Combination therapy efficacy of catgut embedding acupuncture and diet intervention on interleukin-6 levels and body mass index in obese patients

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Abstract. Obesity is a major health problem worldwide, affecting more than 500 million adults with an additional 1.5 billion adults classified as overweight. Acupuncture has been recognized as an adjunctive therapy for obesity, and recent evidence suggests its potential to reduce the inflammatory response in adipose tissue, a condition believed to be responsible for obesity-related health problems. Interleukin-6 (IL-6) has been proposed as an important mediator of the inflammatory response in adipose tissue, but the number of studies addressing the issue is still limited. A double-blind, randomized, placebo-controlled trial was conducted with 36 obese patients currently receiving dietary intervention. The patients were randomly allocated into the catgut embedding acupuncture group with diet intervention or the sham (placebo) embedding acupuncture group with diet intervention. Catgut embedding therapy was given twice at CV12 Zhongwan, ST25 Tianshu, CV6 Qihai, and SP6 Sanyinjiao acupoints with two week intervals between procedures. The study endpoints were the IL-6 levels in the blood plasma and body mass index (BMI), measured before and after the intervention. We observed a reduction in the IL-6 levels (mean reduction 0.13 pg/mL, 95% CI: 0.03–0.23) and BMI (mean reduction 0.66, 95% CI 0.43–0.88) in the acupuncture group. The average difference in mean reduction of BMI between the acupuncture and sham groups was 0.34 (95% CI: 0.17–0.52). No difference was found in mean IL-6 reduction between the two groups (95% CI: -0.17 to 0.06). The results suggest that acupoint catgut embedding therapy may help reduce IL-6 levels and BMI in obese patients receiving dietary intervention.

1. Introduction
Obesity is one of the most common medical problems in many developing countries [1]. It is estimated that more than 500 million adults around the world suffer from obesity and 1.5 billion people suffer from being overweight [2,3]. In 2013, the National Health Survey, stated that obesity rates in Indonesia are increasing each year. The obesity rate for adults in Indonesia age 18 and older is 19.7% for men and 32.9% for women. Obesity is defined as a condition in which the amount of adipose tissue in the body is higher than muscle mass (20% or higher than ideal weight) [4]. Obesity can result in the accumulation of adipose tissue which stores triglycerides. Research has also shown that white adipose tissue can produce a bioactive substance called adipokines [5]. Adipose tissue can synthesize and secrete pro-inflammatory cytokines such as leptin, tumor necrosis factor-alpha (TNF-
α), and interleukin-6 (IL-6) [1,5]. Obesity, hence potentially increase insulin resistance and type 2 diabetes [3,5].

An increase in visceral fat storage and adipocyte hypertrophy were commonly linked to the degree of inflammation in obese patients [6,7]. Previous study explained the role of inflammation in adipose tissue, especially inflammation caused by macrophages in obesity. The researchers concluded that macrophages infiltrated the adipose tissue in the weight gaining phase and directly contributed to the inflammation status of the patients. This later caused insulin resistance and obesity in rat and human subjects [3,7,8]. The amount of macrophages increased 4 to 5 times in obese adipose tissue [3,7]. However, the cause of this is not completely clear yet. It is thought that macrophages infiltrate adipose tissue as a response to a stress signal from the adipocytes; later these enlarged and insulin-resistant adipocytes become increasingly stressed in the obese condition [3,9]. Macrophages that infiltrate adipose tissue would then be responsible for various pro-inflammatory cytokines production, such as TNF-α and IL-6 [6,7,9,10]. Inflammation is a necessary physiological response to recover homeostasis, but chronic inflammation or excessive inflammation can have damaging effects [2]. Research on the inflammation process in obesity began in the 1990s, especially motivated by a demonstration of TNF-α expression that showed an increase in the adipose tissue of obese rats [7,11]. The inflammatory source in obesity and its basic mechanism has yet to be fully understood, but pro-inflammatory cytokines have an important role in the process [2].

In obesity, the level of inflammatory cytokines is higher than normal, which contributes to insulin resistance [2,12]. Previous study states that cytokines produced by adipose tissue might be responsible for insulin resistance in obesity. Subcutaneous adipose tissue secretes IL-6, and this secretion might be correlated to a patient’s body mass index (BMI) [1]. There is little evidence stating that obesity is marked by a low level of chronic inflammation, hence the many inflammatory reactions and cytokines which affect IL-6 production regulation [6]. IL-6 is a single polypeptide chain that contains 185 amino acids and forms four α-helixs. In healthy people, IL-6 serum concentration is very low, about 3-4 pg/ml (about 1-9 pg/ml in thin and obese people), but it will increase when there is inflammation [6]. IL-6 is a pleiotropic cytokine which affects the inflammatory reaction and contributes to metabolic syndrome [12]. IL-6 plays an important role in immune response, inflammatory reactions, antibody mechanisms, and hematopoiesis [6]. It also regulates inflammation, decreases lipoprotein lipase activity, and regulates appetite and energy intake in the hypothalamus. IL-6 affects the transition process from an acute inflammatory condition to a chronic inflammatory condition in obesity, insulin resistance, inflammatory bowel disease (IBS), arthritis, and sepsis [2].

Acupuncture has long been used as an adjunctive therapy for obesity. Acupuncture can reduce the inflammatory response by decreasing macrophage infiltration into adipose tissue in obese patients. Macrophages are the source of pro-inflammatory adipokines, and acupuncture can decrease the number of macrophages and levels of IL-6 [10]. Thread embedding acupuncture therapy is a stimulation acupuncture method performed by embedding catgut in various acupoints. Thread embedding acupuncture has advantages over body acupuncture because it uses fewer acupoints, can be performed less frequently, and has a prolonged stimulation effect [13,14]. Extensive research has been done to examine the effects of catgut embedding acupuncture on obesity, but data on its efficacy in reducing levels of IL-6 is still limited. Therefore, this study has been conducted to examine the effects of combination therapy using catgut embedding acupuncture and diet intervention on IL-6 levels and BMI in obese patients.

2. Materials and Methods
This research was approved by Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia and gained approval from the Cipto Mangunkusumo Hospital. All research subjects agreed to participate by signing an informed consent form. All acquired data was guaranteed to be confidential and participation was voluntary without any coercion. The research design was a double-blind, randomized, clinical test with a control. Research was conducted at the General Hospital of the Cipto Mangunkusumo, in Jakarta, Indonesia. The inclusion criteria for the research were that subjects
be 18–60 years old, male or female, have a BMI ranging from 25–29.9, have signed the informed consent, and were willing to participate until the research was completed. The exclusion criteria were as follows: subjects with a casual plasma glucose test >200 mg/dl, in any medical drug therapy or weight loss program, participating in routine workout activity, in anti-inflammatory drug therapy, chronic indigestion (abdominal pain, bloating, distention, defecation disorder, or flatus for more than three months) [15], pain in three or more joints, numb sensation in the morning, nodules in the bones caused by hand arthritis [16], history of liver and kidney disorder, contraindication to thread embedded acupuncture therapy, a medical emergency, pregnancy, malignancy, blood clotting disorder, anticoagulant drugs consumption, history of allergies to animal protein, and infection or wounds on the acupoint site [15]. The catgut embedded therapy in this research was performed using catgut size 3.0, with 1cm and 0.5cm length, by inserting a 21G needle up to 1.5 cm in acupoints CV6 Qihai, CV12 Zhongwan, bilateral ST25 Tianshu, and unilateral SP6 Sanyinjiao up to two times with a two week interval.

The procedure was performed in the following sequence. The patient was asked to lie on their back. Anesthetic cream was smeared on the acupoint catgut embedded locations CV6 Qihai, CV12 Zhongwan, bilateral ST25 Tianshu, and unilateral SP6 Sanyinjiao. Anesthetic cream was smeared 1cm in diameter from the acupoint and left to absorb into the skin for 30 minutes. The operator used sterile gloves. Asepsis and antisepsis were done on the catgut embedded acupoint location with 70% alcohol and 10% povidone iodine. A size 21G needle, which had been prepared with catgut sized 1cm and 0.5 cm beforehand, was inserted in the designated acupoint up to 1.5 cm deep in a perpendicular position. Catgut measuring 1cm was inserted into acupoints CV6 Qihai, CV12 Zhongwan, and ST25 Tianshu, and catgut measuring 0.5cm was inserted into acupoint SP6 Sanyinjiao. After the 21G needle was inserted, the catgut inside the needle was pushed using a blunt acupuncture needle sized 0.30x50 mm. After the catgut was embedded, the acupuncture needle was withdrawn and, at the same time, the 21G needle was also withdrawn. The operator ensured that there were no catgut ends coming out of the acupoint locations. Pressure was put on the catgut embedded location with an alcohol swab until the bleeding stopped. The catgut embedded location was closed using antibacterial gauze dressing and wound dressing. The entire process was performed one by one on each acupoint location. The first catgut embedding was done on acupoint SP6 Sanyinjiao on the left foot and the second catgut embedding was done on the right foot.

The sham embedding procedure was performed in the same sequence as the catgut procedure, but a 21G needle was not inserted with catgut and only light pressure was applied on identical acupoint locations without wounding the patient. The data collected from this research included the IL-6 levels and BMI of the catgut embedded acupuncture therapy group (treatment group) and sham embedded acupuncture therapy group (control). Evaluation was conducted on day-1 (at the starting point of research) and day-30 (at the end of research). The examination of IL-6 levels was done at the Laboratory Riset dan Esoterik Prodia using Quantikine HS ELISA Human IL-6 Immunoassay kit with the immunoassay sandwich enzyme quantitative method.

Statistical analysis of the research output data was done using the SPSS 2.0 program. Data analysis using a statistical test relied on a variable that was analyzed. The numeric variable comparative hypothesis test with normal distribution of unpaired groups was done using an unpaired T-test. If the data distribution was not normal, the data transformation was done first to normalize the data. If the data distribution was normal, an unpaired T-test was used. If after transformation, the data distribution was still not normal, then the Mann-Whitney test was used. The numeric variable comparative hypothesis test with normal data distribution of paired groups was done using the paired T-test. If the data distribution was not normal, the data transformation was done first and if the distribution data was normal, a paired T-test was used. Data transformation to normalize distribution was done using the Lg10 function. The comparative hypothesis test result was $p > 0.05$, which indicates that there would be no significant differences between the compared variable, however if $p < 0.05$, there would be a significant difference between the compared variables [17].
3. **Results and Discussion**

3.1 **Results**

Research was conducted on 36 obese patients who meet inclusion and exclusion criteria. All subjects were randomly allocated into two groups, the catgut embedded acupuncture group (treatment group) and the sham embedded acupuncture group (control group). Each group contained 18 research subjects. A statistical test on the subjects’ early characteristics was done to examine age, gender, weight, abdominal circumference, BMI, and IL-6 levels. There were no significant differences in the subjects’ early characteristics, except for age (Table 1).

| Characteristics          | Catgut Embedded Acupuncture Group (n=18) | Sham Embedded Acupuncture Group (n=18) | Total (n = 36) | p-value |
|--------------------------|------------------------------------------|----------------------------------------|----------------|---------|
| Age (year)               | Average (SD) 36.28 (9.30)                | 43.72 (7.25)                           | 40.0 (9.05)    | 0.01†   |
|                          | Median (Min-Max) 35 (21 – 54)            | 45 (31 – 56)                           | 39.5 (21 – 56) |         |
| Gender                   | Male 3 (16.70)                           | 3 (16.70)                              | 6 (16.70)      | 1.0***  |
|                          | Female 15 (83.33)                         | 15 (83.33)                             | 30 (83.33)     |         |
| Weight                   | 78.70 (7.26)                             | 77.08 (11.90)                          | 77.89 (9.75)   | 0.63*   |
| Abdominal Circumference  | 101 (91.5 – 115)                         | 99.5 (93 – 121)                        | 100 (91.5 – 121)| 0.65**  |
| Early IL-6 Level         | 0.60 (0.19)†                            | 0.53 (0.19)†                           | 0.53 (0.03)†   | 0.43‡   |
| BMI                      | 30.89 (2.99)                             | 31.27 (3.76)                           | 31.08 (3.35)   | 0.74‡   |

* = unpaired T-test; ** = Mann-Whitney test; *** = Chi-Square test; † = Data normalization using Lg10

The last IL-6 average level was 0.47 in the catgut embedded acupuncture group and 0.53 in the sham embedded acupuncture group. The average difference between the two groups was not statistically significant (p = 0.34; CI 95% = -0.17-0.06). The early and final average of the IL-6 level comparison in the catgut embedded acupuncture group was 0.13 and was statistically significant (p = 0.01; CI 95% = 0.03-0.23). The early and final average of the IL-6 level comparison in the sham embedded acupuncture group was 0.01 and was not statistically significant (p = 0.90; CI 95% = -0.10-0.12). The average BMI in the catgut embedded acupuncture group was 30.24, and 31.14 in the sham embedded acupuncture group. The final BMI average difference between the two groups was not statistically significant (p = 0.43; CI 95% = -3.20-1.41). The early and final BMI average difference comparison in the catgut embedded acupuncture group was 0.66 and statistically significant (p = 0.00; CI 95% = 0.43-0.88). The early and final BMI average difference comparison in the sham embedded acupuncture group was 0.34 and statistically significant (p = 0.00; CI 95% = 0.17-0.52).

3.2 **Discussion**

This research was the first in Indonesia to apply an embedded acupuncture technique on obese patients to examine IL-6 levels and change in BMI. The catgut embedded acupuncture technique was chosen because of its advantages over body acupuncture, which include using fewer acupoints, less frequent therapy, and a prolonged acupuncture stimulation effect [13,14]. The prolonged effects of catgut embedded acupuncture therapy can increase and prolong acupuncture stimulation to 18-21 days, as a result of irritation in the catgut embedded tissue [18]. This research measured IL-6 levels in obese patients. Interleukin-6 is a pleiotropic cytokine which affects the inflammatory condition and metabolic syndrome [12]. IL-6 affects the transition process from acute inflammatory condition to...
chronic inflammatory condition in obesity, insulin resistance, inflammatory bowel disease (IBS), arthritis, and sepsis [2]. Hence, inflammatory disease such as IBS and arthritis was removed from the criteria.

Obesity is linked to chronic inflammation and research has shown an anti-inflammatory effect from acupuncture therapy. The SP6 Sanyinjiao acupoint can effectively control weight and decrease triglyceride levels, cholesterol levels, and pro-inflammatory molecules. The ST25 Tianshu acupoint near the thoracic 10 vertebra is at the same height as the adrenal gland. An increase in adrenal gland production can contribute to obesity and later cause an increase in corticosteroids, which can trigger fat catabolism and cause fat redistribution in all areas of the body. Hopefully, acupuncture on this acupoint will help normalize production in the renal gland, so that decreased fat redistribution can occur. The ST25 Tianshu acupoint was proven to decrease weight and increase peroxisome proliferator-activated receptors [8]. The CV12 Zhongwan acupoint is near the thoracic 7 vertebra, which can cause innervation in the stomach and parasympathetic responses and increase bowel peristalsis. A meta-analysis done by Guo et al. showed that the most frequently used acupoints in catgut embedded therapy were ST25 Tianshu, CV12 Zhongwan, ST40 Fenglong, CV6 Qihai, SP15, SP6 Sanyinjiao, and ST36 Zusanli. These acupoints were called anti-obesity acupoints [19].

The results of this research showed that IL-6 levels were lower in the catgut embedded acupuncture group. This result was slightly different than that of Ismail et al., who found that the average IL-6 levels were statistically significant between the treatment and control groups. In their study, Ismail et al. used a longer research period of six months, which might be one factor that affected the results [20]. The BMI delta average result in this research decreased as much as 0.67 in the catgut embedded acupuncture group and 0.34 in the sham embedded acupuncture group. The BMI delta average in the catgut embedded group was 0.33 lower than the final BMI delta average in the sham embedded acupuncture group and was statistically significant (p = 0.02; CI 95% IK: 0.05 to 0.61). This result supports the findings of Guo et al., Zhang et al., and Vivas et al., where the final BMI in the catgut embedded acupuncture group decreased more than the group that did not receive any embedded therapy.

Research on how acupuncture affects the pro-inflammatory cytokines in obesity is still limited. A few experimental studies have shown that the brain and immune system create two paths, an innervation path and a humoral path. The innervation path informs the brain about the inflammation and other tissue damage so that the brain will produce a local inflammation response. There is also research suggesting that stimulation on the peripheral acupoints could be delivered to the central nervous system by two paths: a fast transmission pathway involving vagal afferent nerves and nucleus tractus solitarii, and a slow transmission pathway involving cytokines. The stimulation activates the adrenal-neuroendocrine axis so that there is an increase in the secretion of catecholamines. Catecholamines and β2 receptors bond in the immune cell, causing TNF-α, IL-1β, and IL-6 pro-inflammatory cytokine levels to decrease, and also causing the anti-inflammatory cytokine IL-10 to increase. Acupuncture could cause the expression of IL-6 pro-inflammatory cytokines to be inhibited by recovering the Th1 and Th2 balance. Another activated immunomodulation path is the cholinergic anti-inflammatory pathway. Acetylcholine and nicotinic acetylcholine receptors bonding (α7nAChR) on macrophages would inhibit pro-inflammatory cytokines synthesis, but would not inhibit anti-inflammatory cytokines. The cholinergic anti-inflammatory path shows that acupuncture creates a physiologic mechanism that acts on the anti-inflammatory effect and antipyretic activity mediated by the regulation of certain cytokines such as IL-6 and IL-1 [21].

Acupuncture could decrease the inflammatory response in adipose tissue by decreasing MCP-1. Existing data show that increased macrophage infiltration and acupuncture could lower macrophage infiltration so that pro-inflammatory macrophages and adipokine source production could decrease. Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a nucleus cell hormone receptor known to affect the metabolism of lipids, glucose, and mesenchymal cell regulation, and has a proven anti-inflammatory effect [14,22]. Acupuncture is proven to increase PPAR-γ coactivator 1α activation, which functions as a transcript coactivator to PPAR-γ. PPAR-γ could inhibit pro-inflammatory
cytokines released from macrophages [14]. This mechanism could decrease the release of IL-6 pro-inflammatory cytokines. The ST25 Tianshu acupoint was proven to decrease weight and increase PPAR [9].

Ligand-activated transcription factor is a part of PPAR which is involved in inflammation regulation and metabolic syndrome in obesity. PPAR-\(\gamma\) is considered a main regulator of adipogenesis and has been widely studied for its role in obesity. PPAR-\(\gamma\) is mostly expressed in human adipose tissue, but can also be found in other organs, such as skeletal muscles, lungs, and the colon. PPAR-\(\gamma\) target gene promotes adipocyte differentiation, fat storage, and glucose metabolism involving lipase lipoprotein and adiponectin. PPAR-\(\gamma\) in liver is involved in triglyceride homeostasis and protects other tissue from triglyceride accumulation and insulin resistance. There are two molecular mechanisms of the PPAR-\(\gamma\) anti-inflammatory effect: inhibiting the pro-inflammatory transcript factor such as STAT, NF-\(\kappa\)B, and protein-1 activator (AP-1); or preventing the elimination of complex co-repressor from the gene so that there is inflammation in the gene transcript suppression. PPAR-\(\gamma\) could overturn the macrophage infiltration condition and reduce inflammation in the gene expression. PPAR-\(\gamma\) could reduce inflammation to activated macrophages by disturbing the NF-\(\kappa\)B path signal [7]. Further, pharmacology therapy for treating obesity has been used for over a hundred years. In the past, there were many limitations to the available drugs. Since obesity is a chronic disease, pharmacology therapy was recommended for long term usage, though this resulted in many side effects. These drugs have several serious side effects and teratogenic risks, which make them unsafe for pregnant women or women who are planning to become pregnant. Otherside effects include tachycardia, change in vision, change in mood, insomnia, headache, confusion, constipation, and dry mouth [19]. The advantage of catgut embedded acupuncture therapy is that there are no side effects, unlike the pharmacotherapy used for treating obesity. Additionally, acupuncture can decrease the inflammatory response in adipose tissue [10].

4. Conclusion
The results suggest that acupoint catgut embedding therapy may help to reduce IL-6 levels and BMI in obese patients receiving dietary intervention.

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