ABSTRACT

Background: Diabetes mellitus (DM) case continues to rise as WHO reported that the number of adults suffering from DM had almost quadrupled since 1980 to 422 million cases. Laser acupuncture for DM therapy is performed by perpendicularly attaching laser probes to the body, and the laser light was directed to the acupuncture point until reaching the appropriate dose. It can be used as a single acupuncture point or more in accordance with meridian theory in Chinese medicine. For the treatment of type 1 diabetes, the selected acupuncture point is the BL20, which represents the pancreas. A previous study reported that this acupuncture point could reduce fasting blood glucose (FBG) levels. The FBG level and immunohistochemistry of pancreatic tissue were measured in the 13th day by cutting tail. Pancreas tissue were took to be further microscopically evaluated.

Methods: Twenty-two male Rattus norvegicus were kept in cages at a temperature of 20-25°C. Time of day and night were divided evenly, each 12hours. Daylight was defined as the time from 6 a.m. to 6 p.m., while the evening was from 6 p.m. to 6 a.m. The study protocol was approved by the ethics committees of the Faculty of Medicine, Airlangga University, Surabaya, Indonesia.

The study was performed by perpendicularly attaching laser probes to the body, and the laser light was directed to the acupuncture point until reaching the appropriate dose. Fifty percent of this time was used on the first day, and the remaining time was used on the second day. Laser acupuncture was performed on the 13th day, which was defined as the time from 6 a.m. to 6 p.m., while the evening was from 6 p.m. to 6 a.m.

Results: The FBG level and immunohistochemistry of pancreatic tissue were measured in the 13th day by cutting tail. Pancreas tissue were took to be further microscopically evaluated.

Conclusion: This finding indicated that laser acupuncture at BL20 point could reduce FBG level, increase pancreatic beta cell and wider Langerhans area in type 1 diabetes mellitus.

Original Article

INTRODUCTION

Diabetes mellitus (DM) case continues to rise as WHO reported that the number of adults suffering from DM had almost quadrupled since 1980 to 422 million cases. This significant rise is highly associated with increased type 2 DM cases and other risk factors, including overweight and obesity. There are many therapies to overcome DM. One kind of them is complementary medicine such as acupuncture. Laser acupuncture is now commonly used since it is painless, more convenient to be tested for small experimental animals and more comfortable to use in babies compared to needle acupuncture.

Laser acupuncture for DM therapy is performed by perpendicularly attaching laser probes to the body, and the laser light was directed to the acupuncture point until reaching the appropriate dose. It can be used as a single acupuncture point or more in accordance with meridian theory in Chinese medicine. For the treatment of type 1 diabetes, the selected acupuncture point is the BL20, which represents the pancreas. A previous study reported that this acupuncture point could reduce fasting blood glucose (FBG) levels. The study did not expose the pancreatic beta cell. In this study, we conducted laser Al-Ga-In-P at BL20 point to stimulate pancreatic beta cell.

There are some lasers, including Aluminium-Gallium-Indium-Phosphorous (Al-Ga-In-P) laser that produces visible light (red). The Al-Ga-In-P laser is more convenient to use at room temperature, allowing the researcher to direct the laser as in manual use (needle acupuncture).

METHODS

Twenty-two male Rattus norvegicus were kept in cages at a temperature of 20-25°C. Time of day and night were divided evenly, each 12hours. Daylight was defined as the time from 6 a.m. to 6 p.m., while the evening was from 6 p.m. to 6 a.m. The study protocol was approved by the ethics committees of the Faculty of Medicine, Airlangga University, Surabaya, Indonesia.

Streptozotocin (STZ) was injected intraperitoneally to create an experimental rat of type 1 DM (T1DM). The STZ dose was 60 mg/kg BW, and was prepared in a solution of 0.1M citrate buffer (pH = 4.5). We used a low pH of STZ solution to make the active substance was not easily inactivated. We obtained STZ from Merck Tbk, Chemical division (Catalogue no. 572201-1GM, batch B56981). The FBG level was measured using Johnson-Johnson One-Touch Strip test (Lifescan Inc., a Johnson & Johnson Company, Milpitas, CA.

Original Article

Keywords: laser acupuncture, BL20, type 1 diabetes mellitus

Cite this Article: Abdurachman, Suhariningsih, Rubiyanto, A. 2019. Laser acupuncture at BL20 Point Stimulate Pancreatic Beta cell in type 1 diabetes mellitus. Bali Medical Journal 8(1): 241-246. DOI:10.15562/bmj.v8i1.1436

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ABSTRACT

Background: Diabetes mellitus (DM) case continues to rise as WHO reported that the number of adults suffering from DM had almost quadrupled since 1980 to 422 million cases. Laser acupuncture for DM therapy is performed by perpendicularly attaching laser probes to the body, and the laser light was directed to the acupuncture point until reaching the appropriate point. Fasting blood glucose (FBG) levels were measured in the 13th day by cutting tail. Pancreas tissue were took to be further microscopically evaluated. Results: The FBG level and immunohistochemistry of pancreatic beta cell between the two groups were compared using independent t-test and Spearman’s correlation test (p <0.05). Rats in L2 showed a significant decrease in FBG level, a significant increase pancreatic beta cell and Langerhans area. Conclusion: This finding indicated that laser acupuncture at BL20 point could reduce FBG level, increase pancreatic beta cell and wider Langerhans area in type 1 diabetes mellitus.

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There are some lasers, including Aluminium-Gallium-Indium-Phosphorous (Al-Ga-In-P) laser that produces visible light (red). The Al-Ga-In-P laser is more convenient to use at room temperature, allowing the researcher to direct the laser as in manual use (needle acupuncture).
95035 U.S.A. No. SMC4212QT) in 48 hours after STZ injection.\textsuperscript{12,13} The cut-off level for T1DM was 300 mg/dl.\textsuperscript{5,14}

T1DM-induced rats randomly divided into two groups. Rats in control group (L1, n=11) did not receive laser, while rats in treatment group (L2, n=11) received laser. Rats in treatment group were radiated every two days, six times consecutively for 12 days, starting in 24 hours after the rats developed T1DM.\textsuperscript{5} In control group, laser probes attached to the rats with similar time interval as in the treatment group, but they were not radiated.

Rats placed inside a rectangular plastic box, with 15 cm thick, 33 cm wide and 40 cm long. The plastic box was covered in wire gauze to maintain the oxygen for rats. Wire gauze also used as a way of giving drinks into the box. Rice hulls were placed at the bottom of the box to make urine to be easily absorbed. Wire gauzed was placed between the hulls and rats in accordance with the size of the wire used as a cover. The rat’s body was not in direct contact with urine in the hulls. The wire was placed at ±3cm from the base of the plastic box. The hulls were replaced every morning.

The laser light was perpendicularly directed to the bilateral BL20-point until reaching 0.3 joules.\textsuperscript{5} Laser type was indium gallium aluminium phosphorous (In-Ga-Al-P). FBG level was measured in day 13. The blood samples were obtained from the rat’s tail.\textsuperscript{9} The rat’s pancreas was taken and fixated to be further microscopically evaluated.

Special immunohistochemical staining technique used indirect method,\textsuperscript{15} using Insulin Ab-6 (INS04 + INS05) Mouse Monoclonal Antibody. We used Olympus BX 50 microscope F4. 7A09746.2.8/1.8 50/60Hz (Olympus Optical Co., Ltd., Japan), equipped with Digital Panasonic Color CCTV Camera model wv-CL350/A. No. 4YB10201 (Matsushita Communication Industrial Co., Ltd., Japan). To measure Langerhans area, we used UTHSCSA Image Tool Version 3.0 February 2002 (Manual revision 5) and WinFast Wizard for PVR/FM, version 5.13.1.2002.

Beta cell percentage was calculated based on the number of beta cells in one islet of Langerhans and divided by the total number of cells in the same islet. We observed three fields of views in each preparation. The field of view was randomly selected.

\textbf{RESULTS}

The minimum and maximum of FBG level changes in L1 were 287 mg/dl and 599 mg/dl, respectively (395.09±87.26). The minimum and maximum of FBG level changes in L2 were 97 mg/dl and 413 mg/dl, respectively (263.63±91.51).

The results showed that average FBG level in L2 was normal (263.63 mg/dl), while average FBG level in L2 was above 300 mg/dl (395.09 mg/dl). The independent t-test on final FBG level showed a significant FBG difference between L1 and L2. This finding indicated that final FBG level in L2 was different from L1.

\textbf{Pancreatic Beta cell percentage}

The minimum and maximum beta cell percentage in L2 were 0.0\% and 79.2\%, respectively (29.96±21.42). On the other hand, the minimum and maximum beta cell percentage in L1 were 0.0\% and 60.7\%, respectively (12.3±15.77).

This finding indicated that beta cell percentage in L2 was more than twice bigger than L1, as evidenced by a larger percentage obtained by

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Control group (L1): A: 200X magnification B: 400X magnification Insulin gave a positive reaction (black arrow), outer boundary of Langerhans islet (white arrow)}
\end{figure}
L2 than L1 (29.96% vs 12.3%), with a difference of 17.66%. The independent t-test on beta cell percentage showed a significant difference between L1 and L2 (p < 0.0001).

Langerhans area
Langerhans area (pixel²) in L2 were 5023 and 49875 respectively (18364.69±10351.56). On the other hand, the minimum and maximum Langerhans area in L1 were 18.64 and 24941, respectively (5881.69±4888.24).

This finding indicated that Langerhans area in L2 was three times wider than L1, as evidenced by a wider area found in L2 than L1 (29.96% vs 12.3%). The independent t-test on Langerhans area showed a significant difference between L1 and L2 (p <0.0001).

Correlation between variables
Table 5 showed a negative correlation between FBG level and beta cell percentage (r = -0.604; p <0.001). This finding indicated that the higher the beta cell percentage, the higher the insulin production and the lower the FBG level. Negative correlation was also found between FBG level and Langerhans area (r = -0.459; p <0.05). It could be inferred that the higher the FBG level, the narrower the Langerhans area. There was a positive correlation between beta cell percentage and Langerhans area (r = 0.253; p <0.05). This indicated that the higher the percentage, the wider the Langerhans area. The strongest correlation was (p <0.001) was found between FBG level and beta cell percentage.

Microscopic examination
Microscopic Examinations in L1 (Figure 1) and L2 (Figure 2).

Brown beta cells indicating insulin content were formed in Figure 1 and 2. Langerhans area (white arrow) in L2 was wider than L1, indicating that L2 was more dominant than L1.

DISCUSSION
STZ can cause pancreatic beta cells destruction, causes a decrease in their number and function. A decrease in the number of beta cells can be observed using immunohistochemistry. According to Halban (2001), beta cells specifically produce insulin hormone. Under normal conditions, this function cannot be taken over by another cell. Extracellular glucose level can directly describe the function of beta cells.

We found that laser therapy significantly increased beta cell percentage (p <0.05) as shown in Table 3. The increase in the number of beta cells in L2 was more than twice bigger than L1 (29.96% vs 12.3%). This increment was also followed by a decrease in FBG level. This indicated that the increasing number of beta cells was functional as it reduced FBG level and produced insulin. Even though most insulin was found in L2, it could not confirm that the produced insulin could function normally.

One of insulin functions is reducing blood glucose level. Therefore, we need to measure blood glucose level to ensure if pancreatic beta cell-produced insulin can function normally. FBG level is measured to eliminate the effect of food intake. Table 2 showed that FBG level in L2 was much lower than FBG in L1 (p <0.05). The value of blood sugar decrease was associated with an increase in the number of beta cells (42%; Table 5).

More than 90% beta cells damage due to necrosis decreases the mass of entire beta cells that subsequently results in decreased space occupied by them in the Langerhans islet. The reduced area of Langerhans islet is very significant, as pancreatic beta cells occupy 80% of the whole islet.
Even though laser therapy at BL20 point was able to increase the number of beta cells, we still needed to trace the origin of these new beta cells, whether it was derived from the remaining beta cells (less than 10%) or other sources (ductus pancreatic). If increased beta cells are derived from the remaining beta cells, this increment is the result of the mitotic process due to its stimulation, therefore, it takes around 11 days to reach beta cell maturation. The matured beta cell is a beta cell that can produce normal insulin and decrease blood glucose level.

Also, if any remaining beta cells could duplicate, it would result in maximum change, and Langerhans islet increases no more than 20%, twice than the original. In this study, we found >30% increase in Langerhans islet.

Figures 2A and 2B showed the therapy effect (compare with L1 in Figures 1A and 1B). Langerhans islet in L1 was narrower than L2. After an extensive measurement using Image Tool, we found a significant difference in Langerhans islet between L1 and L2 (p <0.05). Langerhans area in L2 was three-time wider than L1 (>300%). These results indicated that the newly formed beta cells were the result of stem cell proliferation (stem cells) in ductus pancreatic.

In this study, STZ was a substance that made beta cells underwent necrosis. Necrosis occurred in L1 and L2 was similar because of the same treatment (Table 2). Nevertheless, L2 had a significantly different improvement compared
to L1 (p < 0.000). The only different variable between L1 and L2 was the acupuncture laser stimulation at BL20 point. Acupuncture laser stimulation via BL20 point might be one of the stimulators to stimulate stem cell ductus pancreatic into normal beta cells. Changes caused by laser acupuncture at this point were very significant (p < 0.000).

Increased insulin in immunohistochemical staining also could not confirm that the produced insulin could have normal function as it might increase due to hypertrophy of the remaining beta cells. Therefore, we needed to conduct a correlation test between beta cells, Langerhans area, and beta cell function.

Table 5 showed the results of the statistical test to prove any correlation between Langerhans area and increased beta cell percentage. We found a positive correlation with \( r = 0.464 \), at \( p < 0.05 \). This finding indicated that the higher the beta cells, the wider the Langerhans area. The increase was more than 300%. On the other hand, there was a negative correlation between Langerhans area and decreased FBG level \( (r = -0.464; \ p < 0.05; \text{ Table 5}) \). This finding indicated that the wider the Langerhans area due to increased beta cells, the lower the FBG level. These results were consistent with the correlation between Langerhans area and increased beta cells. The increasing number of beta cells resulted in increased hormone insulin, and the Langerhans area was increasingly wider. The result was a decrease in FBG level. We also found a negative correlation between increased insulin and decreased FBG level \( (r = -0.607; \ p < 0.01) \). The negative correlation indicated that the produced insulin could function normally as it reduced FBG level.

It could be inferred that T1DM condition gradually became normal due to increased beta cell percentage and function. This finding indicated that laser therapy at BL20 point could increase beta cell percentage and function.

Table 5 showed that Langerhans area in L2 was three-time wider than L1 \((18364.69 \text{ pixel}^2 \text{ vs. } 5881.69 \text{ pixel}^2)\), while the beta cell percentage in L2 was more than twice bigger than L1 \((29.96\% \text{ vs. } 12.3\% )\). Increased beta cell percentage was supposedly similar with the increased Langerhans area, particularly when viewed from the results of increased FBG level as the FBG level in L2 only increased to 42% compared to L1.

These unidirectional changes in some variables might be due to the following factors: (1) Pancreatic beta cell percentage was calculated based on their function as an insulin producer. Consequently beta cells that had not produced insulin were not calculated; (2) Insulin in beta cells was not entirely functioning optimally in reducing blood glucose level.

This study still not can exactly prove the origin of beta cell. Further study was recommended to prove that stimulation done by activating stem cell in pancreatic duct.

**CONCLUSION**

Laser acupuncture at BL20 point in type 1 diabetes mellitus could increase beta cell percentage and Langerhans area, and reduce fasting blood glucose level. This study still not can exactly prove the origin of beta cell. Further study was recommended to prove that stimulation done by activating stem cell in pancreatic duct.

**CONFLICT OF INTEREST**

The author declares no conflicts of interest regarding the publication of this article. The author received no funding for this work.

**ACKNOWLEDGMENT**

The author would like to thank the Dean of Faculty of Medicine, Airlangga University, Surabaya, Indonesia, for his generous support. The author would like to thank Prof. Suhariningsih, Ph.D. and Prof. Agus Rubiyanto, Ph.D. for their contributions. Prof. Suhariningsih gave author many pieces of advice in stimulating the acupuncture point. Her dissertation in acupuncture point connecting with voltage support some details of discussion. Prof. Agus Rubiyanto gave author many pieces of advice in laser science; laser was his research in Germany.

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