Original Research Article

Expression and sensitivity of immunohistochemical markers SMA, Desmin, CD10 for uterine leiomyomas - A tertiary care centre experience

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Abstract

Introduction: Leiomyoma is the most common benign tumor of the uterus which usually presents with menorrhagia, pain in abdomen or both. In extremely rare cases where uterine leiomyoma can be difficult to distinguish from other uterine smooth muscle tumors, immunohistochemistry is used. This study was aimed to study the expression and sensitivity of immunohistochemical markers SMA, Desmin, CD 10 for uterine leiomyomas and to find average number of mitosis in uterine leiomyomas using Ki 67.

Materials and methods: The present study was carried out in the Department of Pathology, Dhiraj General Hospital and Smt. Bhikhiben Kanjibhai Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth, Piparia. A total 50 cases of uterine leiomyomas after its histological diagnosis were evaluated with immunohistochemical markers SMA, Desmin, CD 10 and Ki 67.

Results: SMA expression was seen in all 50 cases of uterine leiomyomas with strong expression in 44 cases (88%). Strong SMA expression was seen more in usual leiomyomas as compared to leiomyomas with secondary changes. Desmin expression was also seen in all the 50 cases of uterine leiomyomas with moderate expression in 26 cases (52%). Weak CD 10 expression was seen in 15 cases of uterine leiomyomas (30%). Ki 67 was expressed very focally in only 3 cases of leiomyomas with mean value of only 0.3% tumor cells.

Conclusions: Leimyomas was most frequently seen in the women in 4th decade. The most common clinical presentation was menorrhagia. SMA and Desmin expression was seen in all the cases with strong and moderate immunoreactivity respectively. SMA expression was found to be more specific than Desmin in uterine leiomyoma. Weak CD 10 and focal Ki 67 were expressed only in few cases and were found to be insignificant.

Key words

Leiomyoma, SMA, Desmin, CD 10, Ki 67.

Introduction

Of all Smooth muscle tumors originating from uterus are leiomyomas (Fibroid) are the most common ones. Benign nature and smooth muscle origination of leiomyomas can be easily documented via histological examination [1]. It occurs in one of every fourth to fifth women of reproductive age group. Leiomyomas are of myometrial origin. Unfortunately, symptomatology is variable and their symptoms depend on the number, size and location of the fibroid. Due to its wide variety of clinical symptoms like menorrhagia, pain in abdomen, urinary disturbances, infertility etc it's one of the major health burdens on the women of our society.

Immunohistochemistry is rarely required for diagnosis and characterization of leiomyoma because it is very common and all pathologists are familiar with its microscopic features.

Various investigators have used wide variety of antibodies such as SMA, Desmin, CD 10, Ki 67, ER, PR, h-caldesmon, vimentin, calponin etc for analyzing the expression of these makers for leiomyomas and in some cases to differentiate leiomyomas from other uterine tumors. In the present study we have included limited number of antibodies like SMA, Desmin, CD 10 and Ki 67 to find the frequency of their expression for leiomyomas.

Smooth muscle actin (SMA) is a protein in humans which is encoded by ACTA gene located on 10q22-q24. Antibodies to SMA recognizes only alpha smooth muscle isoform of actin. It stains smooth muscle cells of the myometrium. SMA is highly sensitive in tumors arising from smooth muscle as leiomyoma.

Desmin is muscle specific type III intermediate filament protein which is encoded by DES gene in humans. Antibodies to desmin do not cross react with other intermediate filaments and stains both striated as well as smooth muscle. It confirms myogenic origin of tumor like leiomyoma.

CD 10 is used as a mesenchymal marker which normally stains the endometrial stroma and do not stain leiomyoma but exhibit focal positivity in some of the leiomyoma.

Ki 67 expression is useful to find out the average number of mitosis in leiomyomas which is usually less than 5cells/10hpf. Strong Ki 67 expression is seen in more than 15 cells/10hpf in leiomyomas.

This study is an attempt to analyze the sensitivity of SMA, Desmin and CD 10 and to find out average number of mitosis using Ki-67 in cases of leiomyomas of uterus received in the department of Pathology.

Materials and methods

A prospective, non interventional and observational study of 50 cases of Leiomyomas of uterus was conducted at Dhiraj General Hospital and Smt. Bhikhiben Kanjibhai Shah Medical Institute and Research Centre, Sumandeep Vidyapeeth, Piparia.

All the leiomyomas were evaluated histopathologically and for SMA, Desmin, CD 10 and Ki 67 expression. Poorly preserved specimens and leiomyomas from any other site except the uterus were excluded from our study.

Details of all patients including serial number, hospital registration number, age, sex, clinical diagnosis, ultrasonography diagnosis and any other special notes were obtained from the case files or from the attending clinicians or by personal communication with the patients and her relatives. The data were recorded for later analysis.

All leiomyomas specimens were processed in the histopathology section of Pathology Department. The specimen were processed in the tissue processor, paraffin blocks were made, 3-5 micron thick sections were prepared & stained with routine Haematoxylin & Eosin staining method [2, 3]. The slides were examined microscopically to identify the histomorphological features of leiomyomas.

Representative sections on specially treated slides were done to demonstrate SMA, Desmin (**Photo - 1, 2**), CD 10 and Ki 67 antigen expression immunohistochemically, using Envision flex IHC kit purchased from DAKO. The results were recorded, analyzed and tabulated.

Photo – 1: showed strong SMA positivity in Leiomyoma (10X, IHC stain).

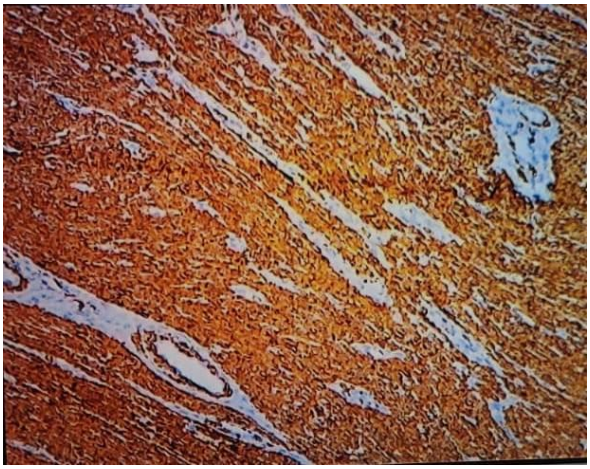
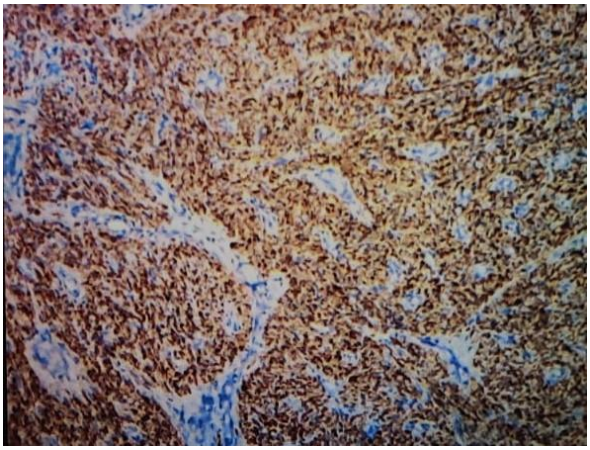


Photo – 2: showed Desmin positivity in Leiomyoma (10X, IHC stain).



Results

The present study was prospective and observational in nature. The study comprises of 50 female patients having leiomyoma of uterus admitted in gynaecology ward. All 50 cases were evaluated histologically. Immunohistochemistry was done by using markers SMA, Desmin, CD 10 and Ki 67. Patient's clinical data were collected from case files or by direct communication with the patients.

In this study, patients with leiomyoma were aged between 20 and 60 years with a mean age of 40.68-7.33 of which majority of patients were in the 4^{th} and 5^{th} decade of life. Forty six percent (46%) patients were in the age group of 41-50 years and 36% were in the age group of 31-40 years (**Table - 1**).

Table – 1: Age distribution.

Age in years	Numbers (n=50)	Total %
21-30	6	12%
31-40	18	36%
41-50	23	46%
51-60	3	6%

In the present study, 32% patients with leiomyoma had only menorrhagia which was the most common symptom, followed by pain in abdomen (28%). Pain in abdomen and menorrhagia together constituted (26%) of patients. Mass per abdomen (8%), amenorrhoea

(4%) and mass per vagina (2%) comprised the rest. In all 86% of the patients either presented with menorrhagia alone or pain in abdomen as sole symptom or both together (**Table - 2**).

Table - 2: Clinical presentation of all individuals included in study groups (n=50).

Clinical presentation	Numbers (n=50)	Total %
Menorrhagia	16+13	32+26=58
Pain in Abdomen	14+13	28+26=54
Pain in Abdomen + Menorrhagia	13	26
Mass per Abdomen	4	8
Amenorrhoea	2	4
Mass per Vagina	1	2

Immunohistochemical analysis of leiomyoma was performed using SMA, Desmin, CD 10 and Ki 67 markers. Hundred percent of (100%) leiomyomas expressed SMA and Desmin whereas 30% leiomyomas expressed focal positivity for CD 10 and 6% leiomyomas expressed weak positivity for Ki 67 (**Table - 3**). SMA was expressed strongly, Desmin moderately whereas CD 10 and Ki 67 were weakly expressed in few cases and negative in most of the other cases.

Highest levels of expression were found in SMA (87.22%) followed by Desmin (60.26%). Very few cases expressed CD 10 (5%) and Ki 67 (0.3%). Furthermore SMA and Desmin expressions were found significant with P value <0.0001 and <0.0001 respectively whereas CD 10 and Ki 67 expression was found to be statistically insignificant. (P value 0.832) (**Table - 4**).

Table - 3: Expression of SMA, Desmin, CD 10 and Ki 67 in all individuals included in study groups (n=50).

Variables	Strong		Moderate		Weak		Negative	
	No.	%	No.	%	No.	%	No.	%
SMA	44/50	88	6/50	12	0/50	0	0/50	0
Desmin	18/50	36	26/50	52	6/50	12	0/50	0
CD 10	0/50	0	1/50	2	14/50	28	35/50	70
Ki 67	0/50	0	0/50	0	3/50	6	47/50	94

Table - 4: Expression of SMA, Desmin, CD 10 and Ki 67 in all individuals included in study groups (n=50).

Variables	SMA	Desmin	CD 10	Ki 67
Sample size	50	50	50	50
Mean value (%)	87.72	60.26	5	0.3
SD	3.25	8.53	2.58	0.34
P value	<0.0001	<0.0001	0.0003	0.832

SMA was strongly expressed in 29 cases of usual leiomyoma as compared to 16 cases of leiomyoma with secondary changes. Moderate expression was found in 3 cases of both types of leiomyoma. Sensitivity of SMA was 100%.

Desmin was strongly expressed in 13 cases of usual leiomyoma as compared to 5 cases of leiomyoma with secondary changes. Moderate expression was found in 14 cases of usual leiomyoma as compared to 12 cases with secondary changes. Sensitivity of desmin was 88%.

CD 10 expression was found in 15 cases whereas Ki 67 was expressed in 3 cases but since

expressions were very weak in case of both markers (<5%), sensitivity could not be derived. SMA expression was significantly high in all cases of leiomyoma as compared to desmin. (P<0.0001) (**Table - 5**).

Table - 5: Comparison was SMA and Desmin expression in all individuals having leiomyoma (n=50).

Variables	SMA	Desmin
Sample size	50	50
Mean value (%)	87.72	60.26
Standard Deviation	3.14	7.47
P value	P<0.0001	

Discussion

Fifty cases of leiomyomas after histopathological examination were evaluated by SMA, Desmin, CD 10 and Ki 67 expression. In the following paragraphs our findings are discussed in view of these of other studies in the field.

Mannem Chethana, et a1. (2010) found that 84% of the females with leiomyomas were in the 4th and 5th decade of life [4]. Dr. Jyoti Nayak, et al. (2012) found that majority of paients with leiomyomas were in 5th decade [5]. Rosario Pinto (1968) found that majority of the cases of leiomyomas were in the 4th & 5th decade with 44.77% in the 31-40 age group and 41.31% in the 41-50 age group [6]. In the present study 46% patients were in 5th decade of life and 36% were in 4th decade of life. Our findings concurred with those of Mannem Chethana, Dr. Jyoti Nayak and Rosario Pinto. These findings dupport the fact that leiomyomas is most prevelant in 4th & 5th decade of life.

Mannem Chethana in her study found that Menorrhagia was the commonest symptom (58%), followed by pain in abdomen (26%) [4]. Dr. Jyoti Nayak found Menorrhagia to be the commonest symptom (40.54%), followed by pain in abdomen (27.02%) [5]. In the present study, Menorrhagia was found to be the most common symptom in patient with leiomyoma (32%), followed by pain in abdomen (28%). Both the symptoms individually or alone were present in 86% of the cases. Our findings concurred with those of Mannem Chetana and Dr. Jyoti Nayak. These findings support the fact that menorrhagia is the commonest presentation of leiomyoma of uterus followed by pain in abdomen.

Liao X evaluated 20 cases of highly celluar leiomyoma and 21 cases of endometrial stromal tumors using Desmin, CD 10 and other antibodies. He found moderate Desmin expression in 19 of 20 cases of leiomyomas where as in 14 of 21 cases of endometrial stromal sarcomas [7]. Peiguo G Chu in her study found moderate Desmin cytoplasmic immunoreactivity in all 10 cases of uterine leiomyomas whereas weak to moderate expression was seen in 8 out of 16 cases of endometrial stromal sarcomas [8].

In the present study, Desmin expression was seen in all 50 cases of uterine leiomyomas with moderate expression in 26 cases while 18 cases showed strong expression with mean value of 60.26%. Our study findings were similar to the studies of Olivia E, Liao X and Peiguo G. Thus from the above findings we can support the fact that Desmin expression is seen in almost all cases of leiomyomas with moderate immunoreactivity. Desmin expression is seen only in 50% cases of endometrial stromal tumors [8].

Peiguo G Chu studied 10 cases of uterine leiomyomas, 16 cases of endometrial stromal sarcomas and 8 cases uterine leiomyosarcomas using SMA, Desmin, CD 10, ER and inhibin. SMA expression was seen strongly in all cases of leiomyomas and in leiomyosarcomas but in endometrial stromal sarcomas only 7 cases out of 16 showed SMA expression [8].

In the present study, SMA expression was seen in all 50 cases of uterine leiomyomas with strong expression in 44 cases with mean value of 87.72% tumor cells. Our findings were similar to studies by Anca Daniela Stanescu [9]. Zhu XQ

[10] and Peiguo G Chu [8]. Thus from our study we can support the fact that strong SMA expression is seen in all the leiomyomas. SMA expression is seen in 43.75% endometrial stromal sarcomas, making it useful marker to distinguish leiomyoma from endometrial stromal sarcomas (**Table – 6**).

Table – 6: Comparison of SMA expression in leiomyomas in various studies.

Various studies	No of cases expressed	Mean value
Anca Daniela Stanescu [9]	100%	92.6%
Zhu XQ [10]	100%	-
Peiguo G Chu [8]	100%	-
Present study	100%	87.72%

In the present study, focal Ki 67 expression was seen in 3 out of 50 cases of leiomyomas. Thus our study showed concurrence with Mayerhofer and Mittal K. Hence Ki 67 is either not expressed or insignificantly expressed in leiomyomas. Thus the level of Ki 67 expression helps in differentiating leiomyoma from leiomyosarcoma [11, 12].

In our present study, CD 10 expression was seen in 15 cases of uterine leiomyomas out of which 14 cases showed weak immunoreactivity with mean value of 5%. Our findings were almost similar to those of Peiguo G Chu and of Olivia E [13]. Thus from our study we can conclude that CD 10 expression is seen in only few cases of leiomyomas with weak immunoreactivity differentiating it from endometrial stromal tumors which show strong CD 10 expression in all the cases (**Table – 7**).

Table – 7: Comparison of CD 10 expression in leiomyomas in various studies.

Various studies	No of cases expressed	Mean value
Peiguo G [8]	20%	<5%
Olivia E [13]	50%	-
Present study	30%	5%

In short, when some other tumors enter the differential diagnosis of uterine leiomyomas, immunohistochemical profile will help in differential diagnosis. In usual practice, such situations exist but will clearly be exceptional.

Conclusion

Leimyomas was most frequently seen in the women in 4th decade. The most common clinical presentation was menorrhagia. SMA and Desmin expression was seen in all the cases with strong and moderate immunoreactivity respectively. SMA expression was found to be more specific than Desmin in uterine leiomyoma. Weak CD 10 and focal Ki 67 were expressed only in few cases and were found to be insignificant.

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