COMPARISON OF HYALURONIDASE INJECTION IN COMBINATION WITH NON-INVASIVE FOCUSED ULTRASOUND VERSUS ULTRASOUND ALONE FOR THIGH FAT REDUCTION

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MASTER OF SCIENCE
IN
DERMATOLOGY

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Thitikarn Teerapancharoen

Thesis Title	Comparison of Hyaluronidase Injection in Combination with Non-Invasive Focused Uultrasound Versus Ultrasound Alone for Thigh Fat Reduction
Author	Thitikarn Teerapancharoen
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Advisor	Lecturer Paisal Rummaneethorn

ABSTRACT

This research has the purpose of studying the effectiveness in the reduction of thigh fat accumulation by using the technique of hyaluronidase injection in combination with non-invasive focused ultrasound which will help break down fat. Moreover, the drug helps to drain fat out through the vascular and lymphatic systems. It is a new way to solve the problem of thigh fat accumulation. This method will help increase the efficacy of treatment without any serious complications by comparison with the existing treatment.

The present research started from bringing together participants in the research project who experienced problem of thigh fat accumulation totaling 18 persons by being treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound and the use of ultrasound alone on each side of the thighs continuously. The treatment was performed 3 times in total, with a one-month interval between each treatment and follow-up of treatment through assessment by measuring the thickness of fat layer with ultrasound and the thigh circumference with a standard tape measure. The comparison was made in relation to the periods before treatment and after treatment for 1 month and 3 months.

The study results show that hyaluronidase injection in combination with non-invasive focused ultrasound is more effective in reducing thigh fat accumulation than the use of ultrasound alone significantly at one and 3 months from both the measurement of fat thickness (p-value < 0.001) and the measurement of thigh circumference (p-value = 0.006). It was found that the side effects caused by treatment are just minor. According to the assessment, most participants in the research have been also satisfied with the treatment method.

In conclusion, hyaluronidase injection in combination with non-invasive focused ultrasound can reduce thigh fat accumulation at a level greater than the use of ultrasound alone with statistical significance. It was found that the side effects are just minimal. Therefore, it may be another alternative of localized fat reduction.

Keywords: Thigh fat/Hyaluronidase/Non-invasive focus ultrasound

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CHAPTER 1

INTRODUCTION

1.1 Background

Excess body fat is a major problem commonly found in today's society. The combination of too high caloric intake and lack of exercise causes many people to suffer from localized fat deposit. The areas most frequently requested for loss of volume are abdomen, saddle bags, flank, love handles, inner and outer thighs, and inner knees (Voss, Siebrecht & Gesunde, 2005). Fatty deposits on the inner and outer thighs are especially resistant to diet and exercise. Many patients become discouraged at the need to lose unwanted pounds without seeing a significant change in the lower body.

The presence of large amounts of body fat affects the body negatively such as increase in risk of coronary artery disease, Stroke, Peripheral vascular disease etc. In addition, increased fat accumulation adds to the problem in terms of their personality and confidence.

In the past, the only way to improve body contouring was the removal of local fat deposits through liposuction or other surgical procedures. Despite many advances in liposuction technique, risk and discomfort remains by its invasive nature such as pain, bruising, swelling, hematoma, wound infection etc. (Grazer & Jong, 2000; Commons, Halperin & Chang, 2001; Matarasso, Swift & Rankin, 2006; Klein, 1995). And post procedure recovery may require extensive downtime and compressing garments.

Nowadays, the non-invasive and minimally invasive technique is used for reducing excess fat and improving body contouring, which is in demand among male and female population such as deep body massage, radiofrequency, carboxytherapy, and light based treatments. These methods are very popular because of their minimal downtime, relative safety, and cosmetic benefit in temporary reduction of cellulite (Avram, 2004;

Sadick & Mulholland, 2004; Van Vliet, Ortiz, Avram & Yamauchi, 2005). However, they are suboptimal for body contouring, as they provide only modest and temporary circumference reduction, require multiple treatments, short-term results and may require maintenance therapy.

Therefore, a new device has been developed that used focused therapeutic ultrasound to reduced adipose tissue in a non invasive manner. This system delivers concentrated energy into a focal volume at a precise depth in the subcutaneous tissue. The system was designed to use mechanical (non-thermal) energy to disrupt fat cells and without damaging neighboring structures such as skin, blood vessels, lymph vessels, muscle, and nerves.

Today, it has been shown that area with density of subcutaneous fat will decrease blood flows and change fibrosis and increase hyaluronic acid 8 times. It makes fat accumulated. The invention of hyaluronidase injection to reduce fat by modifying the permeability of connective tissue through the hydrolysis of hyaluronic acid and decompose material including fiber in hypodermic tissue and helps circulation of adipose tissue by promoting circulation of lymphatic drainage. *

The use of hyaluronidase to reduce the excess fat is an Off-label use. The Food and Drug Administration of the United States (US FDA) has approved hyaluronidase injection as an adjuvant to increase the absorption and dispersion of other injected drugs. The reason why US FDA does not guarantee hyaluronidase for fat reduction, because of the lack of studies that demonstrate the effectiveness of the drug in fat drainage. However, from my literature review, there has never been study on the effectiveness of the injection of hyaluronidase in combination with non-invasive focused ultrasound for Thigh Fat Reduction. The researcher is interested in the injection of hyaluronidase in combination with non-invasive focused ultrasound to increase efficiency of thigh fat reduction. Therefore, this research project is aimed at being the basic information and the option for the patients to choose a safe and effective treatment in the future.

1.2 Objective

To compare the clinical efficacy of the hyaluronidase injection in combination with non-invasive focused ultrasound when compared with ultrasound alone in treatment of thigh fat reduction

1.3 Hypothesis

Hyaluronidase injection in combination with non-invasive focused ultrasound has higher efficacy in thigh fat reduction than the use of ultrasound alone

1.4 The Scope of the Research

Eighteen patients with localized fat deposits around both thighs are randomly assigned to the treatment with the Hyaluronidase injection combined with body contouring by non-invasive focused ultrasound and body contouring by non-invasive focused ultrasound alone on each thigh. The treatments are performed once a month for three months. Clinical improvement of localized fat deposit around thigh is evaluated by ultrasound subcutaneous fat thickness and thigh circumference.

1.5 Limitation of the Research

1.5.1 Limitations in terms of the duration of the study.

1.5.2 Limitations in terms of financing. As a procedural tool have to use consumable equipment, this make a limitation in sample size.

1.6 Operation Definitions

1.6.1 Localized fat deposits around thigh: refers to subcutaneous fat thickness around thigh more than 0.7 cm. (measuring by portable ultrasound before the start of treatment.

1.6.2 Effectiveness in reducing fat: refers to the ability to reduce fat around the thighs by using the reduction in subcutaneous fat thickness and circumference of the thighs for comparison before, 1 month and 3 months after completing 3 treatment sessions. (Subcutaneous fat thickness was evaluated by portable ultrasound and thigh circumference was evaluated by standard measuring tape measured at the mid-vertical between Anterior superior iliac spine (ASIS) and tip of patella in standing position)

1.6.3 Side effects from the procedures: refer to

1.6.3.1 Erythema
1.6.3.2 Edema
1.6.3.3 Burning
1.6.3.4 Bruising
1.6.3.5 Cellulitis
1.6.3.6 hyper and hypopigmentation
1.6.3.7 allergy to hyaluronidase or anesthesia

1.6.4 The pain of the procedures: refers to the pain felt by the patient after the treatment around the thigh either side after each treatment by using Numeric rating scale (NRS, range from 0 to 10, 0 is no pain, 10 is the most pain)

CHAPTER 2

REVIEW LITERATURE

Body fats are very important. Subcutaneous fatty tissue of humans is one of the largest tissues of the body. The main component is fat cells (adipocytes), fascia and vessels, is approximately 9-18% in men and 14-20% in women with normal weight (Hausman, DiGirolamo, Bartness & Martin, 2001). The fat can increase up to 4 times until volume of 60-70% of body weight (Avram, M. M., Avram, A. S. & James, 2005). In addition, the distribution of adipose tissue also change with age.

2.1 Role of Lipid in Human Body

(Vejjabhinanta, Obagi, Singh & Baumann, 2009)

2.1.1 Fat is an energy source for most of the body

2.1.2 Accumulation in fat-soluble vitamins (A, D, E, K) and vitamin A acid

2.1.3 Protect the body from injury by acting as "shock absorber"

2.1.4 Protect internal organ against impact force

2.1.5 Protect body from the physical force such as heat, cold etc.

2.1.6 Fill the gap between the internal organs to make them be in proper position

2.1.7 Prevent body heat loss

2.1.8 Release of cytokines that are essential to function of the body

2.1.9 Synthesis of hormones

2.2 Anatomy of the Adipose Tissue

Hypodermis or Subcutis is beneath deep reticular dermis. Hypodermis can be divided into 3 layers: Apical, Mantle, and Deep layer. (Vejjabhinanta et al., 2009)

Apical layer is under reticular dermis, which is around sweat glands and hair follicles. This layer has blood vessels, lymph vessels and nerves. Its contains high amounts of carotenoids, seen in yellow color. Destruction of this layer causes congestion of bloods (hematoma) and lymph (seroma) and distorted touch sensation (paresthesia). Mantle layer consists of columnar cells, helps to prevent impact force by spreading the force out. Deep layer is beneath mantle layer. The shape of cells in this layer will vary in gender, age, genetics and other areas of the body. Fat cells are in lobules, the area between lobule has fibrous septa.

Adipose tissue is found throughout the body except eyelids, proximal nail fold, penis, scrotum, auricles (except earlobes). The fatty tissue is prominent in the temples, cheeks, chin, nose, abdomen, buttocks, thighs with increase in thickness at palms and soles. Age, gender and lifestyle affect the distribution of fat tissue. Adipose tissue is distributed regularly in newborns. As for the adult, fat in some areas is reduced with an increase in some areas as a result of the hormone. In addition, Males and females have different fat patterns. Male has an Android distribution, accumulation of fat at upper part of body. Women have a gynoid distribution with accumulation of fat in lower part of body, especially abdomen, hips and thighs, shaped like a pear

In elderly, hyper-hypo accumulation of fat can be seen in many areas, such as infraorbital eye bags, buccal fat pad, breast fat in male, increase fat at abdomen buttock and thighs.

Cellulite is a skin alteration often described as an “orange peel”, “mattress”, or “dimpling” appearance on the thighs, buttocks and sometimes lower abdomen of otherwise healthy women. Although some men may get it, 90-98% of cellulite cases occur in women. The uppermost layer of subcutaneous fat has been described as “standing fat-cell chambers” separated by connective tissue. From these fat-cell chambers, small projections of fat cells protrude into the dermis. This unevenness and irregularity of the subcutaneous fat gives skin the ‘bumpy’ appearance we call cellulite.

The reason cellulite is rarely seen in men (obese and non-obese) is because the epidermis, dermis and uppermost part of the subcutaneous tissue is different in males. Men have thicker epidermis and dermis tissue layers in the thighs and buttocks. More distinctively dissimilar, the first layer of fat, which is slightly thinner in men, is assembled into polygonal units separated by crisscrossing connective tissue. The differences in subcutaneous fat cell structure in men and women occur during the third trimester of fetus development and are manifested at birth. Variations in hormones between genders largely explain this skin structure deviation. It has been shown that men who are born deficient in male hormones will often have a subcutaneous fat appearance similar to females (Lockwood, 1991).

2.3 The Ways to Reduce Fat Around the Thighs

Methods in reducing fat around the thighs that are used in the present are the same treatment methods for the reduction of localized fat deposits and elimination of cellulite (Khan, Victor, Rao & Sadick, 2010) which include a variety of methods.

2.3.1 Massage Equipment and a Vacuum (Endermologie, Massage/Suction Technique)

From the principle that the fat accumulation is caused by a deficiency of lymphatic circulation in the area, the use of physical force makes subcutaneous fat have a movement, which is believed to stimulate lymphatic flow and improve the alignment of structures in subcutaneous layer.

The Endermologie is a tool with rollers to massage with a vacuum system to suction the skin. It is intended to be used in massage to make the scar softer and used in doing a standard physical therapy. Later, this has been modified to improve alignment of adipose tissue and shaping.

Chang, Weiseman, Jacoby, Salisbury and Eresk (1998) studied the use of endermologie to reduce fat of patients and found that it can reduce the proportion of patient's body, depending on time and frequency of treatment, no association with change in body weight of the patient.

However, there has never been studied in long-term performance and no clinical research has been studied in patients with a control group and experimental group randomly selected for treatment with the use of endermologie for reducing localized fat deposits. Therefore, it is not possible to conclude as to the extent of usefulness of this equipment in treating excess fat.

2.3.2 Carboxytherapy

Carbon dioxide gas is passed into the subcutaneous fat layer in the area to be treated. Mechanism of action is temporary vasodilatation from changes of pH and improvement of the metabolism, more collaterals occurred lead to the rebuilding of intradermal collagen resulting in skin tone and texture improvement. Improvement of metabolism leads to gradual improvement of the fibrolipodystrophy (cellulite), also helping in releasing of subcutaneous adhesions like older scars. Another action is lipolytic effect resulting from carbon-dioxide expanding and destroying fat cell directly with the occurrence of excess carbon-dioxide in the tissue around it. The blood supply has been created which helps to burn fat by stimulating the body's oxygen (physiologic oxidative lipolytic process).

Brandi et al. (2001) studied the use of carbon-dioxide to reduce localized fat deposit around the thigh, knees and abdomen of the patients. It has been found that treatment with this method is effective in reducing localized fat deposit and does not cause serious side effect from treatment. Side effects may be local burning, oppressive pain, fleeting at the injection site, swelling, redness of the treated area but only temporarily, limb heaviness sensation(no longer than 2 hours), rubor at the injection site, ecchymosis, subcutaneous crepitation. The treated area has increase blood flow, the destruction of adipose tissue. The circumference of thighs, knees, and abdomen of patients decrease significantly.

2.3.3 Mesotherapy or Lipodissolve

Adipolytic therapy is a novel technique that uses subcutaneous injection of phamacologically active, natural detergents to chemically ablate adipose tissue for spot fat reduction. This technique is suitable for non-obese patients with localized fat accumulation.

The active ingredients of lipodissolve formula are a mixture of phosphatidylcholine, a natural substance derived from soybean lecithin, and deoxycholate, a bile salt. Mechanism of action of these compounds is to inhibit the phosphodiesterase enzyme and increase cyclic adenosine monophosphate (cAMP) level, this will cause adipose tissue breakdown.

These drugs were used for injection in the subcutaneous fat layer in the thighs, abdomen, below chin and eye bags (Treacy & Goldberg, 2006). It has been found that the treated area had decrease in fat accumulation. However, this procedure was not standard practice. There may be risks of adverse events like a rash, swelling erythema, ecchymosis, nodule, ulceration and skin necrosis. As for the breakdown of fat by this method, the drug should be standard approved and under the supervision of physicians who are trained for that. (Duncan & Palmer, 2008).

2.3.4 Radiofrequency Device

The mechanism responsible for radiofrequency heating of biological tissue is through the resistance of conductive current flow “tissue dependent”. Different types of tissue have difference in resistance (Fat tissue has the most resistance). When electrons encounter any resistance to their flow, heat is produced at the site of maximum resistance. The extension of the clinical effect depends on the relative electrical resistance of target tissue. It has been used for skin tightening, cellulite reduction, fat reduction, body sculpting and improvement of striae appearance.

2.3.4.1 Bipolar Radiofrequency: two electrode applicators are involved, one for coupling of the RF energy and the other to serve as a return electrode for RF current. The electrical current propagation is limited between the two electrodes, and the depth of penetration is approximately half the distance between the electrodes. Penetration of depth at superficial and constant levels about 1-5 mm. Bipolar RF configurations must use a cooling device in order to prevent epidermal overheating and the potential of burn injuries.

2.3.4.2 Monopolar Radiofrequency: single electrode applicator tip and and a grounding plate. Monopolar RF can transfer energy to the deeper tissue than Bipolar RF (about 5-20mm.). This system must use a cooling device too.

2.3.4.3 Tripollar Radiofrequency: This system works by two negative charges. One positive charge generates energy into a deeper tissue (dermal and subcutaneous at about 20 mm.). It can transmit intense energy, but requires low power source. Tripollar RF does not need to use cooling device

2.3.4.4 Unipolar Radiofrequency (Anolik, Chapas, Brightman & Geronemus, 2009): RF-induced heating of biological tissues by rotational movement of water (dipole) molecules in the alternating electromagnetic field leads to deep tissue heating. Moreover, the system can cause collagen fibers to contract, resulting in skin tightening immediately after treatment.

2.3.4.5 Multipolar Radiofrequency: Rotation of current between the 8 electrodes along the ircle is extremely fast. Each of the electrodes emits RF to the other 7 electrodes at hundreds of thousand times per second which results in the uniform and homogeneous distribution of the heat and fast heating. This system does not need pre/post cooling device.

2.3.5 High Intensity Focused Ultrasound (HIFU)

Recently, high-intensity focused ultrasound (HIFU) technology is being used for performing noninvasive body sculpting by disrupting unwanted adipose cells. There are 2 mechanisms that result in ablating the adipose tissue. One is mechanical effects that disrupt the cell membranes immediately. The other mechanism is heat that destroys additional fat cells at temperatures above 58˚C, and occurs in the focal spot of HIFU. The result is coagulative necrosis and almost immediate cell death within the targeted area, while the surrounding tissue remains mostly unaffected (Ter Haar & Coussios, 2007)

Following HIFU treatment, the dead cells induce a wound healing response, and attract macrophages (along with other cells), which engulf and transport lipids and cellular debris away from the treatment area. Most of the destroyed adipocytes are desorbed within 12 weeks after treatment and 95% are desorbed after 18 weeks. This results in an overall reduction in local fat volume. These changes occur with no significant increase in plasma lip-ids (Fodor, Smoller, Stecco & Desilets, 2006). The wound healing cascade results in attraction of inflammatory cells, followed by fibroblast induction. This, and collagen denaturation by heat, result in new collagen formation which is followed by tightening of septal fibers and skin. Possible side effects are

sensations of prickling, tingling, warmth, heat, discomfort, or pain during treatment, and temporary erythema, ecchymosis, discomfort, paresthesia, and edema after treatment.

2.3.6 Cryolipolysis

This is the new approach to reduce fat in the subcutaneous layer by reducing the temperature of the area to be treated to induced fat cell apoptosis, and then removed by immune system. (Avram & Harry, 2009)

Dover et al. (2009) studied the use of cryolipolysis to reduce fat in the flanks and back of the patients. It has been found that it can reduce fat thickness in the treated area about 22.4% at 4 months after treatment without serious side effects.

Coleman, Sachdeva, Egbert, Preciado and Allison (2009) studied the use of cryolipolysis to reduce the fat and to evaluate the nervous function in the treated area. It has been found that there is a temporary decrease sensation in the treated area and return to normal within 7 weeks, without affecting the nerves in the long-term.

However, this procedure still needs to study about its safety. It cannot be used in patients with disease that can exacerbate when triggered by cooling such as cold urticaria, cryoglobulinemia etc.

2.3.7 Laser-assisted Liposuction

Heat from the laser causes the breakdown of fat tissue during liposuction (Parlette & Kaminer, 2008). Heat energy not only destroys the adipocytes but also causes collagen remodeling, hence giving the skin tightening. This result will be improved in 3-6 months (Badin, Moraes, Gondek, Chiaratti & Canna, 2002; Mordon et al., 2009). Laser-assisted liposuction is applicable in limited areas, such as face, cheeks, upper arms and breasts. However, this technique may cause side effects: burn to skin over the treated area or injury to facial nerve during the treatment which affects facial expression.

2.3.8 Ultrasound-assisted Liposuction

The initial experience with ultrasound-assisted liposuction in treating difficult fibrous area such as gynecomastia and back which are not uniformly responsive to traditional liposuction, has led to evolution and improvement of ultrasound-assisted liposuction technique. The means by which this procedure helps achieve fat contouring

differs from that of traditional liposuction. Ultrasound-assisted liposuction removed fat through a fat emulsification process term “cavitation” whereas traditional liposuction achieves contouring through the mechanical avulsion of fat (Rohrich, Beran, Kenkel, Adams & DiSpaltro, 1998). However, this technique may cause side effects, most common one is lymphatic congestion in treated area. Because ultrasound melt fat into liquid but not properly remove. In addition, there may be numbness after treatment due to damage to nerve receptors in that area.

2.3.9 Liposuction

Liposuction is the most commonly performed esthetic surgical procedure. Developed in Europe in the mid 1970s, it has become the gold standard for the reduction of localized fat deposit when performed with the use of the tumescent local anesthesia technique (Housman et al., 2002; Hanke, Bernstein & Bullock, 1995; Klein, 1993). However, liposuction is a surgical procedure, it has limitations and possible complications. Arrangement of death rates attributable to liposuction has been reported. Complications can be infection, embolism, visceral perforations, seroma, nerve compression, change in sensation, swelling, skin necrosis, fluid imbalance, toxicity from anesthesia, scars and contour irregularities. Conventional liposuction cannot improved stretchmarks and cellulite.

2.4 Body Contouring by Non-invasive Focused Ultrasound

Ultrasound has been proposed for fat reduction for many years. However, external ultrasound devices, usually based on physical therapy machines, have not been proven successful in this regard. Because ultrasonic energy diminishes with the distance from the generator to the target, these non focused energy sources affect the skin more than underlying fat (Zocchi, 1996). The invention of internal ultrasonic devices has been intended for use in liposuction to directly destroy fat cells (Kenkel et al., 1998) .This technology continues to be used today, although internal ultrasonic liposuction has showed a tendency to produce increased complications, such as burns and skin irregularities, without a noticeable difference in results. (Cooter et al., 2001; Igra & Satur,

1997). Meanwhile, the idea that a more focused form of ultrasonic energy might be effectively used externally has continued to simmer in the minds of scientists.

Sound waves can be divided into ultrasonic (above the audible range), infrasonic (below the audible range), and audible (20HC-20,000HC). Ultrasonic waves create compression cycles that exert positive pressure and expansion cycles that exert negative pressure. This pushing and pulling effect can lead to rupture of fat cells and eventually cavitation (Brown, A. B. et al., 2009). Focusing this ultrasonic energy into the deeper fat layers can lead to cavities (Figure 2.2) in the fat and theoretically reduction of the overall thickness of the adipose layer.

The Body contouring by non-invasive focused ultrasound (UltraShape^{TM}) is an exciting, clinically proven, nonsurgical and noninvasive body contouring solution for both men and women. It offers an alternative to patients seeking effective reduction of localized areas of fat deposits without surgery. UltraShape was the first focused ultrasonic device to show noninvasive selective fat cell destruction. The manufacturer, UltraShape, Inc, received a CE mark in July 2005 and a health Canada Medical Device license in May 2007 for its Contour I device. The company initiated a US investigational device exemption (IDE) clinical study in August 2008. To date, it is in clinical use in 57 countries, and over 100,000 patient treatments have been performed with an excellent safety profile.

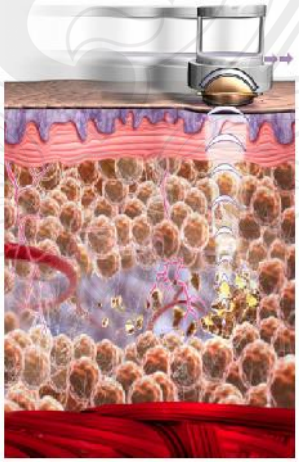
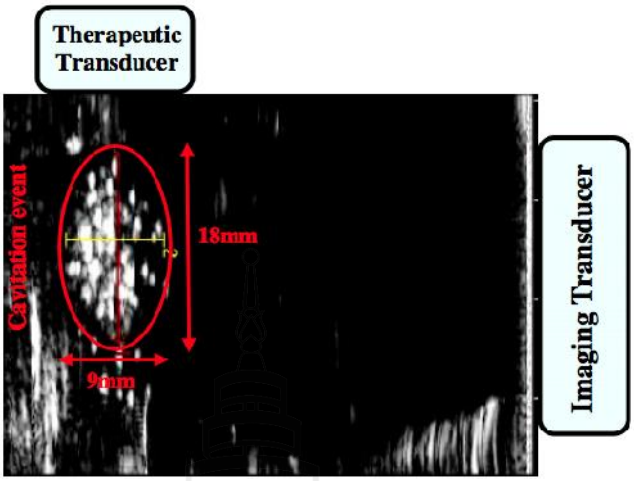


Figure 2.1 Ultrasound Induced Cavitation of Subcutaneous Fat Causing Fat Cell Destruction while Sparing Blood Vessels and Nerve Structures



From Lafon, C., Zderic, V., Noble, M. L., Yuen, J. C., Kaczkowski, P. J., Sapozhnikov, O. A., Chavrier, F., Crum, L. A. & Vaezy, S. (2005). Gel phantom for use in high-intensity focused ultrasound dosimetry. **Ultrasound Med. Biol., 31**(10), 1383-1389.

Figure 2.2 Shown a Cavitation Event Produced by the TFT Operated at a Standard Output Level in a Hydrogel, Mimicking Soft Tissues and Visualized by an Ultrasound Imager

UltraShape features focused ultrasonic energy at a controlled depth uses a nonthermal pulsed wave (Brown, A. B. et al., 2009; Teitelbaum et al., 2007). The mechanical acoustic effects of UltraShape cause selective fat cell disruption without injury to skin, vessels, nerves, or connective tissue (Figure 2.1) (Brown, A. B. et al., 2009; Moreno-Moraga, Valero-Altés, Riquelme, Isarria-Marcosy & de la Torre, 2007). The contents of fat cells are then processed by body's natural metabolic pathways, the same way that fat is processed when one loses weight. The Ultrashape system console houses a power unit, an ultrasound generator, a cooling system, and a computer that orchestrates the overall performance of the system. The device relies on a real time tracking and guidance system that allows treatment only within marked treatment areas and assures that each point is treated only once. This tracking system protects against the

possibility of overtreatment and insures a uniform coverage of the treatment area. Studies to date indicate a low risk of irregularities. (Brown, A. B. et al., 2009; Teitelbaum et al., 2007; Moreno-Moraga et al., 2007).

There are three components to the ultrasound technology that increase the effectiveness and safety to the target cells and surrounding tissues.

2.4.1 **Focused Beam** (Figure 2.3)

Focused ultrasound delivers low energy at the surface but provides a concentrated intensity of energy where the ultrasound waves converge at the focus. This focused energy allows targeting of defined tissues at a controlled depth, while leaving adjacent structures, such as skin, blood vessels, nerves, and muscles, unharmed. Since the effect is focused a specific depth, overlaying skin is not damaged. (Figure 2.4).

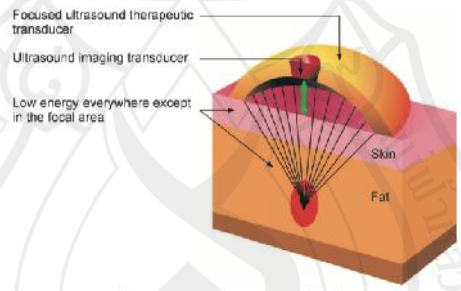


Figure 2.3 Focus Ultrasound Energy

2.4.2 **Pulsed Energy**

The ultrasound wave is delivered in pulses, which allows the generated heat to dissipate before the next pulse begins. This minimizes the temperature rise (0.5 C) within and around the targeted tissues.

2.4.3 **Nonthermal Mechanical Effect**

The energy delivered is transformed into mechanical stresses, which results in better control of tissue effect. The high mechanical stresses remain within the focus and exist only for the duration of energy delivery. This allows the targeting of tissues that are

most susceptible to mechanical disruption, while more resistant structures remain intact.

Moreno-Moraga et al. (2007) studied the efficacy and safety of a novel non-invasive focused ultrasound system (UltraShape Ltd, Tel Aviv, Israel) in reducing localized fat deposits to improve body contours. It has been found that all patients showed significant reduction in subcutaneous fat thickness within the treated area. The mean reduction in fat thickness after three treatments was 2.28 cm. Circumference was reduced by a mean of 3.95 cm. Weight was unchanged during the treatment and follow-up period. No adverse effects were observed.

Studied the safety and efficacy of a focused therapeutic ultrasound device for noninvasive body contouring. It has been found that one hundred sixty four subjects participated in the study. A single Contour I treatment was safe and well tolerated and produced a mean reduction of approximately 2 cm in treatment area of circumference and approximately 2.9 mm in skin fat thickness. The majority of the effects was achieved within 2 weeks and was sustained at 12 weeks. No clinically significant changes in the measured safety parameters were recorded. Seven adverse events were reported, all of which were anticipated, mild, and resolved within the study period.

Studied the safety and effectiveness of a noninvasive, nonthermal, focused ultrasound technology for body contouring. Real-time imaging of acoustic field distribution obtained by the Schlieren system, as well as real-time ultrasound visualization, produced stable cavitation both in water and in the gel phantoms. The area where the effect was visible corresponded to the focal area of energy delivered by the system transducer, as measured by the hydrophone. Histologically stained specimens of skin and subcutaneous fat that were excised from porcine studies (n = 14) following treatments (n = 31) demonstrated fat cell lysis and no observable cellular destruction of adjacent blood vessels, nerves, and connective tissue. No epidermal or dermal changes were observed clinically or histologically.

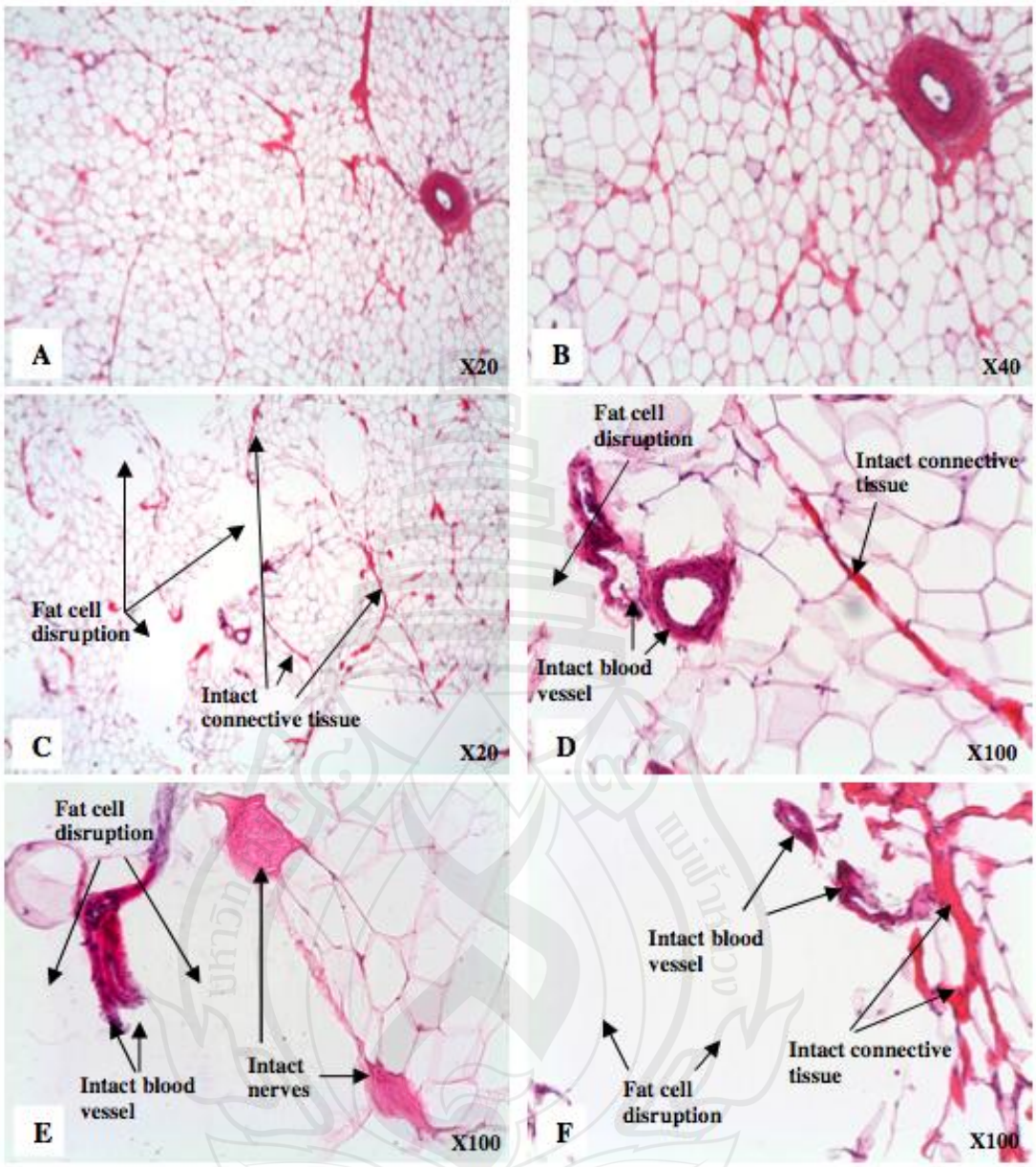


Figure 2.4 Microscopic Evaluation of the Contour I Effect on the Swine Adipose Tissue

Figure 2.4, While intact fat cells are observed in the untreated control (A and B), fat damage is detected in the ultrasound-treated samples (C-F) where ultrasound leads to a selective adipocytes disruption leaving connective tissue (C and D), blood vessels (D-F) or nerve (E) intact.

Fat Clearace Mechanism (Brown, 2005; Brown, S. A. et al., 2009)

Fat, inside fat cells, exists in the form of triglycerides. A triglyceride molecule is composed of three fatty acids attached to a glycerol backbone. When the fat cell membrane is destroyed, triglycerides are released into the interstitial fluid between the cells. The presence of large amounts of triglycerides, in interstitial fluid compartments, has no natural correlation. When being outside the fat cell, triglycerides are normally packaged in discrete lipoprotein particles-a combination of apolipoproteins and lipids, cholesterol, triglycerides, and cholesteral esters. A series of metabolic pathways direct the trafficking of water insoluble molecules of cholesterol and triglyceride through the water-based circulatory system and to the interstitial fluid space1. During the passage through the arteries and interstitial space, lipoprotein-bound triglycerides are catabolized to free fatty acids and glycerol molecules.

A very simplified illustration depicts the normal transport of cholesterol and triglyceride in our circulatory system. Triglyceride (TG) is primarily ingested during the dietary processes [stomach and intestine] and transported by chylomicrons through the capillaries (red arrow) and lymph where a large portion is broken down into free fatty acids and glycerol. Any unprocessed TG in chylomicrons is taken up by the liver. A second source of triglyceride is through production in the liver from excess free fatty acids and glycerol. The important cell type that stores TG as an energy bank or depot is the fat cell, or in more scientific terms-an adipocyte.

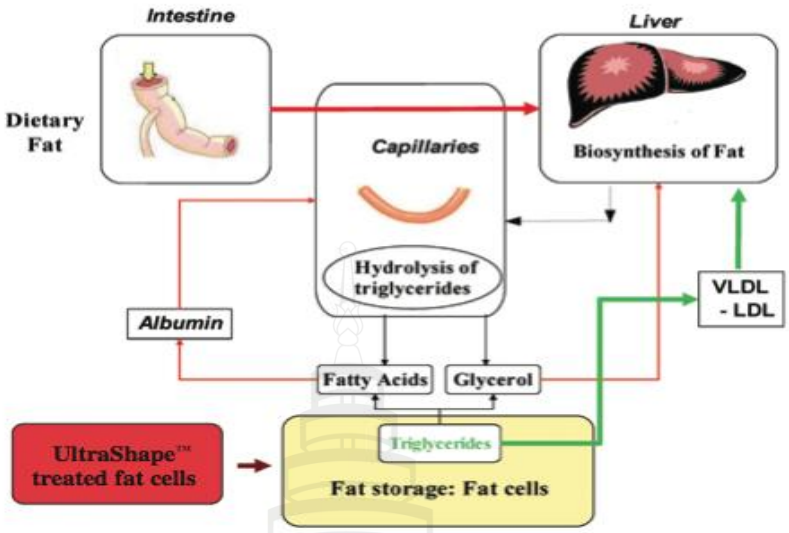


Figure 2.5 Fat Clearance Mechanism

UltraShape (red box) disrupts fat cells by breaking down the cell membranes, causing the release of TG (green) from the cells. A great portion of TG is probably broken into free fatty acids and glycerol because of the enyzme, lipoprotein lipase, on the fat cell membrane walls. (Figure 2.5)

The free fatty acids being relatively insoluble in water bind to albumin and are slowly transported to the liver or other tissues that need these molecules as building blocks or energy. Glycerol is soluble in water and is transported to the liver or to other cells that could use this molecule. The free glycerol equilibrates among both the interstitial fluid compartment (tissue fluids) and systemic (blood) fluid compartments.

If the released TG (green) is not broken down, it may bind to very low density lipoprotein particles (VLDL) found in the lymph. VLDL is further processed to other lipoprotein classes (IDL, LDL) and ultimately transported to the liver for recycling back to free glycerol and free fatty acids.

Safety assessments included laboratory testing, pulse oximetry and liver ultrasound. The laboratory evaluation included complete blood count, serum chemistry, fasting lipids (total cholesterol, HDL, LDL and triglycerides), liver markers and complete urinalysis during the follow up period. No clinically significant changes have been

observed (Figure 2.6). The detailed report on this study is the subject of the peer reviewed article.

Laboratory Study	Study Results
Pulse Oximetry	Normal
Liver Ultrasound	No treatment induced change
Urinalysis	No clinically significant changes
CBC	No clinically significant changes
PT, PTT, INR	No clinically significant changes
Electrolytes, BUN/Cr	No clinically significant changes
LFT's, Bilirubin, Albumin	No clinically significant changes
CPK, Calcium	No clinically significant changes

Figure 2.6 Safety Assessment after Ultrashape Treatment

2.4.4 Hyaluronidase

It is a preparation of purified ovine testicular hyaluronidase, a protein enzyme. The exact chemical structure of this enzyme is unknown. However, the amino acid sequence for the primary structure of the enzyme has been deduced from the sequence of purified peptides. It (hyaluronidase for injection) dehydrated in the solid state under high vacuum with the inactive ingredients listed below, is supplied as a sterile, nonpreserved, white, odorless, amorphous solid. The product is to be reconstituted with Sodium Chloride Injection before use. Each vial of 6200 USP units contains 5 mg lactose, 1.92 mg potassium phosphate dibasic, and 1.22 mg potassium phosphate monobasic. The reconstituted solution is clear and colorless, with an approximate pH of 6.7 and osmolarity of 290 to 310 mOsm.

Hyaluronidase has been legally marketed in the USA since 1948. The approved uses are based on the ability of hyaluronidase to increase the spread and dispersion of other injected drugs. Those uses were preceded by demonstration of the existence of factors in the mammalian testicle that modify the permeability of connective tissue. Subsequent work demonstrated that a factor extracted from testicular tissue enhances the spread of solutions of dyes and toxins injected intracutaneously into experimental animals. The factor was called 'spreading factor' and was identified to be hyaluronidase

(Hobby, Dawson, Meyer & Chaffee, 1941). Spreading factor is also present in, for example, venoms, toxins, bacteria and spermatozoa.

The Extracellular Matrix controls the diffusion and bulk fluid flow of molecules and maintains specific tissue architecture. Within the hypodermis, the Extracellular matrix exists as a network of fibrous proteins within a viscoelastic gel. Structural macromolecules such as collagen and elastin form the basic fibrous building blocks that support adipocytes and vascular structures. Whereas collagen exists essentially as a solid-phase in the Extracellular matrix, glycosaminoglycans and proteoglycans form the hydrated viscoelastic gel-like substance in which the fibrous components are embedded. Glycosaminoglycans create a barrier to bulk fluid flow around the fibrous matrix by way of their viscosity and water of hydration (Bookbinder et al., 2006). Hyaluronic acid (HA), a glycosaminoglycan found extensively in the interstitial matrix and basement membrane. HA, a main component of interstitial gel, inhibits the flow of substances through the tissue. Glycosaminoglycans are complex linear polysaccharides of the Extracellular matrix characterized by repeating disaccharide structures of an N-substituted hexosamine and a uronic acid. These include hyaluronan, chondroitin sulfate, dermatan sulfate, heparan sulfate, heparin and keratan sulfate. Except for hyaluronan, all exist covalently bound to core proteins. The glycosaminoglycans with their core proteins are structurally referred to as proteoglycans.

Despite changes in salt and water intake, the interstitial fluid volume is normally tightly regulated by oncotic and hydrostatic gradients from the blood vascular system and by lymphatic flow (Williams, 1955; Pillwein et al., 1998). The interstitial fluid volume of skin has been calculated at ± 0.4 ml/g of tissue (Pillwein et al., 1998). Albumin is capable of occupying ± 50% of this volume (Eberhart, Weiler & Erie, 2004). Collagen comprises nearly 17% of the extracellular fluid volume of the hypodermis (Pillwein et al., 1998). Although glycosaminoglycans are found at only 1% the concentration of collagen in the skin, they occupy a fluid exclusion volume 10-fold higher than that of collagen on a milliliter H2O/mg basis (Yocum, Kennard & Heiner, 2007). Hyaluronan, the principal glycosaminoglycan of the hypodermis, is a mega-dalton molecule consisting of repeating disaccharide units of N-acetyl glucosamine and glucuronic acid. In contrast to collagen, which has a half-life approaching 15 years (Evered & Whelan, 1988), hyaluronan is

rapidly turned over in the body with a half-life of < 2 days in the skin (Laurent & Fraser, 1992; Rockville, 2004).

The intended pharmacological action of hyaluronidase is to modify the interstitial matrix by enzymatically depolymerizing hyaluronic acid. Hyaluronic acid, a high-molecular-weight glycosaminoglycan, is a major component of extracellular matrix of vertebrates that connects protein filaments, collagen fibers and connective tissue cells (Evered & Whelan, 1988; Laurent & Fraser, 1992). Hyaluronidase splits the glucosaminidic bond between C1 of the N-acetylglucosamine moity and C4 of a glucuronic acid in hyaluonic acid, cause increase penetration between cells. Knowledge of mechanisms involved in the disappearance of injected hyaluronidase is limited (Menzel & Farr, 1998). The clearance of hyaluronidase in the serum occurs with a t1/2 of 2.1 ± 0.2 min, and is followed by inactivation in the kidneys and liver (Menzel & Farr, 1998). The mechanism of inactivation of hyaluronidase after introduction into the dermis remains a matter of speculation. Subcutaneous effects are short lived due to the antagonistic effects of HA synthesis (Frost, 2007) In adult humans, the effects on the interstitial matrix are completely reversed within 48 h (Menzel & Farr, 1998).

The FDA contracted with the National Academy of Science/National Research Council to make an initial evaluation of the effectiveness of over 3400 products that had been evaluated only for safety between 1938 and 1962. This review established the clinical effectiveness of products containing hyaluronidase for three indications: as an adjuvant added to solutions to increase the absorption and dispersion of other injected drugs; for hypo- dermoclysis; and as an adjunct in subcutaneous urography for improving desorption of radio-opaque agents. The finding was based on in vitro enzymatic activity of the products (Rockville, 2004).

Hyaluronidase in treating fat reduction, today, it has been shown that the areas with density of subcutaneous fat will have decrease in blood flows and change of fibrosis, increase in hyaluronic acid 8 times than normal area, thus causing fat accumulation. Hypodermic injection of Hyaluronidase decomposes materials including hyaluronic acid in hypodermic tissue and helps in circulation of adipose tissue by supporting circulation of lymph and enhancement of reuptake for excessive body fluid or blood. It will be helpful for reducing adipose tissue. Yang and Kim (2011) studied the reduction of abdominal circumference by direct hypodermic injection of Hylauronidase into

hypodermic tissue. It has been found that one week after second injection of hyaluronidase, abdominal circumference decreased by 1.81 ± 1.33cm. This study shows statistically significant result to reduce abdominal circumference. ($P<0.05$) Mild adverse reactions such as rubefaction, itching, and pain were observed, but no severe reactions were noticed.

Cheong (2011) studied the change in abdominal circumference after Lipolytic Lymph Drainage (LLD) therapy. LLD is composed of two parts. One is destroying cellulite by local hypodermic injection of Hyaluronidase and the other is moving lipocytes smoothly through lymph massage. Hyaluronidase is used as a medication to destroy fibrous tissue under treatment of lymphedema. It has been found that Abdominal circumference of men decreased from 105.6 ± 9.4cm to 100.86 ± 9.23cm after LLD therapy (a decrease of 4.8 ± 2.4cm) and circumference of women decreased from 90.3 ± 10.3cm to 84.3 ± 8.0cm (a decrease of 6.0 ± 3.8cm). The rate of decrease in women was higher than men.

Hypersensitivity to hyaluronidase or any other ingredients in the formulation is a contraindication. Hyaluronidase should not be injected into or around an infected or acutely inflamed area due to the danger of spreading a localized infection. The most frequently reported adverse experiences have been local injection site reactions. Hyaluronidase has been reported to enhance the adverse events associated with co-administered drug products. Edema has been reported most frequently in association with hypodermoclysis. Allergic reactions (urticaria, angioedema) have been reported in less than 0.1% of patients receiving hysluronidase. Anaphylactic-like reactions following retrobulbar block or intravenous injections have occurred, rarely.

A preliminary skin test for sensitivity to it can be performed. The skin test is made by an intradermal injection of approximately 0.02 mL (3 Units) of a 150 Units/mL solution. A positive reaction consists of a wheal with pseudopods appearing within 5 minutes and persisting for 20 to 30 minutes and accompanied by localized itching. Transient vasodilation at the site of the test, i.e., erythema, is not a positive reaction.

Symptoms of toxicity consist of local edema or urticaria, erythema, chills, nausea, vomiting, dizziness, tachycardia, and hypotension. The enzyme should be discontinued and supportive measures should be initiated immediately. Safety profile: Using enzyme existing in human body (lethal dose =2,000,000 IU)

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Research Design

Randomized controlled clinical trial

3.2 Population and Sample

3.2.1 Populations

Patients, ages between 18-60 years, with localized fat deposits around the thighs

3.2.2 Samples

Patients ages between 18-60 years with localized fat deposit around the thighs, who want to treat their localized fat deposits at Mae Fah Luang University Hospital, Bangkok during September 2012 to February 2013

3.2.3 Sample Size Determination

The sample size was calculated from the formula of one sample, using the ratio of measurement from the previous study (Moreno-Moraga et al., 2007)

From the formula

Assign $\alpha = 0.05$(one-side) , $Z_{0.05} = 1.64$

$\beta = 0.30$, $Z_{0.30} = 0.52$

$$n_{\text{fat thickness}} = \frac{(Z_\alpha + Z_\beta)^2 \sigma^2}{\Delta^2}$$

$$= \frac{(1.64 + 0.52)^2\ 0.80^2}{(0.20 \times 2.28)^2}$$

$$= 14.36$$

$$\approx 15$$

A drop-out rate of 20% is expected, so eighteen patients (= 18) will be recruited.

Note.

n = sample size needed for use in the study

σ = The estimates of the standard deviation found in the population. In this study, a review of the literature, which was 0.80 in fat thickness.

Δ = The difference between fat thickness before and 1 month after last treatment between the groups set by the researcher to be equal to 20% of the average lower. (2.28 in fat thickness)

3.2.4 Selection Criteria

3.2.4.1 Inclusion criteria

1. Healthy patients with localized fat deposits around both thighs.
2. Both males and females, 18-60 years of ages.
3. All subjects are able to participate in the treatment once a month for the duration of three months and can be followed up at 1 month and 3 month after the last treatment.
4. All subjects are required to sign an informed consent form regarding benefits, risks and possible complications of the treatment and the publication of photographs.

3.2.4.2 Exclusion criteria

1. Patients have a subcutaneous fat thickness of less than 0.7 cm around the thighs

2. Patients who had received treatment for excess fat on the thighs by a vendor's tool are as follows

1) Liposuction with prior research

2) Using The Monopolar or Bipolar Radiofrequency in a 6-week period

3) Mesotherapy in a 6-week period

4) Using The Endomology in a 6-week period

5) Carboxytherapy in a 6-week period

3. Patients with significant underlying diseases such as Hypertension, Diabetes Mellitus, Altered states of the immune or inflammatory system, Disorders of connective tissues, Cardiovascular diseases, Renal failure, Metabolic disorder involving abnormal fat metabolism, including but not limited to severe hyperlipidemia or hepatosteatosis, Hepatitis or other liver disease, Epilepsy

4. Patients with Pacemaker, implanted cardiac defibrillator, or other electromagnetic-implanted medical device

5. Patients with History of poor wound healing

6. Patients with infections, Active inflammatory skin disease, open wound in the treatment area

7. Pregnant patients or Lactation

8. Patients with Keloids, hypertrophic scars, or depressed scars in the treatment area

9. Patients with bleeding disorder or coagulopathy or taking drugs that interfere with blood coagulation such as Aspirin, antiplatelet and anticoagulant within six months and NSAIDS within one week before the study

10. Patients with Hypersensitivity to Hyaluronidase or Allergy to anesthesia

11. History of malignant or premalignant lesions in the treatment area

3.2.4.3 Discontinuation criteria

1. Patients with severe complications from the procedure, such as allergic reactions

2. Patients want to withdraw from the research

3. Patients were pregnant during the study

4. Patients' poor compliance with the treatment , unreliable patient

3.3 Tools and Equipment Used in Research

3.3.1 Body contouring by non-invasive focused ultrasound

3.3.2 Hyaluronidase

Mixture protocol: Hyaluronidase 1500 iu + NSS 1 cc + 2% lidocaine (without adrenaline) 1 cc

3.3.3 Before the procedure including

3.3.3.1 Digital scales

3.3.3.2 The height scale

3.3.3.3 The consent form

3.3.3.4 The research data record

3.3.4 The procedure

3.3.4.1 Topical anesthesia (EMLA cream)

3.3.4.2 Cleansing supplies: alcohol, gauzes, cottons

3.3.4.3 Mask

3.3.4.4 Needle no.26

3.3.4.5 Emergency rescue equipment

3.3.4.6 Procedure record

3.3.5 Post procedure

3.3.5.1 pain assessment record (Numeric analogue scale)

3.3.5.2 Side effect record

3.3.5.3 Physical examination record

3.3.5.4 Standard measuring tape

3.3.5.5 Portable ultrasound

3.3.5.6 Bandage

3.3.5.7 Satisfaction record

3.3.6 Digital camera

3.3.7 Statistic program

3.4 Methods

3.4.1 The researcher generates randomization sequence by using “Random Allocation Software” and conceals the sequence in opaque envelopes.

3.4.2 Patients are selected to enroll in the study according to the inclusion and exclusion criteria.

3.4.3 The researcher intensively explains the purpose of the research, process during the study, benefits and possible complications of the treatment.

3.4.4 The patients signed an informed consent form for participation in the study.

3.4.5 The information of the patient is recorded.

3.4.5.1 Name and surname

3.4.5.2 Gender, Age

3.4.5.3 Occupation

3.4.5.4 Frequency of exercise

3.4.5.5 Frequency of the meals

3.4.5.6 History of drug allergy

3.4.5.7 Previous treatment of their localized fat deposits around thighs

3.4.6 The researcher selects the sequence envelop which randomly determines which side of the patient’s thigh to be treated with Hyaluronidase injection in combination with non-invasive focused ultrasound.

3.4.7 Before treatments, the researcher underwent a screening visit including physical examination, weight, height and Body Mass Index(BMI)

3.4.8 Before the treatment procedure

3.4.8.1 Takes a photograph of each patient using digital camera with the patients standing and rotating angle, under constant lightning and standardized distance (1.5 meters between the patient and the camera). Camera height was adjusted to the mid-vertical height of the participant's thighs

3.4.8.2 Measure subcutaneous fat thickness around thigh with a portable ultrasound device between Anterior Posterior Iliac Spine (ASIS) and tip of patella (Figure 3.1), and measured to the nearest millimeter, to confirm the minimal fat thickness for treatment of 0.7 cm and to correct pre-treatment fat thickness

3.4.8.3 Measure thigh circumference with a standard measuring tape at mid-vertical height between Anterior Posterior Iliac Spine (ASIS) and tip of patella (Figure 3.1).

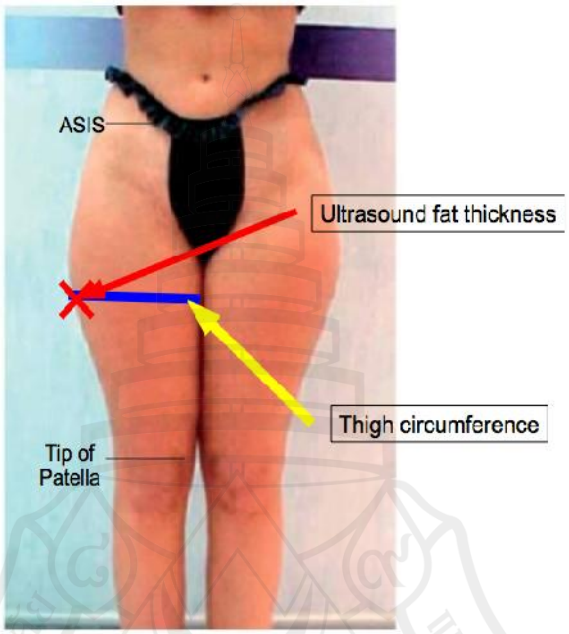


Figure 3.1 Show Landmark for Measuring Fat Thickness and Thigh Circumference

3.4.9 At first treatment session, the researcher has to do a preliminary skin test for sensitivity to hyaluronidase. The skin test is made by an intradermal injection of approximately 0.02 mL (3 Units) of a 150 Units/mL solution on participant's right arm. A positive reaction consists of a wheal with pseudopods appearing within 5 minutes and persisting for 20 to 30 minutes and accompanied by localized itching.

3.4.10 The treatment areas are marked with a skin-marking pen. Then a topical anesthetic cream (2.5% lidocaine/pilocaine, EMLA, APP Pharmaceuticals) is applied to the treatment area with occlusion for one hour.

3.4.11 After that, the anesthetic cream is removed and a skin-compatible treatment oil was applied as a coupling agent during the non-invasive focused ultrasound therapeutic session.

3.4.12 The researcher treated all patients with non-invasive focused ultrasound (Ultrashape^{TM}) bilateral thigh in the same session. After the finish of the Ultrashape procedure, both thighs are cleaned with 70% alcohol.

3.4.13 The researcher injected hyaluronidase to the thigh that previously randomized, subcutaneous injection 1cc/1point (4 points: outer, inner, front, back). On the other thigh with the injection of Normal saline solution (NSS) in the same position.

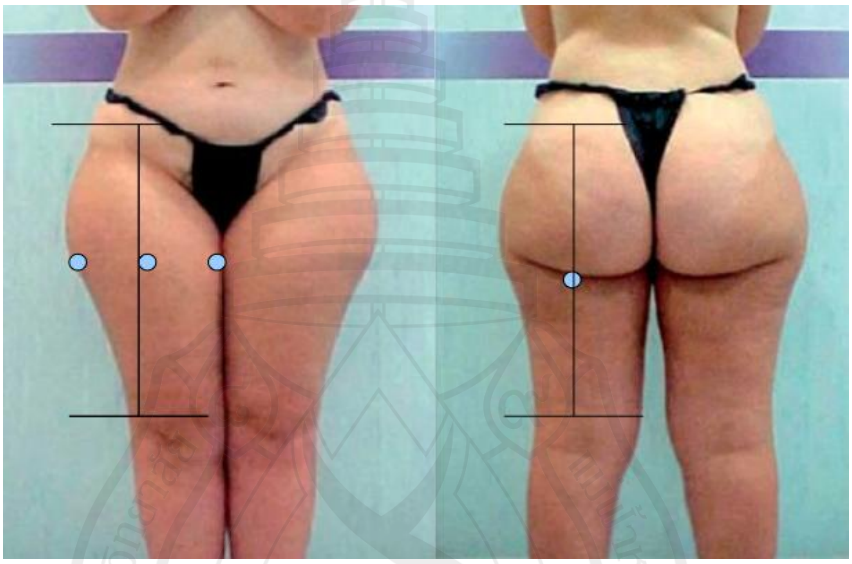


Figure 3.2 Show Injection Point

3.4.14 The researcher does the Compression massage immediately after injection: massage for 100 times, And after injection of 1 set (4 points): massage for 10-20 minutes, to induce the injected drug to flow into the lymphatic vessel, to enhance the flow of lymph fluid. After the procedure, provide pressure with compression bandage on both thighs.

3.4.15 After finishing treatment session, proceed to assessment of side effects and pain score.

3.4.16 The researcher advises the patient to do the following post-treatment self-care : self massage for lymph by direct massage on injected area 10 sets daily (100 times per set), massage for 2-3 days

3.4.17 All patients undergo 3 treatment sessions at 1 month intervals

3.4.18 The patients are given a “side effect record” sheet to record the side effects of treatment including severity and duration of the side effects which are

3.4.18.1 Pain

3.4.18.2 Erythema

3.4.18.3 Itching, rash

3.4.18.4 Ecchymosis

3.4.18.5 Others, such as infection, ulceration, scar formation, post-inflammatory hyperpigmentation and hypopigmentation

If the patients experience any severe side effects, they have to inform the researcher before the next treatment session. The researcher will treat the side effects.

3.4.19 One month and Three month after completion of three treatment sessions, Patients returned for an additional circumference measurement, ultrasound subcutaneous fat thickness and taking a photograph.

3.4.20 The patients are asked to evaluate their satisfaction by means of a questionnaires.

3.4.21 The data is collected and analyzed by statistical methods.

3.4.22 Discussion and conclusion of the study in the written form.

3.5 Outcome Measure & Data Collection

3.5.1 Primary Outcome Measurement

The researcher compares the mean reduction in subcutaneous fat thickness around thighs by portable ultrasound to evaluate the improvement of localized fat deposits around thighs between before treatment, 1 month and 3 months after completing 3 treatment session**s**

3.5.2 Secondary Outcome Measurement

3.5.2.1 The researcher compares the mean reduction of thigh circumference measured by standard measuring tape to evaluate the improvement of localized fat deposits around thighs between before treatment, 1 month and 3 months after completing

3 treatment session

3.5.2.2 Patients are asked to evaluate their satisfaction with the treatments by using the quartile grading scale:

score 0 : unsatisfied

score 1 : very poor satisfied

score 2 : poor satisfied

score 3 : moderately satisfied (fair)

score 4 : quite satisfied (good)

score 5 : most satisfied (excellent)

3.5.2.3 Measurement of adverse effects. The patients are asked to record the following adverse effects after the treatment:

1. pain score, ranging from no pain (0) to the most pain (10)

2. erythema, edema, bruise, burning sensation, itching, rash: duration (days) and severity (range from mild, moderate to severe)

3. Others, such as infection, ulceration, scar formation, post-inflammatory hyperpigmentation and hypopigmentation

3.5.2.4 Patients are asked to evaluate their pain score by using Numeric rating scale (NRS, range from 0 to 10, 0 is no pain, 10 is the most pain) after finished each treatment session.

3.6 Data Analysis

3.6.1 Descriptive Statistics

3.6.1.1 Quality-oriented data (Qualitative data) includes gender, exercise. eating 3 meals, side effects and satisfaction levels The data is concluded in the form of frequency and percentage.

3.6.1.2 Quantity- oriented data (Quantitative data) includes age, weight, height, body mass index, fat thickness, Pain Score and satisfaction scores. The conclusion of data is carried out in the form of mean and the standard deviation, minimum, maximum values.

3.6.2 Inferential Statistics

3.6.2.1 Comparison of the mean of fat thickness (mm) and the mean of thigh circumference (cm) before treatment, 1st and 3rd months after the treatment of three times was completed between the thigh on side treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound versus the thigh on the side treated with the use of ultrasound alone for fat reduction by Repeated Messure ANOVA statistics. The comparison was made before and after treatment in a paired manner in the same group (Multiple comparison) by Bonferoni method.

3.6.2.2 Comparison of the mean change in the 1st month and the 3rd month after the treatment completion of three times with the period before treatment of fat thickness values and high circumference values between the thigh on the side treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound versus the thigh side treated with the use of ultrasound alone for fat reduction by paired t-test statistics.

3.6.2.3 Comparison of Pain Score mean between the thigh on the side treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound versus the side treated with the use of ultrasound alone for fat reduction by Wilcoxon sign rank test statistics.

3.6.2.4 Comparison of side effects caused by the treatment from the first, second times between the thigh on the side treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound versus the thigh side treated with the use of ultrasound alone for fat reduction by Fisher Exact test statistics.

3.6.2.5 Comparison of average scores of satisfaction between the group with thigh on the side treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound versus the group with thigh side treated with the use of ultrasound alone for fat reduction by Wilcoxon sign rank test statistics.

* The level of statistical significance of all tests is determined at $p < 0.05$.

CHAPTER 4

REPORT ON RESEARCH RESULTS

This research with the topic entitled "A comparative study of efficacy in thigh fat reduction between the method of hyaluronidase injection in combination with non-invasive focused ultrasound versus the use of ultrasound alone" is the clinically experimental research with volunteers by the division into two parts. The thigh on one side received the treatment with hyaluronidase injection in combination with non-invasive focused ultrasound device and the other side got the treatment with ultrasound device alone. The treatment was randomly chosen. This research has the purpose of comparing the efficacy of thigh fat reduction between hyaluronidase injection in combination with non-invasive focused ultrasound versus the use of ultrasound device alone. The study was carried out with the sample group of volunteers who came to receive the treatment service at Mae Fah Luang Hospital and were assessed by doctors to have the condition of thigh fat that can be examined with follow-up of treatment totaling 18 persons.

The report on research results is divided into 5 sections as follows:

1. General characteristics of the sample group.
2. Comparison of the mean of fat thickness (mm) and the mean of thigh circumference (cm) in the periods before treatment, 1 month after treatment and 3 months after treatment. The said comparison is between the thigh on the side treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound versus the thigh on the side treated with the use of ultrasound alone for fat reduction.
3. Comparison of the mean change at 1 and 3 months after treatment with the mean before treatment of the values of fat thickness and thigh circumference between the thigh on the side treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound and the thigh on the side treated with the use of ultrasound alone for fat reduction.

4. Number, percentage of side effects arising out of the treatment in the first, second and third times between the thigh on the side treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound and the thigh on the side treated with the use of ultrasound alone for fat reduction.

5. Number, percentage of satisfaction from the thigh on the side treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound and the thigh on the side treated with the use of ultrasound alone for fat reduction.

Table 4.1 Baseline Characteristics (n=18 cases)

Characteristics		Number
Age (years)		
	21 - 30	6
	31 - 40	5
	41 - 50	2
	51 - 60	5
	Mean±SD	37.94±12.27
Gender		
	Male	3
	Female	15
Weight (kg)		
	41 - 50	4
	51 - 60	6
	61 - 70	3
	71 - 80	5
	Mean±SD	59.67±11.10
Height (cm)		
	151 - 160	5
	161 - 170	11
	171 - 180	2
	Mean±SD	163.17±5.39

Table 4.1 (continued)

Characteristics		Number
BMI (kg/m2)		
	Normal (18.5-24.9)	9
	Lower weight(<18.5)	4
	Over Weight(≥25.0)	5
	Mean±SD	22.35±3.60
Exercise		
	Never	2
	Sometime	4
	3 Day/week	12
Food consumption 3 meal/day		18

Table 4.1 shows general data of all 18 participants in the research project. It is found that the sample group in this study had an average age of 37.94 ± 12.27. The minimum age was 22 years and the maximum age was 57 years.

Most participants in the research project were female totaling 15 persons, representing 83.3 percent. The male participants account for 16.7 percent.

Regarding the body mass index, it is found that the mean is 22.35±3.60 kg/m2. Most participants accounting for 9 persons were in the normal range, representing 50.0 percent, followed by the level of Over Weight with the total of 5 persons, accounting for 27.8 percent and the level of Lower weight with four persons, representing 22.2 percent.

Concerning health-related behavior, it was found that most participants totaling 12 persons exercised 3 days /week, representing 66.7 percent. As for eating food, it was found that all participants of the sample group ate three meals /day.

Table 4.2 Compares the Mean of Fat Thickness (mm) and the Average of Thigh Circumference (cm) of Periods before Treatment, after Treatment for 1 and 3 Months between the Thigh Side Treated with the Method of Hyaluronidase Injection in Combination with Non-invasive Focused Ultrasound and the Thigh Side Treated with the Use of Ultrasound Alone (n=18)

Factors	Treatment Group				p-value (between group)
	Hyaluronidase with ultrasound		Ultrasound alone		
	Mean	SD	Mean	SD	
fat thickness(mm)					
Before treatment	46.12	4.56	45.96	4.32	0.914
1 Month	18.55	4.83	27.95	6.22	<0.001
3 Months	18.53	4.80	27.81	6.03	<0.001
p-value (with in group)					
Before vs 1 Month	<0.001		<0.001		
Before vs 3 Months	<0.001		<0.001		
1Month vs 3 Months	1.000		0.46		
thigh circumference(cm)					
Before treatment	56.88	5.40	56.73	5.31	0.934
1 Month	51.73	5.38	53.54	5.27	0.315
3 Months	51.46	5.35	53.30	5.26	0.304
p-value (with in group)					
Before vs 1 Month	<0.001		<0.001		
Before vs 3 Months	<0.001		<0.001		
1Month vs 3 Months	<0.001		<0.001		

Table 4.2 compares the mean of fat thickness (mm) and the average of thigh circumference (cm) before treatment, after treatment for 1 and 3 months between thigh side treated with hyaluronidase injection in combination with non-invasive focused ultrasound versus thigh side treated with the use of ultrasound alone.

It was found that before treatment when measuring the fat thickness of thigh with ultrasound, the thigh side treated with hyaluronidase injection in combination with non-invasive focused ultrasound had the average of fat thickness equaling 46.12 mm. and standard deviation equaling 4.56. The thigh side treated with ultrasound alone had the average of fat thickness equaling 45.96 mm and a standard deviation equaling 4.32.

At one month after treatment completion of three times, it was found that the thigh on the side treated with hyaluronidase injection in conjunction with non-invasive focused ultrasound for fat reduction had fat thickness representing the average of 18.55 mm and standard deviation equaling 4.83. The thigh on the side treated with ultrasound alone for fat reduction had the average of fat thickness equaling 27.95 mm and a standard deviation equaling 6.22.

At three months after treatment completion of three times, it was found that the thigh on the side treated hyaluronidase injection in combination with non-invasive focused ultrasound for fat reduction had fat thickness equaling the average of 18.35 mm. and a standard deviation of 4.80. The thigh on the side treated with the use of ultrasound alone for fat reduction had the average of fat thickness equaling 27.81 mm. and a standard deviation equaling 6.03.

When comparing the average of fat thickness (mm) before treatment, after treatment for 1 and 3 months between the thigh on the side treated with hyaluronidase injection in combination with non-invasive focused ultrasound for fat reduction with the thigh on the side treated with ultrasound alone , it was found that before treatment there was no statistical difference between the thigh on the side treated with hyaluronidase injection with ultrasound and the thigh on the side treated with ultrasound alone. But after treatment in the 1st and 3rd months, it was found that the group treated with Hyaluronidase with ultrasound had the average of fat thickness less than the thigh side treated with Ultrasound alone.

Such difference is statistically significant at $p < 0.001$ in both months.

On the part of thigh circumference, it was found that before treatment on the measurement of thigh circumference with a tape measure, the thigh on the side treated with hyaluronidase injection in combination with non-invasive focused ultrasound for fat reduction had the average of thigh circumference equaling 56.88 cm. and standard deviation equaling 5.40. The thigh on the side treated with ultrasound alone for fat

reduction had the average of fat circumference equaling 56.43 cm. and standard deviation equaling 5.31.

At one month after the treatment completion of 3 times, it was found that the thigh on the side treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound had the average of thigh circumference equaling 51.73 cm. and a standard deviation equaling 5.38. The thigh side treated with the use of ultrasound alone for fat reduction had the average of thigh circumference equaling 53.54 cm. and a standard deviation equaling 5.27

At three months after the treatment completion of three times, it was found that the thigh on the side treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound had the average of thigh circumference equaling 51.46 cm. and standard deviation equaling 5.35. The thigh on the side treated with the use of ultrasound alone for fat reduction had the average of thigh circumference equaling 53.30 cm. and standard deviation equaling 5.26.

When comparing the mean of thigh circumference(cm) before treatment, after treatment for 1 and 3 months between the thigh on the side treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound versus the thigh on the side treated with the use of ultrasound alone , it was found that there was no statistical difference before treatment and after treatment in the 1st and 3rd months between the thigh treated with Hyaluronidase with ultrasound and the thigh on the side treated with Ultrasound alone for fat reduction.

For the mean of fat thickness during treatment at different months within the groups that received the same treatment including the groups treated with Hyaluronidase with ultrasound and Ultrasound alone, the average of fat thickness at 1 month after the treatment completion of three times is different from the period before treatment. The average of fat thickness at 3 months after the treatment completion of 3 times is also different from the period before treatment. The difference is statistically significant with p-value equally,

i.e. < 0.001. As for 3 months after the treatment was completed one time, there is no statistically significant difference from the mean at three months after the treatment was completed three times in the two treatment groups. Concerning the mean of thigh circumference during treatment at different months in the groups that received the same

treatment, it was found that both groups treated with Hyaluronidase with ultrasound and Ultrasound alone had the difference in average of fat thickness in every month with the same p-value equaling <0.001.

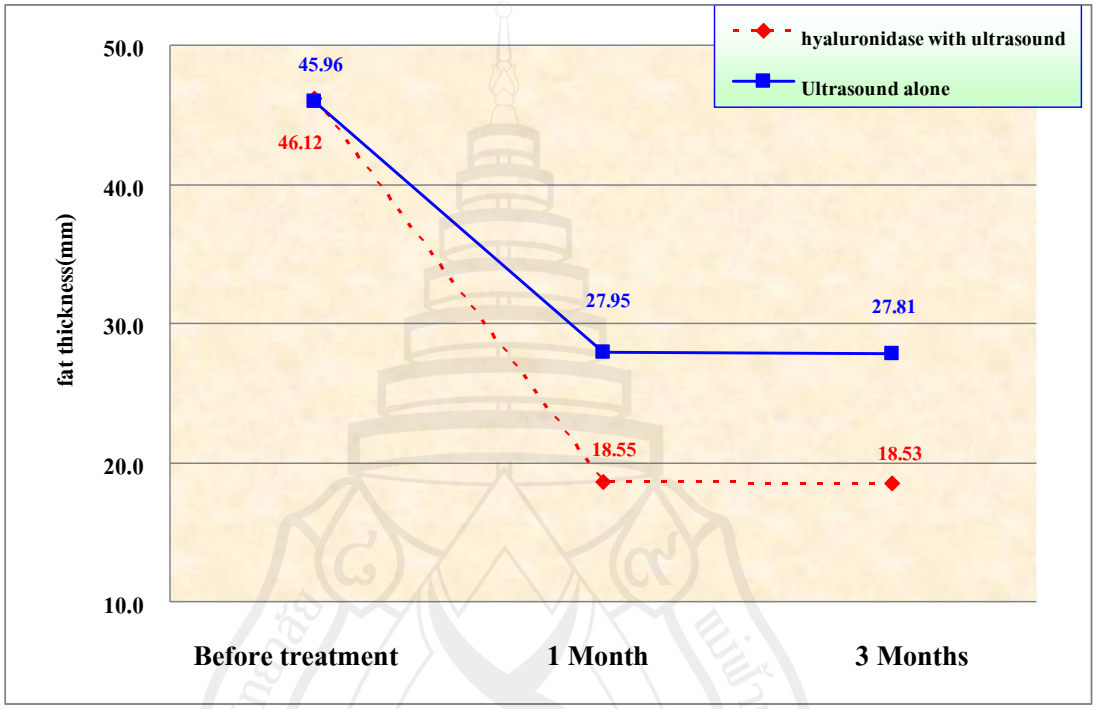


Figure 4.1 Compares the Mean of Fat Thickness before Treatment, after Treatment for 1 and 3 Months between the Thigh on the Side Treated with the Method of Hyaluronidase Injection in Combination with Non-invasive Focused Ultrasound Versus the Thigh Side Treated with the Use of Ultrasound Alone for Fat Reduction

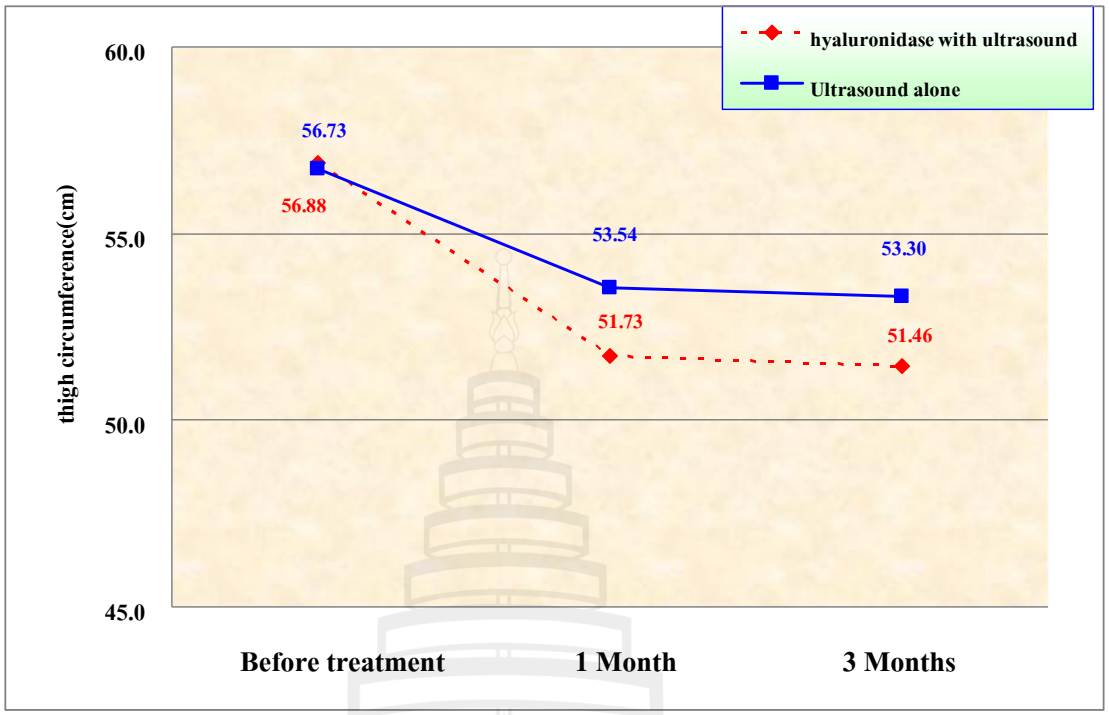


Figure 4.2 Compares the Mean of Thigh Circumference(cm) before Treatment, after Treatment for 1 and 3 Months between the Thigh on the Side Treated with the Method of Hyaluronidase Injection in Combination with Non-invasive Focused Ultrasound Versus the Thigh Side Treated with the use of Ultrasound Alone

Table 4.3 Compares the Average Change in the First and Second Months with the Period before the Treatment of Fat Thickness and the Thigh Circumference between the Thigh on the Side Treated with the Method of Hyaluronidase Injection in Combination with Non-invasive Focused Ultrasound Versus the Thigh Side Treated with the Use of Ultrasound Alone for Fat Reduction

	Treatment Group				**p-value**
	Hyaluronidase with ultrasound		**Ultrasound alone**		
	Change	**%Change**	**Change**	**%Change**	
Fat thickness (mm)					
1 Month - Before treatment	-27.57±3.77	-60.10±8.33	-18.01±3.57	-39.71±9.49	<0.001
3 Months - Before treatment	-27.59±3.63	-60.16±8.21	-18.15±3.29	-40.00±8.98	<0.001
3 Month - 1 Month	-0.022±0.26	-0.11±0.51	-0.14±0.51	-0.37±1.50	0.364
High circumference (cm)					
1 Month - Before treatment	-5.15±3.22	-8.97±5.20	-3.19±1.11	-5.64±1.98	0.01
3 Months - Before treatment	-5.42±3.19	-9.45±5.18	-3.43±1.08	-6.07±1.95	0.01
3 Month - 1 Month	-0.27±0.25	-0.53±0.47	-0.24±0.17	-0.45±0.31	0.469

Note. significant at $p<0.05$

Table 4.3 Comparison of mean change at 1 and 3 months after treatment was completed three times with the average before treatment of fat thickness and high circumference between the thigh on the side treated with the method of hyaluronidase injection in combination with non-invasive focused ultrasound and the thigh on the side treated with the use of ultrasound alone for fat reduction.

Regarding the mean of fat thickness that changes in the first month from the period before treatment, there is a decrease of 27.57±3.77 on average for the group treated with Hyaluronidase with ultrasound while the group treated with Ultrasound alone, the decrease was only 18.01±3.57. Such difference has statistical significance with $p<0.001$,

the same as in the third month which found that fat thickness decreased more for the group treated with Hyaluronidase with ultrasound than the group treated with Ultrasound alone with statistical significance with p <0.001.

Likewise, as for the results in terms of thigh circumference, it was found that the mean that changes from the period before treatment in the first and 3rd months of the group treated with Hyaluronidase with ultrasound, the mean of high circumference decreases greater than the group treated with Ultrasound alone in a statistically significant manner with the same p=0.006 for two months.

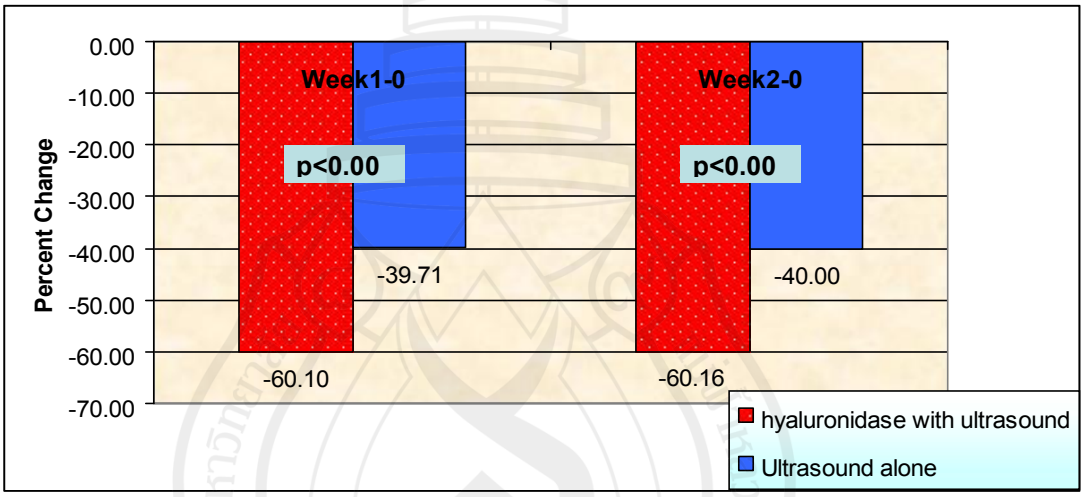


Figure 4.3 Compares the Average Change in 1 and 3 Months with the Period before Treatment of the Fat Thickness between the Thigh on the Side Treated with the Method of Hyaluronidase Injection in Combination with Non-invasive Focused Ultrasound Versus the Thigh Side Treated with the Use of Ultrasound Alone for Fat Reduction

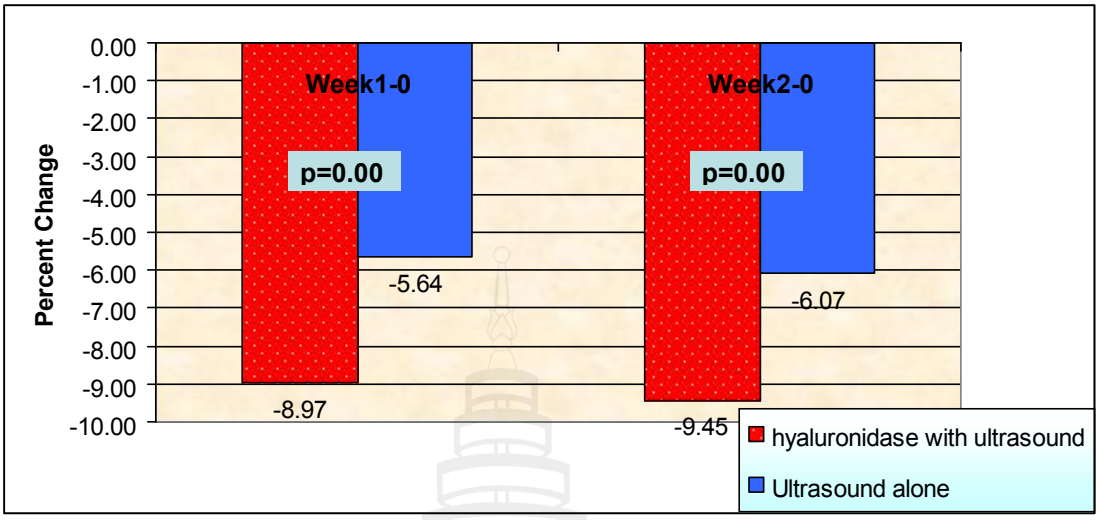


Figure 4.4 Compares the Mean Change in the 1st and 3rd Months after the Treatment Completion of 3 Times with the Mean before Treatment of Thigh Circumference between the Thigh on the Side treated with the Method of Hyaluronidase Injection in Combination with Non-invasive Focused Ultrasound Versus the Thigh on the Side Treated with the Use of Ultrasound Alone for Fat Reduction

Table 4.4 Mean, Standard Deviation, Minimum and Maximum of Body Weight, Change of Body Weight and Percentage of Body Weight Change of Volunteers (n=18)

Weight	Mean	SD	Min	Max
Weight (kg)				
Before treatment	59.67	11.10	45.0	77.0
1 Month	59.64	11.11	45.0	77.0
3 Months	59.64	11.13	45.0	77.0
Change of weight (kg)				
1 Month - Before treatment	-0.03	0.36	-1.0	1.0
3 Months - Before treatment	-0.03	0.40	-1.0	1.0
Percent Change of weight				
1 Month - Before treatment	-0.05	0.64	-1.7	1.8
3 Months - Before treatment	-0.05	0.69	-1.7	1.8

Table 4.4 shows mean standard deviation, minimum and maximum body weight, change in body weight and percentage of change in body weight of volunteers. It is found that all 18 volunteers have the slight decrease in the average of body weight after treatment at several months. The values of mean before treatment , after treatment for 1 and 2 months are 59.67, 59.64 and 59.64, respectively with a slight decrease , that is to say approximately 3 points, accounting for only 0.5 percent of body weight that decreases.

Table 4.5 Number, Percentage of Side Effects Caused by Treatment of the 1st, 2nd and 3rd Times

Side effect	Hyaluronidase with ultrasound			Ultrasound alone		
	Tx1	**Tx2**	**Tx3**	**Tx1**	**Tx2**	**Tx3**
Redness	2(11.11%)	1(5.56%)	1(5.56%)	1(5.56%)	1(5.56%)	1(5.56%)
Blister	0(0%)	0(0%)	0(0%)	1(5.56%)	0(0%)	0(0%)
Burning sensation	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Itching	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Rash	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Bruising	3(16.67%)	1(5.56%)	1(5.56%)	0(0%)	0(0%)	0(0%)
Hyperpigment	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Hypopigment	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Total	**5(27.78%)**	**2(11.11%)**	**2(11.11%)**	**2(11.11%)**	**1(5.56%)**	**1(5.56%)**

Table 4.5 shows the number, percentage of side effects caused by treatment of the first, second and third times. It was found that in treatment of the first time, there were five participants who experienced side effects, accounting for 27.78 percent. Three participants had the symptom of bruising and two participants had redness. As for treatment in the second and third times, two participants in the research had side effects, representing 11.11 percent. One person had the symptom of bruising and one person had the symptom of redness. The symptom of bruising occurred in the location where hyaluronidase injection was performed. As for the treatment with ultrasound alone, it was found that in the first treatment two participants in the research had side effects, accounting for 11.11 percent. In this connection, one person had the symptom of redness and the other had blisters. As for the second and third times of treatment, it was found that one participant in the research had side effect of redness, representing 5.56 percent. All side effects that occurred had severity at just level 1, that is to say only minor symptoms

and can be cured naturally. There were no symptoms of burns, rashes, itching, darker skin or white spots in the thighs of both sides of the research participants who received the two treatment methods.

Table 4.6 Compares Pain Score between the Group with the Thigh on the Side Treated with the Method of Hyaluronidase Injection in Combination with Non-invasive Focused Ultrasound Versus the Group with the Thigh on the Side Treated with the Use of Ultrasound Alone for Fat Reduction

Pain Score	Treatment Group (n=18)		p-value
	Hyaluronidase with ultrasound	Ultrasound alone	
Treatment Time 1			0.001
Mean±SD	2.61±1.72	0.44±0.51	
Min-Max	0-5	0-1	
Treatment Time 2			0.006
Mean+SD	1.11±1.23	0.00±0.00	
Min-Max	0-3	0-0	
Treatment Time 3			0.006
Mean+SD	1.11±1.23	0.00±0.00	
Min-Max	0-3	0-0	

According to Table 6 showing the comparison of Pain Score between the group with the thigh on the side treated with hyaluronidase injection in combination with non-invasive focused ultrasound versus the thigh on the side treated with ultrasound alone, it is found that for all treatment times the group treated with hyaluronidase with ultrasound has the average of Pain Score higher than the group treated with Ultrasound alone. Such difference is statistically significant with the values of p at the 1st, 2nd and 3rd times equaling 0.001, 0.006 and 0.006, respectively.

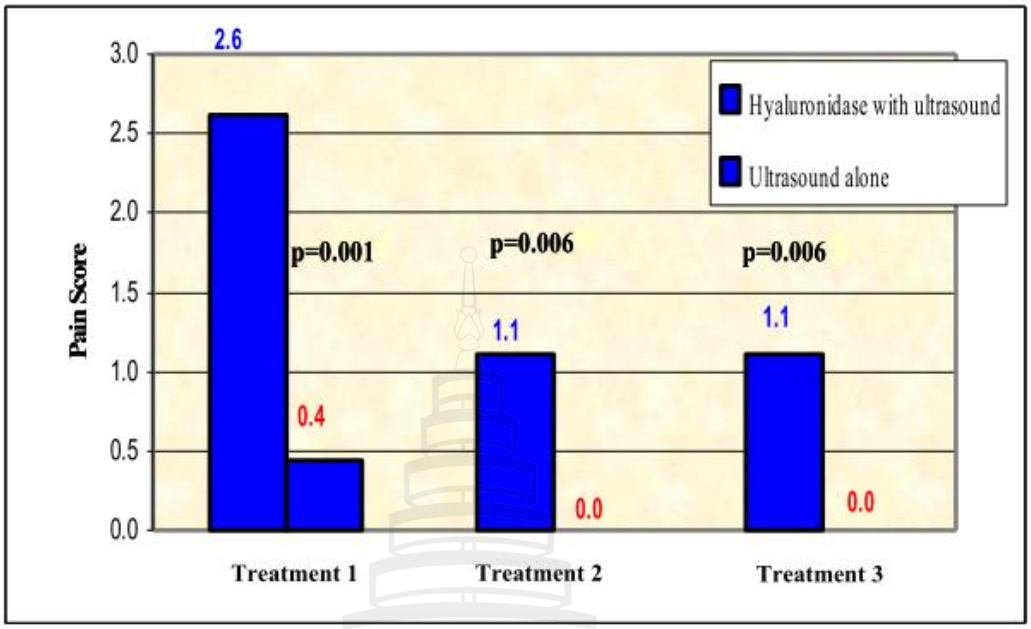


Figure 4.5 Compares Pain Score between the Group with the Thigh on the Side Treated with Hyaluronidase Injection in Combination with Non-invasive Focused Ultrasound Versus the Thigh on the Side Treated with Ultrasound Alone for Fat Reduction

Table 4.7 Compares the Satisfaction between the Group with the Thigh Side Treated with Hyaluronidase Injection in Combination with Non-invasive Focused Ultrasound Versus the Group with Thigh Side Treated with Ultrasound Alone for Fat Reduction

Satisfaction	**Treatment Group (n=18)**		**p-value**
	Hyaluronidase with ultrasound	**Ultrasound alone**	
1	1(5.6%)	2(11.1%)	
2	2(11.1%)	5(27.8%)	
3	4(22.2%)	9(50.0%)	
4	8(44.4%)	2(11.1%)	
5	3(16.7%)	0(0.0%)	
Mean±SD	**3.56±1.10**	**2.61±0.85**	**<0.001**

According to Table 7 showing the comparison of satisfaction between the group with the thigh on the side treated with hyaluronidase injection in combination with non-invasive focused ultrasound versus the group with thigh side treated with ultrasound alone for fat reduction, it is found that the group treated with Hyaluronidase with ultrasound expresses the average of satisfaction higher than the group treated with Ultrasound alone. Such difference is statistically significant with p <0.001.

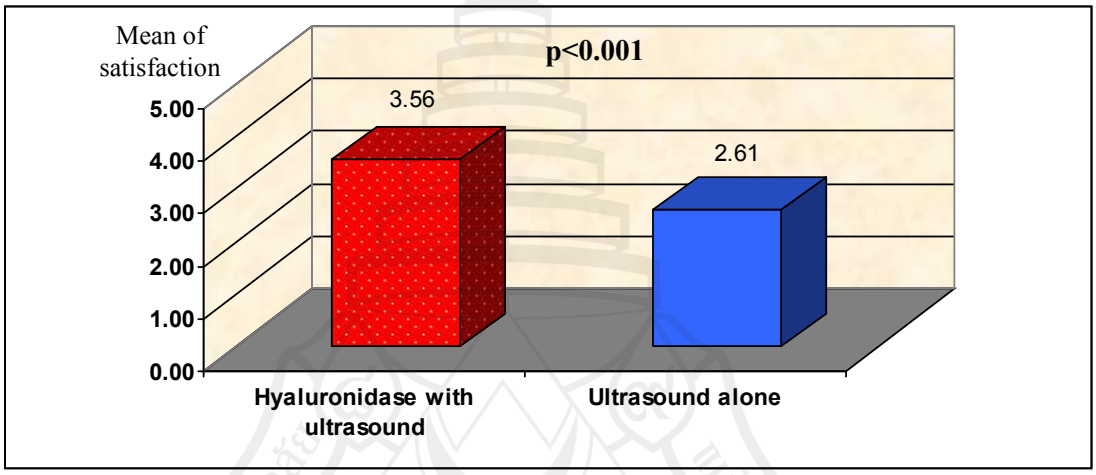


Figure 4.6 Comparison of Satisfaction between the Group with the Thigh on the Side Treated with Hyaluronidase Injection in Combination with Non-invasive Focused Ultrasound Versus the Group with Thigh Side Treated with Ultrasound Alone for Fat Reduction

CHAPTER 5

CONCLUSION, DISCUSSION OF RESULTS AND SUGGESTIONS

5.1 Discussion of Results

The present research is characteristic of clinically experimental study by means of randomized treatment to study the comparison of effectiveness in thigh fat reduction between hyaluronidase injection in conjunction with non-invasive focused ultrasound versus the use of ultrasound alone as well as to examine the side effects and satisfaction from the treatment of participants in the research project.

Excess fat is a major problem that is more common in today's society. Excess fat originates from several risk factors. It is found that the presence of a large amount of fat accumulation in the body negatively affects the body such as in terms of increasing the risk of coronary thrombosis, stroke, fat accumulation in thighs. This is considered as one reason that prevents the body from being well-proportioned and poses the problem in terms of a person's personality and confidence.

In former times the only way to reduce fat and adjust the body shape to be well-proportioned was the treatment of abnormal fat accumulation by means of Tumescent liposuction. This is one way that can help to reduce fat and eliminate excess fat quickly with effectiveness. It has been the aesthetic surgery that has been growing in popularity, but with some limitations nevertheless such as side effects arising from surgery : large open wounds necessary for putting cannula, the lack of Hemostatic process (hemostasis), rehabilitation after surgery, pain , lack of effectiveness of firming up after surgery, infection of the wound and the need for recovery which takes a relatively long time after surgery and fastening in the area where liposuction is performed.

Nowadays, there are many ways to reduce excess fat without surgery such as massage (Deep body massage), the use of high frequency sound waves (Radiofrequency), the use of carbon dioxide (Carboxytherapy) (Brandi et al., 2001). These methods have been quite popular due to safety, less recovery time and the capacity to reduce orange peel skin arising from cellulite. However, these methods require more frequent and numerous treatments and can reduce fat and firm up temporarily only.

As a result, the invention has come up by bringing ultra-sound waves (Focus ultrasound) for use in reducing fat without surgery by sending low power down to the target tissue so as to destroy fat cells (Adipocyte) directly without damaging other structures such as blood vessels, nerves and muscles in the treated area and does not cause damage to the skin in the treated area as well. After the fat cells are broken up by ultrasound waves, various compounds in the fat cells will be eliminated away from the body through vascular and lymphatic systems. The fat reduction in this way is found to be safe, effective with a relatively short recovery period and no need for frequent treatments.

Currently, there have been studies which found that the areas with dense fat will include the decrease in the circulation of blood in such areas with the occurrence of membrane. It is also found that there is an increase in Hyaluronic acid 8 times the normal area. These factors will lead to the increased accumulation of fat in such areas even more. Therefore, Hyaluronidase drug has been brought for injection in the areas with the presence of fat accumulation. Hyaluronidase is an enzyme extracted from sheep''s testicles which serves to break down hyaluronic acid and help the circulation of lymphatic and vascular systems, hence draining the fat away from the areas with fat density better.

Therefore, the researcher has had the concept to conduct a comparative study of the effectiveness between the method of hyaluronidase injection in combination with non-invasive focused ultrasound for thigh fat reduction versus the use of ultrasound alone to be basic information and the option to choose a safe and effective treatment with maximal benefits for patients in the future.

5.2 Discussion of General Information

General data of the sample group includes the following details.

Gender: It is found that there are more female participants than male participants in this research. The female participants represent 83.3 percent and male participants account for 16.7 percent.

Age: Participants in this research project were aged between 22 years to 57 years, representing 37.94 percent on average. For the body mass index, participants in the research had the body mass index equaling the average of 22.35 mainly in the normal range, representing 50.0 percent, followed by Over Weight, accounting for 27.8 percent and Lower weight, representing 22.2 percent.

As for the Underlying disease, all 18 participants in the research had no underlying disease or drugs used on a regular basis in accordance with the criteria for selecting the sample population.

Regarding the health behavior, it is found that most participants exercised 3 days/week, representing 66.7 percent.

About the diet, all participants in the research project ate 3 meals/day.

As for history of the excess fat treatment, no volunteers had a history of treatment of excess fat in thighs before.

Based on the above information, it can be concluded that the group of volunteers in general had similar characteristics and healthy condition in accordance with the suggestions of standards in the selection of patients for treatment of localized fat reduction.

5.3 Discussion of Experimental Results

5.3.1 Research Results of Comparative Analysis Related to the Thickness of Thigh Fat in Different Periods of Time

The present research has been designed for comparing the effectiveness in fat reduction of the two methods with the same participants in the research project, thus

reducing different confounding factors which affect the experiment such as weight change, personal activities.

In terms of the difference in fat layers and the distribution in thighs of both sides that is different, the researcher has made correction by means of choosing at random types of treatment and thigh sides of volunteers. The results of statistical research have helped confirm that both thigh sides before treatment did not differ in size with statistical significance from both thigh circumference and fat thickness.

As for body weight of participants in the research project that may change and affect fat accumulation, there was the calculation of average weight of participants in the research project for comparison of before and after treatment at 1 and 3 months with the analysis by paired t-test statistics. It has been found that there is no statistically significant difference. Therefore, this is not likely to have interfering effects on the interpretation of the research findings.

One month after treatment, it has been found that the reduction of thigh fat accumulation by means of hyaluronidase injection in combination with non-invasive focused ultrasound versus ultrasound alone can reduce the size of thigh circumference and thigh fat thickness of volunteers with statistical significance. If considering the average of the reduction of thigh circumference and thigh fat thickness by using both methods, it has been found that hyaluronidase injection in combination with non-invasive focused ultrasound can reduce thigh circumference of 5.15 centimeters on average and fat thickness at 27.57 mm on average while the reduction of thigh fat by using ultrasound alone can reduce thigh circumference of 3.19 cm on average with the reduction of fat thickness at 18.01 mm. on average. The statistical analysis with Paired t-test has found that there is statistically significant difference.

After treatment in the third month, it has been found that the reduction of thigh fat accumulation by means of hyaluronidase injection in combination with non-invasive focused ultrasound versus the use of ultrasound alone can reduce the size of thigh circumference and thigh fat thickness of volunteers with statistical significance. Considering the mean of reduction of thigh circumference and thigh fat thickness by using both methods, it has been found that the method of hyaluronidase injection in combination with non-invasive focused ultrasound can reduce thigh circumference of 5.42 cm. on average and fat thickness at 27.59 mm. on average while reduction of thigh

fat by using ultrasound alone can decrease thigh circumference of 3.43 cm on average with the average reduction of fat thickness at 18.15 mm. The statistical analysis by using Paired t-test has shown statistically significant difference.

However, when comparing the mean of fat thickness at 1 and 3 months after treatment was completed 3 times, the average fat thickness decreased slightly for the reduction of thigh fat accumulation by means of hyaluronidase injection in combination with non-invasive focused ultrasound and using ultrasound alone (hyaluronidase injection in combination with non-invasive focused ultrasound with reduction at 0.02 mm. and using ultrasound alone with reduction at 0.14 mm.) with no statistically significant difference nevertheless.

However, the comparison of mean of thigh circumference at 1 and 3 months after treatment was completed 3 times, it has been found that the mean of thigh circumference decreased slightly for reduction of thigh fat accumulation by means of hyaluronidase injection in combination with non-invasive focused ultrasound and using ultrasound alone (hyaluronidase injection in combination with non-invasive focused ultrasound with reduction at 0.27 cm. and using ultrasound alone with reduction at 0.24 cm.) with statistically significant difference. Fat thickness at 1 and 3 months does not differ, but thigh circumference is different. This may be due to the fact that after the completion of treatment 3 times from one month to 3 months, the fat thickness of thigh is the same, ie the effectiveness in thigh fat reduction does not increase. But the thigh circumference decreases due to the effectiveness of firming up. This is because the use of non-invasive focused ultrasound also involves the heat transfer into the skin layer, possibly causing the contraction of collagen. However, the effectiveness of firming up has not been studied in this research. There may be additional study in the future.

When analyzing the results of the study, it can be seen that the treatment by means of hyaluronidase injection in combination with non-invasive focused ultrasound and the use of ultrasound alone can reduce thigh fat accumulation significantly on measurement of thigh circumference and fat thickness. But the thigh side treated with hyaluronidase injection in combination with non-invasive focused ultrasound can reduce thigh fat accumulation at the level higher than the treatment with the use of ultrasound alone with statistical significance.

On measurement of thigh circumference and fat thickness for comparison of periods before and after the end of treatment at 1 and 3 months, but when compared to 1 month and 3 months after the end of treatment, it is found that the effectiveness of reducing thigh fat accumulation does not increase. This can be seen from fat thickness that does not differ. As for thigh circumference that decreases significantly, this is possibly caused by the effectiveness of firming up which must be studied further.

The above information shows that the treatment by method of hyaluronidase injection in combination with non-invasive focused ultrasound has the effectiveness of reducing fat higher than the treatment by using ultrasound alone.

When compared with the study of Moreno et al who explored the efficacy of non-invasive focused ultrasound to reduce fat accumulation in different areas of the body for comparison of periods before treatment and one month after the last treatment with all treatments performed in three times, 1-month interval between each treatment, it is found that at one month after the treatment was completed, the average that decreases of fat thickness and thigh circumference differs with statistical significance . This is consistent with the treatment by using non-invasive focused ultrasound in this research. However, due to the absence of study in conjunction with hyaluronidase injection, it is not feasible to compare as to which method has higher effectiveness of treatment. Moreover, the research of Moreno did not follow up at 3 months after cessation of treatment. So, the comparison can not be done as to the extent of increase or decrease related to the effectiveness of fat reduction.

When compared with the study of Yang and Kim who studied hyaluronidase injection in abdominal surface, the average waist circumference was found to be at 1.81 cm. However, due to the absence of study in combination with non-invasive focused ultrasound and absence of study of fat reduction in the same location with this research, so it is not feasible to compare the effectiveness of treatment.

5.3.2 Study Results of Side Effects and Pain

According to the study of side effects caused by treatment through hyaluronidase injection in combination with non-invasive focused ultrasound. It was found that in treatment of the first time, there were five participants who experienced side effects, accounting for 27.78 percent. Three participants had the symptom of bruising and two

participants had redness. As for treatment in the second and third times, two participants in the research had side effects, representing 11.11 percent. One person had the symptom of bruising and one person had the symptom of redness. The symptom of bruising occurred in the location where hyaluronidase injection was performed. As for the treatment with ultrasound alone, it was found that in the first treatment two participants in the research had side effects, accounting for 11.11 percent. In this connection, one person had the symptom of redness and the other had blisters. As for the second and third times of treatment, it was found that one participant in the research had side effect of redness, representing 5.56 percent. All side effects that occurred had severity at just level 1, that is to say only minor symptoms and can be cured naturally. There were no symptoms of burns, rashes, itching, darker skin or white spots in the thighs of both sides of the research participants who received the two treatment methods.

In comparison with the research in the past, there was a study of using Ultrashape conducted by Moreno et al in 30 patients for reduction of fat accumulation in different areas of the body. It was found that two persons had pain during treatment and one person had blisters. But the symptoms were only minor and can be cured naturally in 3 weeks. In view of the study results, it was found that these two studies produced similar results in terms of side effects and the duration of healing process. But as for the research of Yang and Kim with hyaluronidase injection in abdominal area, it was found that there was the occurrence of side effects that are minor, that is to say the symptoms of bruising, itching and pain during injection. This research found only the symptom of bruising which can be cured by itself within 7-10 days, but did not find the symptoms of itching and pain in any way.

The pain arising from treatment is the feeling of pain rated by patients (Numeric Rating Scale: NRS) after treatment with different methods on each side. The study results have shown that the level of pain in the thigh on the side treated by method of hyaluronidase injection in combination with non-invasive focused ultrasound is higher than the pain in the thigh side treated by using ultrasound alone with statistical significance. The pain in the thigh on the side treated by method of hyaluronidase injection in combination with non-invasive focused ultrasound is mainly caused by injecting hyaluronidase. The participants would feel pain when the needle was placed into. But after the injection was completed, the pain would be gone. The pain caused by

the action of device often occurs in areas where there is less fat and adjacent to the bone. The participants in the research would feel pain and heat and the pain will go away after changing the location of operation.

Such data shows that the reduction of thigh fat accumulation by means of hyaluronidase injection in combination with non-invasive focused ultrasound is more effective than using ultrasound alone. The method of hyaluronidase injection in combination with non-invasive focused ultrasound includes higher likelihood of the occurrence of side effects and pain nevertheless, due to the need to inject hyaluronidase. But side effects are just minor symptoms and can be cured naturally. Moreover, the pain will disappear immediately after the completion of treatment at a time.

5.3.3 Results of Assessing the Participants' Satisfaction in the Research Project

It has been found that as a whole participants in the project of reducing the thigh fat accumulation by method of hyaluronidase injection in combination with non-invasive focused ultrasound mainly expressed satisfaction at a relatively high level (good), accounting for 44.4 percent, followed by satisfaction at a moderate level (fair), representing 22.2 percent. It has been revealed that participants in the research were satisfied with the thigh on the side treated with hyaluronidase injection in combination with non-invasive focused ultrasound in a level higher than the thigh on the side treated with ultrasound alone with statistical significance.

5.4 Conclusion

The reduction of thigh fat accumulation by method of hyaluronidase injection in combination with non-invasive focused ultrasound is more effective than using just ultrasound alone but involves a slightly higher level of pain and side effects nevertheless. Therefore, it may be an alternative for the treatment of thigh fat accumulation. Or it may be the treatment in conjunction with other techniques of localized fat reduction currently available.

5.5 Suggestions

5.5.1 It is wise to study the efficacy of treatment in the longer term such as 6 and 12 months in order to get the long-term treatment results as well as the recurrence of localized fat accumulation.

5.5.2 It is suggested to study the efficacy of reducing fat accumulation by method of hyaluronidase injection in combination with non-invasive focused ultrasound in other areas of the body too.

5.5.3 It is wise to study the effectiveness of treatment in each period to verify treatment results that can be seen after which particular time of treatment.

5.5.4 There should be a study of different concentrations of hyaluronidase drug more to get the maximum concentration that can produce the best treatment results as possible without causing the side effects arising from the drug.

5.5.5 There should be a measurement of effectiveness in treatment by using the high-precision device to gauge the thickness of fat layer such as CT Scan (Computed tomography scan) or MRI (Magnetic resonance imaging) to calculate and compare the results in more detail.

5.5.6 There should be a further investigation into the efficacy of firming up of the two treatment methods to get the results of treatment in several aspects in order to be useful for the decision to choose the approach to the treatment of localized fat accumulation further.

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