

Effects of Acupuncture Therapy at the Mingmen (GV4) and Zusanli (ST36) Points on the Expression of Transforming Growth Factor Beta 1 in Mice with Lupus Nephritis Model



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Received: 2023-09-30

Accepted: 2023-11-22

Published: 2023-12-27

INTRODUCTION

Systemic lupus erythematosus is a medical condition that is chronic and associated with autoimmune inflammation. In this condition, the individual's immune system attacks his body tissues. This disease is a complex disorder involving various factors such as genetics, environment, and interactions between these factors, which then contribute to changes in epigenetic mechanisms.¹ This triggers abnormalities in the immune system, such as dysfunction of dendritic cells, B cells, and T cells. Ultimately, immune tolerance in genetically susceptible individuals will

be lost, leading to aberrant autoimmunity activation.²

The incidence of SLE varies in various countries. A systematic study in the Asia-Pacific region recorded an annual incidence rate of around 0.9 to 3.1 cases per 100,000 population. According to the findings of the Lupus Foundation of America, there are roughly 1.5 million instances of lupus in the United States, with a global estimate of at least five million cases. Every year, it is estimated that there are around 16,000 new cases of lupus.³ In Indonesia, the exact number of individuals with Lupus is unavailable. Based on epidemiological research carried

out by Professor Handono Kalim and his team in Malang, survey results show that the prevalence of SLE is around 0.5% of the total population.⁴

The overall prognosis of SLE has improved significantly since the mid-20th century. Nevertheless, SLE mortality and morbidity rates continue to increase, even after the implementation of treatment. This increase is mainly related to the emergence of manifestations of lupus nephritis in patients with SLE. The results of research conducted in advanced medical care facilities reported a 9-fold increase in the risk of death due to lupus nephritis.⁵

ABSTRACT

Introduction: Systemic Lupus Erythematosus (SLE) is a chronic autoimmune inflammation involving various factors and autoantibodies targeting cell components, resulting in epigenetic changes. Lupus nephritis is one of the clinical manifestations of SLE. Nowadays, it is known that acupuncture could inhibit disease development, including chronic nephritis, chronic diarrhea, lumbago adnexitis, impotence, enuresis in children, myelitis, and endometritis. Thus, this study aimed to examine the effect of improving Mingmen (GV4) and Zusanli (ST36) point acupuncture therapy on the expression of Transforming Growth Factor Beta 1 (TGFβ1) in lupus nephritis model mice.

Method: It was a laboratory experiment on mice (*Mus musculus*) lupus nephritis model with a post-test only with a control group design. The research samples were divided into four groups, with seven mice in each group, consisting of a control group (KN), a negative control group given pristane (K), treatment group 1 (P1); given pristane and methylprednisolone, and a treatment group 2 (P2); given pristane and acupuncture at the Mingmen (GV4) and Zusanli (ST36) points. This acupuncture needle insertion was carried out thrice a week for 12 insertions during the intervention. The bivariate analysis was done using Kruskal Wallis.

Results: There were 28 samples examined in this study. we found that the TGFβ1 expression was high in group P2 (72.86±11.13), after that followed by group P1 (65.71±25.73), N (60.71±25.24), and K (5.88±5.35). According to the bivariate analysis, it showed a significant difference in TGF-β (p= 0.010).

Conclusion: Mingmen (GV4) and Zusanli (ST36) point acupuncture therapy improves TGFβ1 expression. Acupuncture therapy triggers inhibition of TGF-β1 expression in lupus nephritis model mice.

Keywords: acupuncture, mingmen point, zusanli point, TGFβ1, lupus nephritis.

Cite This Article: Suparyanti, E.L., Purwanto, B., Wasita, B., Handayani, S. 2024. Effects of Acupuncture Therapy at the Mingmen (GV4) and Zusanli (ST36) Points on the Expression of Transforming Growth Factor Beta 1 in Mice with Lupus Nephritis Model. *Bali Medical Journal* 13(1): 317-321. DOI: 10.15562/bmj.v13i1.4871

Lupus nephritis represents one of the most serious expressions of SLE, usually appearing within five years of diagnosis. High blood pressure, increased urea levels, and kidney failure often accompany symptoms of lupus nephritis. Around 60% of cases will progress to lupus nephritis in patients with SLE who are in advanced stages. The fibroblastic molecule, transforming growth factor $\beta 1$ (TGF- $\beta 1$), plays a crucial role in the development of kidney injury caused by lupus nephritis. Moreover, TGF- $\beta 1$ plays a crucial role in controlling autoimmune responses. Patients with elevated disease activity and significant organ damage exhibited a decrease in overall TGF- $\beta 1$ levels. The fact that the severity of kidney injury is associated with a decrease in serum TGF- $\beta 1$ levels indicates a role for TGF- $\beta 1$ in developing the pathogenesis of kidney injury due to lupus nephritis.⁶ Therapy for lupus nephritis aims to restore normal kidney function or inhibit the progression of kidney damage. One form of complementary therapy that can be used to achieve this goal is acupuncture.⁷

The basic principle of acupuncture is to insert a needle into the skin. The needle moves from the epidermis to the dermis and hypodermis layers. This will stimulate the pituitary hypothalamus to release beta-endorphins, which have the effect of the principle of acupuncture therapy, which involves providing stimulation to acupuncture points located in certain areas of the body. Mingmen (GV 4) is the Governing Vessel Meridian (GV) acupuncture point under the Lumbar II spinal process. This acupuncture point is employed in the treatment of chronic nephritis, persistent diarrhea, lumbago, adnexitis, impotence, enuresis in children, myelitis, and endometritis. The method of inserting the acupuncture needle is at a slight upward angle, 0.5 – 0.8 cm deep.⁸ Meanwhile, the Zusanli acupuncture point (ST36) is located on the foot, around 3 inches or 4 fingers from the knee.⁹ Zusanli (ST-36) is called “The stomach Meridian of Foot Yang-Min” due to gastrointestinal. This point is often chosen in clinical practice as a handy location for overcoming fatigue, strengthening the immune system, and having an analgesic effect.¹⁰ With this as a foundation, the researchers sought to investigate the

impact of enhancing acupuncture therapy at the Mingmen (GV4) and Zusanli (ST36) points on the expression of Transforming Growth Factor Beta 1 (TGF $\beta 1$) in a model of lupus nephritis in mice.

MATERIAL AND METHODS

Study Design

This laboratory experiment uses a post-test only with a control group design. This research uses mice (*Mus musculus*) as an experimental animal model to explore lupus nephritis. Researchers gave treatment to predetermined samples, namely mice divided into groups in the laboratory. Furthermore, the comparison results are based on the treatment status, consisting of a control group and a treatment group.

Location and time of research

This research was carried out from October 2021 – January 2022 at UPT. Universitas Sebelas Maret Integrated Laboratory, Biomedical Laboratory and Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Sebelas Maret. Surakarta, Central Java.

Research Subjects/Samples

The population in this study was Balb/C mice. The inclusion criteria applied in this study included Balb/C mice of the male gender, aged 6-8 weeks, and with a body weight between 20-30 grams. They must be healthy without disease symptoms (including normal eating patterns and activities). Active, and feathers that don't

fall out), follow a standard diet pattern. Then, the exclusion criteria included mice with physical abnormalities or defects and mice that died during the research. The sample size for this study was determined using the Federer formula, and the calculation yielded a minimum requirement of $n \geq 6$. This study had four groups, so 28 mice were randomly selected to be distributed into the four groups. So, each group was determined to consist of seven mice.

The group consisted of four groups, namely the control group, which received no treatment (N). The negative control group solely administered 0.5 ml of pristane intraperitoneally (K), treatment group 1 received 0.5 ml of pristane intraperitoneally along with 0.5 ml of methylprednisolone at a dose of 0.5 mg/kg body weight of mice (P1), and treatment group 2 received 0.5 ml of pristane intraperitoneally and underwent acupuncture procedures at the Ming men (GV4) and Zusanli (ST36) points (P2).

Research Procedure

The sample of each group will be given therapy according to their groups. After completion of the intervention the sample will be assed the TGF- β expression to Understand the impacts of acupuncture treatment on the Mingmen (GV4) and Zusanli (ST36) points on the expression of Transforming Growth Factor Beta 1 in a mouse model of lupus nephritis. This acupuncture needle insertion procedure was conducted three times a week for a total of 12 insertions during

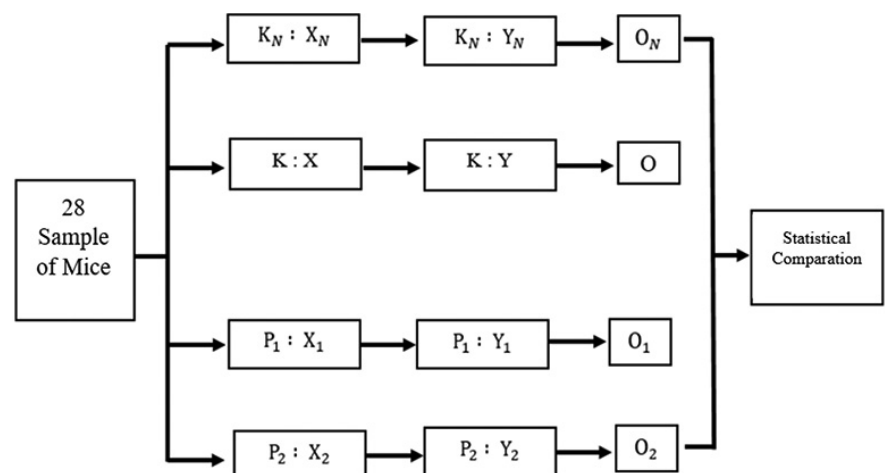


Figure 1. Research design scheme

the intervention period. After the treatment, the mice were terminated by inhalation using ether. The right and left kidney organs were examined for TGF β 1 expression immunohistochemically. Then, the TGF β 1 expression results of each group were compared with the normal control group as a basis for comparison. After completion of the treatment, the parameter was assessed. The results of TGF β 1 expression in the cytoplasm of renal glomerular cells compared to the control group, which was assessed as a normal group.

Statistical Analysis

In the study, the Shapiro-Wilk test was employed as the normality test due to the sample size being less than 50. The results of the normality test revealed a significance value below 0.05, signifying that the data does not follow a normal distribution. Consequently, nonparametric analysis will be utilized to assess the relationship between variables. The nonparametric test used to analyze unpaired numerical variables >2 groups is the Kruskal-Wallis test for the bivariate analysis.

RESULTS

In this study, The Shapiro-Wilk test was chosen as the normality test in consideration of a sample size less than 50. Results from the normality test indicate a significance value below 0.05, suggesting that the data deviates from a normal distribution. Therefore, nonparametric analysis will be used to evaluate the relationship between variables.

For the bivariate analysis, the Kruskal-Wallis test was done. Regarding our study, we observed elevated TGF β 1 expression in group P2 (72.86 \pm 11.13), followed by group P1 (65.71 \pm 25.73), N (60.71 \pm 25.24), and K (5.88 \pm 5.35). Bivariate analysis revealed a notable disparity in TGF- β (p= 0.010). This p-value finding is lower than 0.05. It was indicated that the alternative hypothesis (H1) is accepted. The TGF- β expression was seen by the immunohistochemical examination (Figure 2).

DISCUSSION

A study by Xing et al. 2012 revealed a decrease in TGF β 1 concentrations in

Table 1. Normality test Shapiro-Wilk

Group of Mice	p
N	0.001
K	0.001
P1	0.001
P2	0.482

N (Received no treatment), K (The negative control group, which only received 0.5 ml of pristane intraperitoneally), P1 (Treatment group 1, which received 0.5 ml of pristane intraperitoneally and 0.5 ml of methylprednisolone 0.5 mg/kg body weight of mice), P2 (Treatment group 2, which was given 0.5 ml of pristane intraperitoneally and underwent acupuncture procedures at the Ming men (GV4) and Zusanli (ST36) points)

Table 2. TGF- β expression in each group

Group of Mice	N	TGF- β expression (Mean \pm SD)	p
N	7	60.71 \pm 25.24	0.010*
K	7	5.88 \pm 5.35	
P1	7	65.71 \pm 25.73	
P2	7	72.86 \pm 11.13	

*Significant p-value <0.05; N (Received no treatment), K (The negative control group which only received 0.5 ml of pristane intraperitoneally), P1 (Treatment group 1, which received 0.5 ml of pristane intraperitoneally and 0.5 ml of methylprednisolone 0.5 mg/kg body weight of mice), P2 (Treatment group 2, which was given 0.5 ml of pristane intraperitoneally and underwent acupuncture procedures at the Ming men (GV4) and Zusanli (ST36) points)

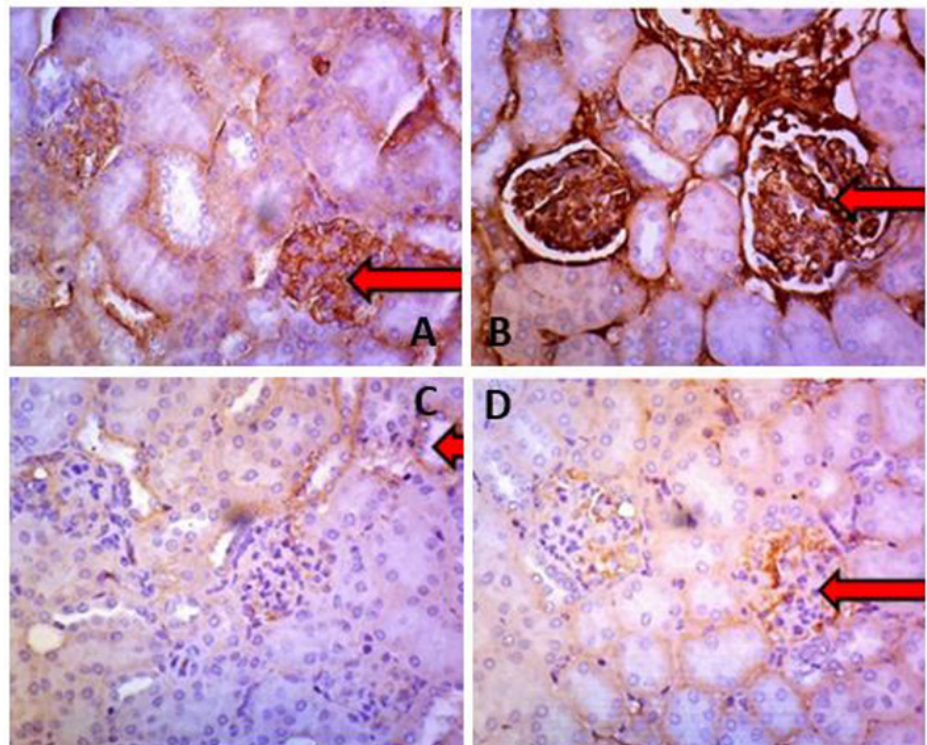


Figure 2. The evaluation of TGF- β expression by the immunohistochemical examination. The TGF- β expression can be seen by 40x magnification with (red arrow). N (Received no treatment), K (The negative control group, which only received 0.5 ml of pristane intraperitoneally), P1 (Treatment group 1, which received 0.5 ml of pristane intraperitoneally and 0.5 ml of methylprednisolone 0.5 mg/kg body weight of mice), P2 (Treatment group 2, which was given 0.5 ml of pristane intraperitoneally and underwent acupuncture procedures at the Ming men (GV4) and Zusanli (ST36) points).

serum in SLE patients.¹¹ Meanwhile, there was a notable increase in TGF β 1 levels in the urine of individuals diagnosed with lupus nephritis. The results of this study suggest that a reduction in the quantity of Treg cells correlates with a decline in serum TGF β 1 levels and an elevation in urinary TGF β 1 levels among individuals with lupus nephritis. TGF β 1 levels in serum may play an essential role in developing renal disorders. In contrast, high levels of TGF- β 1 in urine may be used as a useful biological indicator to predict the development of lupus nephritis. In line with our study showed. Regarding our study, in our findings, we observed elevated TGF β 1 expression in group P2 (72.86 \pm 11.13), succeeded by group P1 (65.71 \pm 25.73), N (60.71 \pm 25.24), and K (5.88 \pm 5.35), and this finding showed a statistically significant ($p= 0.010$). The high expression of TGF- β 1 in the group intervention and low level in the control group indicate that acupuncture could inhibit the development of kidney injury due to lupus nephritis This finding is in line with the fact that the severity of kidney injury is associated with a decrease in serum TGF- β 1 levels, indicating a role for TGF- β 1 in the development of the pathogenesis of kidney injury due to lupus nephritis.⁶

The basic principle of acupuncture is the insertion of needles into the skin, where the needles will move from the epidermis to the dermis and hypodermis layers. Next, interactions between the epidermis and mesenchymal layers occur, activating the ectodermal-mesodermal process. The results of this process affect the levels of skin mediators, one of which is TGF- β .¹² The use of acupuncture therapy will induce a decrease in TGF- β expression, and the use of SB 431542 can strengthen this effect which inhibits TGF- β activity. In this context, SB 431542, which has blocked TGF- β , will reduce the release of inflammatory factors.¹³

This sustained decrease in TGF- β levels will stimulate differentiation, proliferation, maturation, and increased function of various immune cells, including CD8+, CTL, NK cells, DC, macrophages, Th1, Th2, and Tfh. On the other hand, decreasing TGF- β can also inhibit iTreg, Th9, and Th17 differentiation with the

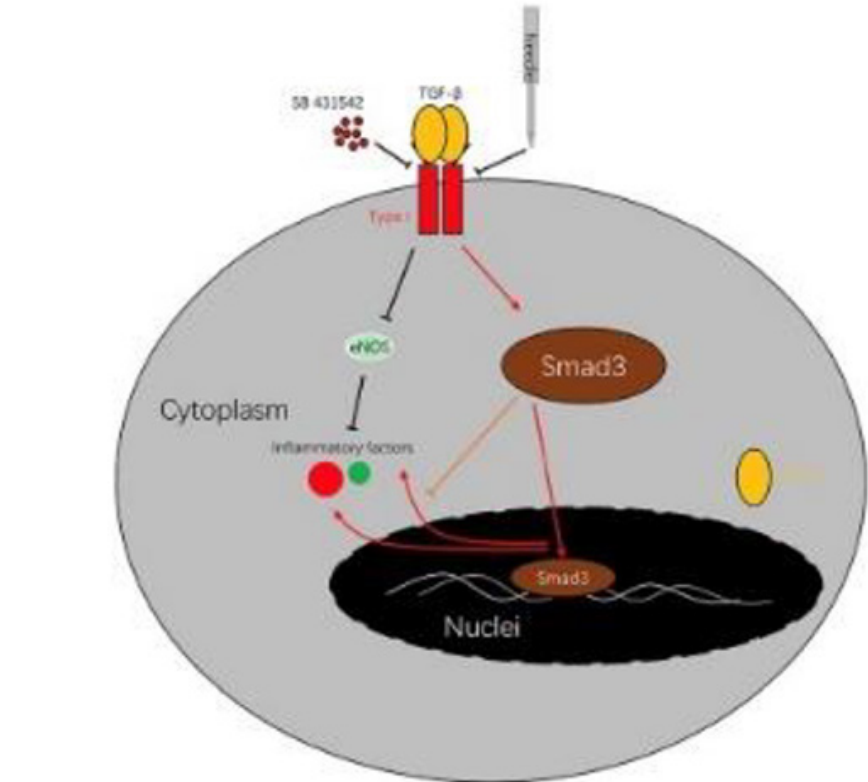


Figure 3. Mechanism of acupuncture in reducing TGF β 1 expression.¹³

help of other cytokines. Next, iTregs will activate Th17 and Tfh. Meanwhile, nTreg cells produced by the thymus will transform into Th1, Th2, Th17, and Tfh cells, with these nTreg cells playing an essential role in immunosuppressive functions. Low TGF- β levels can also reduce IL-6 production, stimulating the differentiation of naive T cells into Th17 cells. Th17 cells have a role in producing IL-17 as a pro-inflammatory cytokine. Reducing levels of these proinflammatory cytokines can ultimately inhibit immune system dysfunction that often contributes to the continuation of inflammatory conditions.¹⁴

This study had several limitations, including not controlling external variables such as stress levels in the experimental animals. Apart from that, this research is a pre-clinical experiment in the initial stage, using mice as subjects with a model of lupus nephritis, but does not yet involve dose variations in acupuncture injections. This research did not involve a group of lupus nephritis model mice that received a combination of methylprednisolone and acupuncture.

However, this study describes the effect of acupuncture at the Mingmen (GV4) and Zusanli (ST36) points on reducing TGF β 1 expression. The study also compares with the intervention of methylprednisolone, one of the conventional therapeutic approaches employed in treating lupus nephritis.

CONCLUSION

Acupuncture therapy at the Mingmen (GV4) and Zusanli (ST36) points can reduce TGF β 1 expression in lupus nephritis model mice, statistically significant.

ACKNOWLEDGMENTS

The author thanks the Doctoral Study Program in Medical Sciences, Eleven University Faculty of Medicine, Sebelas Maret University, Surakarta.

DISCLOSURE

Author Contribution

All authors contributed to this article

Funding

This research uses personal funds without funding from other parties

Conflict of Interest

The authors declare that there is no conflict of interest

Ethical Clearance

This study has been approved by the research ethics committee of faculty Medicine Universitas Sebelas Maret by number 92/UN27.06.6.1/KEP/EC/2021

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