noted.

Day 21: Increased stromal edema is seen.

Day 22: Peak stromal edema occurs on this day.



Photomicrograph 5: Endometrium in mid secretory phase showing maximum intraluminal secretions [(14)]

Late secretory phase (Day 23 - 27)

Day 23: Predecidua is seen surrounding the spiral arterioles.

Day 24: Predecidua is seen bridging multiple vessels.

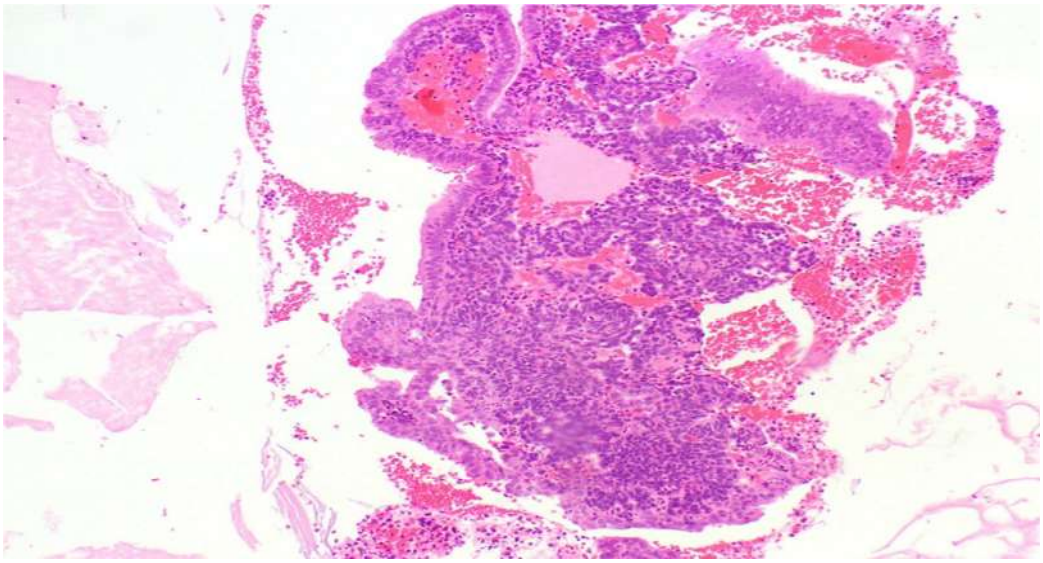
Day 25: Thin band of predecidua is noted beneath the endometrial surface.

Day 26: Thick bands of predecidua is seen beneath the surface

Day 27: Abundant predecidua is seen expanding downward from endometrial surface. Increased number of stromal granulocytes are also noted.

Menstrual phase

Endometrial stromal breakdown is seen with dense round aggregates of stromal cells admixed with inflammatory cells and blood. Papillary syncytial metaplasia is common and is thought to be a reparative response. [14]

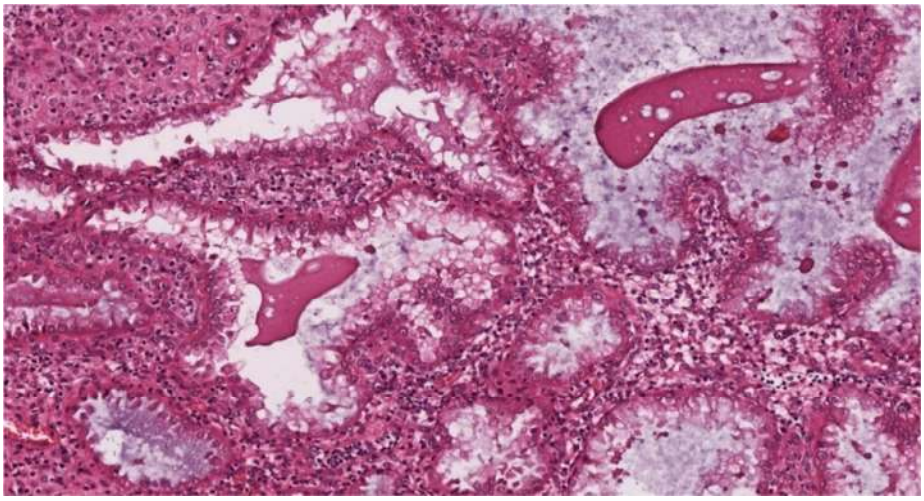


Photomicrograph 6: Low power image showing endometrial stromal breakdown suggestive of menstrual phase. [14]

Gestational changes

Gestational changes are characterised by two changes- 1.Decidual change in which the stromal cells gain abundant eosinophilic cytoplasm and appears polygonal with distinct cell borders;

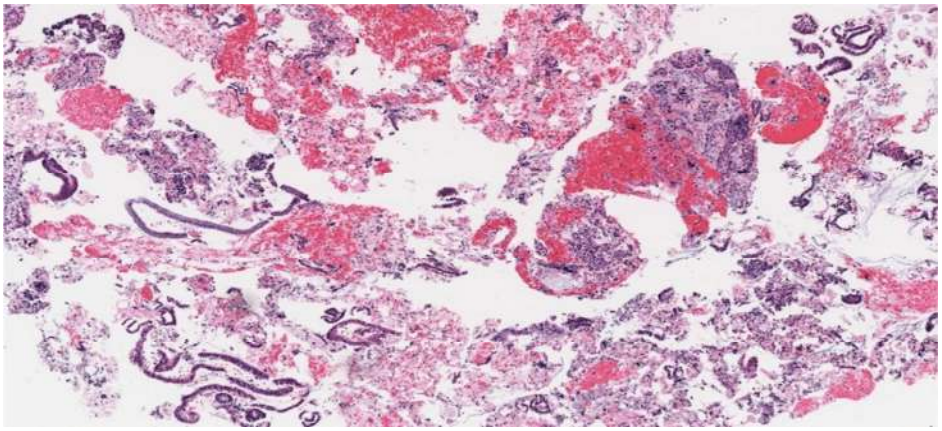
2.Arias-Stella reaction is seen in glandular cells. The cells show nuclear enlargement and hyperchromasia with abundant eosinophilic vacuolated cytoplasm. Hobnail appearance is also noted with cells protruding into glandular lumen. [14]



Photomicrograph 7:Endometrial stroma showing decidual change with Arias-Stella reaction in glands. (14)

Atrophy

This type of endometrium is commonly seen in postmenopausal women due to estrogen withdrawal.Glands are lined by inactive low columnar to cuboidal cells. Glands are often detached from stroma, forming hairpin structures and may show cystic change. (14)



Photomicrograph 8: Low power image of endometrium showing changes suggestive of Atrophy. (14)

MYOMETRIUM

It is comprised of short fascicles of smooth muscle interspersed with large, thick walled blood vessels.[14]

UTERINE LEIOMYOMA

Uterine Leiomyomas commonly called fibroids are benign mesenchymal tumors derived from smooth muscle. They are the most common pelvic neoplasm in women. They are benign monoclonal tumors originating from the smooth muscle cells of the myometrium. The pathogenesis of leiomyomas is not well understood and genetic predisposition, environmental factors, steroid hormones and growth factors important in fibrotic processes and angiogenesis all play a role in the formation and growth of uterine fibroids. [15]

The disease is heterogeneous and different fibroids within the same uterus may have different etiologies. Leiomyoma related effects on the function and structure of the endometrium have been implicated in the pathogenesis of excessive bleeding in the myomatous uteri, and there is evidence of both histologic changes in the endometrium and endometrial vasculature in these uteri. [15]

EPIDEMIOLOGY

The overall incidence of uterine leiomyomas is between 4% - 11% but in rises to about 40% in women over the age of 50 years. Most leiomyomas are detected in middle aged women. Although uncommon in women less than 30 years of age, the youngest case on record is 13 years. Some leiomyomas apparently shrink after menopause, although their frequency does not appear to have reduced. [16]

SITE

The uterine corpus is the most commonest site of origin of leiomyomas. Less common sites include vulva, vagina, cervix, broad ligament and ovary. Based on their location leiomyomas are generally classified into three types - Intramural, Submucosal and Subserosal. The conventional teaching is that Submucosal leiomyomas produce metrorrhagia because of the endometrial ulceration, the Intramural leiomyomas result in menorrhagia as they interfere with myometrial contraction and the Subserosal leiomyomas usually remain asymptomatic. (17)

Submucosal tumors often result in secondary endometrial changes which range from gland distortion to atrophy and ulceration. They may fill the endometrial cavity and emerge from the cervical canal as polypoid growths (Myoma Nascens). Under these circumstances their surface is usually ulcerated and infected, the gross appearance thus stimulating a malignant neoplasm. (16)

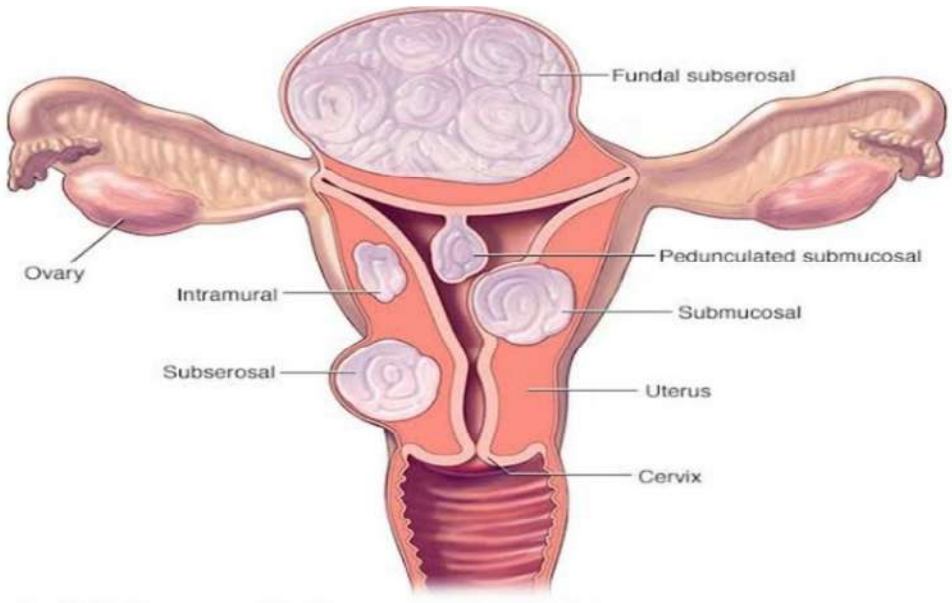


Fig 7: Common sites of origin of uterine fibroids (17)

ETIOPATHOGENESIS

Although the cause or causes of fibroids are unknown, the scientific literature now contains a sizeable body of information pertaining to the epidemiology, genetics, hormonal aspects, and molecular biology of these tumors.

Uterine fibroids are monoclonal tumors [(18)], and increasing evidence indicates that they arise from a single myometrial stem cell (MMSC) [(19,20)]. MMSCs constitute a small proportion of the total population of cells and express specific surface markers that distinguish them from the bulk of other cells [(21)]. The plasticity of MMSCs during development and tissue maintenance permits the acquisition of mutations or aberrant cellular reprogramming via epigenetic mechanisms. Consequently, normal MMSCs can be converted into tumor-initiating stem cells (TICs) that are able to initiate fibroid development.

The most common genetic drivers associated with their development are somatic mutations present in exons 1 and 2 of the MED12 gene, which encodes a subunit of the mediator complex, a co-activator involved in the transcription of nearly all RNA polymerase II-dependent genes [(22)]. In addition to MED12 mutations, which account for ∼70% of UFs [(23,24)], a proportionally smaller fraction of UFs is thought to arise from genetic alterations leading to the overexpression of high-mobility group AT-hook 2 (HMGA2, ∼20%), biallelic inactivation of fumarate hydratase (FH, ∼2%) [(25)], and disruption of the COL4A6 locus (∼3%).

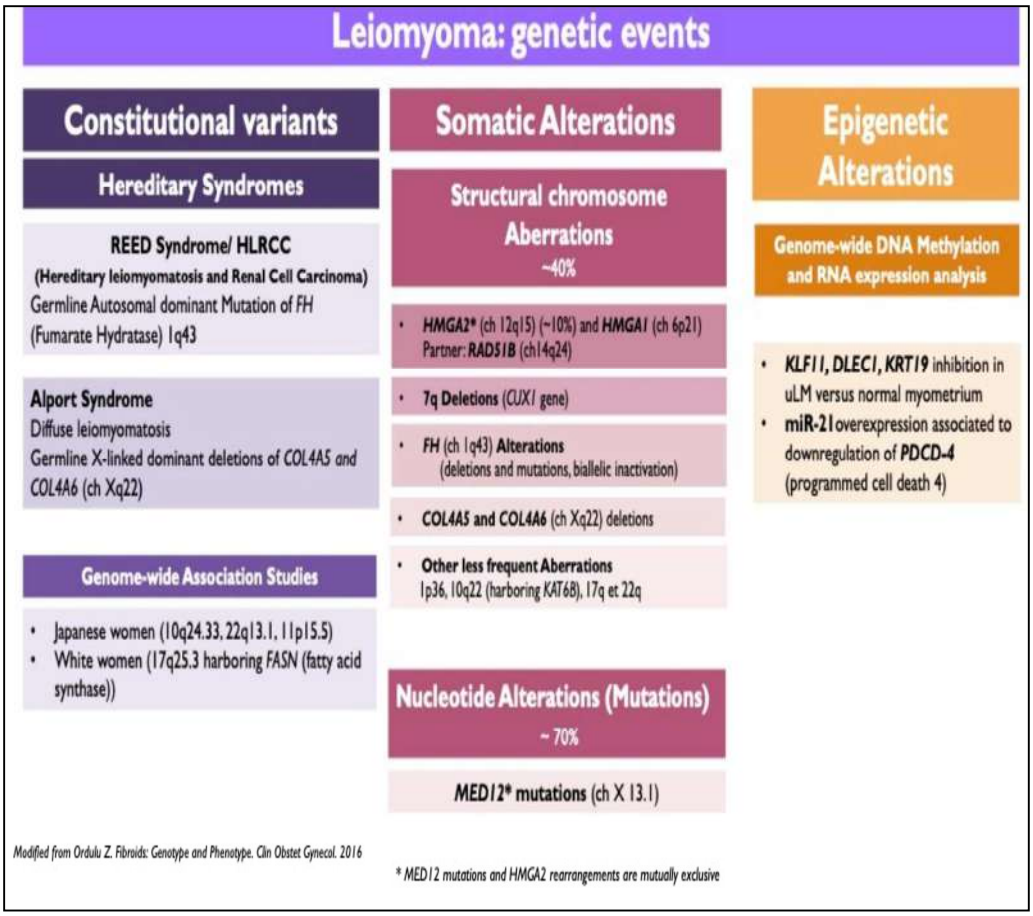


Fig 8: Genetic events related to the origin and proliferation of leiomyomas [26]

In considering the development of uterine leiomyomas, the factors that may be related to tumorigenesis can be subdivided into four categories: predisposing or risk factors, initiators, promoters, and effectors. [27]

RISK FACTORS

Table 1: Risk factors affecting the development of uterine leiomyomas [(27)]

Factor	Risk of Leiomyomas	Reference
Early menarche	Increased	Marshall et al. 1998 [(28)]
Nulliparity	Increased	Parazzini et al. 1996[(29)]
Age (late reproductive years)	Increased	Marshall et al. 1997[(30)]
Obesity	Increased	Ross et al. 1986[(31)]
Tamoxifen	Increased	Deligdisch 2000 [(14)]
Increasing parity	Decreased	Lumbiganon et al. 1996[(32)]
Menopause	Decreased	Samadi et al. 1996 [(33)]
Smoking	Decreased	Parazzini et al. 1996 [(34)]

HORMONAL EFFECTS : The growth of Leiomyomas is affected by the hormonal mileu. Leiomyomas contain estrogen and progesterone receptors which can be demonstrated biochemically and immunohistochemically. They may increase in size during estrogen therapy and most decrease in size when the patient is treated with a Gonadotropin releasing hormone agonist. Progestin, Progesterone, Hormone replacement therapy, Clomiphene use and pregnancy occasionally are associated with a rapid increase in the size of leiomyomas and sometimes cause degenerations. [(35-37)]

INITIATORS OF TUMORIGENESIS

The most important aspect of the etiology of fibroids—the initiator(s)—remains unknown. Several theories have been advanced; One hypothesis states that the increased levels of estrogen and progesterone result in an increased mitotic rate which may contribute to myoma formation by increasing the likelihood of somatic mutations [(38)].Another favors an inherent abnormality in the myometrium of those who develop fibroids, based on the significantly increased levels of ER in the myometrium of fibroid uteri [(39)]. A predisposing genetic factor has also been suggested by others on the basis of ethnic and familial predilections [(40)].

Another interesting theory postulates that pathogenesis of uterine leiomyomas may be similar to a response to injury [(41)] in a manner analogous to the development of keloids (hypertrophic scars) following surgery. One avenue of potential injury might be ischemia associated with the release of increased vasoconstrictive substances at the time of menstruation.

After vascular injury, basic fibroblast growth factor (bFGF) is critical to smooth muscle proliferation, and this factor is also overexpressed in leiomyomas [(42)].

Finally, injury related to menses is worthy of consideration in view of the universality of menstruation and the commonality of fibroids. When we consider the various risk factors, including those that have been attributed in the literature to increased exposure to unopposed estrogens,such as early menarche and nulliparity, we observe that such patients also experience more menstrual cycles than their counterparts.[(27)]

Of equal uncertainty in the genesis of fibroids is the role of genetic and/or epigenetic changes. The possibility of hereditary genetic predisposition to fibroids cannot be excluded at this time. On the other hand, evidence has been presented, though limited in scope, that karyotypic changes may occur secondarily during the evolution or aging of some fibroids [(26)].

Although many theories have been postulated, the most important piece of the fibroid puzzle, the initiator(s), remains unsolved. Further elucidation of the genetic and molecular changes will provide insights into the pathobiology of these tumors and may offer clues to initiating conditions responsible for such changes.

Karyotypic abnormalities have been identified in approximately 40% of surgically removed uterine leiomyomas. The most common of these are the translocation t(12;14) and the deletion of 7q; however, these abnormalities do not exclude submicroscopic mutations of a more universal nature, which will require molecular demonstration. There may be more than one genetic pathway to the formation of fibroids. Phenotypic fibroid variants are probably related to chromosomal differences, either from the outset or as a result of clonal evolution. [(26)]

PROMOTERS & EFFECTORS

Estrogen and progesterone appear to be promoters of fibroid growth, acting in concert. Thus, estrogen upregulates both ERs and PRs during the follicular phase, followed by progesterone-induced mitogenesis during the luteal phase. The deficiency of the estrogen metabolizing enzyme 17β-hydroxy- steroid dehydrogenase in fibroids may be responsible for the accumulation of estradiol in these tumors and its consequent growth- promoting effects. Likewise, the overexpression of estradiol 4-

hydroxylase seems highly significant, as resulting metabolites possesses long-acting estrogenic activity.[43]

The levels of several growth factors and their receptors are increased in fibroids. TGF-β3 and bFGF may be especially important in the pathogenesis of these tumors in view of their combined mitogenic effect and promotion of extracellular matrix production. [44] EGF appears to be significant, as it is the only characterized growth factor, other than TGF-β3, with elevated expression during the luteal phase, when leiomyoma mitotic activity is maximal. IGF-I almost certainly plays an important role because of its potent mitogenic capacity and the overexpression of both the peptide and its receptor in leiomyomas. Growth factors may be the mediators or effectors of sex steroid upregulation, but a primary dysregulation of one or more growth factors must also be considered.[45]

CLINICAL FEATURES

About 25% of women are symptomatic while the remainder are usually coincidental findings. The clinical presentation of leiomyomas depends on their size and location. Leiomyomas cause many signs and symptoms the most common of which is pain followed by a sensation of pressure and abnormal uterine bleeding. In some cases infertility is also attributed to their presence. Rarely pedunculated leiomyomas can undergo torsion, infarction and separation from uterus. Secondary infection may also occur in some cases. [46]

DIAGNOSIS

This can be established by the resection of the whole uterus (Hysterectomy), by resection of the leiomyoma if accessible by curetting (if submucosal) or by

myomectomy (if subserosal),followed by a histopathological examination and confirmation of the diagnosis of a leiomyoma.

Ultrasound or magnetic resonance imaging (MRI) guided core biopsy of the leiomyoma is a promising new procedure. [47]

PROGNOSTIC FACTORS

Uterine leiomyomas are benign tumors that have high rates of recurrence, especially after myomectomy. Fumarate hydratase deficient leiomyomas are known to recur more frequently and hence genetic counseling should be recommended. [48]

TREATMENT

Asymptomatic patients do not require therapy usually. However symptomatic leiomyoma may be treated by different modalities depending on severity and duration of symptoms and whether family has been completed or not. [48]

Medical treatment includes progestins / levonorgestrel intrauterine system, gonadotropin releasing hormone (GnRH) analogs or aromatase inhibitors. Ulipristal acetate is widely used in conservative treatment of uterine leiomyomas but rejected by the FDA due to risk of liver toxicity.

Surgical options include Hysteroscopic resection, myomectomy and finally hysterectomy.

Interventional treatments like uterine artery embolization and radiofrequency myolysis can also be tried. [48]

GROSS FEATURES

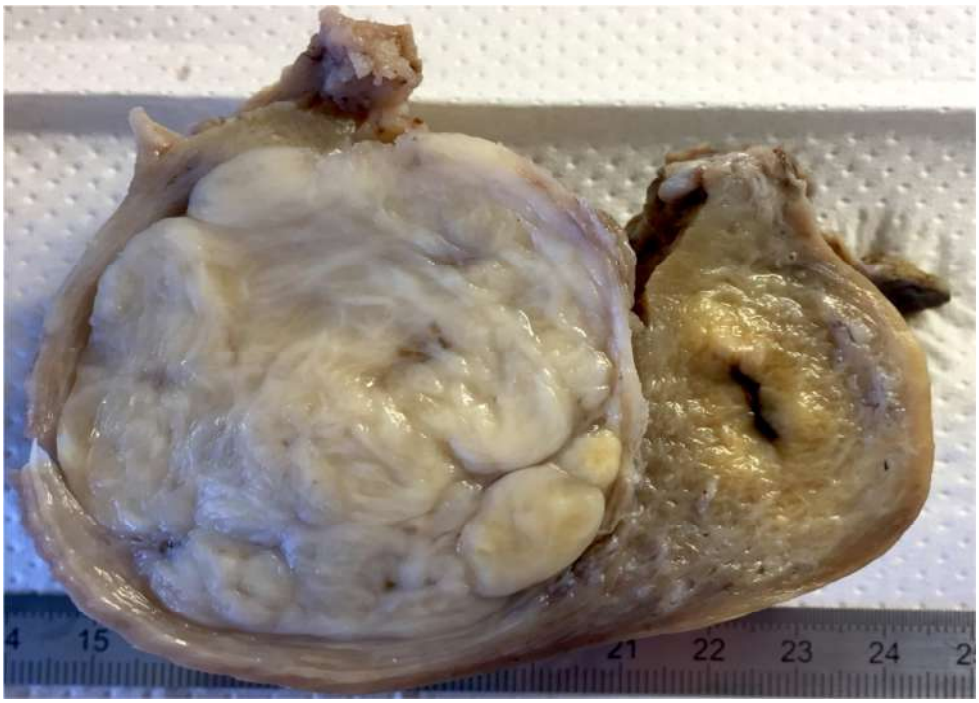


Fig 9: Cut section of the uterus reveals a well defined unencapsulated tumor with bulging, firm, whorled white cut surface-Leiomyoma

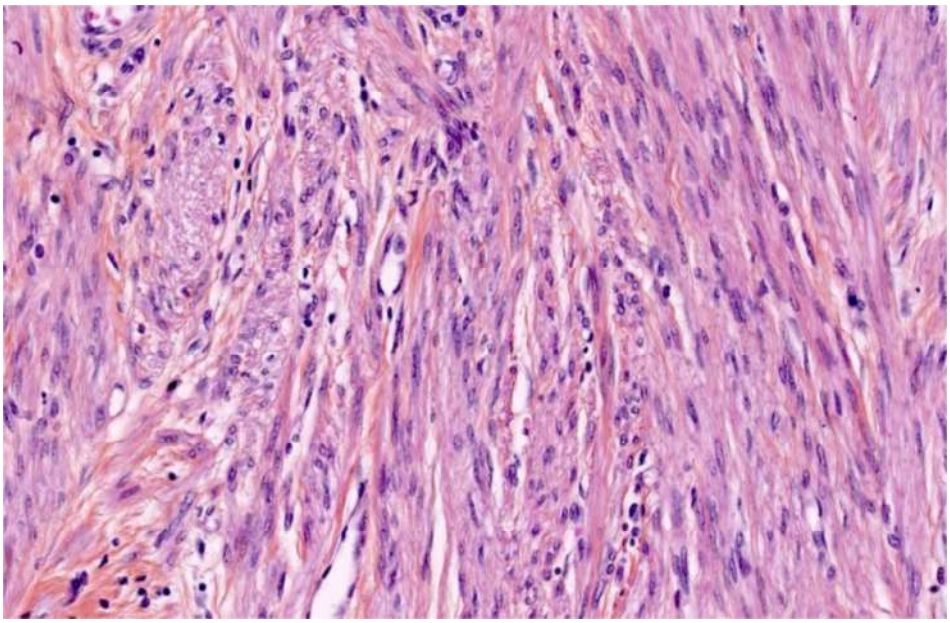
Leiomyomas are firm and spherical and bulge above the surrounding myometrium from which they are shelled out. The cut surfaces are white to tan and show a whorled pattern. Despite the variety of histological subtypes, the gross features usually remain the similar. [(5)]

It is commonly altered by degenerative changes. Hemorrhage(dark red areas) and necrosis(demarcated yellow areas) can be observed in leiomyomas particularly if they are large or occur in women who are pregnant or undergoing high dose progestin therapy. The damaged smooth muscle is replaced eventually by firm white or collagenous tissue. Cystic degeneration also occurs and some leiomyomas become extensively calcified. [(49)]

MICROSCOPY

90% of leiomyomas are the conventional types although histological subtypes exist. The Subtypes include: Cellular Leiomyoma ,Leiomyoma with bizarre nuclei, Fumarate hydratase (FH) deficient, Mitotically active, Hydropic, Apoplectic, Lipoleiomyoma, Epithelioid, Myxoid, Dissecting leiomyoma and Diffuse leiomyomatosis. [(5)]

Conventional leiomyomas have well defined borders comprising of intersecting fascicles of monotonous spindle cells with indistinct borders, eosinophilic cytoplasm, cigar shaped nuclei (with tapered ends) and small nucleoli. Atypia is usually absent or mild with rare mitoses (in general < 5/10 high power fields). Blood vessels have thick walls. They may also show hyalinization, calcification or cystic change. [(5)]



Photomicrograph 9: Fascicles of spindle cells with cigar shaped nuclei-Conventional leiomyomas [(17)]

SUBTYPES:

- **Cellular leiomyomas** have increased cellularity (ie more cellular than the background myometrium). The cells have scant cytoplasm without increased mitotic activity and atypia.They may have irregular borders. However, Highly cellular leiomyoma is not a WHO diagnosis. [17]
- **Leiomyoma with bizarre nuclei** show cells with bizarrely shaped, hyperchromatic, multilobulated nuclei with nuclear pseudoinclusions arranged in a multifocal to diffuse distribution in a background of a typical leiomyoma.Low mitotic activity (< 5 mitoses/10 high power fields) is usually noted along with absence of tumor cell necrosis. Vasculature is usually variable showing staghorn vessels, thick walled vessels, fibrinoid necrosis of vessel walls and/or luminal vascular obliteration. The diagnostic key for leiomyomas with bizarre nuclei is the presence of intermixed normal spindled smooth muscle cells. [50]
- **Fumarate hydratase deficient leiomyoma** show alveolar edema with staghorn or hemangiopericytoma-like vasculature with a chain-like growth of tumor cells. These cells have spindle or epithelioid cells morphology with ovoid nuclei and prominent eosinophilic nucleoli surrounded by perinucleolar halos. Rhabdoid / eosinophilic cytoplasmic inclusions can also be seen in these cells. These leiomyomas may also include multinucleated cells and cells with bizarre nuclei. [5]
- **Mitotically active leiomyoma** are spindle cell leiomyomas without atypia or tumor cell necrosis with increased mitotic activity (the mitotic cutoff varies according to the authors 6 - 14 mitoses/10 hpf or 6 - 15 mitoses/10 hpf) [17]

- **Hydropic leiomyoma** the tumor cells are separated by watery or eosinophilic and proteinaceous fluid, resulting in a trabecular, nested architecture
- **Apoplectic leiomyoma** have a central zone of hemorrhage and necrosis with increased mitotic activity in its periphery or myxoid changes (zonation phenomenon)
- **Lipoleiomyoma** is a tumor composed of smooth muscle cells mixed with variable number of mature adipocytes.
- **Epithelioid leiomyoma** contain tumors cells more than 50% which have round or polygonal morphology with eosinophilic or clear cytoplasm arranged in a nested or trabecular pattern with no cytologic atypia or tumor cell necrosis. The mitotic count is <2 mitoses/10 hpf.
- **Myxoid leiomyomas** are hypocellular tumors in which the cells are separated by myxoid matrix composed of glycosaminoglycans (Alcian blue positive) occupying ≥ 50% of the overall tumor volume. They have no cytologic atypia, mitoses or tumor cell necrosis. The most important criteria for their diagnosis is the presence of well circumscribed borders. [(5)]
- **Dissecting leiomyoma** comprise of nodules of smooth muscle cells dissecting the myometrium. They occasionally show hydropic changes and intravenous extension. These tumors are also called cotyledonoid leiomyoma if they extend outside the uterus.
- **Diffuse leiomyomatosis** are leiomyomas with innumerable diffuse, poorly circumscribed, tumor nodules in the myometrium,with low mitotic count and no atypia or tumor cell necrosis. [(5,17)]

Degeneration

As fibroids enlarge, they outgrow their blood supply causing various types of degeneration, most commonly, Hyaline (60%), Cystic (4%), Red(3%), Calcareous (4%) or Sarcomatous degeneration (0.1-0.8%). The typical clinical findings and ultrasonographic appearance of uterine fibroids are obscured by the degenerative changes.

IMMUNOHISTOCHEMISTRY

Most leiomyomas stain positively for Desmin, h-caldesmon, Smooth muscle actin,Transgelin, Estrogen and progesterone receptors & WT1.However smooth muscle markers can be weak in epithelioid and myxoid leiomyomas. [5,7,17]

Up to 40% of leiomyomas are CD10 positive (especially cellular leiomyomas).

FH loss of expression and 2SC positivity may be useful to identify fumarate hydratase deficient leiomyoma.FH loss of expression is specific of FH deficient leiomyoma but its sensitivity is low with different results across different leiomyomas in the same patient and for these reasons, the clinical value of FH staining is limited. [51]

Most leiomyomas show patchy or negative p16 (except in leiomyomas with bizarre nuclei, where it can be diffusely positive) and negative p53 typically wildtype (except in leiomyomas with bizarre nuclei, where it can show aberrant expression) [52]

MOLECULAR / CYTOGENETICS

About 70% of conventional leiomyomas harbor MED12 mutations and nearly 40% show HMGA2 / HMGA1 rearrangements, COL4A5 / COL4A6 deletions or FH mutations. [53]

Other less frequently seen alterations include 7q22 deletion involving CUX1 gene, 22q deletion with DEPDC5 gene and 1p deletion with NPHP4 gene. [54]

As recent as 2019, a novel t(10;17)(q22;q21) KAT6B-KANSL1 fusion has been observed and described in cellular leiomyoma. [55]

In leiomyomas with bizarre nuclei, the genomic profiles and genomic index can be more complex compared with that of classic leiomyoma. At a molecular level, leiomyomas with bizarre nuclei are separated in 2 subtypes: FH abnormal and TP53 and RB1 abnormal.Bi-allelic inactivation of FH has been described in up to 33% of leiomyomas with bizarre nuclei.For FH deficient leiomyomas, the FH mutation may be somatic or germline. [56]

DIFFERENTIAL DIAGNOSIS

1. **Smooth muscle tumor of uncertain malignant potential (STUMP):**

   i. Uterine spindle cell STUMP

   ii. Myxoid STUMP: have 1mitotic figure/10 hpf in the absence of cytologic atypia and tumor cell necrosis

   iii. Epithelioid STUMP: ≥ 2 mitotic figures/10 hpf and < 4/10 high power fields in the absence of cytologic atypia and tumor cell necrosis.

2. **Leiomyosarcoma** - A cellular neoplasm with moderate to severe cytologic pleomorphism having areas of hemorrhage and coagulative necrosis with mitotic activity generally greater than 5 to 10 mitotic figures/hpf. This neoplasm usually has infiltrating borders.

3. **Endometrial Stromal nodule** (vs Cellular leiomyoma) show diffuse arteriolar vascularization and absence of thick vessels at the center of the lesion. They also don't show a fascicular pattern of growth but have smaller bland spindled cells resembling endometrial stroma and are positive for CD10 with occasional cells positive for SMA. [5,15,16,17]

MAST CELLS

The original description of connective tissue mast cells were given by Paul Ehrlich in 1877 and since then a lot of information about them have been accumulated in literature. [57]

ORIGIN

The origin of mast cells have been a subject of controversy for more than a century. It is described that they appear late in the development of human embryo. Over the course of many years, a variety of cells have been postulated to be the precursors of mast cells including undifferentiated mesenchymal stem cells, pericytes and basophils, all of which were dismissed after further observation, research and experiments.

It is now known that human mast cells are derived from pluripotent hematopoietic stem cells in the bone marrow and mature under the influence of CD34

and CD117 in the presence of other distinct growth factors provided by the microenvironment of the tissue where they reside. [58]

DIFFERENTIATION

The maturation and differentiation of mast cells is locally controlled [59]. Fibroblasts are thought to be important in promoting mast cell maturation in skin and connective tissues [60].IL-4 and IL-10 are capable of inducing the proliferation and differentiation of mast cells. Interleukin 3 (IL-3) production by T lymphocyte is implicated in mast cell precursor maturation and T lymphocytes may promote mast cell division[61] .

DISTRIBUTION:

During the course of intrauterine life, small cells containing a few metachromatic granules are observed at sites of extra medullary haemopoiesis. At birth a fairly abundant number of mast cells are observed in the liver, eye, blood vessels and later in all the subcutaneous connective tissues [62]. In the adult however, mast cells are located in and around almost all tissues around blood vessels ,where they tend to aggregate in small groups[63]. The highest concentration of mast cells is present in the skin, respiratory tract, gastrointestinal tract, urinary bladder, lymphoid system, serosal surfaces and the uterus .They are conspicuously absent in the retina and CNS.[64]

STRUCTURE:

i. LIGHT MICROSCOPY:

Mast cells are not visualized with most of the conventional stains. On light microscopy, in routine H and E stained sections, mast cells resemble fibroblasts or histiocytes. They are also confused with plasma cells or immature granulocytes [(65)].

Special stains like Toluidine blue and Giemsa are essential for their proper evaluation. On light microscopy, with special stains, mast cells appear large, rounded, ovoid or spindle shaped but being sluggish they may assume different shapes. They lack basal lamina and may have short pseudopodia because they are wandering cells [(66)]. Their nucleus is small, inconspicuous, ovoid in shape and is often masked by the cytoplasmic granules. The cytoplasm contains numerous coarse and dense granules which are refractile, water soluble and exhibit metachromasia . Mast cells vary in size from 5 to 15 μm [(67)].

Based on fixation and staining character, mast cells show considerable heterogeneity in certain human tissue. The more familiar type of cell is called the connective tissue mast cell (CTMC). This CTMC is a large cell and possesses a round, unilobed nucleus. The cytoplasm is densely packed with intensely violet, metachromatic granules .This type of cell is found to be resistant to fixatives including 4% formalin.

The second type of cell is called the mucosal mast cell (MMC). It can be demonstrated only after fixation with special fixatives like basic lead acetate or Carnoy's fluid. This cell is smaller, more sparsely granulated; and it stains reddish rather than violet with basic dyes [(68)].

Depending on the age of the mast cells two types are identified on light microscopy.

Type I: is the young mast cell which stains orthochromatically (blue) & homogeneously with Toluidine blue and does not give the periodic acid schiff reaction.

Type II: is the mature mast cell. It stains metachromatically (violet) and with granularity on Toluidine blue staining and gives a positive periodic acid schiff reaction [(69)].

ii) <u>ELECTRON MICROSCOPY</u>:

Although light microscopy after special stains is adequate for the identification of mast cells in tissues, electron microscopy is essential to detect the pregranular and degranulating stages which are likely to be overlooked on light microscopy [(70)].

On electron microscopy, the nucleus of mast cells is seen to lie more or less central and it has a clumped chromatin pattern. The mast cells are irregular in outline with clear cut cytoplasmic processes extending from them. The cytoplasm is packed with granules and shows very few small mitochondria. The Golgi apparatus plays an important role in the synthesis and sulphation of acid mucopolysaccharides [(71)].

Golgi apparatus is well developed in mast cells but ribosomes and endoplasmic reticulum are in moderate amounts. These features suggest a fully differentiated cell in which very little active synthesis or energy expenditure is taking place [(72)].

The most characteristic subcellular components of mast cells are their secretory granules . They vary in size from 0.2 to 0.8 μm and are bounded by a unit membrane . The matrices of these granules have varying patterns such as whorls, scrolls and particulate or lamellar structure [(73)]. However some empty vacuoles representing discharged granules and a few disintegrating granules are also seen [(74)].

STAINING REACTIONS & DETECTION TECHNIQUES:

Different staining methods are employed to demonstrate mast cells because the granules are not identified on routine H and E stain.

The staining of mast cells with basic dyes depends on the cationic nature of their granules.

PRINCIPLE:

The principle of the staining reactions with basic thiazine dyes like Toluidine blue or methylene blue is that one amine (histamine) is replaced by another amine (the dye) in the heparin histamine complex. Heparin is a sulphated mucopolysaccharide and therefore it gives a metachromatic reaction.

The various methods of staining mast cells and their respective staining reactions are indicated in Table II [(75,76)].

Table 2: Various detection methods of mast cells [75,76]

Human mast cell markers and mast cell detection techniques

Markers	Cell type	Staining technique	Additionally stained structures	Notes
1. Sulfated glycosaminoglycans (including heparin)	Mature serosal mastocytes, a part of mucosal mastocytes	**Toluidine blue** **Methylene blue** Thionin **Safranin O** Basic brown Methylene green Azure A May-Grünwald–Giemsa	Macrophages, basophilic leukocytes, goblet cells, Clara cells	Toluidine blue and methylene blue staining is the easiest and most effective method to detect most mastocytes
2. Heparin	Mature serosal mastocytes	**Berberine** **Avidin** Acridine orange Immunohistochemistry	Basophilic leukocytes For avidin — any cells containing biotin	Berberine, avidin are highly selective markers of heparin, but berberine does not cause fluorescence in human skin mastocytes
3. Chondroitin sulfate E	Mucosal and immature mastocytes	**Alcian blue** Astra blue	Components of connective tissue intercellular substance	Frequently used in combination with safranin O for simultaneous detection of mucous and serosal mastocytes
4. Tryptase	Serosal and mucosal mastocytes	Leder's reaction **Immunohistochemistry**	None	Tryptase immunohistochemistry is the most effective technique to detect human mastocytes Leder's reaction reveals an active enzyme alone
5. Chymase	Serosal mastocytes	Leder's reaction **Immunohistochemistry**	None	Leder's reaction reveals an active enzyme alone
6. c-kit (CD117)	Mastocytes	Immunohistochemistry	Stem cells, germinal cells, epithelial cells, interstitial cells of Cajal, melanocytes and basal skin cells, neurons, gliocytes	Also used as a tumor marker
7. Immunoglobulin receptor E (FcεRI)	Mastocytes	Immunohistochemistry	Basophilic leukocytes, monocytes, eosinophils, epidermal Langerhans cells	
8. Histamine	Mastocytes (primarily, serosal)	Immunohistochemistry Histochemistry	Some neurons, histamine-containing epithelial endocrine cells of the stomach, basophilic leukocytes, neutrophils, monocytes, macrophages, dendritic cells	
9. Serotonin	Mastocytes (primarily, serosal)	Immunohistochemistry Histochemistry	Neurons, gustatory receptor cells, glomus cells of carotid bodies, enterochromaffin cells, trophoblast, decidual cells, keratinocytes, fibroblasts, melanocytes, platelets	
10. Vascular endothelial growth factor receptor-1 (VEGFR-1)	Mastocytes	Immunohistochemistry	Endothelial vascular cells, epithelial cells of the uterus and bronchi, type 2 pneumocytes, cytotrophoblasts, interstitial cells of testicles, T lymphocytes, monocytes, micro- and astroglia, neurons	Also used as a tumor marker

N o t e: the most effective markers for mast cell detection exhibiting the highest specificity are highlighted in bold; numbers 6–10 represent less selective mast cell markers, their usage can result in obtaining erroneous results.

PHYSIOLOGICAL ROLE OF MAST CELLS & MAST CELL MEDIATORS

It is known that mast cells are involved in the regulation of a variety of physiological functions, including vasodilation, angiogenesis, bacterial, and parasite elimination. In addition, they regulate functions of many cell types, such as dendritic cells, macrophages, T cells, B cells, fibroblasts, eosinophils, endothelial cells, and epithelial cells. Since, mast cells generate and release multi-potent molecules, like histamine, proteases, prostanoids, leukotrienes, heparin, and many cytokines, chemokines, and growth factors to name a few, they have the capacity to be involved in regulating the functions of many organs and tissues. One of the mostly studied functions of the mast cell is its role in vascular and bronchial homeostasis. Mast cells also play a significant role in the regulation of bone growth, remodeling, and mineral homeostasis. [(1)]

ANGIOGENESIS :

Mast cells secrete pro-angiogenic factors, such as VEGF, bFGF, TGF-beta, TNF-alpha, and IL-8. In addition, they release proteases and heparin which release pro-angiogenic factors that bind to heparin. Histamine, released by mast cells, induces permeability of the microvasculature that also induces angiogenesis. There is also evidence of mast cells enhancing angiogenesis in tumor growth [(77)].

HOMEOSTASIS:

Mast cells serve as a first line of defense against antigens entering the body due to their location in the skin and mucosa. They are important in the differentiation of follicular helper T cells via ATP signaling. As a result, mast cells play a role in IgA maturation and overall homeostasis of the gut bacteria [(78)].

INNATE AND ADAPTIVE IMMUNITY:

Mast cells recognize harmful antigens by binding to pathogens directly or associating with PAMPs on the mast cell surface commonest of which are the TLRs and receptors for complement. Once the antigen binds to the receptors on the mast cell, it causes the release of inflammatory mediators, which increase vascular permeability, increase fluid accumulation, and recruit immune cells, such as eosinophils, NK cells, and neutrophils. Additionally, mast cells directly produce antibacterial products, such as cathelidcidins, defensins, and psidins. Mast cells also contribute to antiviral responses by recruiting CD8+ T cells, which produce IFN-α and IFN-β [(79,80)].

Mast cells are also involved in adaptive immunity. They process and present antigens via MHCI and MHCII [(81)] and can activate dendritic cells that also function as antigen- presenting cells. When mast cells are stimulated through TLF-7, they release IL-1 and TNFα, which causes dendritic cells to move from their location in the skin and go to local lymph nodes and activate cytotoxic T cells. Additionally, mast cells release TNFα, which can activate cytotoxic T cells directly [(82)].

ACTIVATION AND MEDIATOR RELEASE:

Activation of mast cells occurs when an antigen crosslinks IgE molecules that are bound to FcєRI on the surface of the mast cell. FcєRI receptor for IgE has an affinity 100 times greater for the Fc of IgE than of IgG. Because of this, IgE is found bound to the FcєRI receptors on the mast cell even when there are no antigens present. As a result, this makes the response of the mast cell to an antigen very fast. FcєRI signaling causes protein kinase Syk activation which is then phosphorylated

and the products cause calcium release which results in degranulation of the mast cells, lipid mediator production, and cytokine production.

Degranulation occurs a few seconds after crosslinking and results in release of the inflammatory mediators that are stored in the granules [(83)] which attract leukocytes (eosinophils, basophils, Th2 lymphocytes, neutrophils) to the inflammatory site and amplify the inflammatory response [(67)]. After degranulation, mast cells resynthesize the mediators and repopulate granules [(84)].

TLR4 can be activated by LPS from Gram-negative bacteria. This causes cytokine production without degranulation. When TLR2 is activated by peptidoglycan, this results in degranulation and cytokine production [(85)].

IgE-mediated activation by FcϵRI causes degranulation and synthesis of many immune mediators, such as eicosanoids and cytokines,etc. When the mast cell is activated, it immediately releases prepackaged granules.

Tryptase, chymase, cathepsin G, and carboxypeptidase are proteases stored in prepackaged granules that activate metalloproteases in the extracellular matrix. Activation of the metalloproteases breaks down extracellular matrix proteins and remodels the connective tissue matrix.

Chymase cleaves fibronectin and collagen by activation of MMPs.

β-tryptase has been shown to cleave IgE once the mast cell has been activated to down regulate the allergic response [(86)].

Histamine and heparin are involved with vascular permeability and smooth muscle contraction. Histamine works through three different receptors (H1, H2, H3).

Stimulation of H1 receptors, found on smooth muscle cells and endothelial cells, induces the classic allergic reaction. This results in increased vascular permeability and activation of smooth muscle cells resulting in contraction, constriction of airways, and mucous secretion [84].

TNFα, also stored in the mast cell granules activates macrophages, endothelium, and cytokines. TNF-α binds to endothelial cells and results in increased adhesion molecule expression. Leukocytes can bind to these adhesion molecules and then are brought to the site of inflammation [87,88].

Other molecules are synthesized and released after the mast cells have been activated. IL-3, IL-5, and GM-CSF are involved with eosinophil production and activation.

CCL3 is a chemotactic factor for macrophages and neutrophils. Eicosanoids (prostaglandins, leukotrienes, and thromboxanes) are produced by catalytic conversion of arachidonic acid by the action of phospholipase A2 on membrane phospholipids. Eicosanoids act at the local area of mast cell degranulation.

Mast cells express COX1 and COX2, which converts arachidonic acid into prostaglandins and thromboxanes[89]. Prostaglandins increase vascular permeability and attract neutrophils. Leukotrienes are involved with smooth muscle contraction, airway constriction, and mucous secretion [90].

Platelet-activating factor is released after mast cell activation that acts as a chemotactic factor for leukocytes, and activates neutrophils, eosinophils, and platelets[91]. All of the mediators released upon activation results in increased vascular permeability, smooth muscle contraction, and airway constriction.

REVIEW OF MAST CELLS IN THE HUMAN UTERUS

The presence of mast cells in the human uterus was first established in the endometrium by D'Erchia in the year 1897. [92]

The earliest study on uterine mast cells was done by Fox J E and Abel M in 1965 who concluded that mast cells in the myometrium were constant and did not change with the phases of the menstrual cycle. However the mast cells in the muscular layer of the cervix showed changes with different phases of the menstrual cycle. [93]

In their study in 1991 Drudy L,Sheppard B and Bonnar J observed that although there were no significant changes in the number of mast cells with different phases and that higher number of mast cells were seen during the menstruation phase than any other phase. [94]

Mori A ,Zhai Y L et al in 1997 used Immunohistochemical stains for tryptase and chymase to further assess the heterogeneity of mast cells in human uterus and concurred that the inner half of the myometrium had the greatest number of mast cells while the endometrium had the least number. [95]

In 2000 Sivridis E et al studied the density and distribution of mast cells in human uterus using biotin labeled lectins in conjunction with avidin biotin peroxide method to detect the metachromatic granules of mast cells better. Their findings were in agreement with all previous studies on the subject. They also postulated on the role of mast cells in menstruation and their relationship with Intra uterine devices. [96]

Uterine mast cells are increased during pregnancy and may be important in reproductive processes. Nerita S et al [97]reported IgE independent mast cell activation which augmented contractility of the human myometrium.

Zaitsu M et al [98]observed that mast cells expressed estrogen receptors and that estradiol induced their migration to the uterus and further degranulation.

Vasiadi M et al [99] and Guhl S et al [100] observed that mast cells also express progesterone and testosterone receptors activation of which had an inhibitory effect while the activation of the oxytocin receptors prevented serotonin uptake and increased serotonin availability.

REVIEW OF MAST CELLS IN UTERINE LEIOMYOMA

The first study on mast cells in different uterine neoplasms was done in 1965 by Fox and Abel who concluded that a considerable variation existed in the numbers of mast cells in the leiomyomatous tumors .The average counts were however below those of the corresponding adjacent normal tissue.It was also noted that the average counts in leiomyosarcomas were about twice as those in leiomyoma. [93]

Contradictory findings were however reported by later authors who studied the mast cells in uterine leiomyomas. In 1994, Maluf and Gersall reported a decreasing trend in the mean number of mast cells with advancing age in leiomyomas and adjacent myometrium. [101]

In the same year, D'souza et al also brought forth the suggestion that malignant stroma was not a favorable field for mast cells and that their presence indicated the benign nature of the lesion. [102]

In 2001,Yavuz et al studied the role of values of intratumoral mast cell counts and compared it with Ki-67 immunoreactivity index in the differential diagnosis of uterine smooth muscle tumors.They concluded that the intratumoral mast cell count in uterine leiomyosarcoma was significantly lower than that of atypical leiomyomas [(103)].

Zhu X G et al in 2003 conducted a study using various immunohistochemical markers like Desmin, Smooth muscle actin, Calponin, h Caldesmon,ER,PR,CD10 to differentiate Endometrial stromal sarcoma and Cellular Leiomyoma. It was found that mast cell counts were significantly higher in the cellular leiomyoma group than in the endometrial stromal sarcoma group. [(104)]

With regard to the relationship between extent of angiogenesis and number of mast cells in treatment of leiomyomas,no conclusive agreement has been reached with authors like Ribatti D et al (2007) reporting that tryptase and leptin in the secretory granules of mast cells may contribute to leiomyoma associated angiogenesis and antagonists to these can help in treatment [(105)]. However this is in contrast to Erol AYG et al who in 2011 found no correlation between the same [(106)].

In 2013,Jiang L et al reviewed the role of mast cells in gynecological neoplasms based on peer reviewed literature and research studies. They observed that some authors reported a higher concentration of mast cell counts in association with poor prognosis [(107)]. However various others found a significant and positive correlation between high mast cell concentration and good prognosis [(101-104)].

Gousuddin M et al in 2015 compared and studied the significance of mast cells in both benign and malignant lesions of uterus and cervix. They inferred that mast cells were significantly increased in inflammatory and benign lesions as

compared to malignant lesions. It was also noted that Intramural leiomyomas showed high mast cell counts [(108)].

In 2015 Abeyratne NVA et al studied the distribution of mast cells in uterine leiomyoma versus in myometrium and their relationship to the morphology of leiomyomas as well as clinical presentation. They found no linear connection between the size of leiomyomas and mast cell counts. It was also noted that the mast cell counts were lower in leiomyomas with degenerations and in the atrophic endometrium[(4)] .

Vedi et al in 2016 studied the mast cell profile in myometrial lesions in 577 patients. Their findings supported those of many previous authors quoted above. The study pointed out a significant inverse correlation between the mean number of mast cells with increasing age in leiomyoma as well as the adjacent myometrium. Based on the progressively decreasing mast cell counts from benign to malignant lesions of the myometrium, the authors also concurred that the mast cell counts appear to be inversely proportional to the degree of dysplasia and may act as a good prognostic indicator of the benign nature of the lesion [(109)].

METHODOLOGY

The present study was conducted in the Department of Pathology, Jawaharlal Nehru Medical College, Belgaum and Dr.Prabhakar Kore Charitable Hospital and medical research center, Belgaum.

Patient identities were concealed and approval from KAHER University's Institutional Ethical Research Committee was obtained prior to conducting this study (Ref no. MDC/DOME/120).

STUDY DESIGN: Observational Study

STUDY PERIOD: 3 years; 2 year retrospective from January 2019 to December 2020 and 1 year prospective study from January 2021 to December 2021.

STUDY POPULATION: Hysterectomy specimens of Uterine leiomyoma patients admitted to KLE'S Dr.Prabhakar Kore Charitable Hospital and retrospective tissue blocks retrieved from archives of the department, all together a total of 100 cases, formed the source of data.

INCLUSION CRITERIA: All hysterectomy specimens of Uterine leiomyoma

EXCLUSION CRITERIA: Hysterectomy specimens of patients operated for lesions other than Uterine leiomyoma were excluded.

SAMPLE SIZE:100

For retrospective samples, detailed clinical history was retrieved from the patient records and tissue blocks were archived from the Histopathology section of the

Department of Pathology. In the prospective cases the data was collected by interviewing the patient and operated specimens processed.

SAMPLING PROCEDURE

Following gross examination, the myometrium in each hysterectomy specimen was histopathologically examined for uterine leiomyomas. Two sections each were taken, from the leiomyoma and its adjacent myometrium. These sections were processed routinely using formalin fixation and paraffin embedding. The blocks thus obtained were then cut into 4µm sections and stained with Hematoxylin and Eosin stain and 1% Toluidine blue stain.

Cases were categorized according to age groups, presence of degeneration, site of leiomyoma and mast cell density.

MAST CELL COUNTING

The slides stained with Toluidine blue were scanned at 10x magnification and areas where maximum mast cells were seen, were then focused under 40x magnification. Mast cells were then counted manually, under 40X magnification for 10 consecutive fields in each slide in these areas. Mast cell density(MCD) was calculated as the mean number of mast cells per high power field in leiomyoma and in the adjacent myometrium. Number of MCs/10hpf in leiomyomas with degenerations was also recorded and compared.

STATISTICAL ANALYSIS

The data was entered in Microsoft Excel 2013 and statistical analysis was carried out using SPSS17 package. Percentages and mean values were calculated and compared using Pearson's Chi-square test, ANOVA and Karl Pearson correlation coefficient. "p" value of less than 0.05 was accepted as indicating statistical significance at 95% confidence interval.

RESULTS

The present study was conducted at the Department of Pathology, Jawaharlal Nehru Medical College, KAHER Belgaum. All the cases were evaluated for Hematoxylin and Eosin stain followed by 1% Toluidine blue stain and mast cells analyzed.

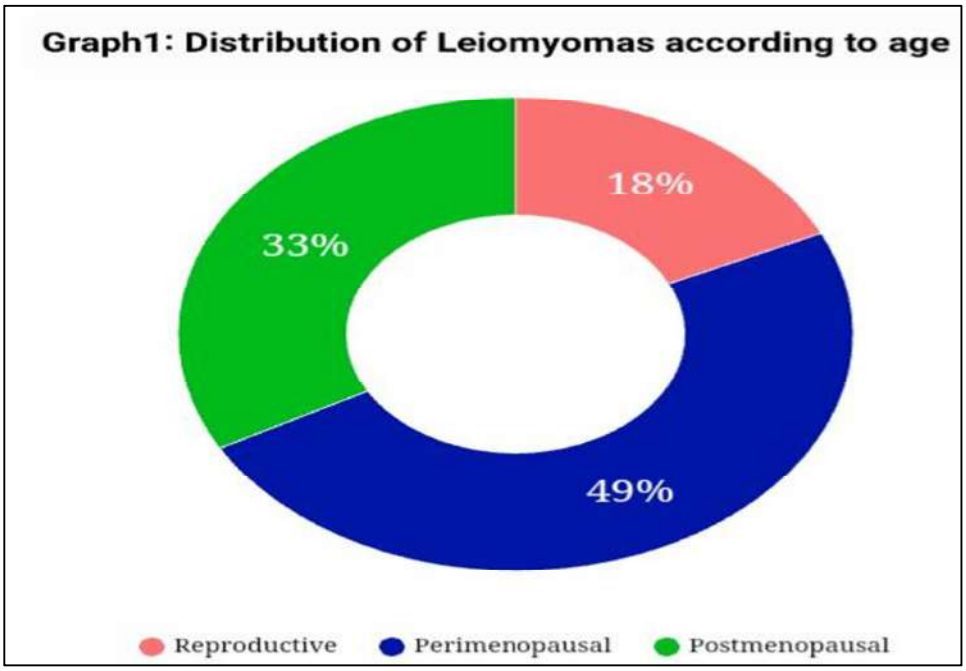
100 hysterectomy specimens of Uterine leiomyoma, were examined with relation to their distribution according to age groups, location & mast cell counts in comparison with the adjacent myometrium.

Mast cells were counted under 40X magnification for 10 consecutive fields in each slide in areas where maximum mast cells are seen- mast cell counts/10hpf; Mast cell density(MCD) was calculated as average of 10hpf.

Uterine leiomyomas showed a perimenopausal age preponderance with 49% of the cases in the ages between 40-50 years, the mean age being 45.94 ±16 years. The lowest number of Leiomyomas were found in the reproductive age group (**Table 3, Graph 1**).

Table 3: Distribution of Leiomyomas according to age

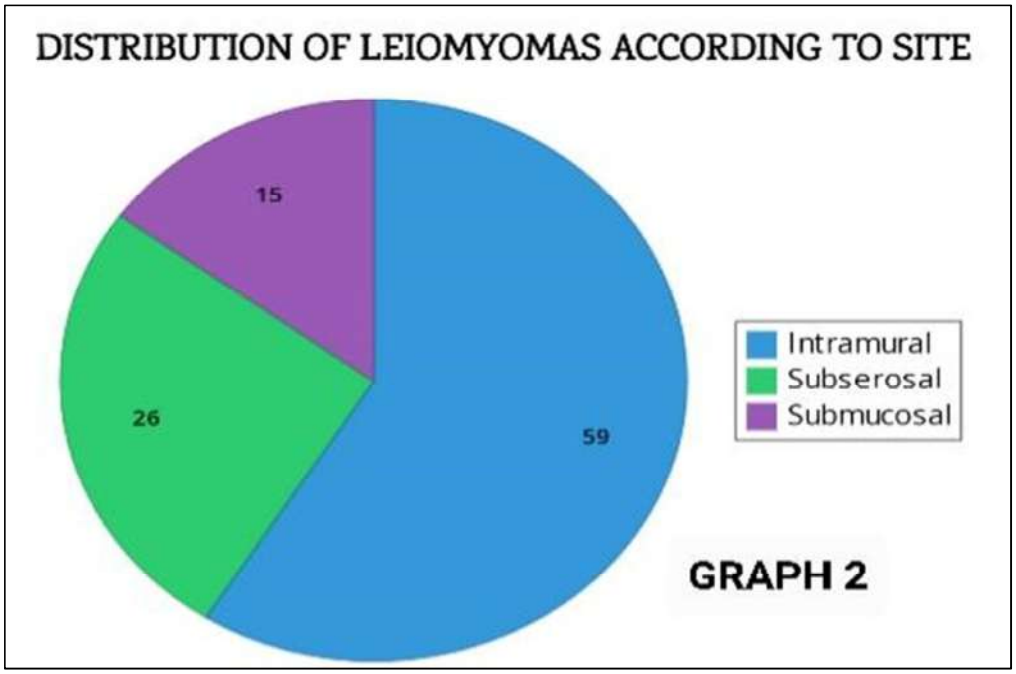
AGE GROUPS	REPRODUCTIVE (<40YRS)	PERIMENOPAUSAL (40-50YRS)	POSTMENOPAUSAL (>50YRS)	TOTAL
NO.OF CASES	18	49	33	100
PERCENTAGE OF CASES	18	49	33	100



The maximum number of leiomyomas in this study,ie 59% were found to be intramural in location. Only 15 were submucosal in origin which was the lowest amongst the three groups.**(Table 4,Graph 2)**

Table 4:Distribution of Leiomyomas according to site

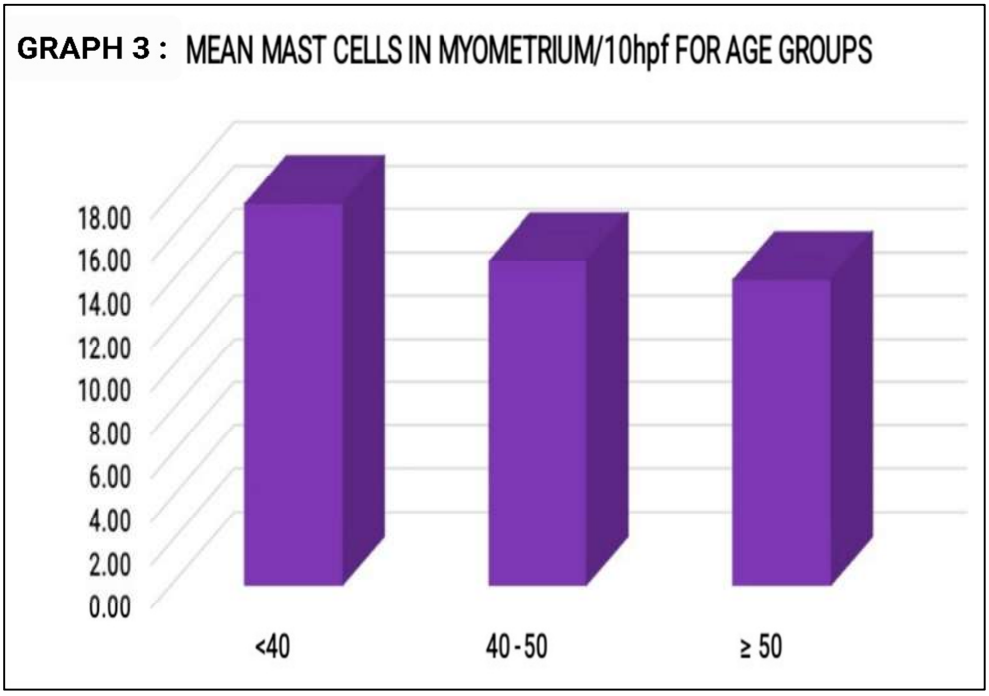
SITE	INTRAMURAL	SUBSEROSAL	SUBMUCOSAL	TOTAL
NO.OF CASES	59	26	15	100
PERCENTAGE OF CASES	59	26	15	100



The myometrial mean mast cell counts/10hpf in the reproductive age group(17.59+/-9.02) was far surpassing those in the perimenopausal(14.94+/-6.39) and postmenopausal age groups(14.06+/-5.66).This was found to be statistically significant with a p value of 0.0458 (**Table 5, Graph 3**)

Table 5 : Distribution of myometrial mast cells/10hpf

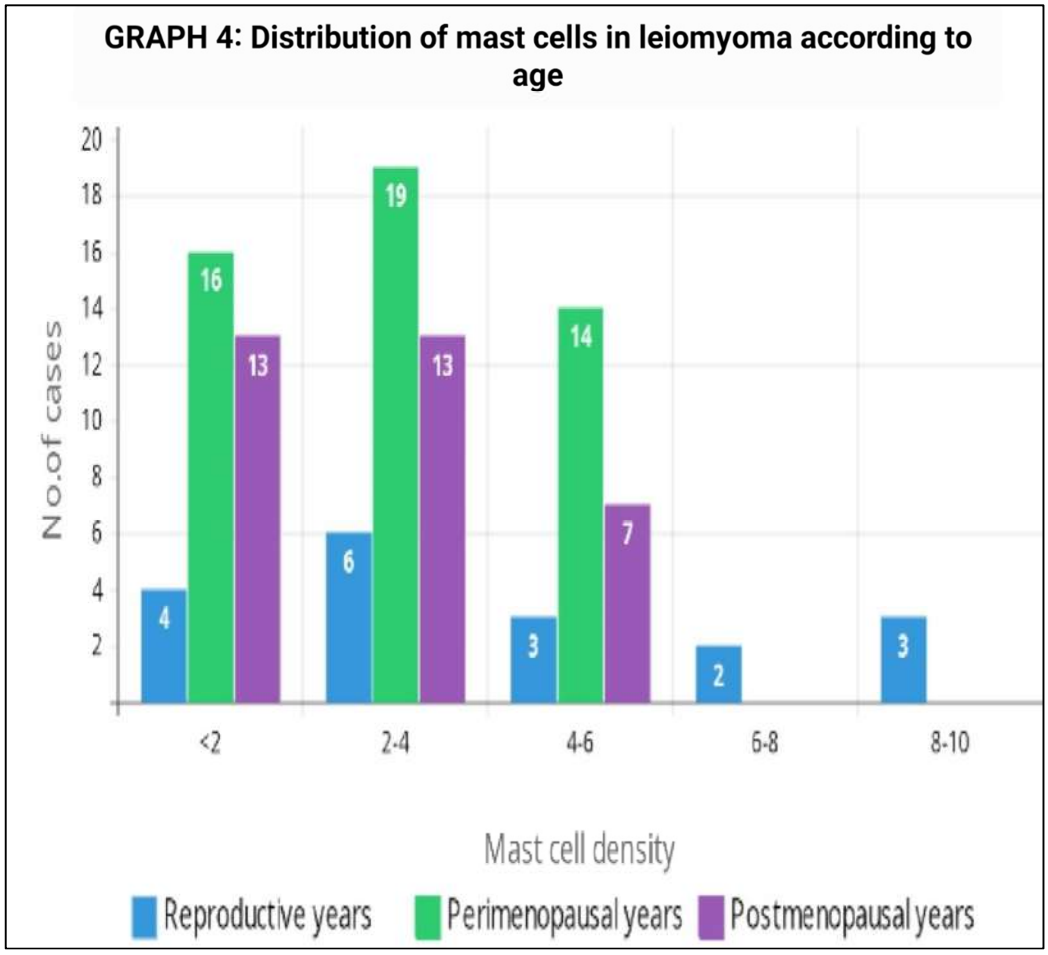
AGE	MEAN	S.D	MIN MC/10hpf	MAX MC/10hpf	p value
<40	17.59	9.02	8	36	0.0458
40-50	14.94	6.39	5	36	
>50	14.06	5.66	6	28	



An inverse correlation was found between the mast cell density and increasing age. The greatest mast cell density (of 8-10 cells) were found in the reproductive age group. However, maximum number of lesions had a mast cell density in the range of 2-4 cells in all three age groups. Using the Chi square test, a significant association between different age groups and classes of average mast cell density of leiomyomas was noted, having a p value of 0.0205.**(Table 6, Graph 4)**

Table 6: Distribution of mast cells in leiomyoma according to age

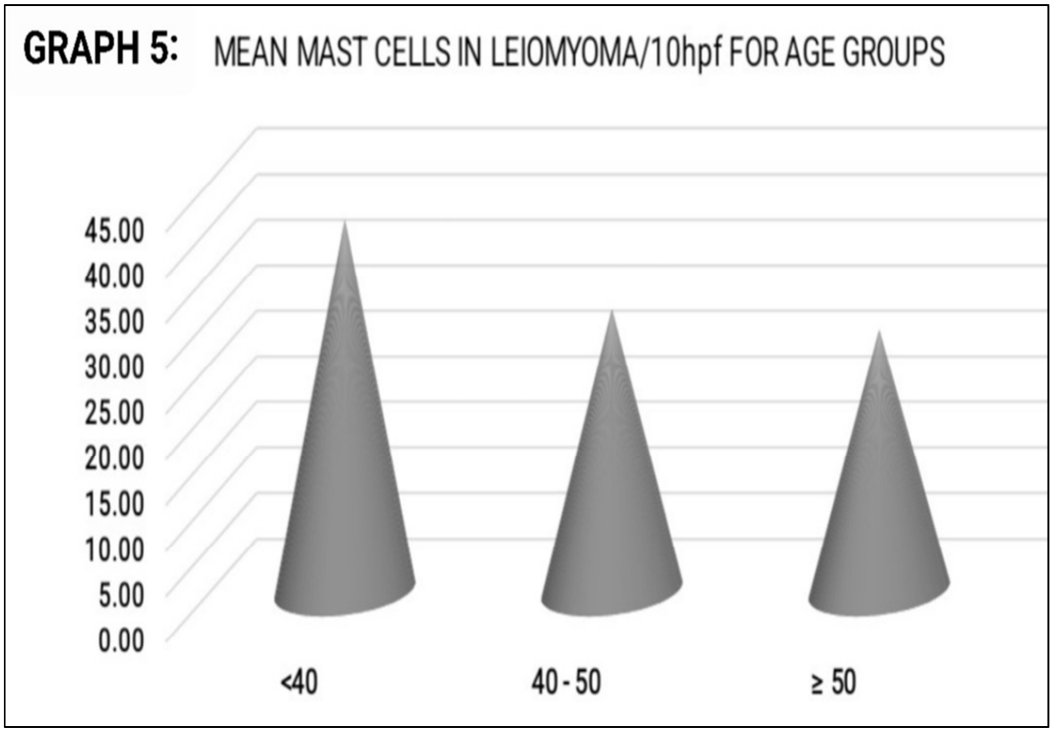
MAST CELL DENSITY	<2	2-4	4-6	6-8	8-10
REPRODUCTIVE (<40YRS)	4	6	3	2	3
PERCENTAGE OF CASES	4	6	3	2	3
PERIMENOPAUSAL (40-50YRS)	16	19	14	--	--
PERCENTAGE OF CASES	16	19	14	--	--
POSTMENOPAUSAL (>50YRS)	13	13	7	--	--
PERCENTAGE OF CASES	13	13	7	--	--



The average mast cell counts/10hpf in leiomyomas according to age groups also showed a highly significant correlation (p value 0.0006 using ANOVA).The mean mast cell count/10hpf in leiomyomas in the reproductive age group was 40.76±23.14 which is larger than the mean counts in the perimenopausal age group (30.88±12.26) and postmenopausal age group (28.62±9.68). (**Table 7, Graph 5**)

Table 7 : Average mast cells in leiomyoma/10hpf

AGE	MEAN	S.D	MIN MC/10hpf	MAX MC/10hpf	p value
<40	40.76	23.14	20	88	0.0006
40-50	30.88	12.26	18	86	
>50	28.62	9.68	17	48	



The distribution of mast cells in leiomyomas according to site of origin were studied. The maximum number of mast cells were found to be in leiomyomas originating intramurally(80-100/10hpf) while subserosal leiomyomas had the lowest (20-40/10hpf) .This relationship was studied using ANOVA tests and was found to be statistically significant(p value 0.0016) **(Table 8)**

Table 8: Distribution of mast cells in leiomyomas according to site of origin

SITE	MEAN	MIN MCD	MAX MCD	P VALUE
INTRAMURAL	45.76	24	88	**0.0016**
SUBSEROSAL	34.88	22	60	
SUBMUCOSAL	28.62	20	40	

The distribution of mast cells in uterine leiomyoma in comparison to the adjacent myometrium showed a clear twofold increase with an average mast cell count in leiomyoma (of 31.79/10hpf) which was double the average mast cell count in the adjacent myometrium (15.09/10hpf).

The distribution of mast cell counts in Leiomyoma range from less than 20/10hpf to 80-100/10hpf. Thirty eight percentage of Leiomyomas had a mast cell count of 20-40/10hpf whereas majority (65%) of the mast cell counts in the adjacent myometrium were less than 20/10hpf. **(Table 9)**

Table 9: Distribution of mast cells in leiomyoma and adjacent myometrium

NO.OF MAST CELLS/10HPF	NO.IN LEIOMYOMA	% OF CASES	NO.IN ADJACENT MYOMETRIUM	% OF CASES
<20	33	33	65	65
20-40	38	38	35	35
40-60	24	24	--	--
60-80	2	2	--	--
80-100	3	3	--	--
TOTAL	100	100	100	100

The Karl Pearson correlation coefficient(r=0.8008) was used to compare the mast cell density in myometrium and leiomyomas.

This correlation coefficient is a measure of the strength of a linear association between two variables and is denoted by **r.** This is unit less and hence may be used to compare different bivariate distributions as well. Its value is usually between +1 and -1.

A value greater than 0 indicates a positive association ie as the value of one variable increases, so does the value of the other variable. A value less than 0 indicates a negative association.

The mast cell counts in leiomyoma was higher than in the adjacent myometrium which was statistically highly significant (p<0.0001) (**Table 10**)

Table 10: Karl Pearson correlation coefficient

	r	P VALUE	INFERENCE
MCD MYOMETRIUM & LEIOMYOMA	0.8008	<0.0001	HIGHLY SIGNIFICANT

Although leiomyomas with degeneration were only 10 among the total 100 leiomyomas, 80% of these had a mast cell density of less than 2 cells while a majority(67.7%) of the leiomyomas without degeneration showed a mast cell density of 2-4 cells.**(Table 11)**

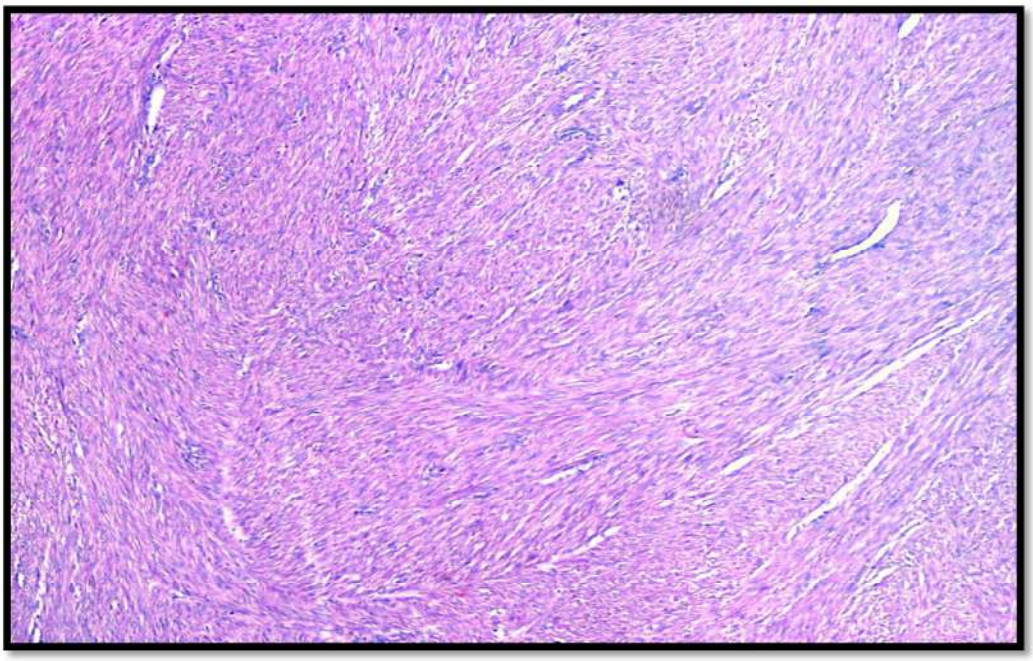
Table 11 : Distribution of mast cells in leiomyoma with degenerations and without degeneration

MAST CELL DENSITY	NO.OF CASES OF LEIOMYOMA WITH DEGENERATION	%	NO.OF CASES OF LEIOMYOMAS WITHOUT DEGENERATION	%
<2	8	80	-	-
02-Apr	2	20	61	67.78
>4	-	-	29	32.22
TOTAL	10	100	90	100

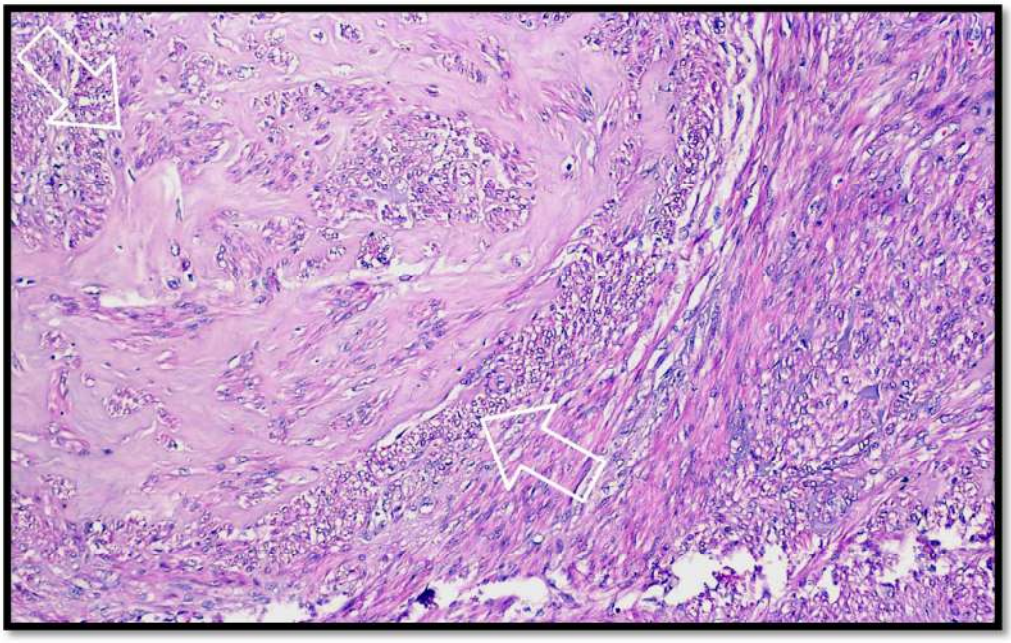
The mean mast cell density of the leiomyomas with degeneration was 1.85+/-0.10 while that of leiomyomas without degeneration was 3.33+/-1.46.This was found to be statistically very significant with a p value of 0.0019. (**Table 12**)

Table 12 : Mean mast cell density of the leiomyomas with degeneration vs without degeneration

MEAN	SD	MIN	MAX	MEAN	SD	MIN	MAX	p Value	Inference
1.85	0.1	1.7	2	3.33	1.46	2	8.8	0.0019	Significant

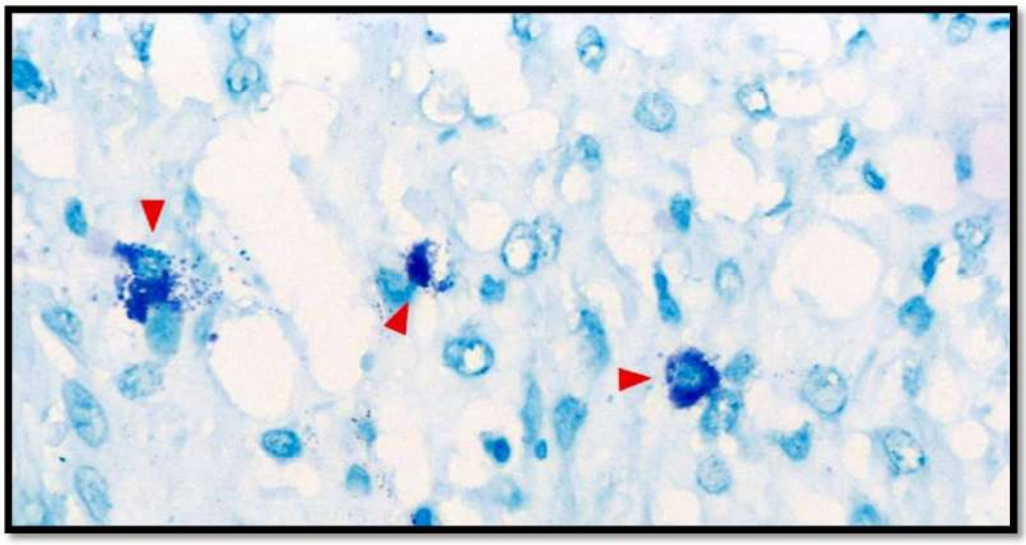


Photomicrograph 10 : Leiomyoma without degeneration (H&E,10x)

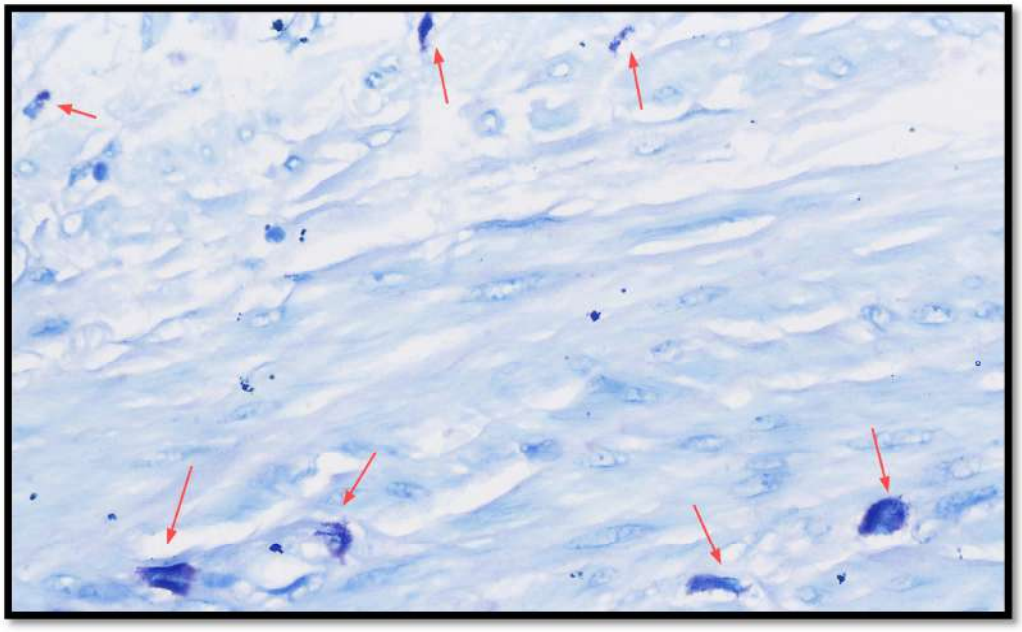


Photomicrograph 11: Leiomyoma with degeneration-White arrows indicate hyaline degeneration (H&E, 20x)

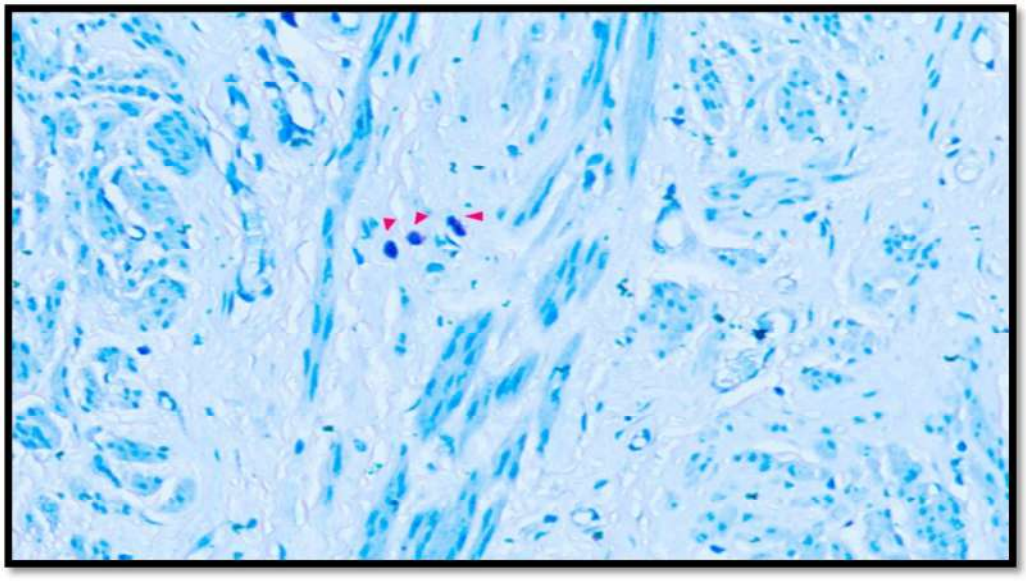
Photomicrograph 12: Myometrium – Black Arrows indicate mast cells (1% Toluidine blue, 40x)



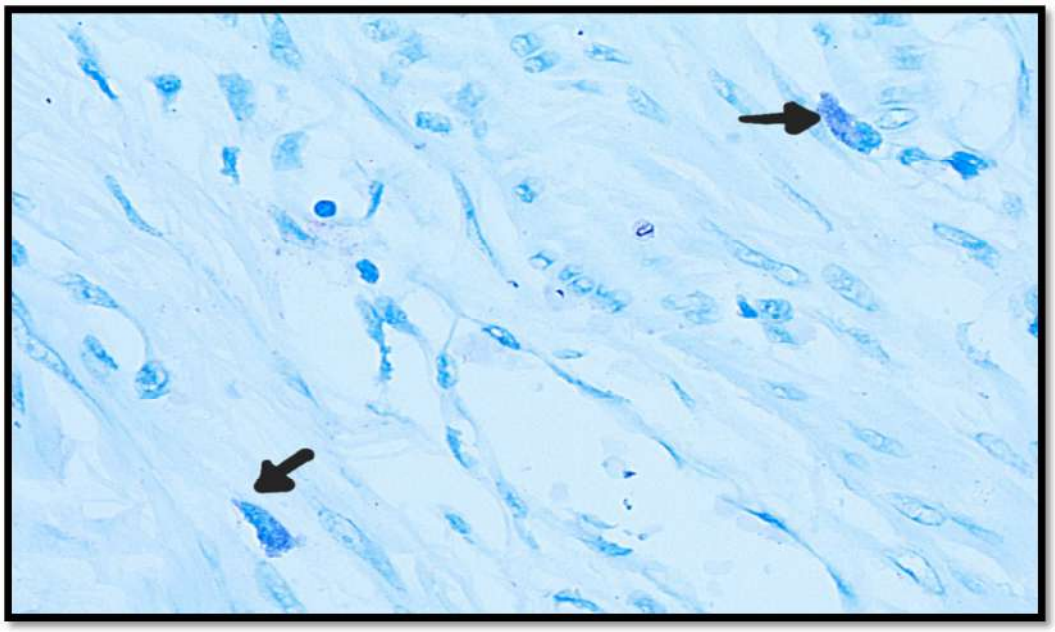
Photomicrograph 13: Leiomyoma without degeneration- Red arrow heads indicate mast cells- Mast cell count=3/hpf (1% Toluidine blue, 40x)



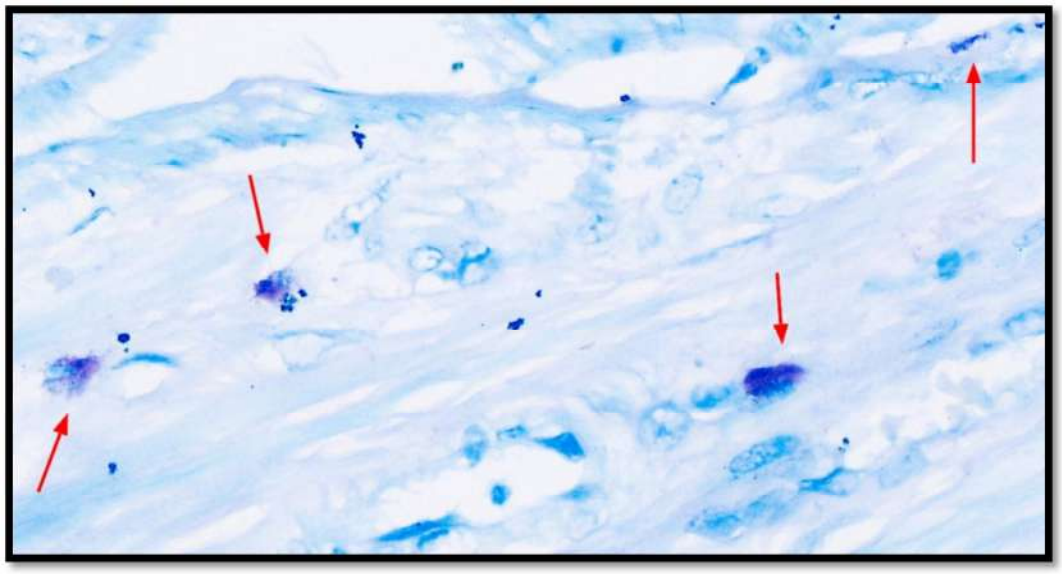
Photomicrograph 14: Leiomyoma without degeneration- Black arrows indicate mast cells- Mast cell count=7/hpf (1% Toluidine blue, 40x)



Photomicrograph 15: Leiomyoma with degeneration- Red arrow heads indicate mast cells(1% Toluidine blue, 20x)



Photomicrograph 16: Leiomyoma with degeneration- Black arrows indicate mast cells-Mast cell count=2/hpf (1% Toluidine blue, 40x)



Photomicrograph 17: Leiomyoma without degeneration- Red arrows indicate mast cells with reddish purple granules-Mast cell count=4/hpf (1% Toluidine blue, 40x)

DISCUSSION

The literature regarding the distribution and potential relationship between mast cell and various conditions of the myometrium is scarce with several authors differing with respect to the conclusion. The human uterus is considered to be relatively rich in mast cells when compared to the other tissues of the body. The present study has been done in an effort to throw more light on these and understand the distribution of mast cells in uterine leiomyoma.

Mast cells were stained using Toluidine blue, a metachromatic stain as this gave a good contrast and stained the granules bright purple-pink.

Our study noted that Uterine leiomyomas showed a perimenopausal age preponderance with 49% of the cases in the ages between 40-50 years, the mean age being 45.94 years. These findings were concurrent with those of Ulin M et al [(110)] and Santoro N et al [(111)] who reported that uterine fibroids peak in the perimenopausal years and decline following menopause.

Similar observations were put forward by Wise A.L and Laughlin-Tommaso [(112)] who reported that uterine leiomyomas tend to peak during premenopausal years and decline in the postmenopausal years. Ghosh S et al[(113)] also reported that uterine fibroids represent the most common mesenchymal tumor in premenopausal women.

Studies done by Casini et al[(114)] ,Pritts et al[(115)] and Gousuddin et al[(108)] reported that Intramural leiomyomas are the most common site of origin of uterine leiomyomas which was consistent with the findings of the present study in which 59% of the cases were Intramural in origin.

There exists no consensus regarding the minimum number of mast cells reported in either the uterine myometrium or leiomyomas with different authors proposing varied ranges.

In the current study, the mean mast cell counts/10hpf in the myometrium in the reproductive age group(17.59/10hpf) was far greater than that in perimenopausal age group(14.94/10hpf) and lowest in postmenopausal(14.06/10hpf) age group. A similar finding was emphasized by Mori et al[(95)]and Drudy L et al[(94)] who found that the lowest number of mast cells were seen after menopause. This was, however, in contrast with a study by Fox et al[(93)] who reported that the number of mast cells in the myometrium was fairly constant across different age groups.

An inverse correlation was found between the mast cell density and increasing age in the present study. The greatest mast cell density (of 8-10 cells) were found in the reproductive age group. These findings were similar to those of Maluf and Gersell[(101)]who observed a decreasing trend in the mean number of mast cell with advancing age in leiomyoma as well as in the normal myometrium, which were synchronous with the findings of the present study.

Similar findings were observed by Orii et al[(116)]who also reported decreasing number of mast cells in leiomyomas with increasing age concomitant with the general atrophy of the uterus that occurs with ageing and particularly following menopause. These observations lend evidence to the proposition that there exists an inverse correlation between the mast cell count and advancing age which may be attributed to the role of estrogen and progesterone at the physiologic levels.

However, this is in contrast to the observations of Fox et al[93] and Maluf et al[101] who are of the view that there is no difference in the mast cell population in the leiomyomas, between the patients in reproductive and postmenopausal age groups.

The distribution of mast cells in leiomyomas according to site of origin was also evaluated in the present study and the maximum number were found in the Intramural leiomyomas. Gousuddin et al[108] observed that mast cells were significantly higher in benign lesions and that Intramural leiomyomas showed the highest mast cell count, a finding which supported the present study.

Our study revealed an exponential increase, ranging from 2.1:1 to 8:1 in the distribution of mast cells in uterine leiomyoma, when compared to the adjacent myometrium which was statistically significant (p value<0.001) .The myometrium, in a majority of cases(65%) had less than 20 mast cells/10hpf.

These observations were concordant with those of multiple authors including Maluf and Gersell [101]who reported that the mast cell counts were consistently higher in the leiomyomas than in the myometrium, in ratios varying from 3:1 to 12:1.

However, contrasting findings were reported by Fox & Abel[93] in their study on mast cells in uterine myometrium and leiomyomatous neoplasms, which concluded that the average values for leiomyomatous tumours were significantly below those of normal myometrium.

Despite the existence of a variety of degenerations in uterine leiomyoma, only hyaline degeneration was observed in our study. Of the 100 cases of leiomyomas only 10 had degenerations.

Eighty percent of these showed a much lower mast cell count (<20 cells/10hpf) as compared to a majority (67.78%) of leiomyomas without degeneration which revealed average mast cell counts in the range of 20-40 cells/10 hpf. The mean mast cell density of leiomyomas with degeneration and that of leiomyomas without degeneration was compared and was found to be statistically significant.

These findings were in conformity with those of Abeyratne NVA et al who reported that mast cell counts were lower in leiomyomas with hyaline degeneration[4]

CONCLUSION

The distribution of mast cells in uterine leiomyoma were analyzed in 100 hysterectomy specimens in comparison with the adjacent myometrium. The distribution of mast cells in leiomyoma with degenerations versus those without degeneration were also assessed.

An inverse correlation was found between the mast cell density and increasing age with the vast majority of mast cells found in perimenopausal age group.

The mast cell counts in uterine leiomyoma originating intramurally far outweighed those found in submucosal or subserosal regions.

The distribution of mast cells in uterine leiomyoma in comparison to the adjacent myometrium revealed a clear twofold increase.

The distribution of mast cell counts in Leiomyoma range from less than 20/10hpf to 80-100/10hpf.

Leiomyomas with degeneration were found to have a much lower mast cell density in comparison to leiomyomas without degeneration.

Based on the observations of this study, progressively decreasing mast cell counts were observed as we move from the uterine leiomyoma to the adjacent normal myometrium. This study also found a decline in the number of mast cells when a leiomyoma undergoes degeneration.

LIMITATIONS

1. COVID 19 posed several constraints to the collection of samples due to lesser number of hysterectomies being performed and hence only 100 cases could be studied.

2. Our study did not include any leiomyosarcomas and hence an opinion on mast cells in leiomyoma vs leiomyosarcoma cannot be given.

SUMMARY

1. The current study was an observational study which included 2 years retrospective and 1 year prospective data. The hysterectomy specimens of uterine leiomyoma patients admitted to KLE'S Dr.Prabhakar Kore Charitable Hospital were the source of data. For retrospective cases , data as well as tissue blocks, were archived from the histopathology section of the department.

2. Cases were categorized according to age groups, presence of degeneration, site of leiomyoma and mast cell density.

3. Two sections each were taken, from the leiomyoma and its adjacent myometrium. These sections were processed routinely and the blocks thus obtained were then cut into 4µm sections and stained with Hematoxylin and Eosin stain and 1% Toluidine blue stain.
4. Mast cells were then counted manually, under 40X magnification for 10 consecutive fields in each slide in these areas. Mast cell density(MCD) was calculated as the mean number of mast cells per high power field in leiomyoma and in the adjacent myometrium. Number of Mast cells/10hpf in leiomyomas with degenerations was also recorded and compared.
5. Of the 100 cases, 18 cases were in the reproductive age group,56 cases in the perimenopausal age group and 26 cases in the postmenopausal age group. An inverse correlation was found between the mast cell density and increasing age.

6. A vast majority, that is, 59 cases were Intramural in location while 26 were Subserosal and 15 were submucosal.
7. The distribution of mast cell counts in Leiomyoma range from less than 20/10hpf to 80-100/10hpf.
8. The distribution of mast cells in uterine leiomyoma in comparison to the adjacent myometrium showed a clear twofold increase with an average mast cell count in leiomyoma of 31.79/10hpf which was double the average mast cell count in the adjacent myometrium 15.09/10hpf.
9. Although leiomyomas with degeneration were only 10 among the total 100 leiomyomas, 80% of these had a mast cell density of less than 2 cells while a majority(67.78 %) of the leiomyomas without degeneration showed a mast cell density of 2-4 cells.

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ANNEXURE I – CONSENT FORM

INFORMED CONSENT

STUDY ON THE DISTRIBUTION OF MAST CELLS IN UTERINE LEIOMYOMA -A HOSPITAL BASED OBSERVATIONAL STUDY

Purpose of the study: You are being asked to enroll in this study as you are eligible for participation in this study. If you undergo Hysterectomy for a uterine lesion you will be included in this study.

The purpose of this study is to evaluate the distribution and concentration of mast cells in various leiomyomata and find a correlation with the clinical profile. This study will help in understanding the prognosis based on the nature of the lesion.

Procedure: During this study, you will be asked questions regarding age and menstrual history and you are supposed to answer to the best of your knowledge. The principal investigator of the study is Dr. Sneha Soney under the guidance of Dr. Hema Shantagiri (guide).

If you agree to enroll yourself in this study, you will be interviewed regarding your present, past and family history and your clinical manifestations.

Risks and benefits: There are no risks involved in taking part in this study and benefit is we will be able to judge the nature and prognosis of the disease which is essential for providing appropriate treatment.

Alternatives: Taking part in this study is voluntary. You may choose not to take part in this study or if you decide to take part now, you can later change your mind and withdraw from the study. The study doctor or sponsor may terminate your participation in this study anytime.

Privacy and confidentiality: All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study will be published but your identity will be confidential in any publication. No information about you or information provided by you during research will be disclosed to other without your written permission except:

1. In emergency to protect your rights and welfare.
2. If required by law.

Financial incentives for participation: You will not be paid / offered any gift /incentives for participating in this study.

Authorization to publish results: The results of this study would be forwarded to the KLE University, Belagavi as a part of requirement towards the completion of MD degree, review and publishing.

Questions: In case you have any questions related to the study in future you can contact:

1. If you have any queries about your rights as a study subject, you may call Dr. Harsha Hegde, Chairman of J.N. Medical College Institutional Ethical Committee of Human Subjects Research, Ph No- 9448113403, at J.N. Medical College, Belagavi

CONSENT STATEMENT

` I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any legal rights by signing this form. My signature below indicates that I have read, or it has been read to me, this entire consent form and have had all my questions answered.

In case of the queries during the study or in future you may contact following person.

Principal Investigator: --------

Guide : ----------

Name of the participant:

(signature/thumbprint)

Name of the witness : (signature)

Name of the investigator: (signature)

Date:

Address:

Phone no

ANNEXURE II

PROFORMA

PATIENT HISTORY

Name : Age : IP no. :

Brief clinical history:

Menstrual history:

EXAMINATION FINDINGS

CLINICAL DIAGNOSIS :

RADIOLOGICAL INVESTIGATIONS:

BLOOD INVESTIGATIONS:

HISTOPATHOLOGICAL DIAGNOSIS :

1. Hematoxyline and Eosin staining :
2. Toluidine blue staining :
3. Mast cell counts and density:

ANNEXURE III - STAINING PROCEDURES

STANDARD HAEMATOXYLIN AND EOSIN STAIN FOR PARAFFIN SECTIONS PROCEDURE:

1. Dewax sections in xylol, hydrate through graded alcohols to water.
2. Stain in Harris Haematoxylin for 5-10 minutes(Regressive stain)
3. Wash well in running tap water until the sections “blue”(5 minutes or Less)
4. Differentiate in 1% acid alcohol (1% Hcl in 70% alcohol) for 5-10 seconds.
5. Wash well in running tap water until sections are again “blue”

   (5 minutes or less)
6. Stain in 1% Eosin for 10 minutes.
7. Wash in running tap water for 1-5 minutes.
8. Dehydrate through alcohols, clear in xylol mount in DPX.

Results:

Nuclei-blue black

Cytoplasm-varying shades of pink

Muscle fibres-Deep Pink – Red

Red blood cells – Orange / Red

Fibrin – Deep Pink

Source: Bancroft J. D., Stevens A.Theory and Practice of Histological Technique, 4th edition. Edinburg:Churchill Living Stone;1996. P.104

TOLUIDINE BLUE STAINING PROTOCOL FOR MAST CELLS

Solutions:

- Toluidine Blue Stock Solution:

Toluidine blue O (Sigma) ---------- 1 g

70% alcohol ---------- 100 ml

Mix to dissolve.

- Sodium Chloride (1%):

Sodium chloride ---------- 0.5 g

Distilled water ---------- 50 ml

Mix to dissolve (make this solution fresh each time). Adjust pH to 2.0~2.5 using glacial acetic acid or HCl.

- Toluidine Blue Working Solution (pH 2.0~2.5):

Toluidine blue stock solution ---------- 5 ml

1% Sodium chloride, pH 2.3 ---------- 45 ml

Mix well. The pH should be around 2.3 and less than 2.5

Make this solution fresh and discard after use. pH higher than 2.5 will make staining less contrast.

Procedure:

1. Deparaffinize and hydrate to distilled water
2. Stain sections in toluidine blue working solution for 2-3 minutes
3. Wash in distilled water – 2 mins
4. Dehydrate quickly in 95% ethanol and Clear in Xylene – 2 mins
5. Mount slides with coverslips using DPX

Results: Mast cells ---------- violet/red purple.

Background ---------- blue

Sources Sheehan D, Hrapchak B.Theory and Practice of Histotechnology,2nd Edition. Ohio:Battelle Press;1980.p282-4

ANNEXURE IV – MASTERCHART

SLIDE NO	BIOPSY NO.	AGE	DIAGNOSIS	DEGENERATION	SITE	MCD Myometrium	Avg MC MYOMETRIUM/hpf	MAST CELLS IN MYOMETRIUM/10hpf	Avg MC LEIOMYOMAS/hpf	MAST CELLS IN LEIOMYOMA/10hpf	MCD LEIOMYOMA
1	212/19	40	Leiomyoma		Intramural	2	2	20	3	30	3
2	1488/19	42	Leiomyoma		Subserosal	3	3	30	5	50	5
3	411/19	35	Leiomyoma		Intramural	1	1	10	3	30	3
4	412/19	44	Leiomyoma		Intramural	1	1	10	2	20	2
5	473/19	41	Leiomyoma		Intramural	1	1	10	2	20	2
6	492/19	36	Leiomyoma		Intramural	2	2	20	4	40	4
7	427/19	51	Leiomyoma		Subserosal	2	2	20	3	30	3
8	1080/19	48	Leiomyoma		Subserosal	1	1	10	2	20	2
9	374/19	50	Leiomyoma		Intramural	1	1	10	2	20	2
10	1089/19	32	Leiomyoma		Subserosal	1	1	10	2	20	2
11	484/19	48	Leiomyoma		Subserosal	2	2	20	4	40	4
12	422/19	55	Leiomyoma		Subserosal	1	1	10	2	20	2
13	801/19	48	Leiomyoma		Intramural	2	2	20	4	40	4
14	801/20	45	Leiomyoma		Subserosal	1	1	10	2	20	2
15	427/20	38	Leiomyoma		Intramural	2	2	20	6	60	6
16	551/19	52	Leiomyoma		Intramural	1	1	10	2	20	2
17	1088/19	53	Leiomyoma		Subserosal	2	2	20	3	30	3
18	984/19	47	Leiomyoma		Subserosal	1	1	10	3	30	3
19	1088/20	34	Leiomyoma		Intramural	1	1	10	2	20	2
20	806/19	45	Leiomyoma		Intramural	2	2	20	4	40	4
21	802/20	46	Leiomyoma		Subserosal	1	1	10	2	20	2
22	1080/19	51	Leiomyoma		Subserosal	1	1	10	3	30	3
23	1081/20	37	Leiomyoma		Subserosal	1	1	10	2	20	2
24	1085/19	38	Leiomyoma		Intramural	1	1	10	2	20	2
25	351/19	43	Leiomyoma		Intramural	2	2	20	4	40	4
26	1083/19	44	Leiomyoma		Subserosal	2	2	20	3	30	3
27	1084/20	47	Leiomyoma		Intramural	2	2	20	4	40	4
28	302/20	36	Leiomyoma		Intramural	2	2	20	4	40	4
29	862/19	46	Leiomyoma		Subserosal	1	1	10	2	20	2
30	1078/19	43	Leiomyoma		Subserosal	1	1	10	2	20	2
31	1074/20	44	Leiomyoma		Subserosal	1	1	10	2	20	2

32	523/20	42	Leiomyoma		Intramural	2	2	20	3	30	3
33	525/19	49	Leiomyoma		Intramural	2	2	20	4	40	4
34	212/20	51	Leiomyoma		Subserosal	1	1	10	2	20	2
35	474/20	45	Leiomyoma		Intramural	2	2	20	4	40	4
36	200/20	33	Leiomyoma		Intramural	2	2	20	3	30	3
37	210/20	50	Leiomyoma		Subserosal	0.8	0-1	8	2-3/	26	2.6/
38	907/19	52	Leiomyoma		Intramural	2	2	20	4-5/	47	4.7/
39	302/21	36	Leiomyoma		Subserosal	1.5	1-2/	15	2-3/	28	2.8/
40	492/21	46	Leiomyoma		Intramural	0.8	0-1/	8	2-3/	25	2.5/
41	907/21	44	Leiomyoma		Intramural	2	2	20	4-5/	48	4.8/
42	1077/19	40	Leiomyoma		Intramural	0.9	0-1/	9	2	20	2
43	352/20	45	Leiomyoma		Subserosal	1.8/	1-2/	18	3-4/	39	3.9/
44	551/19	47	Leiomyoma		Intramural	1	1	10	2	20	2
45	561/20	50	Leiomyoma	hyaline degeneration	Subserosal	0.7	0-1/	7	1-2/	18	1.8/
46	428/21	42	Leiomyoma		Intramural	2	2	20	4	40	4
47	800/21	50	Leiomyoma		Intramural	2.6	2-3/	26	4-5/	48	4.8/
48	569/20	44	Leiomyoma		submucosal	1.5	1-2/	15	2-3/	26	2.6/
49	1070/21	52	Leiomyoma		Intramural	2.8	2-3/	28	4-5/	48	4.8/
50	1492/20	44	Leiomyoma		Intramural	1.6	1-2/	16	2-3/	26	2.6/
51	870/21	35	Leiomyoma		submucosal	0.8	0-1/	8	2-3/	28	2.8/
52	550/21	47	Leiomyoma		Intramural	1.6/	1-2/	16	2-3/	24	2.4/
53	1000/21	46	Leiomyoma	hyaline degeneration	Subserosal	0.5	0-1/	5	1-2/	18	1.8/
54	190/21	55	Leiomyoma		Intramural	1.8	1-2/	18	2-4/	34	3.4/
55	777/21	38	Leiomyoma		Intramural	1	1	10	3	30	3
56	1022/20	50	Leiomyoma		submucosal	2	2	20	4	40	4
57	300/20	44	Leiomyoma		Intramural	2	2	20	4	40	4
58	431/21	39	Leiomyoma		Intramural	3.6/	3-4/	36	8	78	8
59	722/21	48	Leiomyoma		submucosal	0.8/	0-1/	8	2-3/	28	2.8/
60	946/21	43	Leiomyoma		Intramural	1.6/	1-2/	16	4-5/	45	4.5/
61	907/21	33	Leiomyoma		Intramural	3.2/	3-4/	32	8-9/	88	8.8/
62	411/21	56	Leiomyoma		submucosal	1.4/	1-2/	14	2-3/	26	2.6/
63	447/21	52	Leiomyoma		Subserosal	1.6/	1-2/	16	2-3/	26	2.6/
64	1002/21	45	Leiomyoma	hyaline degeneration	submucosal	0.8/	0-1/	8	1-2/	18	1.8/
65	767/21	46	Leiomyoma		Intramural	1.7/	1-2/	17	3	30	3
66	484/20	51	Leiomyoma		submucosal	1.9/	1-2/	19	3-4/	38	3.8/
67	1044/20	54	Leiomyoma		Intramural	1.5/	1-2/	15	2-3/	24	2.4/
68	264/21	38	Leiomyoma		Intramural	2	2	20	4-5/	45	4.5/
69	455/20	50	Leiomyoma		Intramural	2	2	20	4	40	4
70	464/20	49	Leiomyoma		Intramural	2.6/	2-3/	26	3-4/	36	3.6/
71	351/21	54	Leiomyoma		submucosal	1.5/	1-2/	15	2	20	2
72	1089/20	47	Leiomyoma		Intramural	2	2	20	4	40	4
73	1081/21	51	Leiomyoma		Intramural	2	2	20	4	40	4
74	302/21	49	Leiomyoma		Subserosal	1.6/	1-2/	16	4	40	4
75	862/21	37	Leiomyoma		Subserosal	1.4/	1-2/	14	3	30	3

76	1076/20	53	Leiomyoma		submucosal	0.8/	0-1/	8	2	20	2
77	1084/21	55	Leiomyoma		Intramural	1.4/	1-2/	14	3-4/	36	3.6/
78	523/20	46	Leiomyoma		Intramural	1.2/	1-2/	12	3-4/	34	3.4/
79	453/21	52	Leiomyoma	hyaline degeneration	submucosal	0.9/	0-1/	9	1-2/	18	1.8/
80	210/21	55	Leiomyoma		submucosal	1	1	10	2	20	2
81	473/21	50	Leiomyoma		Intramural	1	1	10	2	20	2
82	212/19	48	Leiomyoma	hyaline degeneration	submucosal	0.8/	0-1/	8	1-2/	19	1.9/
83	200/21	43	Leiomyoma		Intramural	1	1	10	2	20	2
84	907/21	55	Leiomyoma		Intramural	2	2	20	4-5/	45	4.5/
85	302/21	47	Leiomyoma		Intramural	1.4/	1-2/	14	3-4/	34	3.4/
86	492/21	49	Leiomyoma		Intramural	1.2/	1-2/	12	2-3/	26	2.6/
87	624/21	36	Leiomyoma		Intramural	3.4/	3-4/	34	8-9/	86	8.6/
88	2012/20	48	Leiomyoma		Intramural	1.6/	1-2/	16	3	30	3
89	1851/20	53	Leiomyoma		submucosal	1.4/	1-2/	14	2-3/	28	2.8/
90	624/20	38	Leiomyoma		Intramural	3.6/	3-4/	36	8-9/	86	8.6/
91	409/21	53	Leiomyoma		Intramural	1.2/	1-2/	12	2-3/	24	2.4/
92	802/21	50	Leiomyoma		submucosal	1.2/	1-2/	12	3	30	3
93	1851/21	49	Leiomyoma		Subserosal	0.8/	0-1/	8	2-3/	24	2.4/
94	0027/21	45	leiomyoma	hyaline degeneration	Intramural	0.6/	0-1/	6	1-2/	18	1.8/
95	0046/21	42	Leiomyoma	hyaline degeneration	Intramural	0.8/	0-1/	8	1-2/	19	1.9/
96	0048/21	52	Leiomyoma	hyaline degeneration	Intramural	0.6/	0-1/	6	1-2/	17	1.7/
97	0018/20	54	leiomyoma	hyaline degeneration	Intramural	0.7/	0-1/	7	2	20	2
98	390/21	48	leiomyoma		submucosal	1.4/	1-2/	14	3	30	3
99	432/21	43	Leiomyoma		Intramural	1.2/	1-2/	12	3	30	3
100	123/20	52	Leiomyoma	hyaline degeneration	Intramural	0.8/	0-1/	8	2	20	2
		45.94						15.09		31.79	