Indian J.Sci.Res. 7 (1): 347-351, 2014

ISSN: 0976-2876 (Print)
ISSN: 2250-0138(Online)

STEREOLOGICAL STUDY OF HISTOPATHOLOGICAL INDICES OF UTERINE LEIOMYOMA IN PRE AND POST-MENOPAUSAL PERIOD

ZAHRA HEIDARI[a], HAMIDREZA MAHMOODZADEH-SAGHEB[b], NARJES DAHMARDEH[c1] AND AZAM ASEMI-RAD[d]

[ab]Genetic of non-communicable Diseases Research Center Zahedan University of Medical Sciences, Zahedan, Iran
[cd]Department of Histology, Faculty of Medicine, Zahedan University of Medical Sciences, Zahedan, Iran

ABSTRACT

Stereological study of histopathological indices of uterine leiomyomas in pre and post-menopausal periods. In this descriptive-analytical study, pre and post-menopausal leiomyoma samples (n= 10 per group) were collected from women who have undergone hysterectomy or myomectomy in Emam Ali hospital, Zahedan, Iran. From each tumor three slices were selected using SURS method and embedded in paraffin wax. Then 10-15 SURS sections were selected from each block and stained with Masson's Trichrome. The length and width of the smooth muscle cells (SMC) and their nuclei calculated and Volume of cytoplasm, nucleus and N/C ratios of SMCs, volume density of muscular and connective tissue (CT) and blood vessels were estimated using Cavalieri's principle. Data were analyzed using non-parametric Mann-Whitney U test. ($p<0.05$) was considered as significant level. The length and width of the SMC and its nucleus, volume of cytoplasm and nucleus of SMC in post-menopausal showed a significant decrease ($p<0.05$). Volume fraction of muscular tissue and vessels were significantly increased in post-menopausal tumors ($p<0.05$). A significant decrease was observed in the volume of CT in post-menopausal group ($p <0.001$). The stereological changes of uterine leiomyomas in post-menopause period, could explain the reduction of tumor size after menopause.

KEYWORDS: Uterine Leiomyoma, Stereology, Menopause, Smooth Muscle Cell

Uterine leiomyomas are commonest benign tumors of female genital tract [1] and occur in 20-25 percent of women of reproductive age [2]. Microscopically, uterine leiomyoma are characterized by bundles of SMC connected to a large amount of fibrous connective tissue [3]. Almost 20-30% of women over 35 years old experience uterine myoma which is diagnosed clinically [4]. Over 60% of Afro-American and about 40% of white non-pregnant 35-years old women have leiomyomas that can be diagnosed via imaging [5]. In case of patients presenting symptoms, the commonest complaints include abnormal uterine bleeding, iron deficiency anemia, increase in the volume of menstrual blood, and menstrual disorders [1]. Mechanism of bleeding related to uterine leiomyomas is still unknown [6].

Leiomyomas are estrogen-dependent tumors. Their growth is related to estrogen cycle [7]. Throughout post-menopausal period or other estrogen-shortage states the growths of these tumors slows down [8]. During reproductive period, the maximum growth of leiomyomas occurs when estrogen secretion is in highest level [7]. The first treatment for uterine fibroids is surgery as myomectomy or total hysterectomy [6]. 600000 hysterectomies are reported annually in U.S [7]. Leiomyomas are usually reduced to half of their original size in post-menopausal period [6].

Their rapid growth in young women who do not have children and old women in post-menopausal period can be a sign of malignancy [7]. Painful leiomyomas in post-menopausal women should be considered as leiomyosarcoma [6]. No clinical finding or imaging modality can reliably distinguish leiomyomas from leiomyosarcomas. The diagnosis of uterine leiomyosarcoma is based on its histological properties rather than appearance [9]. The similarity of clinical symptoms of leiomyosarcoma to common uterine leiomyomas poses difficulties for its pre-operative diagnosis. The diagnostic problems of these tumors have been partly eliminated by histological investigations, immunohistochemical studies and molecular pathology [10]. In recent years, novel technologies, modern image analysis systems and stereological techniques have been able to present valid relations via diagnostic criteria of tumors [11]. In this regard, considering lack of quantitative data on cellular-histological features of these tumors, in this study, it was tried to compare the quantitative cellular parameters, such as the volume of cytoplasm, the volume of nucleus, and nucleus to cytoplasm (N/C) ratio and stereological parameters of uterine leiomyomas including the volume fraction of

[1]**Corresponding author**

fibrous connective tissue, smooth muscle tissue, and blood vessels between pre and post-menopausal period.

MATERIALS AND METHODS

In this cross-sectional, descriptive-analytical study, 10 samples of pre and post-menopausal uterine leiomyoma were collected from women who have undergone hysterectomy or myomectomy at Emam Ali hospital, Zahedan, Iran. From each sample 3 slices were taken using systematic uniform random sampling (SURS) method. The slices were processed and embedded in paraffin wax. To make sections, the first section where microtome blade completely touched leiomyoma tissue was selected as the first random section. Then in 40 µm intervals, 4 µm-thick sections were prepared from each block. 10-15 sections were selected via SURS and stained using Masson's trichrome method [12].

Then, the stereological grid with organized points was thrown on the projected histological images and the volumes of each component were estimated using Cavalieri's principle. On each sampled section five to seven fields were selected in a SURS manner by movement of the microscope's stage in X and Y directions with the aid of vernier scale of the stage of a projection microscope (Olympus, Japan) [13]. The SMC are fusiform and their longitudinal axis parallels to the axis of their elliptical nucleus. To measure the length and width of the SMC and their nucleus, the longitudinal (x) and transverse (y) axes were drawn on stereological grade and then, using a digital caliper the measurement of SMCs axes was done on projected histological pictures [14].The volume weighted mean volume (Vv) of SMC and its nucleus was estimated using the mean diameter of SMC and nucleus (length and width) by this formula:

$$\text{est}V_v = \frac{\pi}{3} \cdot \ell_0^3 \cdot F$$

Where, Vv is the volume weighted mean volume, l_0^3: the mean of the cubed measured length (diameter) and F is (1/Magnification)3.

To calculate the relative volume of muscle tissue and CT and blood vessels following formula was used:

$$Vv = \frac{P(part)}{p(ref)} .100(\%)$$

Where, P (part) and P (ref) are the number of test points falling in tissue components, and the reference space, respectively [15].

Data were analyzed using SPSS-19 and the non-parametric Mann-Whitney U test. A value of ($p<0.05$) was considered significant.

RESULTS

The mean age of pre-menopausal group was 35±5.8, while the mean age of post-menopausal group was 50.2±1.61. The mean length and width of SMC in uterine leiomyomas in post-menopausal samples showed a significant decrease compared to pre-menopausal group ($p<0.05$). There was a significant decrease in the mean width and length of nuclei of SMC in post-menopausal uterine leiomyomas compared to pre-menopausal samples ($p<0.05$). The cytoplasm volume of smooth muscle cells and the volume of their nuclei in post-menopausal leiomyomas were significantly smaller than pre-menopausal group ($p<0.05$) (Table 1). SMC and its nucleus diameter in post-menopausal leiomyomas was smaller than pre-menopausal group. The volume fraction of muscle tissue in post-menopausal uterine leiomyomas showed a significant increase relative to pre-menopausal samples ($P<0.05$). There was a significant decrease in the volume fraction of CT of post-menopausal uterine leiomyomas compared to pre-menopausal groups ($p<0.05$). The volume fraction of blood vessels of post-menopausal uterine leiomyomas was significantly higher than that of pre-menopausal group ($p<0.05$) (Table 1).

Table 1: Histological indices of pre and post-menopausal uterine leiomyomas

	Pre-menopause	Post-menopause	p-Value
Width of SMC(μm)	46.07±0.06	35.19±0.7	0.001**
Length of SMC(μm)	266.9±10.3	108.6±2.3	0.001**
Width of SMC nucleus(μm)	19.82±0.6	16.59±0.2	0.001**
Length of SMC nucleus(μm)	93.44±1.8	43.82±0.8	0.001**
SMC mean diameter(μm)	52.14±1.98	27.26±0.59	0.001**
nucleus mean diameter(μm)	21.75±0.39	11.19±0.19	0.001**
Volume of SMC nucleus(μm3)	11370.8±801.01	1503.6±70.3	0.001**
Volume of SMC cytoplasm(μm3)	141626.9±18431.6	19342.3±1178.6	0.001**
Volume of SMC(μm3)	170989.2±19595.7	21133.9±1169.2	0.001**
Volume fraction of MT (%)	60.48±1.96	82.81±0.84	0.0001**
Volumefraction of CT (%)	37.53±3.08	17.17±0.84	0.001**
Volume fraction of blood vessels (%)	5.66±0.36	9.98±0.76	0.001**

Abbreviation: MT, muscle tissue.
Values are given as mean ± SD.

DISCUSSION

Based on the findings of this study, the length and width of SMC and volume weighted volume of SMC and its nucleus, in post-menopausal uterine leiomyomas showed significant decrease compared to those of pre-menopausal leiomyomas. The volume fraction of blood vessels in post-menopausal leiomyomas is significantly higher than pre-menopausal group. Significant increase in the volume fraction of muscle tissue of post-menopausal leiomyomas was observed compared to pre-menopausal leiomyomas. The volume fraction of CT in post-menopausal uterine leiomyomas showed a significant decrease relative to pre-menopausal groups.

Cramer et al. investigated the size of myoma cells according to state of menopause. Their study revealed that the size of myoma cells in post-menopausal group is significantly smaller than pre-menopausal group. Also, the proportion of nucleus size to cytoplasm was significantly larger in the myoma of pre-menopausal women. Recent studies have shown that destruction of fibrous tissue in leiomyomas can be the main factor affecting the contraction of uterine leiomyomas post menopause [16]. The findings of the present study are in line with outcomes of two studies cited above. Decrease in the volume fraction of connective tissue observed in our study can be in this argument. In any account, dependence of leiomyomas to sex hormones indicates that any change in these hormones, particularly estrogen, can play a significant role in their growth or decline [17]. Thus, the women are exposed to lack of estrogen and decline of sex hormones in post-menopausal period [7] which can be a reason for the small size of uterine leiomyoma in post-menopausal period compared to pre-menopausal samples. This atrophy is on smooth muscle cells and connective tissue, but it is more obvious in CT.

In the present study, the volume fraction of blood vessels in uterine leiomyoma was significantly larger in post-menopausal group. In a study conducted by Aitken et al. stereological methods were used for investigating blood vessels of uterine leiomyomas and normal myometrium. The findings revealed thick blood vessel walls in normal myometrium while they were not observed in small leiomyomas. The tissues of leiomyomas involved thin vascular walls [18].

Weston et al. studied blood vessels of uterine leiomyomas in pre and post-menopausal women. They indicated that the density of myometrial vessels increased in post-menopausal period while the density of vessels in leiomyomas remains unchanged compared to pre-menopausal group. Their findings showed that increase in vascular density of myometrium in post menopausal period might lead to complete increase in vascular compartments [19]. On the other hand, many studies suggest that stimulating effects of estrogen might be exerted on the growth of leiomyomas through cytokines, growth factors or apoptotic factors. It has been shown that endothelial growth factor and its receptors play a role in the growth of leiomyomas and stimulating angiogenesis in these tumors [20]. Recently, angiogenesis and perfusion are considered as factors controlling the growth tumors, especially malignant ones [21]. Growth factors contribute to pathology of leiomyoma and regulating angiogenesis which is essential for tumor growth [22]. The endothelial growth factor A affects leiomyoma's

growth by accelerating formation of new vessels feeding the tumor. Since using estrogen-reducing factors in leiomyoma does not suppress this factor, it appears that it activity is not estrogen-dependent [23]. Increasing expression of vascular endothelial growth factor has been shown in uterine leiomyosarcomas compared to uterine leiomyomas [24]. Since uterine leiomyosarcomas usually occur in post-menopausal period, and increasing expression of vascular endothelial growth factor has been proved in this period, the vascularization of uterine leiomyomas in post-menopausal period observed in this study can be justified. One of the limitations of this study was the small sample size. It is suggested that studies be done in several clinics on a larger number of pre and post-menopausal patients with uterine leiomyomas. Also, it is recommended to compare stereological indices of these cases with pre and post-menopausal leiomyosarcomas so that the morphological features of uterine tumors can be investigated more comprehensively and malignancy parameters can be traced, and a treatment and follow-up program can be developed according to the trend of the illness.

ACKNOWLEDGEMENT

The authors are grateful of the helps of Research and Technology Deputy, Zahedan University of Medical Sciences which made this study possible. We also thank the respected staff of Histology Department, Faculty of Medicine who sincerely cooperated in the process of this study.

Conflict of interest

The authors have no conflicts of interest.

REFERENCES

Weintraub J, Yemin A. Endovascular Treatment of Uterine Fibroids. Touch Briefings 2011; 63-9.

Lumsden M. Modern management of fibroids. Obstet Gynaecol Reprod Med 2010; 20(3): 82-86.

Assessment of Uterine Leiomyomas in the Fibroid Growth Study (FGS) using Histological Sampling Methods. http://www.docstoc.com/docs/52054207/Assessment of Uterine -Leiomyomas-in-the-Fibroid-Growth-Study. pdf. Published 2010. Accessed Jul 15, 2012.

Fasih N, Prasad Shanbhogue AK, Macdonald DB, Fraser-Hill MA, Papadatos D, Kielar AZ, et al. Leiomyomas beyond the uterus: unusual locations, rare manifestations. Radiographics 2008; 28(7): 1931-48.

Laughlin Shannon K, Baird Donna D, Savitz David A, Herring Amy H, Hartmann Katherine E. Prevalence of uterine leiomyomas in the first trimester of pregnancy: an ultrasound-screening study. Obstet Gynecol 2009; 113(3): 630-5.

Lefebvre G, Vilos G, Allaire C, Jeffrey J. The management of uterine leiomyomas. J Obstet Gynaecol Can 2003; 25(5): 396-418.

Lilic V, Djordijevic B, Stanojevic Z, Goran Lilic AP, Sonja Pop-Trajkovi RZ. Genesis, Clinical Presentation, Diagnosis and Treatment of Uterine Myomas. Acta Fac Med Naiss 2007; 24(1): 33-40.

Bulun SE, Zeitoun K, Sasano H, Simpson ER. Aromatase in aging women. Semin Reprod Endocrinol 1999; 17(4): 349–58.

Scott J, Karland BY, Gibbs RS, Haney AF, editors. Danforths obstetrics and gynecology. 9th ed. Philadelphia: Lippincott Williams & Wilkins; 2003.

Ip PP, Cheung A. Pathology of uterine leiomyosarcomas and smooth muscle tumors of uncertain malignant potential. Best Pract Res Clin Obstet Gynaecol 2011; 25(6): 691-704.

Şahin O, Gundogdu C, Unal B, Aslan H, Şahin B, Kaplan S. A new approach of diagnosis of leiomyoma and leiomyosarcoma: A stereological study. J Exp Clin Med 2009; 26(3): 119-123.

Heidari Z, Mahmoudzadeh-Sagheb H, Hashemi M, Rigi-Ladiz MA. Quantitative Analysis of Interdental Gingiva in Chronic Periodontitis Patients With Transforming Growth Factor-β1 29C/T Gene Polymorphisms. J Periodontol 2014; 85(2): 281-9.

Heidari Z, Mahmoudzadeh-Sagheb H, Moudi B. A quantitative study of sodium tungstate protective

effect on pancreatic beta cells in streptozotocin-induced diabetic rats. Micron 2008; 39(8): 1300–5.

Tonar Z, Kochova P, Janacek J. Orientation, anisotropy, clustering, and volume fraction of smooth muscle cells within the wall of porcine abdominal aorta. Applied and Computational Mechanics 2008; 2(1): 145–156.

Mahmoudzadeh-Sagheb HR, Heidari Z, Noori-Mugahi MH. A stereological study of the effects of lithium on morphology of submandibular gland. Pak J Biol Sci 2006; 9(4):746-749.

Cramer SF, Marchetti C, Freedman J, Padela A. Relationship of myoma cell size and menopausal status in small uterine leiomyomas. Arch Pathol Lab Med 2000; 124(10): 1448-53.

Newbold RR, Moore AB, Dixon D. Characterization of Uterine Leiomyomas in CD-1 Mice Following Developmental Exposure to Diethylstilbestrol (DES). Toxicol Pathol 2002; 30(5): 611-16.

Aitken E, Khaund A, Hamid SA, Millan D, Campbell S. The normal human myometrium has a vascular spatial gradient absent in small fibroids. Hum Reprod 2006; 21(10): 2669-78.

Weston GC, Cattrall F, Lederman F, Vollenhoven BJ, Rogers PA. Differences between the pre-menopausal and post-menopausal uterine fibroid vasculature. Maturitas 2005; 51(4): 343–348.

Ciavattini A, Giuseppe JD, Stortoni P, Montik N, Giannubilo SR, Litta P, et al. Uterine Fibroids: Pathogenesis and Interactions with Endometrium and Endomyometrial Junction. Obstet Gynecol Int 2013; 2013.

Walocha JA, Litwin JA, Miodonski AJ. Vascular system of intramural leiomyomata revealed by corrosion casting and scanning electron microscopy. Hum Reprod 2003; 18(5): 1088-1093.

Di Lieto A, De Falco M, Pollio F, Mansueto G, Salvatore G, Somma P, et al. Clinical response, vascular change, and angiogenesis in gonadotropin-releasing hormone analogue-treated women with uterine myomas. J Soc Gynecol Investig 2005; 12:123–8.

Gentry CC, Okolo SO, Fong LF, Crow JC, Maclean AB, Perrett CW. Quantification of vascular endothelial growth factor-A in leiomyomas and adjacent myometrium. Clin Sci (Lond) 2001; 101: 691–695.

Hong T, Shimada Y, Uchida S, Itami A, Li Z, Ding Y, et al. Expression of angiogenic factors and apoptotic factors in leiomyosarcoma and leiomyoma. Int J Mol Med 2001; 8(2): 141-8.