Digital Comparisons of Traditional Acupuncture Therapies of Japan, Korea and China-Special Reference to Brain Topography, Hormone and Peripheral Leukocyte

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Abstract

Objective: TCM had established in China and it spread along with firstly Far East countries and modified and included in each country as ethnic medicine. In this trial we discussed the procedures and effects of traditional acupuncture therapies in China, Korea and Japan by selecting 10 normal volunteers after informed and consented.

Volunteer and Method: For the digital comparison of effect and accurate comparison, brain, hormone and peripheral leukocyte linkage were the approved scale of QOL Three of well-trained traditional acupuncture therapists were selected and recommended and they figured out the same subjects at random order with intervals of at least two months of cool down. Every acupuncturist picked up their diagnosis and points selection for moderate the vital sign. Each judgement factors were selected as brain topography, serum adrenalin and peripheral leukocyte subset.

Results: Brain topography, emotional hormone/adrenalin, peripheral leukocytes were compared according to the impact from each volunteer. China, Korea and Japan were in order to each criteria of judgement. Although variations of data were quite large, these results clearly indicated that acupuncture therapies have made up among three countries in building up to the ideal therapy. With the brain topography, we had set up the check point for each 52 detector points for evaluation. After the left leg stimulation for example by GB34, China, Korea and Japan were 22/52, 16/52 and 12/52 points in order.

Conclusion:

1) Brain topography, emotional hormone/adrenalin, peripheral leukocytes were compared according the impact from each volunteer.

2) Chinese style was most impacted style for acupuncture to all the menu, NIRC, hormonal and leukocyte regulation.

3) Korean one revealed the smart regulation in NIRC and hormonal regulation.

4) Japanese one was a week impact but effective in each check system, brain and peripheral lymphocyte subset.

5) With NIRS, Korean and Japanese one probed back flash phenomenon just after draining the needle.

6) For infection control, all three acupuncture system selected single use disposable system.

Keywords: Acupuncture; QOL; Digital Presentation; Brain Topography; Drain; Back Flash; Emotional Hormone; Total Leukocyte; Leukocyte Subset; Chinese; Korean and Japanese Traditional Acupuncture

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Abbreviation

BF: Back Flash: real-time increase of NIRS impact just after drain a needle; HFrEF: Heart Failure and Reduced Ejection Fraction; NIRS: Near Infrared Resonance System; QOL: Quality of Life; RCT: Randomized Clinical Trial; TCA: Traditional Clinical Acupuncture; TCM: Traditional Chinese Medicine

Introduction

The disease structure was different from the 1950's, and the acute infectious disease disappeared at present, and the chronic disease called the lifestyle related illness arose instead. In Our Country, cancer and cardiovascular disease (Include the circulation of brain) were the leading causes of death [1-4]. Although it is well understood by people that these diseases do not develop suddenly on a certain day, many people do not try to scope with them until they become suffered. The latent period of this abnormality is expressed as "unhealthy" in oriental medicine and is a state of "Half-Disease and Half-Health". The abnormality which contracts disease after a long time means that the period of "unhealthy" is long, and it can be said that there are sufficient opportunities of "Undefined Complaint". Treatment of "unhealthy" is unsuitable for drugs. You can't keep giving Western medicine to people who don't know when they will get sick. In addition, although some TCM are effective for "Opportunistic Infection", it is difficult to administer them as well as Western medicines considering that they are pharmaceutical products.

It is well understood that lifestyle-related diseases, especially diabetes, hyperlipidemia and gout, are associated with abnormalities in metabolic functions, and food habits are particularly involved. The improvement of life style is important for the prevention, and the control of the inflammation is also important, since it is accompanied by the inflammatory reaction.

Along with lifestyle-related diseases, the number of patients with osteoarthritis, a chronic disease associated with aging, has been increasing reflecting the senile society. This disease is a difficult abnormality to treat because it shows dyskinesia with joint abnormality with aging, organic change and inflammation of the nose and joint [5,6].

It is commonly recognized that acupuncture and moxibustion therapies are developed in China, and it repeated in some test system and established its special medical technic as traditional medicine. Thereafter it spread to worldwide as traditional Chinese Medicine (TCM). The diagnosis and procedure of TCM have been well established and recently various re-evaluation of TCM has been conducted [7-11]. The majority of practitioners have learned acupuncture therapy in China or Chinese doctors in the different countries, so TCM way of acupuncture therapy may be considered as only one acupuncture therapy in the world. During the process of development and worldwide distribution, it has modified in different countries with their nature, social customs, foods and other things. Thereafter the term "acupuncture and moxibustion therapies" includes several different process of diagnosis and treatment in them, although appearance of the differences has not been scare sly noticed the researcher of clinical trials of acupuncture in the Western world. In Japan, acupuncture therapy has long been applied as the main medicine until Edo era (the middle of 19 century), and it is usually established by its use of needling tube with fine needles and soft manipulation without de-qi sensation [9-12]. It has developed for the requirement of the patients. Recently, more modernized acupuncture based on physiology and anatomy of the body has been applied cooperate with Western medical doctors.

On the contrary, modern clinical approach of acupuncture have reported an important evidence of efficacy and safety of acupuncture manipulation, and the majority of acupuncture retrospectively used in the trials is based on TCM theory of acupuncture points selection and manipulations [13]. The mega studies designed in German clearly reported that real TCM style acupuncture has a statistically eminent effect compared with conventional and/or standardized Western therapies [14-16]. Ton the economical stand point also demonstrates the cost-benefit of acupuncture therapy in German acupuncture project [15,16]. The major issues to be emphasized now is that there is no statistically significant difference between real TCM acupuncture group and those of sham acupuncture (minimal acupuncture) group, that is no specific effect of acupuncture therapy was proven by high quality RCTs trialed in German. In that project, minimal acupuncture is characterized by shallow needling to non-acupuncture points without de-qi sensation. As the major interest of clinical trials of acupuncture in the Western world have learned and trained TCM as common fundamental acupuncture therapy, so the location of puncture points and stimulation of particular de-qi sensation is regarded to be fundamentally important. Then the German trials select the minimal

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acupuncture as sham intervention might be reasonable. Then Prof. Yamaguchi proposed a project called TCA (three countries' acupuncture) project to investigate the different therapeutic properties of traditional acupuncture in Japan, Korea and China. The protocol of the present project conducted was determined through several meetings and discussions among the researcher in China, Korea and Japan.

Purpose of the Study

The purpose of TCA project is to clarify the different characteristics of traditional acupuncture procedures in three countries and it may help to understanding and development of potential roles of acupuncture therapy in the future CAM.

Methods

Subjects and practitioners

Subjects were healthy young subjects with informed consent (male, average 21 years, 20 - 25, n = 10). Special reference was made to select in order not to try acupuncture since then. This projects were planned in April, June and August in 2009 at Kanazawa. Acupuncture practitioners were well-experienced traditional acupuncture therapists in Japan, Korea and China (their clinical experiences were over 20 years). The purpose of the treatment was to regulate self-defense system and to make better QOL in each volunteer. The concrete procedures of diagnosis and treatment were memorized on the chart in detail, and the processes of diagnosis and treatment were recorded by digital apparatus with permission of the volunteers and practitioners. The subjects were treated by three practitioners in three different styles of acupuncture treatments at cooling down for about 2 months or more. The experiments usually started in a clinic at 19:00 in each time to avoid the circadian rhythm in adrenalin contents and leucocyte subset number [17,18]. The trials were opened on April, June and August. The average treatment time was about 30 mins. The procedures of diagnosis and treatments were recorded by three video cameras with permission of the subjects and practitioners. The issue of this trial was approved by the Ethics Committee of Kanazawa Medical School with written informed and consented (Figure 1).
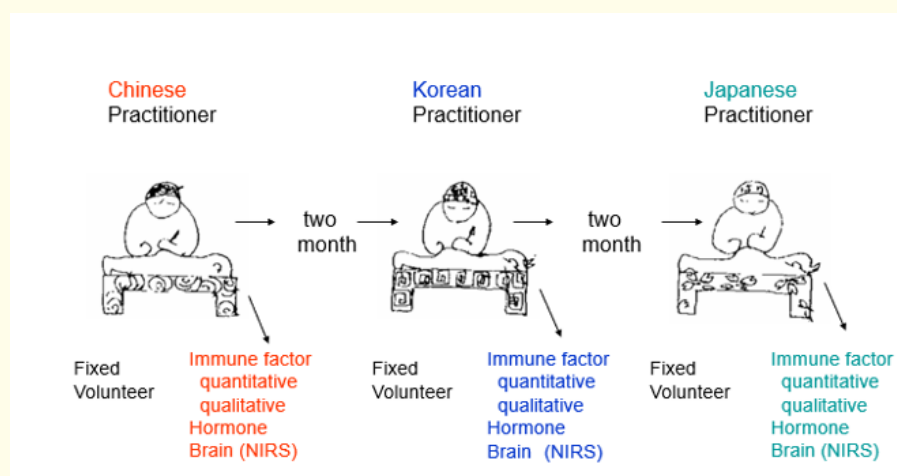

Figure 1: Schematic Diagram of this Study; Volunteers were healthy young subjects with informed consent (male, average 21 years, 20 - 25, n = 10). Special reference was made to select in order not to try acupuncture since then. The subjects were treated by three practitioners in three different styles of acupuncture treatments at cooling down for about 2 months or more.

Brain topographical representation by NIRS system

So as to suggest the effect of acupuncture impact to the brain site, NIRS system was employed in this trial. The trial system of photo was shown in figure 2, suggesting the real positioning of acupuncturist, volunteer and the engineer for digital imaging. Functional near-infrared spectroscopy is a noninvasive functional neuroimaging technique measuring cortical absorption of near-infrared light to estimate local concentration. In this system, the changes of oxygenated (HbO_2) and deoxygenated hemoglobin by acupuncture therapy. Hemodynamic

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responses depicting brain activation comprise task-evoked HbO_2 increases and lower amplitude deoxygenated hemoglobin decreases. An ETG-4000 Optical Topography System (Hitachi Medical Co, Tokyo) captured activation across 52 prefrontal locations (channels), spatially defined according to the international 10-5 system of probe placement. The probes were placed on each participant's head by the same investigator, after which the participants were given 10 minutes warm-up time to become familiar with the box trainer and get used to wearing the device. The experiment was started immediately. This machine consisted with 52 detector of NIRS impact and judging the efficacy of acupuncture therapy for brain effect with a least damage to volunteer (ETG-4000, Hitachi High-Technologies, Co, Ltd, Tokyo).
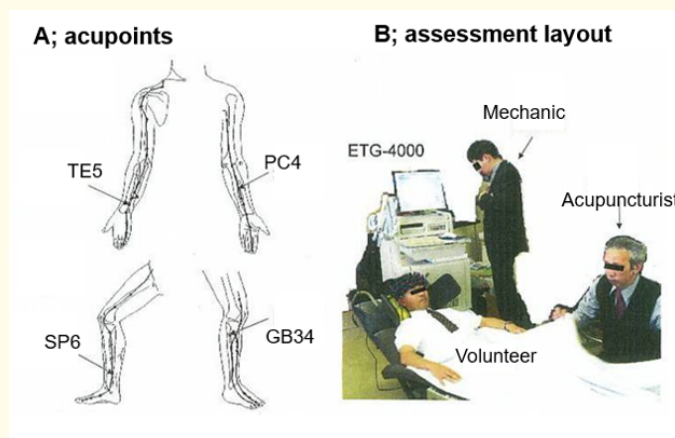
Figure 2: *Practical Set up of this study; The trial system of photo was shown in figure 2, suggesting the real positioning of acupuncturist, volunteer and the engineer for digital imaging.*
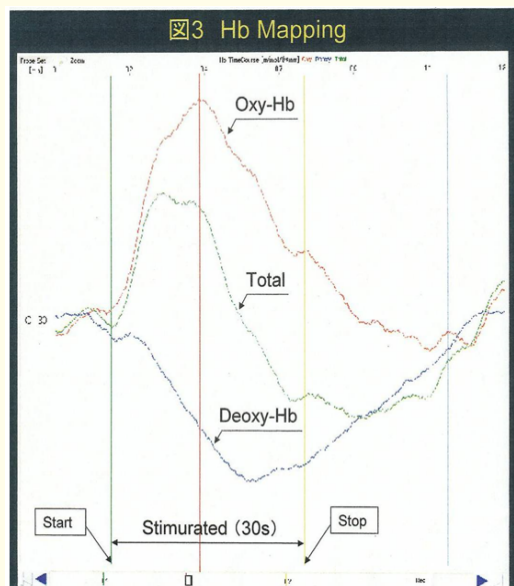
Figure 3: *Digital Comparison System in this Study; NIRS probes covered the prefrontal cortex and premotor cortex activation. 52 multichannel probe holders were used. These consisted of 8 illuminative and 8 detective probes arranged alternately at an inter-probe distance of 3 cm, resulting in 8 channels (CH) per set. This machine consisted with 52 detector of NIRS impact and judging the efficacy of acupuncture therapy for brain effect with a least damage to volunteer (ETG-4000, Hitachi High-Technologies, Co., Ltd., Tokyo). This machine can digitally shown NIRS level in each detector.*

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Measurement of white blood cell composition and leukocyte subsets, granulocyte, lymphocyte

The total and differential leukocyte counts were measured by the automated hematology analyzer XE-2100 (Sysmex, Inc., Kobe, Japan). The levels of catecholamines were measured by high performance liquid chromatography (HPLC) system (Tosoh Co. and Hitachi High-Technologies Co, (ToKyo). The immune system is a totally integrative system. It includes brain, endocrine and immune system. For example, an immune system contains various cells, tissues and organs that protect organisms against potentially.

We sampled peripheral blood from the 10 volunteers before and after the therapy, at the same time on each day, in consideration of circadian rhythm of leukocyte. These subjects participated in this study after giving their informed consent. Measurements of the total and differential leucocyte counts and 3 catecholamines levels in the peripheral blood. We depended on the laboratory of Ishikawa Prefecture Preventive Medicine Association about the total and differential leukocyte counts and the levels of 3 catecholamines (adrenaline, noradrenaline, and dopamine) in the peripheral blood from the volunteers. The total and differential leukocyte counts were measured by the automated hematology analyzer XE-2100 (Sysmex, Inc., Kobe, Japan). The amount of catecholamines were calculated by high performance liquid chromatography (HPLC) system (Tosoh Co. and Hitachi High-Technologies Co., Japan).

Adrenalin contents

Blood samples were collected by nurse before acupuncture treatment and 24 hours after the treatment. The blood samples were analyzed conventional procedures and measured the total leukocytes (WBC: white blood cell) number and ratio of granulocytes and lymphocytes. The catecholamine content (adrenalin) was also measured by ELISA (Enzyme-Linked Immuno Sorbent Assay).

Statistical analysis

The data were expressed as mean +/− standard deviation. The WBC is number of cells, granulocytes and lymphocytes were shown as % of total leukocytes and adrenalin content was expressed by pg (pikogram/ml). Group comparison of data was performed by ANOVA and post hoc multiple test. Difference of point selection between two countries was analyzed by chi-square test (Statview, 5.0, SAS). The correlation between baseline data and ratio of each WBC composition and adrenalin were also calculated.

Results

Diagnosis and treatment of three countries

Volunteers attended in this pilot trials were ten healthy young male school students, and they were diagnosed by three traditional acupuncturists in each treatment session and point selections were issued by personalized way. Korean acupuncturist clearly classified based on Sasang constitution theory, a traditional Korean medical theory [14-16]. Nine subjects were classified into Soeum-in type (n = 4), Soyang-in type (n = 1), Taeum-in type (n = 3) and Tayang-in type (n = 0) by Sasang constitutional medicine, and deficiency of responsible meridians were pointed out. The point selection was conducted by base on the Saam acupuncture theory, and the points located in the periphery were frequently used. Supplement or drain technique on the selected points were restrictedly performed.

Chinese acupuncturist employed pulse and tongue diagnosis then selected the points. No TCM diagnosis of condition was performed as the subjects were basically healthy young students. The relatively thick acupuncture needles were used and inserted deep in tissues and de-qi sensation frequently revealed by the needle manipulation.

Japanese acupuncturist did careful palpation of the skin and deep tissues to detect reaction points, no pulse diagnosis was employed. The needling procedure was gentle manipulation without any subjective impact called de-qi. He selected the points regardless of the precise location of acupuncture points so no information regarding selection of the acupuncture points. But rough location of needling points was recorded. The extremely different characteristic of the Japanese style acupuncture from that of C and K treatment was confident. All practitioners used the single-use disposable acupuncture needle in sterile package.

Point selection of Korean and Chinese acupuncturists

The puncture points selected by Korean and Chinese practitioner were summarized in table 1, for all the volunteers in this project. The total numbers of acupuncture points selected were similar but the actual points used were quite different between C and K treatments.

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Total number of acupuncture points selected in C and K treatment were 83 (4 - 17, mean of 9.2/subject) and 75 (6 9, mean of 8.3/subject), and less and regular number of points selection were observed in K treatment. The acupuncture points selected in C and K treatments were obviously different, and only 5 acupuncture points (ST36, V12, LI11, LR3, SP2) were selected in both K and C treatment, although the volunteers were the same and a total of 44 acupuncture points were selected in K and C treatment. The major points selected in C and K treatments were listed in table 4.

	Points	Range	Mean
China	83	6 - 9	9.22
Korea	75	4 - 17	8.33

Table 1: *Point selection by K and C practitioners.*

This table summarizes the acupuncture points selected by Korean and Chinese practitioner in all subjects of this study. The total numbers of acupuncture points used were similar but the actual points used were quite different between C and K treatments. Total number of acupuncture points used in C and K treatment were 83 (4 - 17, mean of 9.2/subject) and 75 (6 - 9, mean of 8.3/subject), and less and regular number of points selection were observed in K treatment.

Brain topographical representation by NIRS system

In order to suggest the effect of acupuncture impact to the brain site, NIRC system was employed in this trial. The trial system of photo was shown in figure 4-6, suggesting the real positioning of acupuncturist, volunteer and the engineer for digital imaging. The diagnosis room was kept silent and dark so as to avoid any stimulus including cell phone. The starting point of impact had been kept zero in each country before therapy.

As a result of each results in the same volunteer were shown in table 4. From these figures, the impact of NIRC in volunteer were Chinese, Korean and Japan in order. With the brain topography, we had set up the check point for each 52 detector points for evaluation. After the left leg stimulation for example by GB34, China, Korea and Japan were 22/52,16/52 and 12/52 points in order. For the acupoint SP6, impact point were 18/52,16/52 and 14/52 in order.

Back flash phenomenon by NIRS

NIRS could easily compare the impact of each acupuncture by this system during needling. With this simultaneous indication, the impact by three country shown that the Japanese (GB34) and Korean (SP6, GB34) looked so weak and rarely shown by NIRS. However, we found first that there could be shown the back flash phenomenon (BFP) when the needle drained from the acupoint by NIRS (Figure 4a/p6, Figure 4b/p7, Figure 5a/p8, Figure 5b/p9, Figure 6a/p10, Figure 6b/p11).

Points	1	2	3	4	5	6	7
China	BL23	ST36	SP6	CV4	KI3	GV4	CV12
Korea	SP3	CV12	ST36	LI4	SI2	HT7	SI3

Table 2: *Rank of the acupuncture points frequently used in K and C treatments.*

The acupuncture points used in C and K treatments were obviously different, and only 5 acupuncture points (ST36, V12, LI11, LR3, SP2) were used in both K and C treatment, although the subjects were the same and a total of 44 acupuncture points were used in K and C treatment. The major points used in C and K treatments were listed in this table.

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|	Mean	SD	n
Leukocytes			
China	64.78	12.04	9
Korea	69.25	12.30	8
Japan	64.78	15.80	9
Lymphocytes			
China	39.91	9.49	9
Korea	31.21	7.23	8
Japan	37.12	10.03	9
Granulocytes			
China	55.54	9.61	9
Korea	64.76	8.29	8
Japan	58.98	11.26	9
Adrenalin			
China	51.67	21.05	9
Korea	57.00	16.21	8
Japan	61.78	29.04	9

Table 3: *Total scoring of acupuncture style in three countries.*

Subjects were healthy young subjects with informed consent (male, average 21 years, 20 - 25, n = 10). Special reference was made to select in order not to try acupuncture since then. This projects were planned in April, June and August in 2009 at Kanazawa. Acupuncture practitioners were well-experienced traditional acupuncture therapists in Japan, Korea and China (their clinical experiences were over 20 years). The purpose of the treatment was to regulate self-defense system and to make better QOL in each volunteer.
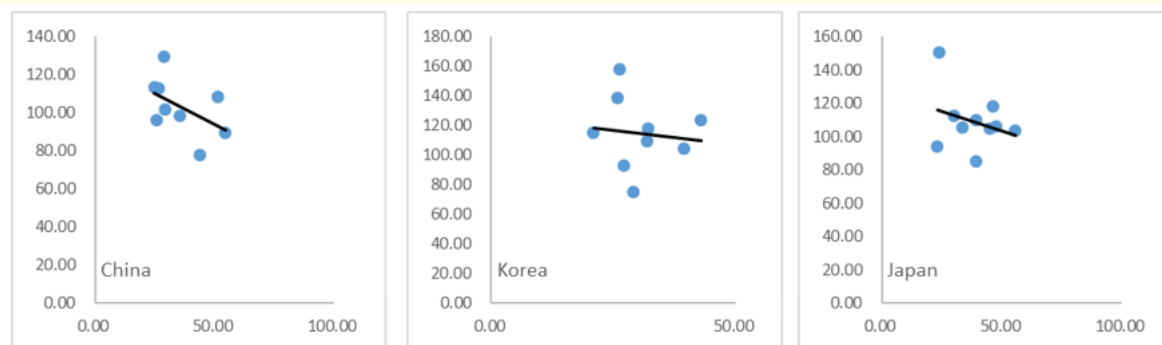
	Before	Needling		Just after withdrawn	
		SP6	GB34	SP6	GB34
China	---	18/52	22/52	ND	ND
Korea	---	16/52	16/52	14/52	16/52
Japan	---	14/52	12/52	16/52	7/52

***Table 4:*** *NIRC scoring of acupuncture style in three countries.*

Subjects were healthy young subjects with informed consent (male, average 21 years, 20 - 25, n = 10). Special reference was made to select in order not to try acupuncture since then. This projects were planned in April, June and August in 2009 at Kanazawa. Acupuncture practitioners were well-experienced traditional acupuncture therapists in Japan, Korea and China (their clinical experiences were over 20 years). The purpose of the treatment was to regulate self-defense system and to make better QOL in each volunteer.
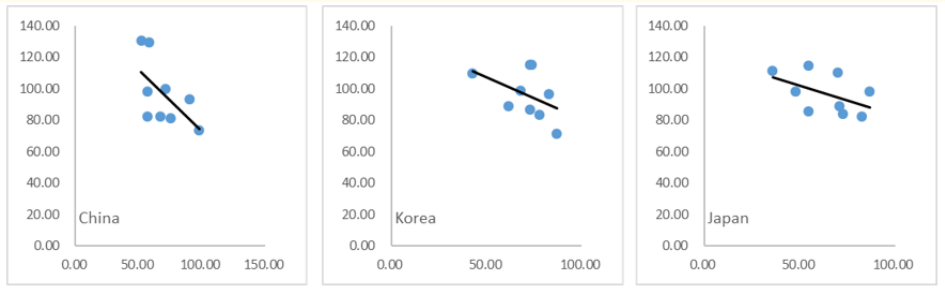
Figure 4: *Changes of WBC after one acupuncture session; Blood samples were collected by nurse before acupuncture treatment and 24 hours after the treatment. The blood samples were analyzed conventional procedures and measured the total leukocytes (WBC: white blood cell) number and ratio of granulocytes and lymphocytes. The catecholamine content (adrenalin) was also measured by ELISA (Enzyme-Linked Immuno Sorbent Assay).*

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Figure 5: Changes of Lymphocyte numbers after one acupuncture session; Blood samples were collected by nurse before acupuncture treatment and 24 hours after the treatment. The blood samples were analyzed conventional procedures and measured the total leukocytes (WBC: white blood cell) number and ratio of granulocytes and lymphocytes. The catecholamine content (adrenalin) was also measured by ELISA (Enzyme-Linked Immuno Sorbent Assay).
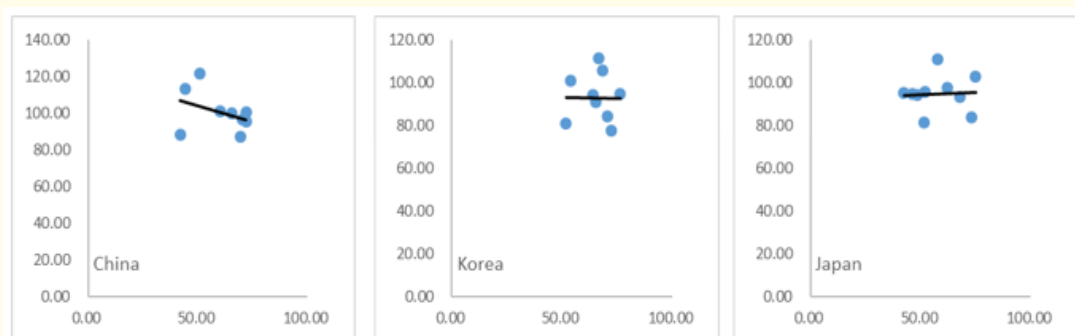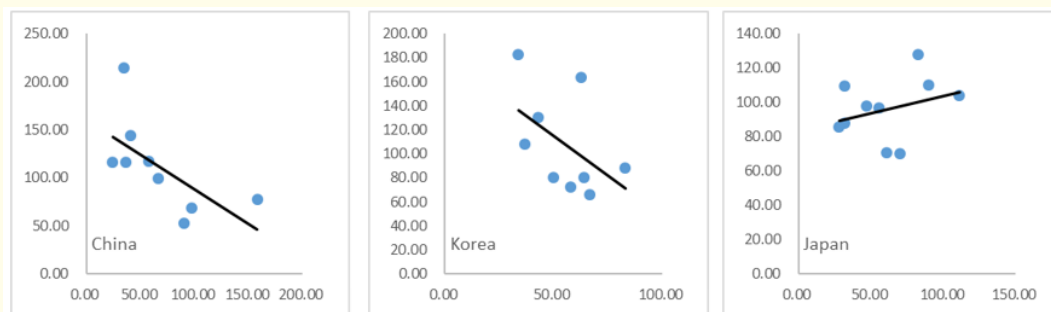

Figure 6: Changes of granulocyte numbers after one acupuncture session; Blood samples were collected by nurse before acupuncture treatment and 24 hours after the treatment. The blood samples were analyzed conventional procedures and measured the total leukocytes (WBC: white blood cell) number and ratio of granulocytes and lymphocytes. The catecholamine content (adrenalin) was also measured by ELISA (Enzyme-Linked Immuno Sorbent Assay).

Adrenaline level

Subjects were healthy young subjects with written informed and consented. They had no experience of acupuncture treatment. The experimental design used was cross-over and each subject was randomly allocated to Chinese, Korean and Japanese acupuncture in order of their registration. One subject received three different treatments. The experiments were conducted in April, June and August in 2009 at Kanazawa. The average level of three countries were shown in figure 7, indicating Japanese one was the best value but other two, China and Korea were the next and the same level. However, the regulatory ability was in order, China and Korea but Japanese one was the discrete vector of regulation. Relative changes in adrenalin contents are demonstrated (Figure 7). Regarding adrenalin contents, three treatments have different influences. In C treatment, increase of the contents with large variation in C treatment whereas slight reduction with small variation in J treatment. K treatment has no changes as the mean value although its variation was large itself.

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Figure 7: Changes of Serum Adrenalin after one acupuncture session; The average level of three countries were shown, indicating Japanese one was the best value but other two, China and Korea were the next and the same level. However, the regulatory ability was in order, China and Korea but Japanese one was the discrete vector of regulation. We depended on the laboratory of Ishikawa Prefecture Preventive Medicine Association about the total and differential leukocyte counts and the levels of 3 catecholamines (adrenaline, noradrenaline, and dopamine) in the peripheral blood from the volunteers. The total and differential leukocyte counts were measured by the automated hematology analyzer XE-2100 (Sysmex, Inc., Kobe, Japan).

WBC/leukocyte number

The total WBC number, lymphocyte (%), granulocyte (%), serum adrenalin content (pg/ml) were measured before treatment as baseline. The baseline data of nine subjects who complete three acupuncture therapies received were listed in figure 8.
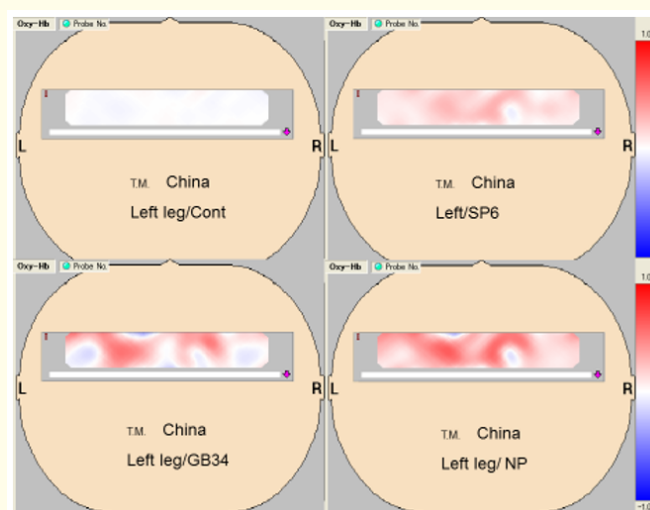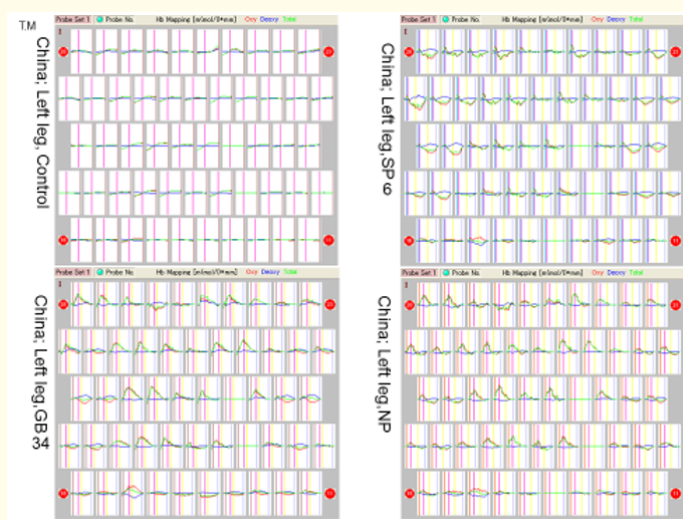

Figure 8: Brain Topographical Representation by NIRS System by China; So as to suggest the effect of acupuncture impact to the brain site, NIRS system was employed in this trial. The trial system of photo was shown in figure 2, suggesting the real positioning of acupuncturist, volunteer and the engineer for digital imaging. Functional near-infrared spectroscopy is a noninvasive functional neuroimaging technique measuring cortical absorption of near-infrared light to estimate local concentration. In this system, the changes of oxygenated (HbO$_2$) and deoxygenated hemoglobin by acupuncture therapy. Hemodynamic responses depicting brain activation comprise task-evoked HbO$_2$ increases and lower amplitude deoxygenated hemoglobin decreases.

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There was no significant difference among three groups (China, Korea and Japan) in their baseline data although individual variations tended to be large. The regulative effect was eminent in China style but other two, Korea and Japan was little or no effect for this regulation

Granulocyte

The total WBC number, lymphocyte (%), granulocyte (%), serum adrenalin content (pg/ml) were measured before treatment as baseline. The baseline data of nine subjects who complete three acupuncture therapies received were listed in figure 9. One subject was dropout for his personal reason. There was no significant difference among three groups (China, Korea and Japan) in their baseline data although individual variations tended to be large. The regulative effect was eminent in China style but other two, Korea and Japan was little or no effect for this regulation.

Figure 9: Brain Topographical Representation by NIRS System by China; An ETG-4000 Optical Topography System (Hitachi Medical Co, Tokyo) captured activation across 52 prefrontal locations (channels), spatially defined according to the international 10-5 system of probe placement. The probes were placed on each participant's head by the same investigator, after which the participants were given 10 minutes warm-up time to become familiar with the box trainer and get used to wearing the device. The experiment was started immediately. This machine consisted with 52 detector of NIRS impact and judging the efficacy of acupuncture therapy for brain effect with a least damage to volunteer (ETG-4000, Hitachi High-Technologies, Co, Ltd, Tokyo).

On granulocytes ratios are basically opposite pattern of those of lymphocyte. Only C treatment increased to 108.8% and K and J treatments reduced to 95.1 and 95.5, respectively. There are significant difference among the countries (ANOVA, p = 0.026). The negative slope of regression lines was observed in C treatments and positive slopes were obtained in K and J treatments. The negative slope of regression lines was observed in C treatments and positive slopes were obtained in K and J treatments.

Lymphocyte ratio

The total WBC number, lymphocyte (%), granulocyte (%), serum adrenalin content (pg/ml) were measured before treatment as baseline. The baseline data of nine subjects who complete three acupuncture therapies received were listed in figure 10. One subject was dropout for his personal reason. There was no significant difference among three groups (China, Korea and Japan) in their baseline data although individual variations tended to be large. The regulative effect was eminent in China style but other two, Korea and Japan was little or no effect for this regulation. The effects of C, K and J treatments on the lymphocytes were summarized in figure 3 and 4 influences of acupuncture treatments on lymphocytes differ among the countries. In C treatment tended to decrease the relative value to 91.1%,

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whereas K and J treatments increase to 109.5 and 108.6%, although no statistical significance observed (one way ANOVA, P = 0.0784). The variation of SD among the countries is similar.
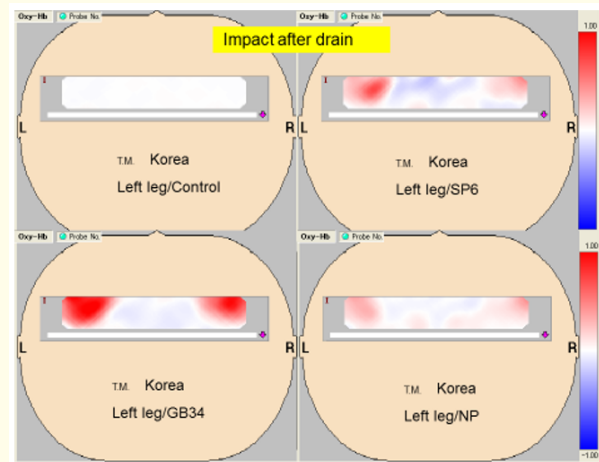
Figure 10 summarizes the relation between the baseline data and degree of changes in percentages of lymphocytes ratios after the treatment. In C and J treatments, negative regression lines, whereas K treatment produce.
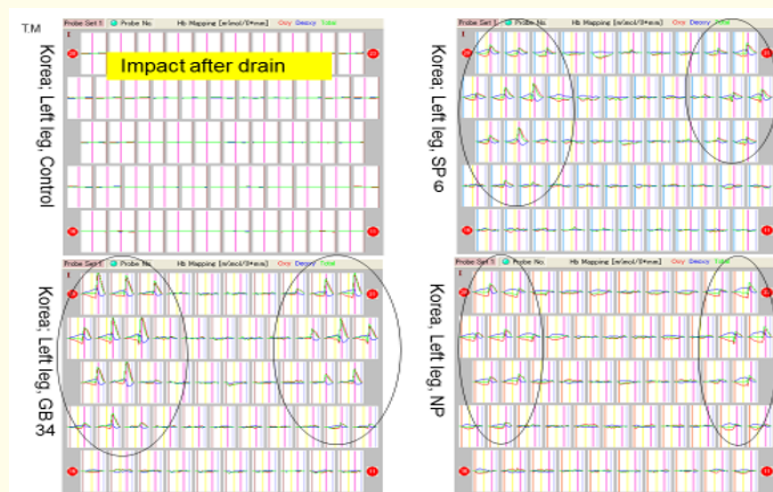

Figure 10: *Brain Topographical Representation by NIRS System by Korea. So as to suggest the effect of acupuncture impact to the brain site, NIRS system was employed in this trial. The trial system of photo was shown in figure 2, suggesting the real positioning of acupuncturist, volunteer and the engineer for digital imaging. Functional near-infrared spectroscopy is a noninvasive functional neuroimaging technique measuring cortical absorption of near-infrared light to estimate local concentration. In this system, the changes of oxygenated (HbO2) and deoxygenated hemoglobin by acupuncture therapy. Hemodynamic responses depicting brain activation comprise task-evoked HbO2 increases and lower amplitude deoxygenated hemoglobin decreases.*
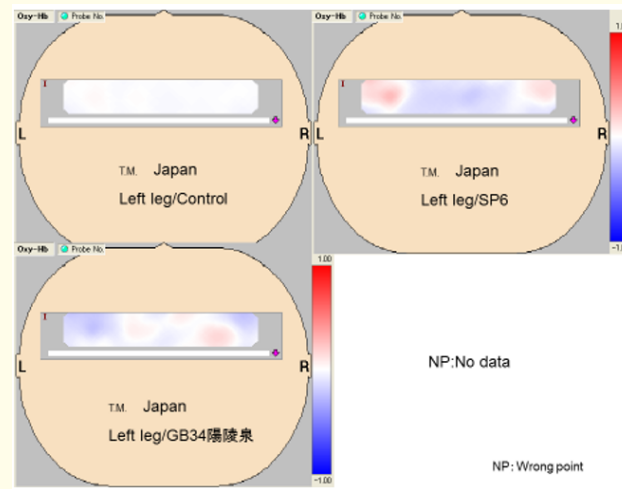

Figure 11: *Brain Topographical Representation by NIRS System by Korea; An ETG-4000 Optical Topography System (Hitachi Medical Co, Tokyo) captured activation across 52 prefrontal locations (channels), spatially defined according to the international 10-5 system of probe placement. The probes were placed on each participant's head by the same investigator, after which the participants were given 10 minutes warm-up time to become familiar with the box trainer and get used to wearing the device. The experiment was started immediately. This machine consisted with 52 detector of NIRS impact and judging the efficacy of acupuncture therapy for brain effect with a least damage to volunteer (ETG-4000, Hitachi High-Technologies, Co, Ltd, Tokyo).*
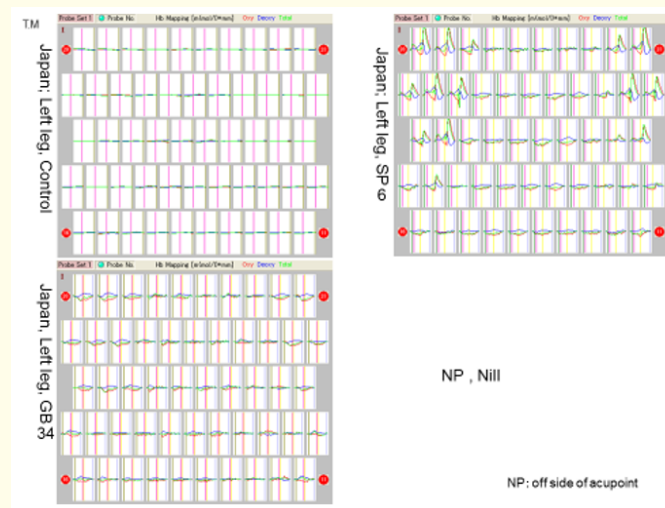
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Figure 12: Brain Topographical Representation by NIRS System by Korea after Drain the Needle; So as to suggest the effect of acupuncture impact to the brain site, NIRS system was employed in this trial. The trial system of photo was shown in figure 2, suggesting the real positioning of acupuncturist, volunteer and the engineer for digital imaging. Functional near-infrared spectroscopy is a noninvasive functional neuroimaging technique measuring cortical absorption of near-infrared light to estimate local concentration. In this system, the changes of oxygenated (HbO2) and deoxygenated hemoglobin by acupuncture therapy. Hemodynamic responses depicting brain activation comprise task-evoked HbO2 increases and lower amplitude deoxygenated hemoglobin decreases.

Figure 13: Brain Topographical Representation by NIRS System by Korea after Drain the Needle; So as to suggest the effect of acupuncture impact to the brain site, NIRS system was employed in this trial. The trial system of photo was shown in figure 2, suggesting the real positioning of acupuncturist, volunteer and the engineer for digital imaging. Functional near-infrared spectroscopy is a noninvasive functional neuroimaging technique measuring cortical absorption of near-infrared light to estimate local concentration. In this system, the changes of oxygenated (HbO2) and deoxygenated hemoglobin by acupuncture therapy. Hemodynamic responses depicting brain activation comprise task-evoked HbO2 increases and lower amplitude deoxygenated hemoglobin decreases.
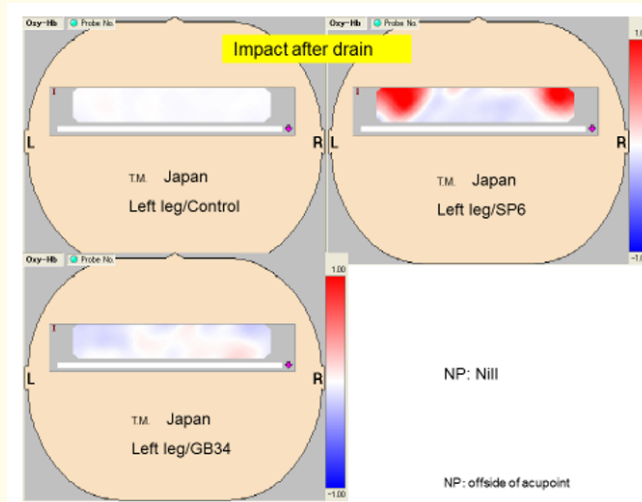
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Figure 14: Brain Topographical Representation by NIRS System by Japan; So as to suggest the effect of acupuncture impact to the brain site, NIRS system was employed in this trial. The trial system of photo was shown in figure 2, suggesting the real positioning of acupuncturist, volunteer and the engineer for digital imaging. Functional near-infrared spectroscopy is a noninvasive functional neuroimaging technique measuring cortical absorption of near-infrared light to estimate local concentration. In this system, the changes of oxygenated (HbO2) and deoxygenated hemoglobin by acupuncture therapy. Hemodynamic responses depicting brain activation comprise task-evoked HbO2 increases and lower amplitude deoxygenated hemoglobin decreases.
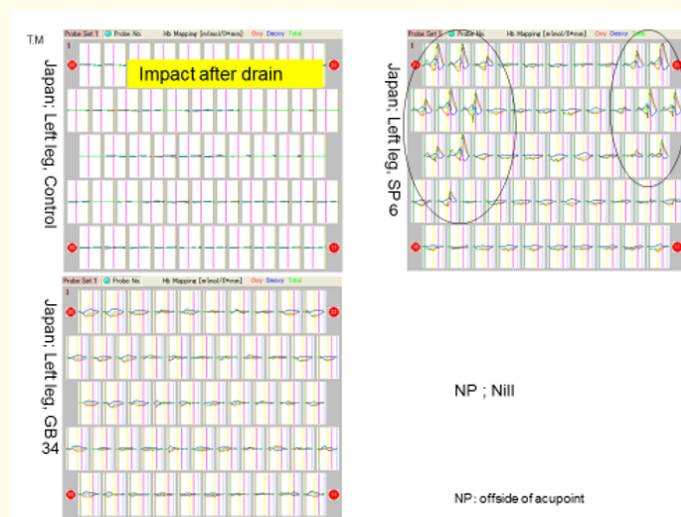

Figure 15: Brain Topographical Representation by NIRS System by Japan; An ETG-4000 Optical Topography System (Hitachi Medical Co, Tokyo) captured activation across 52 prefrontal locations (channels), spatially defined according to the international 10-5 system of probe placement. The probes were placed on each participant's head by the same investigator, after which the participants were given 10 minutes warm-up time to become familiar with the box trainer and get used to wearing the device. The experiment was started immediately. This machine consisted with 52 detector of NIRS impact and judging the efficacy of acupuncture therapy for brain effect with a least damage to volunteer (ETG-4000, Hitachi High-Technologies, Co, Ltd, Tokyo).

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Figure 16: Brain Topographical Representation by NIRS System by Japan after Drain the Needle; So as to suggest the effect of acupuncture impact to the brain site, NIRS system was employed in this trial. The trial system of photo was shown in figure 2, suggesting the real positioning of acupuncturist, volunteer and the engineer for digital imaging. Functional near-infrared spectroscopy is a noninvasive functional neuroimaging technique measuring cortical absorption of near-infrared light to estimate local concentration. In this system, the changes of oxygenated (HbO_2) and deoxygenated hemoglobin by acupuncture therapy. Hemodynamic responses depicting brain activation comprise task-evoked HbO_2 increases and lower amplitude deoxygenated hemoglobin decreases.

Figure 17: Brain Topographical Representation by NIRS System by Japan after Drain the Needle; An ETG-4000 Optical Topography System (Hitachi Medical Co, Tokyo) captured activation across 52 prefrontal locations (channels), spatially defined according to the international 10-5 system of probe placement. The probes were placed on each participant's head by the same investigator, after which the participants were given 10 minutes warm-up time to become familiar with the box trainer and get used to wearing the device. The experiment was started immediately. This machine consisted with 52 detector of NIRS impact and judging the efficacy of acupuncture therapy for brain effect with a least damage to volunteer (ETG-4000, Hitachi High-Technologies, Co, Ltd, Tokyo).

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Discussion

With the present pilot study, we set up the effects of three different individualized diagnoses and treatments of traditional ways of Chinese, Korean and Japanese acupuncture on the WBC composition and adrenalin in the same subject. The apparently clear differences of point selection and procedure of acupuncture manipulations among the countries were observed. A total of 44 acupuncture points was selected but only 5 points were common in K and C treatments. Three different acupuncture treatments also induced different tendencies in modification of the WBC counts and lymphocyte and granulocyte ratios and serum adrenalin although no statistically significant as obvious individual variations also observed.

The initial protocol was planned to select the subjects from three countries, but the time schedule of practitioners could not allow the selection from three countries, and small number of Japanese young male volunteers who have no experience of acupuncture could be prepared. The selection of subjects in three countries might be one of the important issue in the future project. The selection of traditional acupuncture practitioners was also important issue. In Japan a practitioner who participated in the previous study [18] was adopted as key member of this project. In Korea, representative of the Korean Acupuncture and Moxibustion Society (KAMS) discussed and recommended the practitioner in this project. In Chinese acupuncture, one Chinese acupuncturist who has acupuncture clinic in Japan was selected. The individual variation of diagnosis of TCM doctors was pointed out [3], so it is important to prepare several practitioners in each country for obtaining sufficient and representative results of each country. The facts that multi-directional changes in the outcome measures could be produced by three different acupuncture treatments suggested the importance of procedures of acupuncture needling. In this project, Chinese acupuncture produced de-qi sensation, and Korean acupuncture frequently used the acupuncture points in the hand. On the contrary, Japanese acupuncture provoked almost no subjective sensation but the practitioner detected slight changes in respiratory movement during his needling technique. These three acupuncture treatments clearly resulted several regulation on the nervous system, hormone and peripheral leukocyte system.in different style of manner.

The major purposes of this project were 1) confirmation of efficacy that digitally responsible system, 2) the comparison of the efficacy between in three country, 3) in a different needle size judging by the factor relevant for brain hormone and peripheral leucocyte. There confirm the reasonable degree of efficacy in all the system employed in this trial. With NIRS system, there confirm the reasonable degree of efficacy in all the system employed in this trial. The impact by NIRS, the impact was C, K and J in order. However the differences was so close and found back flashes were found in K and J. One of hormonal factor, adrenalin was clearly regulated by C and K. However, leucocyte subset regulation, lymphocyte were regulated C and J. Granulocyte subset regulation was only found in C.

The immune system was strongly influenced by various factors. For the accumulation of report were seen that peripheral leukocyte subsets closely linked with hormonal system and adrenal-cortical organ and nervous system in number and function. So there were many report that e efficacy of traditional therapy should be first for the peripheral leucocyte system, immune system.

Abo reported that peripheral leucocyte subset were regulated by catecholamine in a sense with circadian rhythm. Psychological state such as depression and mental stress suppressed the immune capabilities [19] Acupuncture and moxibustion treatment could improve and regulate the immune activity by measuring of lymphocytes and NK cell activities [20-22]. On the contrary, lack of effect of acupuncture on the immune regulatory functions was also reported in [23].

Recently analysis of gene by using cDNA microarray analysis demonstrated participation of several genes which related to the up-regulation of NK cell activity by electro-acupuncture (EA) [23]. Down-regulation of proinflammatory cytokine such as IL-I beta mRNA by EA was also shown [24].

In mice, EA activated the spleen NK cells and IFN-gamma level also increased, and EA also increased beta endorphin level and its effects were antagonized by naloxone [26-29].

Anti-inflammatory effect of acupuncture was suggested to be mediated by vagal nerve stimulation and deactivation of macrophages [21] and the fact that existence of acetylcholine detectors on the lymphocytes [24,30] suggested the importance of tune of autonomic nervous system on the immune activity.

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Our previous study clearly demonstrated that one of Japanese-style acupuncture treatment induces prolonged increases of cytokines and shift of ratios of granular cells and lymphocytes depending on the previous condition of the subjects. The increases of IFN-gamma and IL-I6 and other leukocytes with various CD marker including NK cells were observed. These promoting influences of acupuncture on the immune systems and reciprocal effects on lymphocytes and granulocytes might be a possible explanation of bio-regulatory function of acupuncture [18,30,31]. Then measurement of ratios of the lymphocytes and granulocytes proposed as a useful outcome measures in complementary and alternative medicine (CAM) [17,23-26].

In this project, apparent regulation were seen in all three practitioner for total white blood cells, leukocyte in a reversal dose dependent manner. For the lymphocyte regulation, Chinese one was eminent but other two, significant change was not seen. For quantitative regulation of granulocyte, Chinese style was only proved to positive.

Next, we tried to adrenaline level, the intermediate position of blood –brain connection, all the system were effective for the regulation. However, vector of regulation was the same in China and Korean, but different vector in Japan. This was an important finding of this trial to need further stud, including the number of specimen for test.

The large variation of baseline data in small sample size might be a possible cause of the discrepancy of results. Health conditions of the subjects, young university students, varied during the relatively long periods (4 months) accompanied with seasonal changes of circumstances, so the present data could not be assumed as the simple results of J treatment. The similar problems were also found in the characteristics of the modulating potentials of K and C acupuncture treatments. Another issue was the subject used in the present study. The traditional acupuncture procedures and its theoretical concepts might developed based on their subjects in each country, so the facts that the subjects in this study were Japanese young students might also be a possible cause of large variation of the results in K and C treatments.

The results obtained in the present study were not conclusive but we got important information about the variety of acupuncture treatments in Japan, Korea and China, and they have potentials to modulate the immune systems and stress level in different way with degree. The information might be useful for understanding the meaning the each system of traditional acupuncture therapy developed in each country.

By the way, TCM are indicated for the treatment of chronic inflammatory pain such as osteoarthritis, but they have risk for infectious disease on the dermatological barrier and are not suitable for repeated use. Therefore, the authors consider that the treatment with materials such as Japanese style is desirable considering the infectious risks. Therefore, we tried to develop a non-transcutaneous acupuncture using materials that are permitted to be used without injection of needle. Presently, preparations containing chondroitin are marketed for the prevention of osteoarthritis, but the problem is that it takes time for the effect to appear. TCM was applied by combining anti-inflammatory and analgesic [32,33].

One case report was indicated that the patient with heart failure was severe risk of infection as well as pumping activities. The acquired immunodeficiency was promised to shoed such a patient. So needless to say, disposable system was necessary to avoid infection especially to hepatitis virus infection. In this condition, Japanese non transcutaneous acupuncture was tried to augment the heart function without risk of injection of needle for at least one year. As a consecutive to the patient who had been in heart failure and reduced ejection fraction (HFrEF).The results proved to the potential increase of stroke volume by echocardiographic assessment, starting phase 33% and to 36, 42, 45% at the following phase, compare to the starting value before this therapy [34-38]. Based on the results of this study, the efficacy of acupuncture was evidenced in our digital presentation system, brain hormone and peripheral leukocyte at least in number. The number of volunteer and qualitative aspect of investigation are expected.

The main purpose of the present study was to identify three different traditional acupuncture treatments by applying to the same volunteer, employing crossover model. However, the remarkable variation of baseline data and lack of control subjects, who received no acupuncture treatments, made it difficult to analyze and discuss the data sufficiently. This clinical study of JCK traditional acupuncture

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was the first attempt in the world and it offered important information to establish the role of acupuncture therapy in CAM as an individualized therapy, and also offer interesting information for the future researches.

Conclusion

As shown in table 3 and 4, we tried to access the effect of acupuncture in three country by digital presentation enabling to make score for judgement. From the Table following conclusion were found:

1) The effective impact for brain function were, China, Korean and Japan in order.

2) With NIRS, K and J probed back flash phenomenon just after draining the needle.

3) The regulational effect for emotional hormone were C and K.

4) The regulational effect for WBC number were C and K.

5) The regulational effect for granulocyte were only C.

6) The regulational effect for lymphocyte number were C and J.

7) Recommended system was disposable needle system.

Conflict of Interest

We declared that there was no conflict of interest in this study.

Acknowledgment

We all appreciated to the relevants, especially for the student volunteers who attended for this trial.

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