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Original Research Article

Immunohistochemical Analysis of Vascular Endothelial Growth Factor Receptor in Human Uterine Leiomyomas in Relation to Endometrial Hormonal Pattern

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Abstract

Aim: To analyze the expression of VEGF and find the significance of upregulation of VEGF in uterine leiomyomas in different phases of endometrium.

Materials and Methods: A total of 50 leiomyoma specimens were selected of which 25 cases showing proliferative phase in endometrium, 25 cases showing secretory phase in endometrium and 10 cases of normal myometrium used as control. Haematoxylin and Eosin slides of all the tissues were evaluated. The formalin fixed, paraffin embedded blocks were sliced in 3-4 µm thickness for IHC. The Avidin Biotin complex (ABC) detection system was used. Immunoreactivity was regarded as positive when brown staining was localized in the cytoplasm of leiomyoma cells.

Results: The intensity of immunostaining in leiomyoma and matched myometrium in both phases of menstrual cycle was observed and graded as 0,1+,2+ and 3+.

Conclusion: VEGF expression in leiomyoma was abundant relative to the normal myometrium of the same individual uterus and that VEGF protein expression in leiomyoma cells predominated in the secretory phase than the proliferative phase. Therefore, both oestrogen E2 and progesterone P4 seemed to induce the tumour growth. VEGF expression was significantly higher in those with myomas compared to those with a healthy myometrium suggesting that VEGF may play a significant role in pathogenesis of uterine myomas. These suggest that VEGF and other angiogenic factors may represent potential targets for the treatment and prevention of uterine fibroids.

Keywords: VEGF Protein; Leiomyoma; Immunohistochemistry.

Introduction

Uterine leiomyoma is the most common benign smooth muscle cell tumor of the myometrium, occurring in as many as 30% of women over 35 years of age [1]. Prevalence rate for uterine fibroids is approximately 1 in 20 or 5 % or 13.6 million people globally. As per the Country/Region Extrapolated Prevalence Population Estimated to have uterine fibroid in India is 53 million in a total population of 1.2 billion [2]. Leiomyomas are a frequent cause of menorrhagia, dysmenorrhea, pelvic pain, reduced fertility, and recurrent pregnancy loss.A growing body of evidence suggests that the action of sex steroids may be mediated in part by local growth factors produced by the target cells [3,4,5]. However, the mechanisms of ovarian steroid hormone actions in the regulation of leiomyoma growth are not well defined yet.

Vascular endothelial growth factor (VEGF) is a signal protein produced by cells that stimulates vasculogenesis

and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate.

Serum concentration of VEGF is high in bronchial asthma and diabetes mellitus [6]. VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels (collateral circulation) to bypass blocked vessels. When VEGF is overexpressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are able to grow and metastasize.

VEGF mRNA and protein expression have been identified in the smooth muscle cells of both normal myometrium and leiomyomas [7,8,9]. The VEGF receptors, VEGFR-1 and VEGFR-2 are also expressed in myometrium [10], leiomyomas and in cellular leiomyomas [9]. A stronger VEGF expression was found in leiomyomas than in adjacent myometrium, indicating that local angiogenesis may be important for the development and growth of these tumours [11] Hong et al. (2001) reported that VEGF is significantly expressed in leiomyosarcoma compared with leiomyoma. VEGF stimulates angiogenic activity which is responsible for actively growing tumours and may enhance the growth of fibroids and disease progression in many carcinomas [12,13,14]. Both E2 end P4 induce VEGF expression in the rodent uterus[15].

Materials and Methods

This study was conducted at the Meenakshi Medical College and Research Institute Hospital, a rural tertiary care hospital. The Institutional Medical Ethics Committee approved this study. Fifty cases were selected from August 2015 until July 2017 and it was diagnosed by a single histopathologist to avoid inter-observer variations.

Uterine leiomyomas and adjacent normal myometrial tissues were obtained from women with regular menstrual cycles who underwent abdominal hysterectomy for medically indicated reasons at Meenakshi Medical College and Hospital. The mean age group was 39 years, and none had received hormonal therapy for at least three cycles before surgery. Informed consent was obtained from each patient before surgery for the use of uterine tissues for the present studies. Each uterine specimen was examined by a pathologist for histological examination and dating of the endometrium. Endometrial tissues were obtained from the extirpated uteri, and the day of the menstrual cycle was determined by endometrial histological dating according to the method of Noyes et al. and the patient's last menstrual period. Histological features of all cases studied with hematoxylin and Eosin. A total of 50 uterine leiomyomas and myometrial tissues were collected of which 50% were from proliferative phase and 50% from secretory phase of menstrual cycle.

Inclusion Criteria

Cases from August 2015 to July 2017 were selected.

Exclusion Criteria

Samples were excluded if accurate menstrual cycle dates could not be assigned, if unexpected pathology was found eg - endometrial hyperplasia and leiomyoma variants.

Immunohistochemical Study

Haematoxylin and Eosin slides of all the tissues were evaluated, and for each case the best paraffin block with highest tumour content were chosen in order to prevent artefacts staining.

These formalin fixed, paraffin embedded blocks were sliced in 3-4 µm thickness for IHC. The Avidin Biotin complex (ABC) detection system was used on specimens of formalin fixed, paraffin embedded tissue section.

Scoring System

Immunoreactivity was regarded as positive when brown staining was localized in the cytoplasm of leiomyoma cells. The intensity of immuno staining was evaluated by repeated staining of the same specimens.

Grading

(-) : No immunostaining

(1+) : Weak but definitely detectable immunostaining

(2+) : Moderate immunostaining

(3+) : Intense immunostaining

The extent was semiquantitatively estimated with a range of 0% to 100%. Percentages were estimated by counting at least 50 cells and then establishing the ratio of immunoreactive cells to total number of cells multiplied by 100; percentages were rounded to the nearest 10%. When less than 10% of cells were positive a score of 0 was used, 10 % to 30% cell positivity was scored as 1 , 31% to 60% positivity was scored as 2 , and more than 60% positive cells was labelled as 3.

Statistical Analysis

Statistical analysis was carried out using SPSS version 19.0 (IBM SPSS, US) software with Regression Modules installed. All data was entered into a Data Collection Proforma Sheet and were entered into Excel (MS Excel 2010). Results are represented in the form of tables and bar charts.

Results

A total of 50 uterine leiomyomas and myometrial tissues were collected of which 25 were from proliferative phase and 25 from secretory phase of menstrual cycle. Hysterectomy specimen with endometrial hyperplasia, leiomyoma variants, ovarian pathology and cervical pathology were not included in the study. Immunohistochemical study was done using VEGF antibody on all 50 cases selected.

Leiomyomas are seen in the women of child bearing age, most commonly occurring in the 4thdecade. The mean age being 39 years. The youngest patient in our study was 25 years old, and the oldest was 54 years old.

Though leiomyoma is a disease of low parity, in our study we have noted it to be more common in para-II women.

In our study, most of the patients presented with menstrual disturbances followed by pain abdomen and dysmenorrhea. Rare symptoms included vaginal mass and infertility. In our study it was noted that the size of the fibroid uterus varied from a few centimeters to 30 weeks of gravid uterus. It is seen that, about 38% were of the size of 16 weeks gravid uterus, 42% were of the size between 16-20 weeks, and huge fibroids of >20 weeks were encountered in 20% of the patients. The heaviest fibroid weighed 3.5 kg.

All the leiomyomata were corporeal, no extrauterine fibroids were encountered. Among the uterine about 98% were in the body & 2% were in cervix. Intramural fibroid was the commonest variety comprising about 74% of the cases, 10% submucous, 14% subserous and 2% cervical fibroid was encountered

In our study we selected our cases in accordance to proliferative and secretory phases of normal menstrual cycle. All other pattern of endometrium was excluded. For our case we selected 50% of each proliferative and secretory phase.

Table 1: VEGF Expression in Leiomyoma & Myometrium (Proliferative phase)

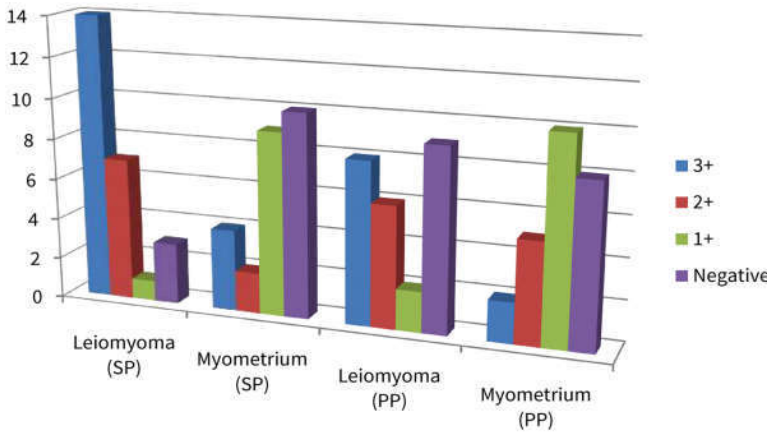
S. No	Cases	VEGF Antibody	
		Positive	Negative
1	Leiomyoma	65%	35%
2	Myometrium	70%	30%

Table 2: VEGF Expression in Leiomyoma & Myometrium (Secretory phase)

S. No	Cases	VEGF Antibody	
		Positive	Negative
1	Leiomyoma	90%	10%
2	Myometrium	68%	32%

Table 3: Comparison of intensity of VEGF expression in Leiomyoma and Myometrium in Secretory phase

S. No	Intensityof VEGF antibody	Proliferative phase	
		Leiomyoma	Myometrium
1	3+	8	2
2	2+	6	5
3	1+	2	10
4	Negative	9	8



Graph 1: Comparison of intensity of VEGF staining in leiomyoma and myometrium in both phases of menstrual cycle

Table 4: Comparison of intensity of VEGF expression in Leiomyoma and Myometrium in Secretory phase

S. No	Intensity of VEGF Antibody	Secretory phase	
		Leiomyoma	Myometrium
1	3+	14	4
2	2+	7	2
3	1+	1	9
4	Negative	3	10

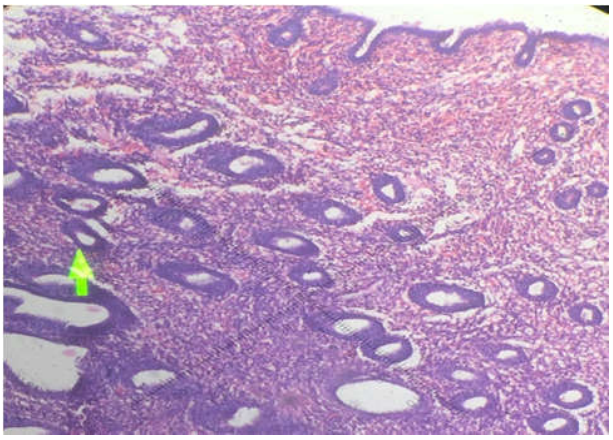


Fig. 1a: Photomicrograph of Proliferative phase (H&E, magnification 400X)

Fig. 1b: Photomicrograph of VEGF expression in leiomyoma in proliferative phase (IHC, magnification 400X)

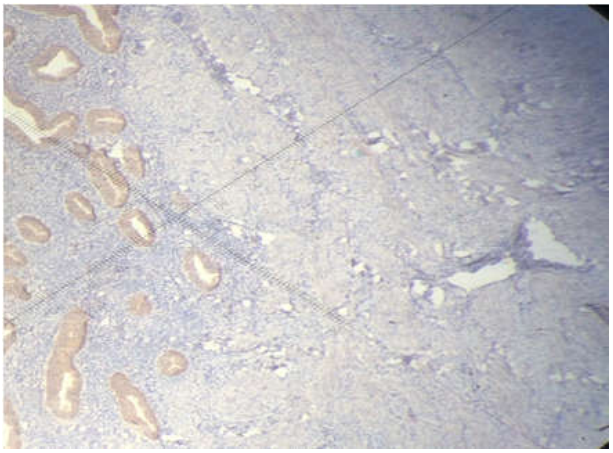


Fig. 1c: Photomicrograph of VEGF expression in myometrium in proliferative phase (IHC, magnification 400X)

Leiomyoma cases showed 65% positivity while normal myometrium showed 70% of positivity (Table 1),(Figure 1a,1b,1c). Thus indicating that VEGF expression remained unchanged in leiomyoma and myometrium.

Leiomyoma cases showed 90% positivity while normal myometrium showed 68% of positivity in secretory phase (Table 2),(Figure 2a, 2b, 2c). Thus indicating that VEGF expression was more prominent in leiomyoma than myometrium.

Comparison of immunohistochemical staining for VEGF protein showed that leiomyoma cells in the secretory phase of the menstrual cycle showed more predominant immunostaining than the leiomyoma cells in the proliferative phase. However, there was not much difference in the intensity of immunostaining for VEGF protein in myometrial smooth muscle cells between the proliferative phase and the secretory phase of the menstrual cycle (Table 3, 4) (Graph 1).

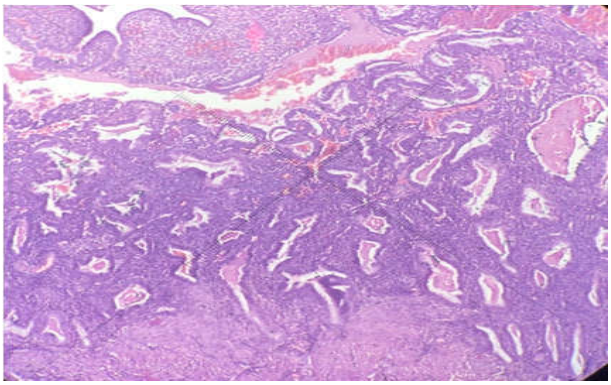


Fig. 2a: Photomicrograph of secretory phase (H&E, magnification 400X)

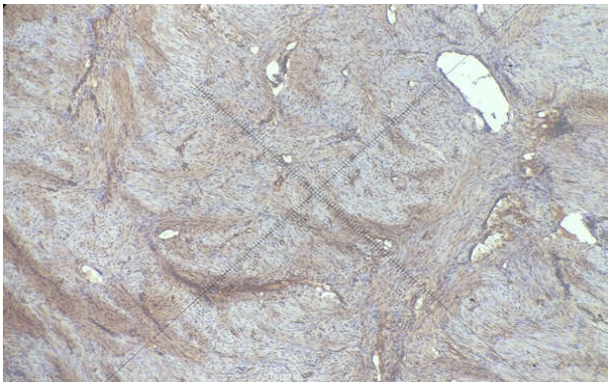


Fig. 2b: Photomicrograph of VEGF expression in leiomyoma in secretory phase (IHC, magnification 400X)

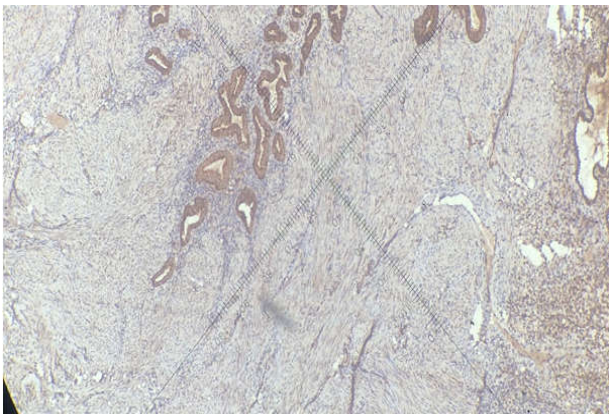


Fig. 2c: Photomicrograph VEGF expression in myometrium in secretory phase (IHC, magnification 400X)

Discussion

Immunohistochemical staining comparison of VEGF showed that leiomyoma cells showed intense immunostaining during secretory phase than during the proliferative phase. However there was no difference in the immmunostaining pattern of VEGF protein in the myometrial smooth muscle cells between the proliferative and secretory phase of the menstrual cycle.

Lewicka et al, found that VEGF expression was found to be increased in the leiomyoma cells in patients with endometrium in the secretory phase compared to patients with endometrium in proliferative phase. It was also revealed that VEGF expression was 2.03 fold higher in those with myomas than those with healthy myometrium. This suggested that VEGF may play a significant role in the pathogenesis of uterine leiomyomas.

In a study by Gentry et al, it was found that VEGF was expressed in 14 out of 18 leiomyoma sections from women without GnRH pretreatment, and in 15 out of 18 of those women with prior treatment. VEGF expression in the adjacent myometrium was much lower being noted in 2 out of 18 sections from women without prior GnRH treatment and 1 out of 18 in women with prior GnRH pretreatment. Moreover, when VEGF expression was present, expression was strong in leiomyomas, but not in adjacent myometrium. The differential expression of VEGF antigen in leiomyomas compared with adjacent myometrium indicates that local angiogenesis may be important in the development and growth of these tumours. GnRHa therapy does not appear to alter this pattern of VEGF expression.

Studies done by Dixon et al, showed that VEGF protein was seen in the cytoplasm of smooth muscle cells of leiomyomas and matched myometrium. In a study by Hyder et al, it was found that VEGF was expressed more during the oestrogenic phase than the progesterone phase of the menstrual cycle. In a study by Sanci et al, it was found that EGFR had mild to moderate immunoreactivity in leiomyoma cells compared to a lesser staining pattern in cellular leiomyoma but a strong immunoreactivity was noted in cells of leiomyosarcoma.

In the same study, it was found that VEGF had moderate immunostaining pattern compared to a lesser staining pattern in cellular leiomyoma in contrast to a stronger immunoreactivity in leiomyosarcoma.

All these results suggests that in both leiomyoma and leiomyosarcomas, angiogenesis factors such as VEGF and its receptors may be involved in tumour angioigenesis.

Conclusion

Based on our data on VEGF staining in leiomyoma and normal myometrium in relation to secretory and proliferative phase of menstrual cycle and the results from the literature on the expression of VEGF we conclude there is an abundance of VEGF expression in leiomyoma relative to the normal myometrium of the same individual uterus and that VEGF protein expression in leiomyoma cells predominated in the secretory phase than the proliferative phase, though there was not much of significant difference in number was noted. Therefore, both oestrogen E2 and progesterone P4 seemed to induce the tumour growth. VEGF expression was significantly higher in those with myomas compared to those with a healthy myometrium suggesting that VEGF may play a significant role in pathogenesis of uterine myomas. These suggest that VEGF and other angiogenic factors may represent potential targets for the treatment and prevention of uterine fibroids.

The limitations of our study using immunohistochemical analysis alone should be acknowledged; for completion, it may require further validation using other methods including cell culture, immunoblotting, molecular and cytogenetic methods for understanding the underlying molecular mechanisms that determine VEGF over-expression and other pathways in development of uterine smooth muscle tumors. It is worth mentioning that, our results may contribute to clarify the sequential evolution in the development and treatment of uterine leiomyoma.

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