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Review Article

Genetic Predisposition and Molecular Mechanism in the Etiopathogenesis of Uterine Leiomyoma: A Review

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Abstract

Uterine leiomyomata (UL) are smooth muscle tumors of the uterus. They are commonly called as fibroids, grossly round, well circumscribed and encapsulated solid nodules. About 20% to 80% of women develop fibroids by the age of fifty. UL cause abnormal uterine bleeding, pelvic pain, pressure, infertility, repetitive abortions. Early menarche, age, obesity, endocrine changes, family history, ethnicity are the main risk factors. In addition to hormonal factors, various gene polymorphisms and variants have been associated with UL susceptibility. Among them somatic variants in second exon of MED12 gene were around 70% - 85% globally, but in the South Indian population there were approximately 40% variants of MED12 and Collagen gene missense variants rs36117715 and rs 2270669 increasing UL susceptibility in women.

Keywords: *Uterine Leiomyomata; Somatic Mutations; Missense Variants; Codon 58*

Introduction

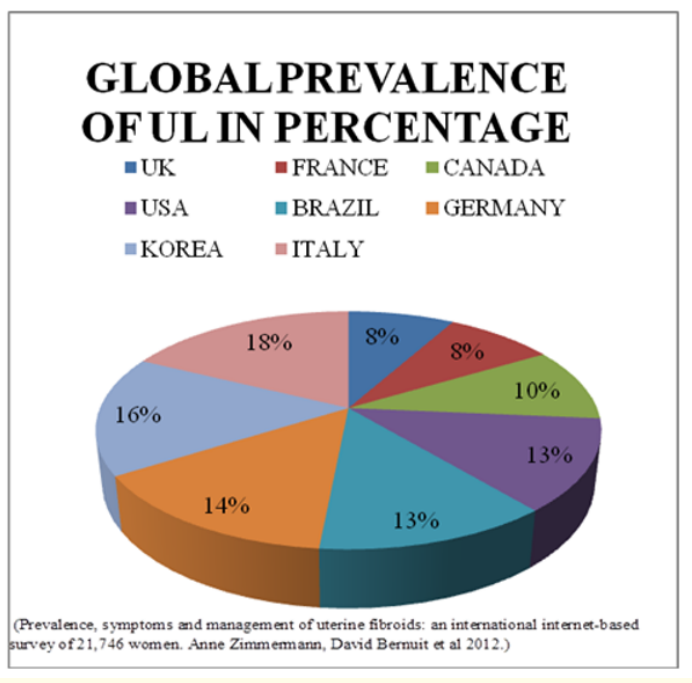
Uterine leiomyomata are smooth muscle tumors of the uterus. They are commonly called as fibroids. In Greek etymology, leios=smooth, myo=muscle and oma=tumor. Other common names are myoma, fibromyoma, fibroleiomyoma. UL are benign, monoclonal neoplasms of the uterus. They generally occur during the third to fourth decades of life in women. Their incidence is 20% to 30% in reproductive age group women. Every one in three hospital admissions of women, for gynecological problems, is due to fibroids. Fibroids are found in multiple locations in the uterus. They can be single or multiple. Majority of the fibroids are asymptomatic, but as they grow in size, they become symptomatic. Fibroids are a major cause for hysterectomy. Despite the fact that uterine leiomyomata, represent the most common gynecologic tumor in women and constitute a significant public health concern, the mechanisms that initiate their growth and pathology are still not completely understood.

Classification: Depending on their location they are classified as follows: Intramural UL develop within the smooth muscle layers of the uterine wall; these are the most common types of fibroids. Sub serosal UL develop on the outer mucosal surface of the uterine wall and grow outwards, and give a knobby appearance to the uterus from outside; they may or may not be pedunculated. Sub mucosal are seen in the muscular layers beneath endometrium of the uterus. Cervical fibroids are present in the wall of the cervical region. Rarely fibroids are also present in the supporting structures like round ligament, broad ligament and uterosacral ligament.

Pathophysiology: They are grossly round, well circumscribed and encapsulated solid nodules, which show whorled appearance. Lesions, the size of a grapefruit or bigger, can be felt by the patient herself through the abdominal wall. Microscopically tumor cells resemble normal cells, with a cigar shaped nucleus, and form bundles in different directions (whorled), uniform in size and shape with scarce mitoses. Three benign variants of fibroids: 1) Bizarre (atypical), 2) Cellular and 3) Mitotically active. Presence of prominent nucleoli with perinucleolar halo indicates the possibility of hereditary leiomyomatosis and/or renal cell cancer syndrome (HLRCC). They occur both in Syndromic (multiple cutaneous uterine leiomyomata, MCUL) and non-Syndromic forms.

Size, color and weight: The size of uterine leiomyoma can vary from few millimeters to as big as filling the entire abdomen. Leiomyoma are pale white, tan to pinkish in color. The largest fibroid so far removed surgically was 17 kg. A few years ago, 84 fibroids weighing about 4 kg, with the largest weighing 1.07 kg were successfully removed from a 40-year-old patient in Hyderabad. This was accomplished in a single operation through minimally invasive low transverse mini laparotomy incision for the first time anywhere in the world. The feat was recognized by the Guinness World Records, Guinness number 417249.

Prevalence: Uterine leiomyomata have been detected in all populations and ethnic groups but with various prevalence rates. About 20% to 80% of women develop fibroids by the age of 50. Globally in 2013 it was estimated that 171 million women were affected. Uterine leiomyoma remains the primary indication for hysterectomy in reproductive age group women and account for more than 2,00,000 hysterectomies in USA. A US study in the year 2012 [1] with randomly selected women between 35 to 49 years, who were screened by self-report, medical record and sonography, the incidence of uterine fibroids by age 35 was 60% among African-American women, increasing to > 80% by age 50, whereas Caucasian women showed an incidence of 40% by age 35, and almost 70% by age 50. The self-reported prevalence of uterine fibroids ranged from a low of 4.5% in UK and 4.6% in France to a high of 9.8% in Italy and 9.0% in Korea. Although prevalence increased with age, Self-reported prevalence in the age group of 40 years and older rose to 14.1% across all countries, ranging from 9.4% in UK (n = 45) to 17.8% in Italy (n = 86).



(Prevalence, symptoms and management of uterine fibroids: an international internet-based survey of 21,746 women. Anne Zimmermann, David Bernuit et al 2012.)

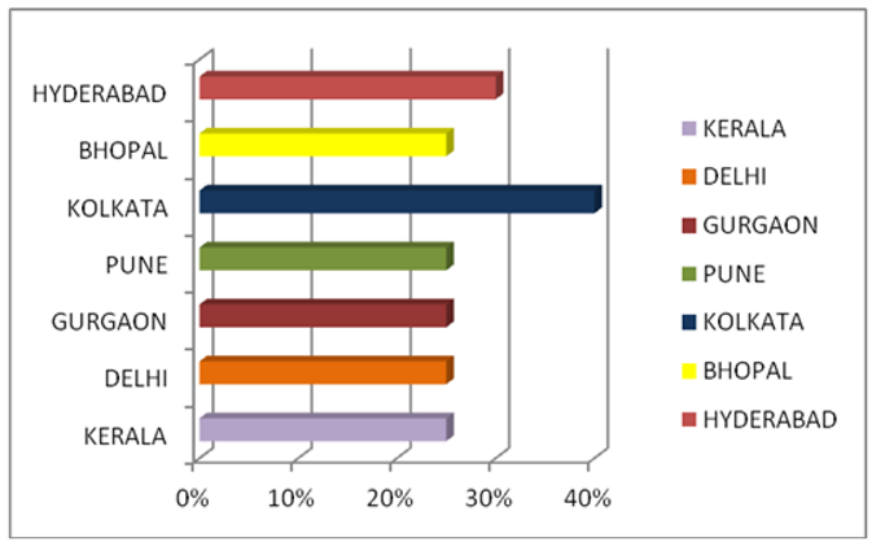


Figure: *Incidence of uterine leiomyomata in India.*

Similar to white women, the incidence rate of uterine leiomyomata in Asian and Hispanic women was up to 30%. Case records of patients with Abnormal Uterine Bleeding (AUB) from the hospitals in Pune, Delhi, and Gurgaon, states that 20 - 25% of women have uterine fibroids. According to Institute of Neurosciences Kolkata states that 20 - 40% of women have fibroids. A hospital-based report of Kerala says UL incidence is around 20 - 25%. In a Bhopal based article incidence of hysterectomies due to leiomyomas was ascribed as 25% by a study conducted in People's College of Medical Sciences and Research Centre. According to an oral reference by Superintendent and Head of the Department of Gynecology, Gandhi Hospital, Hyderabad says, "Removal of fibroids account for 20 - 30 per cent of the operations at this hospital [2].

Symptoms: Uterine Leiomyomata maybe symptomatic or asymptomatic. Large fibroids are associated with hemorrhage, pelvic pain, pressure, dysmenorrhoea, dyspareunia, pregnancy related problems, infertility, repetitive abortions, premature labor interfere in the development of fetus and frequent urination or urinary retention. Uterine Leiomyomata rarely progress to malignancy. They either regress i.e. shrink in size or calcify after menopause [3].

Risk factors: We can only speculate the mechanistic link between predisposing factors and fibroid tumorigenesis, there may be an overlap between one or more factors. The exact cause of uterine leiomyomata is still unknown but a number of factors which may contribute in increasing the risk of fibroid development. These factors are: early menarche, age, obesity, nulliparity, endocrine changes, family history, ethnicity, diet, and vitamin D.

Early menarche: Early menarche has been directly proportional to increased risk of uterine leiomyomata. Statistically the risk is not always significant. An early normal menstrual cycle is usually exhibited by women with fibroids and this regularity of the menstrual cycle may lead to enhanced leiomyoma growth in early reproductive life [4]. There is an increased chance of mutations in genes controlling proliferation, which due to the early onset of menstrual cycles may increase the number of cell divisions that the myometrium normally undergoes during the reproductive years.

Age: Epidemiological studies have shown that an increase in the prevalence of uterine leiomyomata with increase in age during the reproductive years [5]. It is usually seen in middle reproductive year's i.e. third to fourth decades of life. What is not known is that, if the women are in their forties, does it increase the risk of new fibroids? Rapid increase in fibroids could be due to increased growth of already existing fibroids or their symptomatology. Hormonal factors associated with premenopause may be important modulators for fibroid development and growth; also, the cumulative culmination of 20 - 30 years of stimulation by estrogen and progesterone.

Obesity: Ross., *et al*. 1986 reported an association between obesity and increased risk of uterine leiomyomas in a Great Britain study; for every 10 Kg increase in body weight the fibroid risk increased by 21%, similar results were obtained when BMI was considered in UL rather than weight. A 6% increase in UL was observed for each unit increase in BMI. A US study of registered nurses found an increased fibroid risk with increasing adult BMI. For example, a Japanese study reported that women with occult obesity (BMI < 24.0 and percent body fat > = 30%) or women with upper body fat distribution (> 0.80 waist to hip ratio) were at significantly higher risk [6]. This obvious association between obesity and an increased risk of fibroids could be indirectly related to hormonal factors associated with obesity, but other pathologic pathways might also be involved. Excess adipose tissue converts circulating adrenal androgens to estrone thereby increasing it significantly. More unbound physiologically active estrogen is formed due to decreased hepatic production of sex hormone-binding globulin. These two mechanisms most likely have more impact in postmenopausal than premenopausal women. In obese premenopausal women, decreased metabolism of estradiol by the 2-hydroxylation route reduces the conversion of estradiol to inactive metabolites, which could also result in relatively hyper estrogenic state.

Diet: The function of diet has received attention to some extent in the origin of fibroids, in a case control study in Italy. A moderate association was found between the risk of uterine myomas and the consumption of beef, other red meat and ham, whereas a high intake of green vegetables seemed to have a protective effect. The assessment of the association of dietary intake of fruit, vegetables, carotenoids, folate, fiber, and vitamins A, C, and E with UL in the Black Women's Health Study was done the association was stronger for fruit than for vegetables. The inverse association for dietary vitamin A appeared to be driven by preformed vitamin A (animal sources), not provitamin A (fruit and vegetable sources). UL were not materially associated with dietary intake of vitamins C and E, folate, fiber, or any of the carotenoids, including lycopene. Hence women with a good dietary intake of fruit and preformed vitamin A may have a reduced risk of fibroids [7].

Parity: It is inversely associated with fibroids; there is a progressive decline in risk, relative to number of births. Pregnancy reduces the time of exposure to unopposed estrogens whereas nulliparity may be associated with anovulatory, increasing the number of cell cycles of myometrium during the reproductive lifespan that increases the risk of accumulating the mutations in them. Child bearing in the mid reproductive years 25 - 29 provides the women with greatest protection factor against uterine fibroid development [8]. Possibility exists that uterine fibroids are the cause of infertility rather than consequence of it, relative risk of fibroids associated with parity remains the same.

Endocrine changes: Hormonal changes associated with premenopausal women may enhance the development of fibroids during this time. This could be due to cumulative symptomatology by estrogen and progesterone from already existing fibroids in early reproductive years [9].

Family history: Uterine leiomyomata are often found in family members of the patient. In Japanese women, the incidence of positive first-degree family history of myomas among women aged 45 - 54 years was greater than that among controls (31.5% versus 15.2%, respectively, $p < 0.01$). Vikhlvaeya and Sato found that a myoma was discovered in 24.7% of cases, 2.2 times more frequently ($P < 0.001$) among the first-degree female relatives in families with two or more verified myoma cases. There is a high concordance in monozygotic twins than dizygotic twins. Twin studies also indicate that as many as 50 - 60% of uterine myomas are inherited [10]. Myomas can be connected with other syndromes, such as Reed's syndrome, Bannayan-Zonana syndrome and Cowden disease.

Ethnic predisposition: Literature reports that uterine fibroids are more prevalent in black women than white women. A higher incidence of clinically significant fibroids has been reported among African-American women than Hispanics, Asians and Whites. Ethnic predisposition is another causative factor, which shows 3 - 4-fold higher uterine leiomyomata prevalence in black women than in Asian, Hispanic and White women. In an analysis of data from the Nurses' Health Study II. Race does affect incidence and symptom severity of uterine leiomyomata. NIEHS Uterine Fibroid Study demonstrated that the cumulative incidence of fibroids is greater for Blacks than in Whites. NIEHS Fibroid Growth Study showed that there could be a significant variation in fibroid growth rate over a six-month period in black than in white women. African women have larger and higher number of leiomyomata, including earlier age at diagnosis and hysterectomy [11].

Vitamin D: Vitamin D is shown to be protective for breast cancer, is proposed as one of the risk factors evaluated for fibroids [12]. In a study sun exposure was used as a surrogate for vitamin D levels. A reduced uterine leiomyomata incidence of 30% was observed in both black and white women, when correlating women with defined sufficient vitamin D levels, which suggests that adequate vitamin D levels may be important in preventing fibroids.

Genetic predisposition: There are definitely inversions, translocations and insertions in less than 5% of uterine leiomyomata, Trisomy 12 is the genetic basis for fibroids; this statement can be taken up by different views.

Cytogenetic abnormalities: More than 40% of uterine leiomyomata harbor karyotypic abnormalities which are recurrent and tumor specific. In addition to Translocations between chromosomes 6 and 10, translocations between chromosome 12 and 14, is the most common abnormality that occurs in about 20% of leiomyomata. Rearrangements of 12q15 (HMGA2) and 6p21 (HMGA1), 1p36 and10q22, t (12:14) q (q14-q15; q23-24 are present in 20%of karyotypically abnormal leiomyomata. Deletions of chromosomes 3, 6, 7, 13, 17: Del 3(q12-q27), Del 6 (p21), Deletion 7(q22-q32) largest subgroup among leiomyoma and was first described more than 20 years ago, del (q13-q33) in about17% of fibroids [13]. Clonal chromosomal abnormalities were found in five different chromosomes 2, 7,8,12, and 22. Three loci were associated with susceptibility to uterine fibroids, on chromosomes 10q24.33, 22q13.1 and 11p15.5. Monosomy of chromosome 22 is the condition in which one chromosome lacks its homologous partner. Chromosomal aberrations of 6p 21 including deletion, abnormality in as many as 12% uterine leiomyoma. Fine mapping in a large Women's Genome Health study of 25000 women [14], a subset of which were diagnosed with fibroids, discovered a peak signal on chromosome 17 that could be replicated in a cohort of Australian twins. A haplogroup in chromosome 17 is likely to be important in predisposing women to uterine leiomyomata development.

Genetic basis of fibroids: Hereditary defects in the Fumarase hydratase (FH), Birt-Hogg-Dube (BHD) and Tuberous sclerosis complex 2(TSC-2) genes may contribute to the development of uterine leiomyomata in some cases.

Familial aggregation: A number of Studies on familial clustering of fibroids in first degree relatives have been reported. A German study in which fibroids were found to be 4.2 times more common in first-degree relatives of women with fibroids than those without. A Russian study in which a higher incidence of fibroids was found in first-degree relatives and sisters of affected probands than the controls. In a study of Washington State, fibroid patients again were found more likely than the controls to report a history of fibroids in a mother or sister (33.2% vs. 17.6%). The incidence of positive first-degree family history of myoma in Japanese women aged 45 - 54 years with myomas was greater than that among controls (31.5% versus 15.2%, respectively, $p < 0.01$); subjects showed that the risk for myomas was the greatest in women who had both fewer births (parity = 0 or 1) and the positive family history of myomas as compared with those who have both more births (parity = 2) and the negative familiality of myomas (odds ratio = 5.8, 95% confidence interval = 2.3 - 14.6) [15]. Familial clustering of fibroids was 4-5-fold more common in first degree relatives of women with leiomyomata compared with general population, Mother-daughters, sisters, aunt-nieces.

Twin studies: Fibroids are generally monoclonal, which is based on Lyons hypothesis. A study based on comparison between monozygous and dizygous twins reported a 2-fold higher correlation for hysterectomy in monozygotic than dizygotic twins [16]. This finding in

monozygous twins suggests a genetic liability for fibroids. The study did not report the actual incidence of leiomyomata; it may be due to heritable conditions other than fibroids contributed to the observed correlation in twins.

Syndromic occurrence: Uterine leiomyomata have been associated with Reed syndrome (OMIM 150800), characterized by uterine leiomyomata in association with multiple cutaneous leiomyomata, and hereditary leiomyomatosis and renal cell cancer (HLRCC) (OMIM 605839), a cancer syndrome characterized by uterine leiomyomata and papillary renal cell carcinoma. The family histories suggest an autosomal dominant inheritance with incomplete penetrance. Previous reports of several families in England and Finland with multiple uterine and cutaneous leiomyomata, and subset of these with papillary renal cell carcinoma, have independently linked this disorder to a predisposition gene in the region of chromosome 1q42.3-q43. In follow-up studies, mutations were detected only in the fumarate hydratase gene a component of the energy-producing Tricarboxylic acid cycle [17]. Germline heterozygous loss of function mutations of the fumarate hydratase (FH) gene are found in the recessive FH deficiency syndrome and in autosomal dominantly inherited syndrome susceptible to multiple cutaneous and uterine Leiomyomatosis. (MCUL and HLRCC).

Non-syndromic occurrence: Most patients with skin and uterine leiomyoma will have underlying FH mutations. Somatic mutations in FH do not show pronounced effect in non-Syndromic UL compared to their Syndromic counterparts [18]. 77% of women with Hereditary Leiomyomatosis and Renal Cell Cancer syndrome have uterine leiomyoma along with aggressive forms of kidney cancer. Inactivation of the FH gene is unusual for non-Syndromic leiomyomas.

Molecular mechanisms in the etiopathogenesis of uterine leiomyoma: Leiomyoma may arise due to a number of factors as follows:

a) **Epigenetic factors:** The term epigenetic refers to heritable changes in gene expression (active versus inactive genes) that does not involve changes to the underlying DNA sequence; a change in phenotype without a change in genotype. DNA methylation at the fifth carbon of cytosine resulting in 5-methyl cytosine mostly within CpG dinucleotides is involved in various developmental processes by silencing, switching and stabilizing genes. DNA hypomethylation and imbalanced expression of DNA methyltransferases DNMT1, DNMT3A and DNMT3B were found by Li., *et al*. 2003 in human uterine leiomyoma compared with adjacent myometria. Maekawa., *et al*. identified 14 hypomethylated genes and one hypermethylated gene HDAC8 located on the X chromosome in uterine leiomyoma.80% of the genes showed an inverse relationship between DNA methylation status and mRNA expression in uterine leiomyoma and majority of the genes (62%) displayed hypermethylation associated with gene silencing. Three tumor suppressor genes-KLF11, DLEC1, and KRT19-with hypermethylation, mRNA repression, and protein expression in leiomyoma. These results indicate a possible functional role of promoter DNA methylation-mediated gene silencing in the pathogenesis of uterine leiomyoma [19].

b) **Histone modification:** Although lesser important than DNA methylation, it has a critical role in regulation of gene expression. Histone proteins can be modified in many ways in their N-terminal tail, by acetylation, phosphorylation, methylation, ubiquitylation, sumoylation and adenosine diphosphate ribosylation, deamination, and proline isomerization. HDAC6 represses transcription was reported by Wei., *et al*. 2007 its expression and pathogenic role in uterine leiomyoma [20]. This is a regular pattern of increasing HDAC6 and ER –α expression in leiomyoma samples.

c) **MicroRNA**: MicroRNAs are small non-protein-coding RNAs that regulate a number of biological processes by targeting mRNAs for cleavage or translational repression. Several MicroRNAs like Let7, miR-21, miR-93, miR-106b, and miR-200 and their predicted targeted genes are significantly dysregulated in uterine leiomyoma compared with normal myometrium. Their expression seems to be strongly associated with tumor size and race. Elevated leiomyoma miRNA-21 levels are predicted to decrease programmed cell death 4 (PDCD-4) levels. Leiomyoma differ from other tumors in which, loss of PDCD-4 is associated with tumor progression [21].

d) Initiators of tumorigenesis may be subdivided as follows:

A) Ischemia: Ischemia is a restriction in blood supply to the tissues. Injury during menstruation is considered to be one of the reasons for the pathogenesis of fibroids. Ischemia causes release of vasoconstructive substances during menses. Endometrium secretes increased amounts of prostaglandins and vasopressin in patients with dysmenorrhea. Myometrial contraction for end of menstrual bleeding could induce ischemic injury to myometrial smooth muscle cells that might be candidates for the progenitor cells of leiomyoma. In support of this hypothesis, apoptotic, p53 and p21-positive cells have been found in the follicular phase of the menstrual cycle. However, Ki-67 positive cells are mainly observed in the luteal phase of the menstrual cycle. Majority of the injured cells seem to be eliminated in the follicular phase of the menstrual cycle as apoptotic cells or non dividing cells (cell cycle arrested). But even then, some cells may survive injury, acquire a protective mechanism against oxidative stress and apoptosis. Menstruation, ovulation, and parturition, represent a physiologic injury triggering an inflammatory reaction of the uterus. Myofibroblast cells activated by restore tissue homeostasis and the wound healing process. Myofibroblasts are characterized by an increased proliferation, migratory ability, production of cytokines and increased production of interstitial matrix. Inappropriate function of myofibroblasts may cause fibrosis, owing to its inability to regenerate tissue, often regenerating a stiff collagenous scar. Myofibroblastic transformation could occur from different types of cells, because myometrium consists of myometrial smooth muscle cells, connective tissue fibroblasts, stem cells, vascular cells, and a detectable number of bone marrow-derived progenitor cells [22].

B) Environmental carcinogens: Significantly higher sources of carcinogens like Dichlorodiphenyltrichloroethane (DDT) and its metabolites were found in uterine leiomyomatous tissue than in normal myometrium and higher levels of DDT in blood of women with leiomyoma than in those without fibroids. Exposure during critical stages of development can result in permanent molecular and cellular alterations including the formation of uterine leiomyoma [23].

C) Viral infections: Epstein Barr Virus and Human Papilloma Virus have been associated with cutaneous leiomyoma in adults and children effected with AIDS, e.g. Simian virus-40 (SV-40).

Hormonal factor in uterine leiomyoma tumorigenesis: Ovarian steroid hormones appear to promote UL development especially during the reproductive years and regress after menopause [24]. Factors that increase the overall exposure to estrogen and progesterone such as early menarche; nulliparity and obesity lead to increase the risk of UL incidence. Furthermore, treatments with Gonadotropin releasing hormones (GnRH) agonists, which reduce ovarian hormone production, lead to reduction in the size of leiomyoma, but after the therapy is discontinued re-enlargement of leiomyoma occurs. Clinical and Biochemical studies have supported the role of estrogens in leiomyoma growth. In addition, there is increasing evidence to suggest the involvement of progesterone in the pathogenesis of leiomyoma.

Promoters: Both estrogen and progesterone have been implicated as the promoters of UL. Increased levels of estrogen, progesterone act in combination resulting in an increased mitotic rate which may contribute to myoma formation by increasing the likelihood of somatic mutations [25]. They exert their effects on the target cells by binding to specific nuclear receptors: the estrogen receptor ER-α, ER-β and for progesterone PR-α, PR-β.

1. **Estrogen:** Clinical observations that fibroids appear only after menarche, develop during reproductive years, may enlarge during pregnancy, regress following menopause and their risk is greater in nulliparous women (due to greater number of anovulatory monthly cycles) and obese women in whom aromatization of androgens to estrone in the fat leads to suggest that estrogen is the primary promoter of fibroids. Estrogens elicit its physiological effects on target cells by binding to ER-alpha, and ER-beta. In both the receptors protein expression level is higher in leiomyoma than in normal myometrium. Estrogen significantly suppresses p53 protein in cultured leiomyoma cells suggesting it may stimulate leiomyoma growth partially by suppressing normal p53 functions. Estrogen can alter the expression of a large number of genes such as c-fos, c-jun, connexin 43, PR, insulin like growth factor IGF-1,

IGFRs, IGFBP5, A-myb, and MKP [26]. Several authors have demonstrated that leiomyomata over-express aromatase p450, an estrogen synthetase, which catalyses androgens to estrogens, which may play a role in promotion of leiomyoma growth in an autocrine/paracrine mechanism.-

2. **Progesterone:** Progesterone levels are cyclically elevated during the reproductive years and markedly increased during pregnancy. The role of estrogen is to increase the levels of ER and PR in the myometrium, while progesterone decreases the level of ER. This is in accordance with their levels during the menstrual cycle, i.e. in the myometrium both the ER, PR levels rise during the proliferative phase (follicular) and then fall during the secretory (luteal) phase [27]. The fall in PR is related to fall in levels of estradiol during the luteal phase. Mitogenic effect of progesterone is dependent upon prior exposure to estrogen as this increases the concentration of progesterone in myomas. In short during the follicular phase estrogen up regulates ER and PR, which leads to progesterone surge in luteal phase associated with a heightened mitogenic activity and subsequent downregulation of ER and PR. Specifically, progesterone stimulates EGF production while estrogen stimulates EGF receptor production. Since myoma growth is a balance between cell proliferation and apoptosis, the effects of progesterone on apoptosis have been shown to increase the expression of the anti-apoptotic protein Bcl-2 (B-cell lymphoma 2) in cultured leiomyoma cells; where estradiol has no effect. Additionally, progesterone has been found to inhibit the expression of tumor necrosis factor-alpha (TNF-a), an apoptosis-inducing factor and IGF-I expression in cultured leiomyoma cells. Fibroids regress with the administration of antiprogesterone drug, mifepristone (RU-486), further supporting the role of progesterone as a promoter of fibroid growth. SPRMs can induce leiomyoma inhibition in a cell-specific manner through decreased cellular proliferation and increased apoptosis in *invitro* studies [28].

Since fibroids are hormone dependent, steroid hormone pathways have long been targets of investigation, especially selective estrogen receptor modulators (SERMs) and selective progesterone receptor modulators (SPRMs). Clinical studies have shown that SRPMs such as Mifepristone and Asoprisnil induce amenorrhea, decrease leiomyoma size and increase primary apoptosis or inhibit cellular proliferation [29].-

Effectors: The growth factors belong to the effector category. The promoting effects of estrogen and progesterone may occur through the growth factors.

1. **Growth factors:** Growth factors are proteins or polypeptides produced locally by smooth muscle cells and fibroblasts which take part in cellular events like proliferation, ECM synthesis and angiogenesis for leiomyoma growth. Growth factors are associated with the pathogenesis of leiomyomata. Polymorphisms in growth factor genes might play a role in the complex pathogenesis of leiomyoma. Angiogenic growth factors play an important role in mechanisms of fibroid pathophysiology, including abnormal vasculature and fibroid growth and survival [30].

   The following growth factors play a potential role in leiomyoma growth: TGFbeta3, bFGF, bFGFR, EGF, EGFR, PDGF, PDGFR, IGF1, IGF1R and VEGF. The growth promoting effects of estrogen and progesterone on the uterine myometrium and myomas are mediated through the mitogenic effects of growth factors produced by the smooth muscle cells locally. They are essential in controlling the proliferation rate of cells, and over expression of either the growth factor or its receptor may contribute to tumorigenesis. Growth factors and their receptors evoke responses which are complex and dependent on type of cell, differentiation stage of cell and stimuli.

2. **Epidermal growth factor (EGF):** Significantly promotes human leiomyoma smooth muscle cells proliferation. Epidermal growth factor increases DNA synthesis and polyploidization in leiomyoma cells through transient activation of the EGFR-MAPK pathway. EGF and EGF-R mRNA have both been identified in myometrial and leiomyoma cells and immunolocalization of both proteins has also been described in the cytoplasm of smooth-muscle cells of leiomyomata and matched myometrium [31].

3. **Platelet derived growth factor (PDGF):** Stimulates DNA and protein synthesis, increases collagen alpha-1 expression and modulates the rate of cell proliferation in myometrium and leiomyoma cells. NADP oxidase is necessary component of the MAPK pathway activated by EGF and PDGF in leiomyoma cells. PDGF and PDGF-R expression have been documented in both normal myometrial and leiomyoma tissues [32].
4. **Transforming growth factor (TGF-Beta):** It shows bimodal effects on cell proliferation [33], induces up regulation of ECM related genes and decreases production of ECM degradation-related genes. Transforming growth factor beta can activate the MAPK/ERK/Smad pathways and regulate gene expression whose products may influence leiomyoma growth and regression. A selective inhibitor of TGFBR1 decreases the incidence, number and size of these tumors. ECM proliferation, in uterine leiomyomata, is upregulated by the promoter protein TGFβ3 which is known to interact with dermatopontin and thrombospondin. The transforming growth factor beta-(TGF-β), mammalian target of rapamycin (mTOR) and progesterone receptor signaling pathway have been considered important in fibroid growth and as potential therapeutic agents. Over expression of TGF-Beta and to some extent granulocyte macrophage colony stimulating factor (GM-CSF), has been associated with tissue fibrosis throughout the body.
5. **Insulin like growth factor-IGF-1:** Activated receptor tyrosine kinases (RTKs) play an important role in the enhanced proliferation observed in fibroids. Overexpression of RTKs and, in particular, activation of the IGF-IR signaling pathway through Shc/Grb2/MAPK are important in mediating fibroid growth. Insulin like growth factor 1 increases cellular proliferation in uterine leiomyoma through activation of MAPK pathway, also up regulating BCl2 protein expression in leiomyoma cells [34]. Analysis of IGF-2 and COL4A2 mRNA expression levels in uterine leiomyoma with MED12 missense mutations expressed significantly higher levels of IGF-2 mRNA. In contrast MED12 gene status does not appear to affect mRNA expression gene of COL4A2. On the basis of this finding MED12 status stratifies the uterine leiomyoma into two mutually exclusive pathways of leiomyoma genesis, one with IGF-2 over-expression and the other with no IGF-2 activation. IGF-2 over-expression could be therapeutically targeted for non-surgical treatment of leiomyomata.
6. **Basic fibroblast growth factor-bFGF:** Although bFGF is mitogenic for both human uterine myometrial and leiomyoma cells, leiomyoma cells are less responsive to it. Expression of bFGF and its receptors FGFR-1 and FGFR-2 in both leiomyoma and myometrial cells has been amply reported, with more distinct expression of FGFR-1 in the tumors compared with myometrium [35]. bFGF regulates angiogenesis and it can also bind to a component of the extracellular matrix of leiomyomata.
7. **Activin and myostatin:** Activin and myostatin can regulate myometrial cell proliferation. Experimental data have shown that activin-A and myostatin expression levels are higher in leiomyoma. than in adjacent myometrium. But that transcription response is altered in leiomyoma. Activin-A and myostatin were able to increase Smad7 expression levels in myometrium but not in leiomyoma indicating they are less responsive to these ligands [36].
8. **Vascular endothelial growth factor-VEGF-A:** Vascular endothelial growth factor stimulates angiogenesis, which is essential for actively growing tumors. It is the most potent agent known for increasing capillary permeability which could enhance the growth of fibroids by increasing their nutrient supply. Polymorphisms in the VEGF gene may be a useful marker to predict susceptibility to leiomyoma. VEGF-A expression was strong in leiomyomata > or = 20 focal areas/cm^2 but not in adjacent myometrium, this differential expression shows that local angiogenesis maybe important in the growth and development of tumors. Evaluation of VEGF mRNA during leiomyoma growth at different phases of menstrual cycle with RT-PCR resulted in highest VEGF mRNA expression in women with myomas who were in menopause. Among the menstruating patients VEGF expression was significantly higher in women with UL compared to those with a healthy myometrium, suggesting that VEGF may play a significant role in the pathogenesis of uterine leiomyoma. Expression of Vascular endothelial growth factor mRNA is seen in human leiomyomata [37]. Recently it was reported that the 5'-3' UTR-460 polymorphism in the VEGF gene might contribute to the pathogenesis of leiomyoma. It was also observed that VEGF T homozygotes and T allele are associated with higher susceptibility of leiomyoma development. These findings strongly suggest a molecular correlation between VEGF and leiomyoma, and that VEGF is involved in the pathogenesis of

leiomyoma. The growth factors are considered the ultimate effectors of the steroid hormone actions because they have stimulatory or inhibitory effects on cell proliferation and probably leiomyoma formation.

Other factors

1) **Cytokines:** Cytokines which are low molecular weight 10 - 50 kDa proteins released by the cells of the immune system, bind to specific cell surface receptors and send intracellular signals. IL-1, IL-6, IL-11, IL-13, IL-15 [38] which, in synergistic action with TGF Beta IL-11 and IL-13 are over expressed in leiomyoma compared with myometrium.
2) **Chemokines:** Chemokines are small 8 - 10 kDa proteins expressed by almost all nucleated cell types. Chemokines along with their receptors are critical mediators of a variety of biological processes like development, angiogenesis, hematopoiesis and fibrosis [39]. Monocyte chemo attractant protein-1(MCP-1) plays an effective part in inflammatory response of blood monocytes and tissue macrophages. Sozen., *et al*. 2006, found out that MCP-1 at higher levels in myometrium compared with leiomyoma. When leiomyoma cells were treated with anti-MCP-1 neutralizing antibody, proliferation was noted.
3) **Extracellular matrix (ECM) components:** Qualitative and Quantitative abnormalities in ECM components, primarily collagens, fibronectin and proteoglycans are present in UL. ECM is more than 50% in leiomyoma when compared with the corresponding myometrium, and it may serve as a storage for growth factors, cytokines, chemokines, angiogenic and inflammatory response mediators, and proteases produced by tumor cells [40]. Collagens contribute for stability and structural integrity of the tissues. An abnormal collagen fibril structure and orientation were found in leiomyoma. A series of collagen subtypes were tested and COL1A1, 4A2, 6A1, 6A2, 7A1and16A1 were expressed to a greater extent in leiomyoma cells compared with myometrial cells. Uterine leiomyoma derived fibroblasts can stimulate uterine leiomyoma cell proliferation with elevated production of collagen type -1, IGFBP-3, VEGF, EGF, bFGF, PDGF-A and B, TGF-B1, and TGF-B3 and can also activate receptor tyrosine kinases and TGF-B receptor signaling. These findings suggested that leiomyoma growth may in part be mediated by autocrine or paracrine mechanisms. Tumor derived fibroblast cells and leiomyoma cells can promote synthesis of growth factors and activate their signaling pathways that stimulate tumor cell proliferation and production of ECM components [41]
4) **Dermatopontin:** Dermatopontin an extracellular protein that binds to small dermatan sulphate molecules and decorin is expressed in lower levels in leiomyoma. Epidemiologic similarities between leiomyomas and keloids have built a molecular link by observing reduced dermatopontin expression and disordered collagen fibril structure [42].
5) **Proteoglycans:** Proteoglycans are glycosylated proteins that are covalently linked to sulphated glycosaminoglycans and are an important part of the leiomyoma structure. David., *et al*. 2012 found higher amounts of glycoaminoglycans and versican but lower amounts of decorin in uterine fibroids and keloids scars compared with corresponding normal tissues. Several studies reported that the expression levels of versican are higher in leiomyoma than in myometrium [43].
6) **Fibronectin:** Fibronectin is a glycoprotein of the ECM that binds to collagen and integrins. No significant differences between leiomyoma and myometrium at any stage of the menstrual cycle were found by Stewart., *et al*. 1994 found, but Arici and Sozen 2000 found an elevated level of fibronectin expression in leiomyoma compared with autologous myometrium. Tissue remodeling involving "ECM turnover "plays a major role in leiomyoma growth and regression, which is regulated by the combined action of matrix metalloproteinase's (MMPs, a class of proteins that breaks down the ECM and tissue inhibitors of MMPs (TIMPs). Differential expression of matrix metalloproteinases and their tissue inhibitors has been seen in leiomyomata [44].

Gene polymorphisms

Numerous gene polymorphisms have been reported to be associated with the pathogenesis of leiomyoma. A number of Single nucleotide polymorphisms in anti-oxidant and DNA repair genes such as cytochrome CYP P450 c17 alpha gene*A2 allele by Amant F., *et al*. 2004, CYP 1 A1 Ile 462 Val AG, CYP1B1 Leu 432 Val CC genotype by El -Shennawy G A., *et al*. 2011, MSP1 Polymorphism in CYP 1A1 gene by

Barao., *et al*. 2010, Arg280 His polymorphism of XRCCI gene by Yang Y., *et al*. 2009, Deletion polymorphism in GSTM1 by Huang P C., *et al*. 2010 are positively described to elevate the risk of sporadic UL in women. Hormone receptor genes that have been studied are: +331G/A and the V600L single nucleotide polymorphisms in the progesterone receptor Renner S P., *et al*. 2008, Pvu II (T/C) polymorphism by Govindan S., *et al*. 2010, thymine-adenine dinucleotide repeat by Hsieh Y Y., *et al*. 2003 in estrogen receptor genes, CAG repeats in Androgen Receptor gene [45] and Alu insertion polymorphism in Progesterone Receptor gene by Govindan S., *et al*. 2007. For growth factor and cytokine, the following genes have been investigated; A/G polymorphism in exon 9 of IGF2 gene Hsieh Y Y., *et al*. 2010, -308 A/G promoter polymorphism in TNF alpha gene by Hsieh Y Y., *et al*. 2004, 511 C<T polymorphism IL-1 beta gene by Pietrowski., *et al*. 2009, 174 G/C polymorphism in IL-6 by Litovkin KV., *et al*. 2007, 5" UTR -460 Polymorphism in VEGF gene by Hsieh Y Y., *et al*. 2008 are studied in relation to higher genetic susceptibility of UL tumors.

GWAS

Recent genome-wide association study (GWAS)analyzing 457,044 SNPs in 1,607 individuals with clinically diagnosed non-syndromic UL and 1,428 female controls suggested the SNP's (rs7913069, rs12484776 and rs2280543) of three loci on chromosomes 10q24.33, 22q13.1 and 11p15.5 revealed genome-wide significant associations with UL [46].

A few mutations were also studied such as:

- **High mobility group proteins-HMGA2 (locus12q14):** Classical cytogenetics revealed that a subset of uterine leiomyomata harbor recurrent chromosomal rearrangements, such as translocations affecting the HMGA2 gene [47], specifically HMGA1 and HMGA2. Aberrant expression of HMGA2 may affect the expression of growth factors and growth inhibitors, fibroblast growth factor 2 (FGF2) and p19 alternate reading frame (p19Arf), respectively. Moreover, over-expression of HMGA2 in leiomyomata correlates with increased FGF2 levels and tumor size, and repression of the growth inhibitor factor p19Arf. Over-expression of the truncated form of HMGA2 induces myometrial cell transformation toward putative tumor-initiating leiomyoma cells. Complex chromosomal re-arrangements resembling chromothripsis are a common feature of leiomyomata. These re-arrangements are best explained by a single event of multiple chromosomal breaks and random reassembly. The re-arrangements are responsible for tissue-specific changes consistent with a role in the initiation of leiomyoma, such as translocations of the HMGA2 and RAD51B loci (14q24, translocation partner of HMGA2, a DNA repair protein) and aberrations at the COL4A5-COL4A6 locus, and occurred in the presence of normal TP53 alleles. In some cases, separate events had occurred more than once in single tumor-cell lineages. Chromosome shattering and re-assembly resembling chromothripsis (a single genomic event that results in focal losses and re-arrangements in multiple genomic regions) is a major cause of chromosomal abnormalities in uterine leiomyomata. We therefore propose that tumorigenesis occurs when tissue-specific tumor-promoting changes are formed through these events. Chromothripsis has previously been associated with aggressive cancer; its common occurrence in leiomyomata suggests that it also has a role in the genesis and progression of benign tumors.

Fumarate hydratase (FH): FH is an enzyme which catalyzes the reversible hydration/dehydration of fumarate to malate. Germline, heterozygous mutations due to biallelic inactivation in 1q42 lead to recessive FH deficiency syndrome and in autosomal dominantly inherited syndrome susceptible to multiple cutaneous and uterine leiomyomatosis (MCUL and HLRCC). Altered FH accumulation in cells leads to formation of S-C2-Succino cysteine which can be detected by polyclonal antibody [48].

Mediator complex subunit-12 (MED12), locus Xq13.1 chromosome: The Mediator complex is a 26-subunit transcriptional regulator that bridges DNA regulatory sequences to the RNA polymerase II initiation complex. The Mediator complex interacts extensively with the RNA polymerase II enzyme and regulates its ability to express protein-coding genes. The mechanisms by which Mediator regulates gene expression remain poorly understood, in part because the structure of Mediator and even its composition can change, depending upon

the promoter context. Combined with the sheer size of the human Mediator complex (1.2 MDa), structural adaptability bestows seemingly unlimited regulatory potential within the complex. Recent efforts to understand Mediator structure and function have identified expanded roles that include control of both pre- and post-initiation events; it is also evident that Mediator performs both general and gene-specific roles to regulate gene expression. In a study Finnish 2011 study, the somatic mutations resided in exon 2, suggesting that aberrant function of this region of MED12 contributes to tumorigenesis. This analysis confirmed a notable frequency (70%) of somatic MED12 codon 44 region mutations in uterine leiomyomas. Of the 225 tumors, 110 (49%) displayed missense mutations affecting codon 44 which included G44V, G44C, G44R, G44A, G44S, and G44D mutations. In addition, Q43P was found three times. Another hot spot was detected in codon 36. Eleven lesions (5%) all displayed a L36R mutation. Twenty-five tumors (11%) with an insertion-deletion type mutation were also observed.

The genetic evidence suggests that MED12 mutations contribute to the genesis of uterine leiomyomata [49]. Mutations of the Mediator Complex Subunit 12 gene (MED12) have been reported in uterine leiomyomata from American women of varied ethnicities and racial backgrounds, in South African and Finnish women. Mutations in MED12 appear to be common in exon 2, and studies have shown that exon 2 mutations are tissue specific to uterine leiomyomas, rare in other tumors, and may contribute to the development of fibroids. Whole exome sequencing revealed that the gene encoding transcription factor MED12 harbored heterozygous missense mutations caused by single nucleotide variants in highly conserved codon 44 of exon 2. A South Indian study by Ruqia Firdaus., *et al*. 2021 observed MED12 somatic sequence variants in codon 58 and 59 of exon 2 were around 40% comparatively less than the published percentages ranging from 50% - 85% globally [50].

A study of benign and malignant uterine smooth muscle tumors concluded MED12 mutations are common in UL, MED12 protein product may be reduced or lost in complex mutations, MED12 mutations are absent in UL with HMGA2 overexpression and their malignant counterparts, the leiomyosarcoma have a very low rate of MED12 mutations, suggesting they have independent tumorigenic pathways.

Collagen gene deletions in COL4A5 (Xq22.3) cause upregulation of Insulin receptor substrate-IRS4. Aberrant methylation of COL6A3 has been implicated in the development of uterine leiomyoma. Encoded variants or variants of their post translational products may drive the pathogenesis of UL as reported by Aissani B [51]. Some rare cases of UL have COL4A5, COL4A6 germ line mutations. A recent study by Firdaus R., *et al*. 2021 observed that missense variants rs36117715 "T" allele and rs 2270669 "G" allele of COL6A3 increased likelihood with uterine leiomyoma risk. Both these missense variants could be used as biomarkers for identifying women with UL risk [52].

Cut like homeobox (CUX1): Target of 7q alterations was found to be interrupted by inversions. It was the most commonly deleted gene on chromosome 7 and identified as haploinsuffcient tumor suppressor gene in acute myeloid leukemia. Inactivating CUX1 mutations promoted tumorigenesis by activating PI3K-AKTsignalling pathway. chromosomal pericentric and paracentric inversions target the cut-like homeobox 1 (CUX1) gene on chromosomal band 7q22.1 in a way which is functionally equivalent to the more frequently observed del(7q) cases, and which is compatible with a mono-allelic knock-out of the cytogenetic subgroup showing chromosome 14q involvement [53].

Diagnosis: The diagnosis of uterine leiomyomata is by non-invasive imaging techniques and invasive laparoscopy, hysteroscopy, surgical techniques.

Non-invasive imaging technique (Non-surgical approaches)-ultrasonography: Uses sound waves to create a picture of the uterus and other pelvic organs. It is the standard test to detect uterine fibroids.

Magnetic resonance imaging: It is used to take 3D images of the fibroids. The waves are directed at the fibroids through the skin with the help of magnetic resonance imaging. Its costly and risky due to metal objects in the uterus.

Computed tomography scans: It may be used as an alternative imaging device. It's a computer processed combinations of many X ray images taken from different angles to produce a cross sectional image.

Invasive tests: Hysteroscopy procedure visualizes the cavity of the uterus with a thin optic, a hysteroscope, to detect sub mucosal fibroids, polyps.

Laparoscopy: Laparoscope helps to visualize within the abdominal cavity where the abdominal and pelvic organs are seen including the external surface of the uterus.

Hysterosalpingography: It is a special X-ray test. It may detect abnormal changes in the size and shape of the uterus and fallopian tubes but not the number and exact location of leiomyoma (Maria., *et al*. 2017).

Management: Management of uterine leiomyoma depends on the age, symptoms, fertility status of the patient between medical and surgical treatment. Drug treatment for fibroids includes the following options:

- **Medical treatment (Non-surgical):** Birth control pills and other types of hormonal birth control methods-these drugs are often used to control heavy bleeding and painful periods.
- **Gonadotropin-releasing hormone (GnRH) agonists**: These drugs stop the menstrual cycle and can shrink fibroids. They sometimes are used before surgery to reduce the risk of bleeding. Because GnRH agonists/analogs, RU 486 have many side effects, they are used only for short periods (less than 6 months). After a woman stops taking a GnRH agonist, her fibroids usually return to their previous size. Side effects may include headaches, hot flashes, mood swings and vaginal dryness (Friedman., *et al*. 1991).
- **NSAIDS**: Non-steroidal anti-inflammatory drugs can be used to reduce painful menses (Tiago., *et al*. 2010).
- **Progestin-releasing intrauterine device (IUD):** This option is for women with fibroids that do not distort the inside of the uterus. It reduces heavy and painful bleeding but does not treat the fibroids themselves (Sangkomkamhang., *et al*. 2013).
- **Levonorgestrel IUDs:** Provide symptomatic relief by limiting menstrual blood flow.
- **Danazol and Dostinex:** Shrink fibroids effectively but their use is limited due to unpleasant side effects. Safety and side effects can be improved by cautious dosing (RM Moroni., *et al*. 2014).
- **Surgical approaches:** Surgery has for long been the main mode of therapy for the myomas.
- **Myomectomy**: For women who wish to retain the uterus for future pregnancies or other reasons, myomectomy is preferred, they may still be able to have children. Fibroids do not regrow after surgery, but new ones may develop.
- **Hysterectomy**: It is the removal of the uterus. The ovaries may or may not be removed, its done when other treatments have not worked or are not possible or the fibroids are very large. A woman is no longer able to have children after having a hysterectomy. Surgery is the only conclusive treatment for leiomyoma [54].
- **Endometrial ablation**: This procedure destroys the endometrial lining of the uterus. It is performed to treat women with small fibroids (less than 3 centimeters). In the following ways endometrial ablation is done:
- **Uterine artery embolizations (UAE):** UAE in this procedure, tiny particles (about the size of grains of sand-500um) are injected into the blood vessels that lead to the uterus. The particles cut off the blood flow to the fibroid and cause it to shrink. Both UAE and endometrial ablation methods preserve the uterus and reduce the recovery time and morbidity in comparison to surgeries. At times uterine artery ligation is also done to limit the blood supply to the uterus.
- **Laparoscopic cryoablation**: Cryoablation is a process that uses extreme cold (cryo) to destroy or damage tissue (ablation) of solid tumors found in uterus by a cryoprobe.

- **Radiofrequency thermal ablation**: In this minimally invasive technique the fibroid is shrunk by inserting a needle-like device into the fibroid through the abdomen and heating it with radio-frequency (RF) electrical energy to cause necrosis of cells.

Future therapeutic prospects

Gene therapy: Gene therapy is a potentially effective non-surgical approach for the treatment of uterine leiomyoma. Reports showed that adenovirus (Ad)-mediated herpes simplex virus thymidine kinase gene transfer in combination with ganciclovir treatment significantly inhibits the growth of human and rat leiomyoma cells proliferation, and also decreases uterine fibroid volume in an Eker rat model [55]. stem cells paracrine interactions with differentiated cell populations in leiomyoma tissue could lead to the development of therapeutics which temper leiomyoma growth as well as those that eradicate them [56].

Conclusion

Fibroids are the commonest non-malignant abnormal tissue growth of the uterus in reproductive age women. Uterine leiomyomata disease is a multifactorial disorder. Among the various genes, Collagen gene missense variants seem to be the most promising ones knocking the MED12 gene in showing increased association with fibroids in South Indian population. Molecular and epidemiologic studies suggest that genetic factors influence UL development and growth. Involvement of genetic factors in the etiopathogenesis of uterine leiomyoma has been extensively examined which clearly indicates that fibroids do have a genetic basis involved in their growth.

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Conflict of Interest

The authors declare there is no conflict of interest.

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