Clinicopathological association of Uterine Fibroids with *SOX4* gene overexpression

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Abstract:

Uterine fibroids represent the major public problem and the commonest benign tumors. This study was intended to determine the role of *SOX4* gene in induction uterine fibroids, for these purpose 25 patients aged 37-70 years with severe uterine bleeding followed hysterectomy were included in this study. Histopathological of biopsies findings confirm the incidence of uterine fibroids. According the body mass index, the *SOX4* gene expression recorded a high significant increase ($p > 0.01$) :Normal weight =1.74, Overweight =2.94 and Obese=3.81 when compared to control group that recorded in Normal weight =1.08, Overweight =1.04 and Obese=1.04 respectively. On the other hand; the effect of age in *SOX4* gene expression folding revealed there was a highly significant increase on ($p > 0.01$) in group aged less than 45 years (2.6209) when compared to control group (1.0769). In more than 45 years mean folding of *SOX4* gene recorded (3.443) when compared to control group (1.020).

In conclusion, the overexpression of *SOX4* gene participates to form fibroids from myometrium and the prevalence of uterine fibroids increasing with high body mass index and aging.

Keywords: Uterine fibroids , leiomyoma, Body Mass Index , *SOX4*

Introduction:

Uterine fibroids (leiomyoma) are the commonest generous uterine tumors, with an expected frequency of (20-40%) in ladies amid their reproductive age [1,2]. They are monoclonal tumors of the uterine smooth muscle cells and involve a great deal of extracellular system that contain collagen, fibronectin, and proteoglycan [3,4].

The predominance of uterine fibroids varies among (5%-65%) dependent on age, ethnicity, geological zone and nature of imaging system [5]. They can occur as single or various central fibroids or can be diffused [6]. The mechanism for progressing uterine

fibroids is poorly understood. Both hereditary features, for example, mutation and natural factors, such as, obesity have been involved in the development of fibroids [7]. Symptoms linked to fibroids contain draining inconsistencies for instance overwhelming, protracted or irregular periods which may result in iron need, subfertility, subfertility and preterm birth [8].

Sex-determining region Y (SRY) - related high motility group box-4 gene (*SOX4*) is overexpressed in a variety of cancers and disease, It has been appeared to be associated with determination of cell fate and the guideline of embryonic advancement of numerous organ systems including heart [9], Pancreas [10], brain[11] and endometrial cancers [12]. In an ongoing report demonstrated the hypermethylation of CpG advertiser in miR-203 prompting overexpressed of SOX4 quality that recommended the quality might be directed by miRs molecules [13]. The progression of endometrial hyperplasia to endometrial adenocarcinoma was reported by Al-Deresawi *et al* [14] whose improved the role of SOX4 quality overexpression in this induction . Due to its significance in numerous cellular processes, this study was pointed to detect the role of high expression of *SOX4* gene in induction of uterine fibroids.

2: Materials and Methods:

2.1. Subjects:

All cases were obtained from Al-Zahra Teaching Hospital in Wasit Province/Iraq. Patients aged from (37-70) years were included in this study. Twenty five patients suffering from abnormal uterine bleeding followed by hysterectomy and ten healthy individuals as a control group.

2.2. Body Mass Index:

The female body mass index (BMI) was measured according to the following equation: Dividing the weight in kilograms by the height in squared meters (kg/m2) [15]**.** The parameter of body mass index [16] : Underweight ≤18.5, Normal 18.5-24.9, Overweight 25-29.9 and Obesity ≥ 30

2.3. Histopathological Examination:

Histological technique of all tissues was carried out to observe the changes in tissues. Twenty five biopsies of hysterectomy and cortege were concluded in this study [17].

2.4. Gene expression:

One gram of fresh biopsy was crashing by Homogenizer to obtained free cell. Total RNA of all samples was extracted using the TRIzol® LS Reagent according to the manufacturer's instructions. Total RNA was reversely transcribed to complementary DNA (cDNA) using WizScript™ RT FDmix Kit. The procedure was carried out in a reaction volume of 20 µl according to the manufacturer's instructions. The expression levels of *SOX4* gene were

estimated by qRT-PCR . To confirm the expression of target gene, quantitative real time qRT-PCR EV Green assay was used. the program of the reaction was : Initial denaturation: 95°C for 5 minuts (on cycle), Denaturation: 95°C for 30 second, annealing: 59°C for 30 second, Extension :72 °C for 40 second. The mRNA levels of endogenous control gene *GAPDH* were amplified and used to normalize the mRNA levels of the *SOX*4 gene. SOX4 primers sequences are F:5′-AGGATTCAAACGCAACTCAAAT-3, R: 5′-AAAGAAATACGAGGATGGAGCA-3) and sequence of *GAPDH* (F: 5′-AACTTTGGCATTGTGGAAGG-3′ and R: 5′-ACACATTGGGGGTAG GAACA-3′) .

2.5. Statistical analysis:

ΔCT and ΔΔCT were calculated according to the Livak method [18]. This was conducted according to Statical Analysis System-SAS [19] to detect the impact of various factors in considering the parameters. Least significant difference (LSD) test was utilized to compare about between methods. P esteem for all tests was viewed as huge if $p<0.01$

3: Results:

3:1:-Histopathological finding:

Figure (1) showed the made out of uniform smooth muscles cells arranged in whorled anastomosing fascicles. Cytogenetically, smooth muscle cell are spindly, with undefined cytoplasmic borders and a fibrillary eosinophilic cytoplasm. Nuclei are ovoid to lengthen with blunt ends. The chromatin is open and incidentally an inconspicuous nucleus was seen.

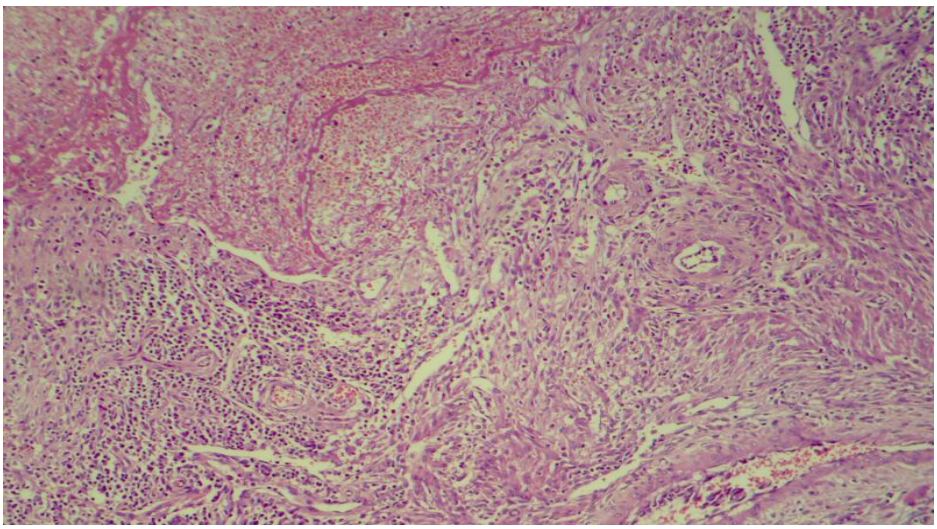

Figure (1): Whorl appearance of uterine fibroids, pattern of smooth muscle bundle separated by well vascularized connective tissue. H & E stained, Power magnification ×40

3.2. Molecular Study:

This study was included *SOX4* gene as an interest gene and *GAPDH* as a housekeeping gene and SOX4 gene as interest of gene

3.2.1. *GAPDH* gene Expression:

There was no significant differences of Ct value of *GAPDH* in subjects and healthy control group (1±0.00). The housekeeping gene used in the present study is shown in table (1). The mean fold of *GAPDH* gene expression in the patients was (1.03 ± 0.11), There is no significant difference in the expression of *GAPDH* in the patients group when compared to the healthy control group. The little variations in gene fold expression between the patients group and healthy group makes *GAPDH* gene useful as a control/reference gene.

Table (1): Comparison of *GAPDH* gene fold expression between study groups

Group	Means Ct of *GAPDH*	2^{-Ct}	experimental group/ Control group	Mean fold of *GAPDH* expression
Uterine fibroids	29.831	E9 1.04	1.04 E9/9.7 E10	1.03 ± 0.11 a
Control	29.946	9.7 E10	9.7 E10/9.7 E10	1 ± 0.00 a
LSD value	---	---	---	0.217 NS
NS: Non-Significant.				

3.2.2- *SOX4* gene expression and Body Mass Index:

The results of effect the BMI in folding of *SOX4* gene expression among control group and patients group were recorded in table (2), the normal weight patient group showed a significant increase (1.74) when compared to normal weight control group (1.o8). The SOX4 gene expression in overweigh patents group showed a significant increase (2.94) when compared to overweight control group (1.4), also there was a significant increase in obese patient group (3.81) when compared to obese control group (1.04).

Table (2): Effect of BMI in folding *SOX4* gene expression in control and patients

BMI	Mean ± SE of folding of SOX4 gene		
	Patients	**Control**	**L.S.D**
Normal	1.74	1.08	0.614
Over weight	2.94	1.04	0.848
Obese	3.81	1.04	0.85
LSD value	1.271	NS	
P-value	0.017	0.53	
P<0.01, NS: Non-Significant			

3.2.3. *SOX4* gene expression and Age:

Table (3) revealed there was no significant difference in SOX4 gene expression in control group (Less than 45 and More than 45), while the gene expression of SOX4 in age group more than 45years showed a significantly increase (3.443) when compared to the less than 45 years group (2.6209)

Table (3): Effect of Age in folding *SOX4* gene in control and patients

Age group (year)	Mean ± SE of folding of *SOX4* gene	
	Patients	**Control**
Less than 45	2.6209	1.07692
More than 45	3.4431	1.02083
LSD value	NS	0.04645
P-value	0.13	0.02
P<0.01, NS: Non-Significant		

Discussion:

Histopathologically, Uterine fibroids are benign soft--tuissues tumors that arise from myometrium. As non-carcinogenic development emerging from myometrium, Uterine fibroids are detached to the normal state of uterine malignancy and uterine cervical disease which more often than not arises from the endometrium or cervical epithelium, individually [20].

An expansion with age in the prevalence of fibroids amid the conceptive age has been shown by a couple of epidemiologic examinations [21]. Concentrates that characterize cases by pathologic end, henceforth constraining cases to those having therapeutic medical procedure [22], have seemed quick addition in uterine fibroid break down among women in their forties.

The Uterine fibroid headway truly accelerate during the late reproductive age, hormonal components related with perimenopause may be basic modulators; then again, the apparent increase in the late conceptive age may just speak to the combined perfection of (20-30) long stretches of incitement by estrogen and progesterone [23].

A few of examinations have found a connection between high BMI and an expanded recurrence of uterine fibroids. In a prospective report from Extraordinary England [24], the peril of fibroids expanded around 21% for each 10-kg increase in body weight; near results were gotten when the BMI was analyzed rather than weight. For a situation control think about from Thailand [20], a 6% expansion in hazard was looked for each unit increase in BMI. A case control think about from Japan [25], in like way reported that women with strange strength or women with overweight allocation were at basically higher risk. In an examination from Boston, Massachusetts [26], 51% of the hysterectomy attested patients with uterine fibroids were overweight, and 16% were fat; this clear relationship among weight and an expanded danger of uterine fibroids may be related to hormonal factors related with heftiness, anyway other pathologic pathways may in like manner be incorporated. A couple of relevant hormonal association with strength are known. A noteworthy increment occurs in the difference in coursing adrenal androgens to estrone by wealth fat tissue. The hepatic formation of sex hormone-confining globulin is reduced, achieving continuously unbound physiologically active estrogen [25].

Gene expression:

The inherent assumption in the use of housekeeping Genes in molecular studies is that their expression remains constant in the cells [27]. One of the most commonly used housekeeping genes in comparison with the gene expression data is *GAPDH* [28]. Robert *et al*. [29] studied the expression of 1,718 genes using qReal-time-PCR and finding there were no significant differences between them and they applied the *GAPDH* as a reference gene in 72 kinds of normal human tissues.

Expression of the *SOX4* gene was highly significant increased ($p<0.01$) in patients with uterine fibroids when compared to the healthy control group. *SOX4* gene expression is up-regulated in many tumor types, with experimental evidence suggesting that this contributes to cellular transformation [30], and/or a metastatic phenotype [31,32]**.** miRs play important role in carcinogenesis by targting tumor suppressor gene or by go about as Oncogenes with raised expression [33]. *miR-203 , miR-129-2 ,*

miR-596 , and miR-618 recognized to bound to the un translated region (3-UTR) of *SOX4* gene insilico investigation of these miRs keep the dimensions of *SOX4* by the degradationof its mRNA. Huang *et al* .,[34.12] detailed that the hypermethylated promoters of *miR-129-2* and *miR-203* lead to *SOX4* overexpression.

A high expression of *SOX4* gene has been accounted for in numerous types of tumor and there is proof of its contributes in cellular transformation [30]; consequently, the role of SOX4 in inducing uterine fibroids is proposed, because the high expression of *SOX4* leads to the transformation of cells covering the endometrium. More than one investigation has detailed aberrant expression of *SOX4* in several cancers, through improving the development and multiplication of endometrial cells and accelerating the advancement of this cells from G0/G1 into S stage [35]. miRs regulates gene expression at the post-transcriptional levels and silencing of *miR-129-2* by methylation process on its promoter leads to overexpression of *SOX4* gene that suggests the role of miRs in endometrial transformation [12].

Conclusions:

Uterine fibroids are a common disease and are progressively treated with option in contrast to hysterectomy. Understanding the varying assortment of ailment in both pathophysiology and symptomatology will prompt focused on treatment for the time being and aversion methodologies in the long-term. The *SOX4* gene participates in induction of uterine fibroids as well as in many histological changes especially when in overexpression status.

References:

1-Ryan, G.L.; Syrop, C.H. and Van Voorhis, B.J.(2005). Role, epidemiology, and natural history of benign uterine mass lesions. *Clin Obstet Gynecol.*48: 312–324.

2-Wallach, E.E. and Vlahos, N.F.(2004). Uterine myomas: an overview of development, clinical features, and management. *Obstet Gynecol.*104: 393–406.

3-Sankaran S, Manyonda IT. Medical management of fibroids. *Best Pract Res Clin Obstet Gynaecol.* 2008;22(4):655–676.

4-Parker, W.H.(2007). Etiology, symptomatology, and diagnosis of uterine myomas. *Fertil Steril.*87(4):725–736.

5-Baird, D.D.; Hill, M.C.; Schectman ,J.M. and Hollis, B.W.(2013). Vitamin d and the risk of uterine fibroids. Epidemiology. 24 (3):447–453.

6-Moorman, P.G.; Leppert, P.; Myers, E.R. and Wang , F. (2013). Comparison of characteristics of fibroids in African American and white women undergoing premenopausal hysterectomy. *J Fertil Steril.* 99(3):768–776

7-Segars, J.H.; Parrott, E.C.; Nagel, J.D.; Guo, X.C.; Gao, X.; Birnbaum, L.S.; Pinn, V,W and Dixon, D.(2014).Proceedings from the third national institutes of health international congress on advances in uterine leiomyoma research: comprehensive review, conference summary and future recommendations. *Hum Reprod Update* J . 12 (3):1–25

8-Moshesh, M.; Olshan, A.F.; Saldana, T. and Baird ,D.(2014). Examining the relationship between uterine fibroids and dyspareunia among premenopausal women in the United States. *J Sex Med.*11(3):800–808.

9-Restivo, A.; Piacentini, G.; Placidi, S.; Saffirio, C.; and Marino B.(2006). Cardiac outflow tract: a review of some embryogenetic aspects of the conotruncal region of the heart. *Anat Rec A Discov Mol Cell Evol Biol.* 288(9):936-943.

10-Wilson, M.;E.; Yang, K.Y.; Kalousova, A.; Lau, J.; Kosaka, Y.; Lynn, F.C. , et al.(2005). The HMG box transcription factor Sox4 contributes to the development of the endocrine pancreas. *Diabetes*. 54(12):3402-3409.

11-Hong, C.S.; and Saint-Jeannet, J.P. (2005). Sox proteins and neural crest development. *Semin Cell Dev Biol*.16(6):694-703

12-Huang, Y.W.; Liu, J.C.; Deatherage , D.E.; Luo, J.; Mutch, D.G.; Goodfellow, P.J *et al*.(2009). Epigenetic repression of microRNA-129-2 leads to overexpression of SOX4 oncogene in endometrial cancer. *Cancer Res*. 69:9038–9046.

13-Al-Deresawi, M.S.; Al-Faisal, A,H. and Al-obaidi,S.R. (2018)a. Role of *SOX4* gene in progression the endometrial hyperplasia to endometrial adenocarcinoma. Iraqi Journal of Biotechnology, 17(2): 82-90

14-Al-Deresawi, M.S.; Al-Faisal, A,H. Al-obaidi,S.R.and Fahad,I.A. (2018)b. Hypermethylation of *miR-203* and overexpression of *SOX4* are new methods for prediction of Endometrial adenocarcinoma. *Pharm. Sci. & Res*.10 (12): 312-8-3132

15- Flegal, K. M.; Graubard, B. I.; Williamson, D. F. and Gail, M. H. (2005).Excess deaths associated with underweight, Overweight, and Obesity. *J American Med Assoc* ; 293(15):1861-1867

16- European Society of Human Reproduction and Embryology(2009): Oxford Journals ,*Oxford University Press*

17- Bancroft, J. D. and Stevens, A. (1999). Theory and Practice of Histological Techniques. 4th edition. Churchill Livingstone. 127-129.

18-Livak, K.J. and Schmittgen, T.D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔ CT Method. *Method*s. 25, 402–408.

19- SAS. (2012). Statistical Analysis System, User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary. N.C. USA.

20- Bulun S. (2013). Uterine Fibroids .*N Engl J Med* ; 369 : 14-34

21-Marshall, LM .; Spiegelman, D.; Barbieri, R.L; Goldman,MB ;Manson,JE, and Colditz, G.A. (1997). Variation in the incidence of uterine leiomyoma among premenopausal women by age and race . *Obstet Gynecol,* 90 :967-973

22- Drayer, S.M. and Catherino ,W.H. (2015).Prevalence, morbidity, and current medical management of uterine leiomyomas.*Int J Gynaecol Obstet;*131:117-122

23- Flake,G.P;Anderson,J and Dixon, D.(2003) Etiology and Pathology of uterine liomyoma: A review . inciromental Health Perspective, 111 (8):1037-1054

24- Khan, A.T.; Shehmar, M. and Gupta, J.K.(2014).Uterine fibroids: current perspectives. *Int J Womens Health*; 6 :95-114

25-Stewart, E.A. (2001)Uterine fibroids: Review .*Lancet ;*357:293-298

26- Doherty, L.; Mutlu, L .; Sinclair, D. and Taylor, H.(2014).Uterine fibroids: clinical manifestations and contemporary management. *Reprod Sc, 2*1: 1067-1092

27-Reboucas E.; Costa J.; Passos M.; Passos J.; Hurk R. and Silva J. (2013). Real Time PCR and Importance of Housekeepings Genes for Normalization and Quantification of mRNA Expression in Different Tissues. *Brazil Arch Biol Technol*. 56: 143-154

28- Barber D. (2005). GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues. *Physiological Genomics*, 21 (3): 389-395.

29- Robert B.; Harmer W.; Coleman A. and Clark B. (2005). GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues. *Physiol Genom*. 21: 389–395.

30- Liu, P.; Ramachandran ,S.; Al-Seyed, M.; Scharer, C.D.; Laycock, N.; and Dalton, W.B., et al .(2006) .Sex-determining region Y box 4 is a transforming oncogene in human prostate cancer cells. *Cancer Res*. 15;66 (8): 4011-4019

31- Liao, Y,L.; Sun, Y.M.; Chau, G.Y.; Chau YP, Lai, T,C. and Wang, J.L., et al.(2008) . Identification of *SOX4* target genes using phylogenetic footprinting-based prediction from expression microarrays suggests that Overexpression of *SOX4* potentiates metastasis in hepatocellular carcinoma. *Oncogene*. 17:122-131

32- Vervoort, S.J.; van Boxtel, R.; and Coffer, P.J. (2013).The role of SRY-related HMG box transcription factor 4 (SOX4) in tumorigenesis and metastasis: friend or foe? *Oncogene*. 32:3397–3409

33-Devor, E.J.; Hovey, A.M.; Goodheart, M.J.; Ramachandran, S., and Leslie, K.K.(2011). microRNA expression profiling of endometrial endometrioid adenocarcinomas and serous adenocarcinomas reveals profiles containing shared, unique and differentiating groups of microRNAs. *Oncol. Rep. 26*, 995–1002

34-Huang,Y.; Kuob,C .; Chenb,J.; Paul, J.; Huangd,T Radera,J.S; and Uyara ,d.S.(2014). Hypermethylation of *miR-203* in endometrial carcinomas. *Gynecol Oncol*. 133(2): 340–345.

35-Sun, R.; Jiang,B.; Qi,H.; Zhang ,X.;Yang,J.;Duan,J.;Li,Y.; and Li,G. (2015). *SOX4* contributes to the progression of cervical cancer and the resistance to the chemotherapeutic drug through ABCG2.*Cell Deat Dise*.2-10