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Effect of ultrasound on subserosal and intramural fibroids in vitro: A quasi-experimental study of physical therapy

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ABSTRACT

The aim of this study was to determine the effect of ultrasound (US) on extra subserosal and intramural uterine fibroids in vitro**.** A quasi-experimental, randomized, pre-post intervention study of physical therapy was conducted. Thirty women, who underwent myomectomy for subserosal or intramural fibroids with any leiomyomas' volumes in the operating room of Bab El-Sharia Hospital, participated in the study. Thirty uterine fibroids whose were collected from participants were randomly divided into three groups (I, II and III), and exposed to ultrasound therapy for 15, 30, and 45 minutes respectively. Leiomyomas volumes were measured using the fluid displacement method before and after the application of US. The samples were kept in saline solution immediately after their excision from operating room to be transferred to physical therapy department in same hospital to apply ultrasound therapy. Results of all study groups (I, II, and III) showed a statistically significant decrease in the volume of subserosal and intramural leiomyoma post US application ($p<0.05$). However, there was no statistically significant difference in the size of the subserosal and intramural leiomyoma between study groups ($p>0.05$). In conclusion, the ultrasound therapy is

effective in shrinking the volume of subserosal and intramural leiomyomas without effect of long duration ultrasound therapy.

KEYWORDS

Ultrasound (US); Uterine Fibroid; Subserosal; Intramural; Vitro.

1. INTRODUCTION

Uterine fibroids, also known as uterine leiomyoma are benign tumors of the smooth muscle of the uterus. Subserosal and intramural uterine fibroids are extra uterine fibroids, which are developed out of the uterine cavity. Subserosal uterine fibroids mainly grow largely under the peritoneal surface of the uterus, while intramural uterine fibroids grow as nodules within the muscular wall of the uterus (Wallach & Vlahos, 2004).

Uterine fibroids commonly affect women in their reproductive age. Various studies found that about 70% of white women and 80% of African women were diagnosed with uterine fibroids during their lifetime (Giuliani, As- Sanie & Marsh, 2020).

Although uterine fibroids are asymptomatic, there are 30% of them symptomatic. Symptoms vary from mild symptoms to sever symptoms (Stewart, Nicholson, Bradley & Borah, 2013), including intermittent irregular bleeding or heavy continuous menstrual bleeding (menorrhagia), longer menstrual period with sever pelvic pain or pelvic crumps, and urgency of urination. Pain during intercourse is more often (Moroni, Vieira, Ferriani, Rosana, Nogueira & Brito, 2015).

The acute complications of uterine fibroids are more serious and dangerous. They lead to significant morbidity that worsens woman's quality of life. These complications include acute torsion of subserosal pedunculated leiomyomas, mesenteric vein thrombosis, acute urinary retention, and acute vaginal or intra-peritoneal hemorrhage (Gupta & Manyonda, 2009).

Treatment of symptomatic fibroids ranges from medication to surgical procedures either myomectomy or hysterectomy (Sukhorukov, 2007). In addition to other procedures differ according to the problem, such as uterine artery embolization (UEA), whose results are more similar to hysterectomy (Gupta, Sinha, Lumsden & Hickey, 2012), but the risk of embolism or septicemia makes the decision difficult (Vashisht, Studd & Carey, 2000). Radiofrequency ablation (RFA) is an ablative procedure that is suggested if child-bearing is preferred, but the procedure is still under investigation (Beauchamp, Evers, Mattox & Townsend, 2012). Endometrial ablation that removes

the endometrial lining of a uterus, mainly targets submucosal uterine leiomyoma but can't reach to subserosal and intramural fibroids (Lethaby, Hickey, Garry & Penninx, 2009).

Magnetic resonance guided focused ultrasound (MRgFUS) is a non-invasive procedure that ensures no incision that able to destroy the fibroid tissues by applying high intensity focus ultrasound (HIFU) waves. The magnetic resonance imaging (MRI) is guiding definitely for the locations of fibroids (Baker, Robertson & Duck, 2001; Hynynen, McDannold, Sheikov, Jolesz & Vykhodtseva, 2005). This based on hyperthermia of leiomyoma tissues resulting in forming necrotic clots (Zowall, Cairns, Brewer, Lamping, Gedroyc & Regan, 2008; Leung, Yu & Cheung, 2014). However, its cost is very high when compared with alternative procedures of treatment for uterine fibroids (Ren, Zhou & Yan, 2009; O'Sullivan, Thompson, Chu, Lee, Stewart & Weinstein, 2009).

These findings push to support the effect of ultrasound on uterine fibroids size through vitro design for subserosal and intramural uterine fibroids leiomyoma to find out the appropriate parameters of ultrasound that can be used in the treatment of extra-uterine fibroids. It will also help to ensure the effectiveness and safety of the using of ultrasound therapy in the shrinking of subserosal and intramural fibroids in vivo. This study aims to determine the effect of ultrasound (US) on extra subserosal and intramural uterine fibroids in vitro**.**

2. METHODS

2.1. Study Design and Participants

A quasi-experimental, randomized, pre-post intervention study was conducted. Before commencing the study, ethical approval was fulfilled from the institutional review board at the Physical Therapy Faculty of Cairo University (NO: P.T.REC/012/002175). It lasted from January 2019 to August 2020. Thirty participants who underwent myomectomy for subserosal or intramural fibroids with any leiomyomas' volumes in the operating room of Bab El-Sharia Hospital, participated in the study. Informed consent was taken from each participant after interpreting the research aims and procedures, ensuring their privacy. Participants were premenopausal women with age ranged from 45 to 50 years old and body mass index of less than or equivalent to 30 kg/m^2. Participants who underwent any less invasive procedures such as uterine embolization, radiofrequency ablation and magnetic resonance guided high intensity focus ultrasound, or had autoimmune diseases, were excluded.

Thirty uterine fibroids which were collected from participants, were randomly divided into three groups (I, II and III). The groups were exposed to ultrasound therapy for 15, 30, and 45 minutes

respectively. The volume of each subserosal or intermural was measured before applying ultrasound (US) to investigate its effect on leiomyomas.

2.2. Randomization

Randomization was carried out by a rotation of uterine fibroids after excision, which determine the duration to which ultrasound will be applied to ablated uterine fibroid (15, 30 and 45 minutes). There were no control samples as it was a quasi-experimental study.

2.3. Outcome Measures

2.3.1 Fluid displacement method

Fluid displacement method was used to measure the volume of irregular objects based on Archimedes' law upon which the validity and reliability of the fluid displacement method depend (Hughes, 2005). It measured the volume of irregular shaped object such as leiomyoma that was developed as round, well circumscribed (but not encapsulated) and solid nodules (Garg, Tickoo, Soslow & Reuter, 2011).

2.3.2. Ultrasonic Device

Ultrasound was applied on subserosal and intramural leiomyoma. Ultrasound device was calibrated before its application to ensure that the ultrasonic power output was similar to the actual output. Ultrasound devices are frequently used by physiotherapists, that ultrasound machines are accurate for safe and effective treatments. Ultrasound accuracy was identified first time in 1956 when the United States established standards for calibration (Schabrun, Walker & Chipchase, 2008). Ultrasound parameters were set up as Table 1 shows.

Table 1. Ultrasound parameters

1. Continuous ultrasound
2. Frequency of 1MHz energy
3. Intensity up to 2 W/ cm^2
4. Durations applied in different 15, 30, 45 min.
5. Ultrasound Head sizes varified in 3 different diameter

MHz (Mega Hertz); W (watt)

Ultrasound was applied after myomectomy. Leiomyomas were kept in the saline solution and taken to be exposed to the ultrasound wave. Each subserosal or intramural uterine fibroids in vitro

was exposed directly to the ultrasound wave. Gel was applied to facilitate the sound head motion that was applied perpendicular on the leiomyoma. Ultrasound was applied for 15 minutes in group (I), 30 minutes in group (II), and 45 minutes in group (III), while ultrasound therapy was applied only once for each uterine fibroid

Fluid displacement method measures all uterine fibroids again after ultrasound application by to record all fibroids size in recording data sheet for data analysis.

2.4. Data Analysis

The search data was processed through the Statistical Package for the Social Sciences (SPSS). The Shapiro-Wilk test was used to determine the distribution of the sizes of the subserosal and intramural leiomyomas, Levene's test to analyze whether there was homogeneity of variances, and the Wilcoxon signed rank test to measure the differences between the two measurement periods for each group. The Kruskal-Wallis H test was used to compare the three groups in the different measurement periods, while the Mann-Whitney U test (post hoc test) was used to determine whether there were statistical differences in the size of subserosal and intramural leiomyomas between the study groups. For all the statistical tests, a p-value of <0.05 was considered statistically significant

3. RESULTS

Prior to final analysis, data were screened for normality assumption, homogeneity of variance, and presence of extreme scores. This exploration was done as a pre-requisite for parametric calculations of the analysis of difference for one continuous variable (Sizes of subserosal and intramural leiomyomas). Sizes of subserosal and intramural leiomyomas weren't normally distributed, as assessed by Shapiro-Wilk's test ($p < 0.05$). There was no homogeneity of variances, as assessed by Levene's ($p < 0.05$) for the same variable. So, the researchers conducted data transformation (using the logarithm). After data transformation, the normality and homogeneity of variance assumptions were violated the parametric assumption.

In the following table (Table 2) we present the median (IQR) values of the sizes of subserosal and intramural leiomyomas for each group before and after ultrasound (US) application.

Table 2. The median (IQR) values of the sizes of subserosal and intramural leiomyomas for each group before and after ultrasound (US) application.

Sizes of subserosal and intramural leiomyomas	Group I Median (IQR)	Group II Median (IQR)	Group III Median (IQR)
Before US	42.93 (318.17)	309.715 (831.077)	89.67 (252.21)
After US	25.25 (183.278)	172.5 (437.653)	43.145 (117.8)

NOTE: IQR (Inter quartile range)

Table 3 shows the level of reduction of subserosal and intramural leiomyomas before and after ultrasound application within study groups.

Table 3. The level of reduction of subserosal and intramural leiomyomas before and after ultrasound application within study groups.

Pre vs. post	Group I	Group II	Group III
Z-value	-2.803	-2.803	-2.803
P-value	0.005*	0.005*	0.005*

As we can see from the results of Table 3, the size of subserosal and intramural leiomyomas decreased significantly in group I compared with the results before ultrasound application ($p < 0.05$). Also, there was significant reduction of size of subserosal and intramural leiomyoma ($p < 0.05$) in group II. Additionally, there was significant reduction of size of subserosal and intramural leiomyoma ($p < 0.05$) in group III.

The following table (Table 4) shows whether there is a significant difference in the size of subserosal and intramural leiomyoma before US application among three groups.

Table 4. The comparison of the size of subserosal and intramural leiomyoma before US application among three groups.

	X^2 value	p value
Before US	3.579	0.167
After US	3.437	0.179

Among three groups, Kruskal-Wallis H test revealed that there was no significant difference of the size of subserosal and intramural leiomyoma before US application ($p > 0.05$). As well as it revealed that there was no significant difference of sizes of subserosal and intramural leiomyomas

among three groups after US application (p >0.05). Also, as the Mann-Whitney U test revealed, there was no statistically significant difference in the size of subserosal and intramural leiomyoma between (group I versus group II), (group I versus group III), and (group II versus group III) before US application with (p>0.05).

Table 5. Comparison of the size of subserosal and intramural leiomyoma between study groups.

	Group I Vs. Group II		Group I Vs. Group III		Group II Vs. Group III	
	U value	**p value**	**U value**	**p value**	**U value**	**p value**
Before US	26	0.07	45	0.705	32	0.174
After US	28	0.096	48	0.88	30	0.131

4. DISCUSSION

In 10–20% of women with uterine fibroids, symptoms are sufficient to prompt them toward surgical solutions, with hysterectomy being the most common. About 200,000 hysterectomies are performed every year in USA to solve problems of uterine fibroids (Jacoby, Autry & Jacobson, 2009). Myomectomy and uterine artery embolization are another option for the treatment of uterine fibroids, but the risk of scar adhesion or thrombotic emboli is usually considered (Camanni, Bonino, Delpiano, Ferrero, Migliaretti & Deltetto, 2010).

Ultrasound is one of electrotherapeutic modalities used in physical therapy filed that has thermal and non-thermal effect when it is absorbed by tissues. Continuous ultrasound converts non thermal energy into heat, which increases deep soft-tissue extensibility and acts as a counter irritant stimulus (Diarmid, Ziskin & Michlovitz, 1996). Therapeutic ultrasound accelerates the healing process, especially in the phases of inflammation and repair, helps in pain relief or any inflammation symptoms (Roebroeck, Dekker & Oostendorp, 1998). In addition, its ability in increasing the extensibility of collagen bands, that facilitates the stretching of scars and resorption of adhesions where US was applied by parameter of continuous mode of 1MHz, intensity: 1.5w/cm and 15 minutes for treatment site as a duration depending on heat production and micro massaging effect of the US wave from slowly moving US head (Mansour, Mohamed, Hegah, Okeel, Youssef & Fouad, 2009).

In this study, the parameter of ultrasound may be similar to commonly used in physical therapy field but the head of US was fixed on leiomyoma tissues to get benefit from the continuous US to

create the stationary field to produce prolonged standing waves of ultrasound that might induce the damage of uterine fibroids tissue cells (Dyson, Pond & Broadben, 1974).

The results of direct application of ultrasound on subserosal and intramural uterine fibroids in vitro showed a statistically significant decrease ($p<0.05$) in the size of subserosal and intramural uterine fibroids in all groups (I, II and III) receiving US that was also observed immediately after the application. That mean all uterine fibroid was exposed for ultrasound has shrinking in its size. Also, the results showed a non-significant decrease ($p>0.05$) in the sizes of subserosal and intramural uterine fibroid between the groups that received ultrasound for 15, 30, and 45 minutes. That mean there were no important effect by increasing the duration of ultrasound application on sizes of fibroids in-between group that can be explained by shock effect of ultrasound on the subserosal and intramural uterine fibroid where the stationary filed of ultrasound cause generation greater amount of heat (hyperthermia) that might lead to cell death of uterine fibroids tissue (Ren, Zhou & Yan, 2009; Leung, Yu & Cheung, 2014).

The results of our study are supported by El-Touny (2015) who reported that the suitable effect of ultrasound therapy on subserosal and intramural fibroids in premenopausal women (Vivo) that was explained by hyperthermia effect of prolonged standing wave of ultrasound, which was created by the effect of the stationary field of ultrasound application that induce cell tissue death by necrosis, cause the reeducation of subserosal and intramural fibroids size in the premenopausal women and consequently its symptoms.

These results are also in line with thermos-ablative effect of magnetic resonance guided focused ultrasound (MRgFUS) that was reported by (Jolesz & Hynynen, 2008). Where the high intensity focused ultrasound (HIFUS) wave is focused produce hyperthermia in targeted fibroids guided by magnetic resonance (MRI), cause destruction of uterine fibroid tissue as a result of rapid deposit of focused ultrasound energy.

Safety of using the technique of US was approved on (Vivo) when the level of tumor marker (cancer antigen 125) in blood serum was tested for women before and after ultrasound exposure as well as beyond 3 months from ending of ultrasound sessions to found no changes in its level after ultrasound application to ensure how much it was safe. Moreover, vaginal swab pathological examination that was performed for women before and after ultrasound exposure, as well as beyond 3 months from ending of US sessions, found no remarkable change in tissue nature of any malignancy signs after ultrasound treatment (El-Touny, 2015).

5. LIMITATIONS

Some degree of limitation should be considered when interpreting the results of this study. Some leiomyomas varied in size and surface area, so they may not have been fully detected by the ultrasound probe. Some postoperative specimens were excluded because of the delayed onset of ultrasound application after excision, as well as the methods of excision of leiomyomas themselves, which are proposed to be performed in future studies.

6. CONCLUSIONS

From the obtained results of this study, we conclude that direct application of ultrasound on subserosal and intramural uterine fibroids in vitro has a great effect in shrinking of its size that was observed immediately after US application rather than the time of exposure to ultrasound. The study helps to ensure the efficacy of US therapy in shrinking the size of uterine fibroids in vivo to open the view for further research on the underlying mechanism of ultrasound therapy through deeper histopathological findings.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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