

Comparison of the Effect of Oestrogen Plus *Foeniculum vulgare* Seed and Oestrogen alone on Increase in Endometrial Thickness in Infertile Women

MAHNAZ YAVANGI¹, SOGHRA RABIEE², SARA NAZARI³, MARZIEH FARIMANI-SANOEE⁴, IRAJ AMIRI⁵, MARYAM BAHMANZADEH⁶, SAEID HEIDARI-SOURESHJANI⁷

ABSTRACT

Introduction: *Foeniculum vulgare* seed is used to treat infertility because of phytoestrogenic properties.

Aim: The present study was conducted to compare the effects of *F. vulgare* plus oestrogen and oestrogen alone on Endometrial Thickness (ET) in infertile women.

Materials and Methods: In this study, 92 people with infertility were enrolled by convenience sampling and randomly divided into treatment case (45 women) and control (47 women) groups. Both groups were administered with oestradiol valerate 2 mg tablet three times a day since the third day of the menstrual cycle. Treatment group was also administered with *F. vulgare* tea on a daily basis. Then, ultrasound was conducted once every three days to determine ET. Other data such as serum

β -hCG level and increase in oestrogen dose were recorded in a checklist. The data were analysed by independent t-test and Chi-square in SPSS version 17.0.

Results: There were no significant differences in mean age, BMI, number of years of infertility, number of children, cause of infertility, positive β -hCG, failure to achieve adequate endometrial thickness, and increase in oestradiol dose between the treatment and control groups ($p>0.05$). The mean number of days to achieve ET of at least 8 mm was 13.1 ± 3.2 in the treatment group and 14.2 ± 3.5 in the control group with no significant difference ($p>0.05$).

Conclusion: *F. vulgare* seed tea was not significantly effective in increasing the ET as compared to oestradiol valerate 6 mg alone.

Keywords: Gynaecological disorders, Infertility, Oestradiol valerate, Phytoestrogen

INTRODUCTION

Infertility is one of the common healthcare issues that many couples worldwide are facing [1]. In Iran, infertility is a common issue associated with several outcomes such as aggression, social stigma, exclusion, and emotional-psychological problems that lead ultimately to adverse effects on quality of life [2,3]. In addition, infertility brings stupendous health care costs for women and their families and leads to economic instability in the health care system [4]. ET is one of the factors that plays an important role in infertility. Women with $6\text{ mm}<ET\leq 10\text{ mm}$ endometrial thickness have greater chance of pregnancy [5].

To achieve successful implementation in Assisted Reproductive Technologies (ARTs), ET should be taken into account to increase fertility likelihood [6,7]. This issue is not considered to deserve much investigation by fertility specialists in treating infertility [8]. Oestrogen therapy is an effective and side effect free approach to treat inadequate ET in folliculogenesis and ovulation [9], because it has been recommended to increase ET using oestrogen supplements in women without adequate ET. Oestrogen supplements have also been investigated for their effects in preventing the complications of ET-reducing infertility drugs such as clomiphene citrate [9,10].

In addition to synthetic drugs, plants are used to treat problems due to infertility including fennel (*Foeniculum vulgare* Mill). *F. vulgare* is from family Apiaceae (Umbelliferae) that occurs perennially in temperate regions and Mediterranean Basin. Because *F. vulgare* seeds have pleasant smell and taste, they are abundantly used

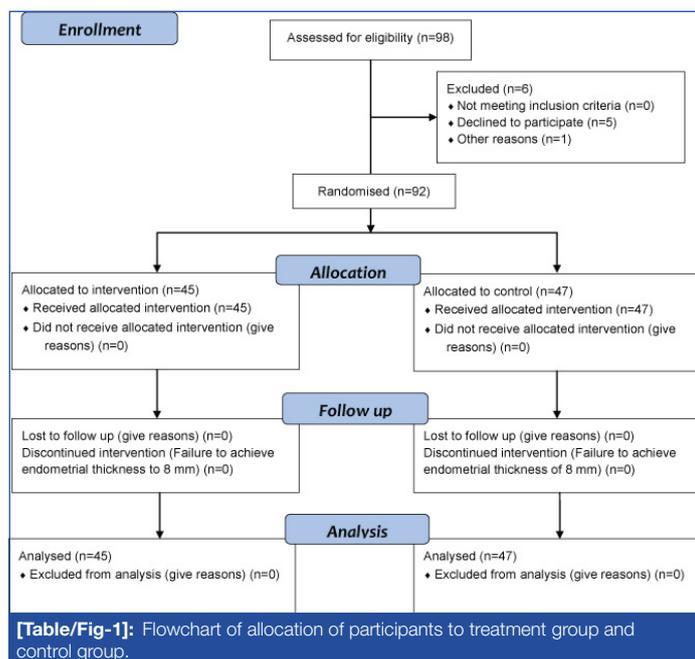
in food industries. This seed is used as stimulant, carminative, anti cancer, and anti-inflammatory in different organs of the body [11,12]. *F. vulgare* aromatic seeds have also certain properties such as dysmenorrhea reducing, menstruation symptoms reducing, delivery facilitating, milk secretion increasing, libido increasing, and premenstrual syndrome symptoms reducing properties. In addition, they are used to treat different gynaecological disorders because they have oestrogenic properties [13-15].

Given that *F. vulgare* is a phytoestrogen, can be a potentially suitable alternative to oestrogen because of being economical and causing fewer side effects, and has many uses in Iranian traditional medicine; hence, we conducted this study to comparatively investigate the effects of oestrogen plus *F. vulgare* and oestrogen alone on ET in infertile women.

MATERIALS AND METHODS

The study population of this randomised clinical trial that was conducted in April 2014 to December 2014 consisted of 98 infertile women with indication of Frozen Embryo Transfer (FET) protocol. Randomisation was done with 92 labeled cards, selected by the patients randomly. Participants were enrolled according to convenience sampling if they met the inclusion criteria including being diagnosed with infertility irrespective of having previous fertility and FET indication. The exclusion criteria were patients suffering from chronic systemic diseases, incidence of severe side effects, failing to follow up the patient carefully, and not volunteering to participate in the study. The patients provided informed consent to participate in

the study after the study protocol was registered in Iranian Registry of Clinical Trials (registration code: IRCT2014120210617N2) and ethical permission was obtained from Hamedan University of Medical Sciences with approval number 16.935.699. Then, they were randomly assigned to two groups, treatment and control, according to random number table [Table/Fig-1].



Both groups were administered with oestradiol valerate 2 mg tablet (Aburaihan Pharmaceutical Co., Tehran, Iran) three times a day since the third day of the menstrual cycle. Treatment group was also administered with *F. vulgare* tea on a daily basis.

To prepare the tea, 50 mL of boiling water was added to 10 gm of *F. vulgare* seed and the resulting solution brewed for 20 minutes [16]. After starting treatment with oestrogen, the patients underwent vaginal ultrasound (Samsung Medison, SonoAce R3, Seoul, South Korea, 6-MHz vaginal transducer) and ET of approximately 1 cm below the the fundus from the external margin of the endometrium contact surface with the myometrium to endometrial stripe outer margin [17] was measured by an experienced radiologist. If the endometria of the patients did not respond to the treatment appropriately based on the findings of the two ultrasounds, the dose of administered oestrogen increased to four, five, or six 2 mg tablets a day, but the amount of the administered *F. vulgare* tea did not change. When ET reached 8 mm or over, FET was conducted by the same protocol and instruments for the patients of the two groups and the parents were followed up for two weeks after the FET to investigate β -hCG result. Serum β -hCG level of over 50 milli-international unit/mL was considered to represent chemical pregnancy [18].

The data on each patient were recorded in a separate checklist including demographic characteristics, the day when ET reached 8 mm, failure to achieve ET of 8 mm, and β -hCG results two weeks after the FET. The data were analysed by descriptive statistics, independent t-test, and Chi-square in SPSS version 17.0.

RESULTS

There was no significant difference in age, body mass index, the number of years of infertility, the number of children, and the mean number of days to achieve adequate (8 mm) ET between the two groups [Table/Fig-2].

Chi-square test indicated no significant difference in cause of infertility, previous surgery (any previous invasive vaginal or abdominal surgery on upper genital tract), positive β -hCG, and failure to achieve adequate ET ($p > 0.05$) [Table/Fig-3].

Variables	Oestradiol valerate plus <i>Foeniculum vulgare</i> (n=45) Mean \pm Standard deviation	Oestradiol valerate (n=47) Mean \pm Standard deviation	p-value
Age (year)	30.7 \pm 5.4	30.1 \pm 6.3	0.716
BMI (kg/m ²)	25.95 \pm 3.25	26.9 \pm 2.6	0.627
Number of years of infertility	6.00 \pm 2.80	6.4 \pm 1.1	0.649
Number of children	0.13 \pm 0.50	0.06 \pm 0.25	0.522
Number of days to achieve adequate ET	13.10 \pm 3.20	14.2 \pm 3.5	0.591

[Table/Fig-2]: Comparison of demographic features between the two groups. BMI: Body mass index ET: Endometrial thickness

Variables	Oestradiol valerate plus <i>Foeniculum vulgare</i>	Oestradiol valerate	p-value (Chi-square)
	No. (%)	No. (%)	
Cause of infertility			
Male infertility	27 (60)	31 (66)	0.765
Tubular	5 (11.1)	3 (6.4)	
Ovular	4 (8.9)	5 (10.6)	
Combined	7 (15.6)	6 (12.7)	
Miscellaneous	2 (4.4)	2 (4.2)	
Previous surgery	9 (20)	8 (17.0)	0.565
β-hCG Positive	6 (13.3)	5 (10.6)	0.718
Failure to achieve ET* of 8 mm	1 (2.2)	2 (4.2)	0.674

[Table/Fig-3]: Comparison of cause of infertility, previous surgery, positive β -hCG, and failure to achieve adequate endometrial thickness between two groups. * Endometrial thickness.

Oestradiol valerate dose	Oestradiol valerate plus <i>Foeniculum vulgare</i>	Oestradiol valerate	p-value (Chi-square)
	No. (%)	No. (%)	
6 mg (baseline dose)	19 (42.2)	15 (31.9)	0.648
8 mg	11 (24.5)	11 (23.4)	
10 mg	8 (17.8)	10 (21.3)	
12 mg	7 (15.5)	11 (23.4)	

[Table/Fig-4]: Comparison of the number of patients requiring increased dose of oestradiol between two groups.

In addition, no significant difference in the number of oestradiol doses was seen between the two groups ($p=0.648$) [Table/Fig-4].

DISCUSSION

The present study was conducted to investigate the effect of *F. vulgare* seed on infertile women's ET to achieve a thickness adequate for fertility. To the best of our knowledge, no study has yet been done to investigate this issue. This study demonstrated that taking 200 mg/dL of *F. vulgare* seed tea per day decreased the mean number of days required to achieve at least 8 mm ET (though statistically insignificant). Sadr Fozlaee S et al., study indicated that treatment with *F. vulgare* helped to regulate the secretion of oestrogen and progesterone and therefore led to increase in ET and decrease in epithelium [19]. A study on menopausal women indicated that phytoestrogens prevented endometrial tissue atrophy and could be used as safe herbal compounds [20]. Studies shows that medicinal plant like *Cimicifuga racemosa* [21] and *Aspalathus linearis* [22] and plant derivatives such as klimadynon [23] and icariin [24] can increase endometrial thickness. Also some of plant formulations such as Xiaoyao powder have oestrogenic effect and can increase cell proliferation in endometrial layer [25].

F. vulgare seed contains certain phytoestrogens such as isoflavones, prenylated flavonoids, and coumestans [26]. *F. vulgare*'s oestrogenic

activity is attributed to a compound named anatole. Trans-anatole comprises over 80% of *F. vulgare* compounds [27-29]. Oestrogenic compounds (like phytoestrogens) increases endometrial thickness by affecting glands and endometrial cells and increasing the blood flow of the functional layer [30,31]. A small amount of alcoholic *F. vulgare* extract causes increase in alkaline phosphatase activity and therefore cell growth and proliferation [31,32].

Inconsistency in the findings of the current study and other studies can be explained by the fact that phytoestrogens (such as isoflavones) largely bind to beta-type oestrogen receptor [33] but alpha-type oestrogen receptor represents the main oestrogen receptor in the endometrium [34,35]. Therefore, low doses of phytoestrogens are likely to be influenced by the method of brewing *F. vulgare* (*F. vulgare* tea), and the bioavailability of *F. vulgare* active compounds (anatole and oestrageole) may be affected by the route of administration (oral or local) or the extract type. Consistently, a number of studies have reported that phytoestrogens have no effect on ET in menopausal women [35-37].

Although, more patients in the treatment group needed increase in dose compared to the control group, the difference was not statistically significant. Besides that, treatment with *F. vulgare* caused increase in the mean rate of chemical pregnancy yet insignificantly. *F. vulgare* antioxidant properties have been reported to cause increase in fertility in mice through destroying cells and facilitating cell growth and folliculogenesis [38].

A study demonstrated that low ET caused difficulty for endometrial glandular growth, disrupted angiogenesis process, and subsequently decreased secretion of vascular endothelial growth factor. More clearly, inadequate endometrial bed leads to weak implantation and vascularisation and therefore early abortion [39]. However, it is not sufficient to determine and exclusively consider ET and prescribe human chorionic gonadotropin to treat infertility, and other factors such as appropriate endometrial tissue (triple-line) and embryo quality should be taken into account [7] as well.

LIMITATION

Not determining the active dose of *F. vulgare* and not isolating phytoestrogenic compounds of this plant are some limitations of the current study. It is recommended to conduct studies with larger sample size and longer follow-up as well as considering molecular, genetic, and histopathological characteristics. Although, there were no clinical side effect in treatment group, but it is recommended that this can be considered in future studies.

CONCLUSION

F. vulgare seed tea caused no significant effect on ET compared to oestradiol valerate 6 mg tablet alone. However, further studies are needed to investigate this issue.

ACKNOWLEDGEMENTS

Hereby, we gratefully thank the Research and Technology Deputy of the Hamedan University of Medical Sciences for cooperating with this study. This article was derived from a research project approved at this deputy.

REFERENCES

- Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. *PLoS Medicine*. 2012;9:e1001356.
- Hasanpoor-Azghdy SB, Simbar M, Vedadhir A. The social consequences of infertility among iranian women: a qualitative study. *Int J Fertil Steril*. 2015;8:409-20.
- Hasanpoor-Azghdy SB, Simbar M, Vedadhir A. The emotional-psychological consequences of infertility among infertile women seeking treatment: Results of a qualitative study. *Iran J Reprod Med*. 2014;12:131-38.
- Dyer SJ, Patel M. The economic impact of infertility on women in developing countries - a systematic review. *Facts, Views & Vision in Ob Gyn*. 2012;4:102-09.
- Habibzadeh V, Nematollahi Mahani SN, Kamyab H. The correlation of factors affecting the endometrial thickness with pregnancy outcome in the IUI cycles. *Iran J Reprod Med*. 2011;9:41-46.
- Momeni M, Rahbar MH, Kovanci E. A meta-analysis of the relationship between endometrial thickness and outcome of in vitro fertilization cycles. *J Hum Reprod Sci*. 2011;4:130-37.
- Chen SL, Wu FR, Luo C, Chen X, Shi XY, Zheng HY, et al. Combined analysis of endometrial thickness and pattern in predicting outcome of in vitro fertilization and embryo transfer: a retrospective cohort study. *Reproductive Biology and Endocrinology*. 2010;8:30.
- Wolff EF, Vahidi N, Alford C, Richter K, Widra E. Influences on endometrial development during intrauterine insemination: clinical experience of 2,929 patients with unexplained infertility. *Fertil Steril*. 2013;100:194-9.e1.
- Satriapod C, Wingprawat S, Jultanasri R, Rattanasiri S, Jirawatnotai S, Choktanasiri W. Effect of estradiol valerate on endometrium thickness during clomiphene citrate-stimulated ovulation. *J Obstet Gynaecol Res*. 2014;40:96-101.
- Liao X, Li Z, Dong X, Zhang H. Comparison between oral and vaginal oestrogen usage in inadequate endometrial patients for frozen-thawed blastocysts transfer. *Int J Clin Exp Pathol*. 2014;7:6992-97.
- Chainy GB, Manna SK, Chaturvedi MM, Aggarwal BB. Anethole blocks both early and late cellular responses transduced by tumor necrosis factor: effect on NF-kappaB, AP-1, JNK, MAPKK and apoptosis. *Oncogene*. 2000;19:2943-50.
- Aggarwal BB, Prasad S, Reuter S, Kannappan R, Yadav VR, Park B, et al. Identification of novel anti-inflammatory agents from ayurvedic medicine for prevention of chronic diseases: "reverse pharmacology" and "bedside to bench" approach. *Curr Drug Targets*. 2011;12:1595-653.
- Rather MA, Dar BA, Sofi SN, Bhat BA, Qurishi MA. *Foeniculum vulgare*: a comprehensive review of its traditional use, phytochemistry, pharmacology, and safety. *Arab J Chem*. 2016;9(Suppl 2):S1574-S83.
- Oktay M, Gülçin I, Küfrevioğlu ÖI. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. *Food Sci Technol*. 2003;36:263-71.
- Ostad SN, Soodi M, Sharifzadeh M, Khorshidi N, Marzban H. The effect of fennel essential oil on uterine contraction as a model for dysmenorrhea, pharmacology and toxicology study. *J Ethnopharmacol*. 2001;76:299-304.
- Badgujar SB, Patel VV, Bandivdekar AH. *Foeniculum vulgare* mill: a review of its botany, phytochemistry, pharmacology, contemporary application, and toxicology. *Bio Med Research International*. 2014;2014:842674.
- El-Toukhy T, Coomarasamy A, Khairy M, Sunkara K, Seed P, Khalaf Y, et al. The relationship between endometrial thickness and outcome of medicated frozen embryo replacement cycles. *Fertil Steril*. 2008;89:832-39.
- Sivalingam VN, Duncan WC, Kirk E, Shephard LA, Horne AW. Diagnosis and management of ectopic pregnancy. *J Fam Plann Reprod Health Care*. 2011;37:231-40.
- Sadr Fozalae S, Farokhi F, Khaneshi F. The effect of metformin and aqueous extract *Foeniculum vulgare* (fennel) on endometrial histomorphometry and the level of steroid hormones in rats with polycystic ovary syndrome. *Qom Univ Med Sci J*. 2015;8:12-19.
- Torella M, Tammara C, Auriemma S, Zurzolo L, Natullo V, Scaffa C, et al. Endometrial survey during phytoestrogens therapy in postmenopausal women. *Minerva Ginecol*. 2008;60:281-85.
- Shahin AY, Mohammed SA. Adding the phytoestrogen *Cimicifuga racemosa* to clomiphene induction cycles with timed intercourse in polycystic ovary syndrome improves cycle outcomes and pregnancy rates - a randomized trial. *Gynecol Endocrinol*. 2014;30:505-10.
- Monsees TK, Opuwari CS. Effect of rooibos (*Aspalathus linearis*) on the female rat reproductive tract and liver and kidney functions in vivo. *South African Journal of Botany*. 2017;110:208-15.
- Kamel HH. Role of phyto-oestrogens in ovulation induction in women with polycystic ovarian syndrome. *Eur J Obstet Gynecol Reprod Biol*. 2013;168:60-63.
- Le AW, Wang ZH, Dai XY, Xiao TH, Zhuo R, Zhang BZ, et al. An experimental study on the use of icariin for improving thickness of thin endometrium. *Genet Mol Res*. 2017;16.
- Gao X, Chang X, Du H, Zhang M, Zhang J, Zhu A. Effect of soothing liver therapy on oocyte quality and growth differentiation factor-9 in patients undergoing in vitro fertilization and embryo transfer. *J Tradit Chin Med*. 2013;33:597-602.
- Shahat AA, Ibrahim AY, Hendawy SF, Omer EA, Hammouda FM, Abdel-Rahman FH, et al. Chemical composition, antimicrobial and antioxidant activities of essential oils from organically cultivated fennel cultivars. *Molecules*. 2011;16:1366-77.
- Singh G, Maurya S, De Lampasona M, Catalan C. Chemical constituents, antifungal and antioxidative potential of *Foeniculum vulgare* volatile oil and its acetone extract. *Food control*. 2006;17:745-52.
- Nakagawa Y, Suzuki T. Cytotoxic and xenoestrogenic effects via biotransformation of trans-anethole on isolated rat hepatocytes and cultured MCF-7 human breast cancer cells. *Biochem Pharmacol*. 2003;66:63-73.
- Tognolini M, Ballabeni V, Bertoni S, Bruni R, Impicciatore M, Barocelli E. Protective effect of *Foeniculum vulgare* essential oil and anethole in an experimental model of thrombosis. *Pharmacol Res*. 2007;56:254-60.
- Zin SRM, Omar SZ, Khan NLA, Musameh NI, Das S, Kassim NM. Effects of the phytoestrogen genistein on the development of the reproductive system of Sprague Dawley rats. *Clinics*. 2013;68:253-62.
- Pelletier G, El-Alfy M. Immunocytochemical localization of oestrogen receptors alpha and beta in the human reproductive organs. *J Clin Endocrinol Metab*. 2000;85:4835-40.
- Mahmoudi Z, Soleimani M, Saidi A, Khamisipour G, Azizoltani A. Effects of *Foeniculum vulgare* ethanol extract on osteogenesis in human mesenchymal stem cells. *Avicenna J Phytomed*. 2013;3:135-42.

- [33] Mueller SO, Kling M, Arifin Firzani P, Mecky A, Duranti E, Shields-Botella J, et al. Activation of oestrogen receptor alpha and ERbeta by 4-methylbenzylidene-camphor in human and rat cells: comparison with phyto- and xenoestrogens. *Toxicol Lett.* 2003;142:89-101.
- [34] Wedren S, Lovmar L, Humphreys K, Magnusson C, Melhus H, Syvanen AC, et al. Oestrogen receptor alpha gene polymorphism and endometrial cancer risk: a case-control study. *BMC Cancer.* 2008;8:322.
- [35] Quaas AM, Kono N, Mack WJ, Hodis HN, Felix JC, Paulson RJ, et al. The effect of isoflavone soy protein supplementation on endometrial thickness, hyperplasia and endometrial cancer risk in postmenopausal women: A randomized controlled trial. *Menopause (New York, NY).* 2013;20:840-44.
- [36] D'Anna R, Cannata ML, Marini H, Atteritano M, Cancellieri F, Corrado F, et al. Effects of the phytoestrogen genistein on hot flushes, endometrium, and vaginal epithelium in postmenopausal women: a 2-year randomized, double-blind, placebo-controlled study. *Menopause.* 2009;16:301-06.
- [37] Nikander E, Rutanen EM, Nieminen P, Wahlström T, Ylikorkala O, Tiitinen A. Lack of effect of isoflavonoids on the vagina and endometrium in postmenopausal women. *Fertil Steril.* 2005;83:137-42.
- [38] Khazaei M, Montaseri A, Khazaei MR, Khanahmadi M. Study of *Foeniculum vulgare* effect on folliculogenesis in female mice. *Int J Fertil Steril.* 2011;5(3):122-27.
- [39] Mahajan N, Sharma S. The endometrium in assisted reproductive technology: How thin is thin? *J Hum Reprod Sci.* 2016;9(1):03-08.

PARTICULARS OF CONTRIBUTORS:

1. Associate Professor, Department of Obstetrics and Gynaecology, Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.
2. Professor, Department of Obstetrics and Gynaecology, Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.
3. Resident, Department of Obstetrics and Gynaecology, Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.
4. Associate Professor, Department of Obstetrics and Gynaecology, Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.
5. Professor, Department of Anatomy, Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.
6. Assistant Professor, Department of Anatomy, Endometrium and Endometriosis Research Center, Hamadan University of Medical Sciences, Hamadan, Iran.
7. MSc, Modeling In Health Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Mahnaz Yavangi,
Associate Professor, Department of Obstetrics and Gynaecology, Endometrium and Endometriosis Research Center,
Hamadan University of Medical Sciences, Hamadan, Iran.
E-mail: m_yavangul@yahoo.com

Date of Submission: **May 16, 2017**Date of Peer Review: **Jul 24, 2017**Date of Acceptance: **Sep 25, 2017**Date of Publishing: **Jan 01, 2018****FINANCIAL OR OTHER COMPETING INTERESTS:** None.