

Qualitative Aspect of Constitutive Regulation by Emotional Hormone in Air Way Inflammation

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Abstract

Background: The purpose of this report was to introduce CAM menu for the aspect of emotional hormone together with quantitative aspect of leucocyte subsets in number and function for regulating airway inflammation. Within some reports, serum adrenaline and dopamine were different from each individual and age, sexuality and personality along with the ratio of emotional hormone. In this article, we would like to show the emotional hormone regulation along with quantitative aspect by CAM menu, even in regulation of allergic symptom for prophylactic and therapeutic.

Methods: Many menu of CAM were tried to the normal subjects that could control the emotional hormone for the aspect regulating air way inflammation. The menu were listed hot-spring hydrotherapy, light exercise, floor heating and some famous decoction of TCM including aroma therapy. 3 Catecholamine were evaluated before and after the CAM menu adjusting the time schedule in order to coincide the circadian rhythm.

Results: A hot-spring hydrotherapy, light exercise, floor heating and some famous decoction of TCM could downly regulated to the adrenaline level whose value showed higher before trial. However, Dopamine level were upregulated to whom levels were showed a few. Within a different type of constitution, both adrenalin-rich type and dopamine-rich type were moderately regulated their constitution for the vector to counterpart.

Conclusion:

- 1) The qualitative regulation of total hormonal level could be assessed by down-regulated, namely tailored scale for each constitution.
- 2) With each CAM proved to regulate the total level of emotional hormone, along with granulocyte and lymphocyte
- 3) With each CAM proved down-regulated for adrenalin level, but up-regulated for dopamine in adult and older age.

Keywords: Hay Fever; Airway Inflammation; Chronic Respiratory Tract Infection; Asthma; Chronic Cough; COPD; Bronchiolitis; Leucocyte Subset; Adrenaline; Dopamine; Hot-Spring Hydrotherapy; Light Exercise; Floor Heating; Blood Cell Supportive TCM

Abbreviations

CAM: Complementary and Alternative Medicine, Beside the western medicine, there are many traditional medicine and/or health promoting menu all over the world; CD: Cluster of Differentiation. Each lymphocyte has name that expressed CD number, for example CD2, CD4, etc.; COPD: Chronic Obstructive Pulmonary Disease; DM: Diabetes Mellitus; Emotional Hormone: Adrenaline, Dopamine and Cortisone were selected as emotional hormones for this trial; FCM: Flow Cytometry; G-Rich Type: The individual that exhibit over 60% of granulocyte in peripheral blood, finding many in young gentleman; HCLH: Head Cooling and Leg Heating System; LCHH: Leg Cooling and Head Heating System; L-Rich Type: The individual that exhibit over 40% of lymphocyte in peripheral blood, finding lot in ladies and senile; QOL: Quality of Life; VAS: Visual Analog Scale

Introduction

There are many kinds of medicine including Ethnic Medicine in the world, therefore it is not many who can receive benefit from the Western medicine like in developing countries. Fork medicines were found elsewhere in the world where native peoples started to live. With a simultaneous report, we would like to concern the constitutional aspect of allergic disease especially for air way inflammation. So the purpose of this report was concern the qualitative presentation for CAM menu that could regulated the constitution according to the life-style related one. The important thing was express as digital way of assessment, not by VAS; visual analog scale *in vivo* rout of approach. Therefore, fine assessment and reviewing is necessary to estimate between the subtype as well as main type of traditional medicine [1-9]. In this and prior article, we tried to present the effect of CAM for respiratory diseases, as a digital presentation of Japanese new CAM style reported as digital methodology and issue modeling. For the fine assessment, especially air way inflammation, Airway epithelial cells play an important role in innate immune functions in the lungs. Airway epithelial cells also exhibit the characteristics of muco-ciliary cells and physically remove pathogens via a process known as the muco-ciliary movement, which involves the trapping of pathogens in the mucus produced in airways under inflammatory conditions and removing the mucus via the movement of cilia present on epithelial cells [10,11]. In that point of view, the regulation of constitution is important by the CAM menu even in hay fever, airway inflammation.

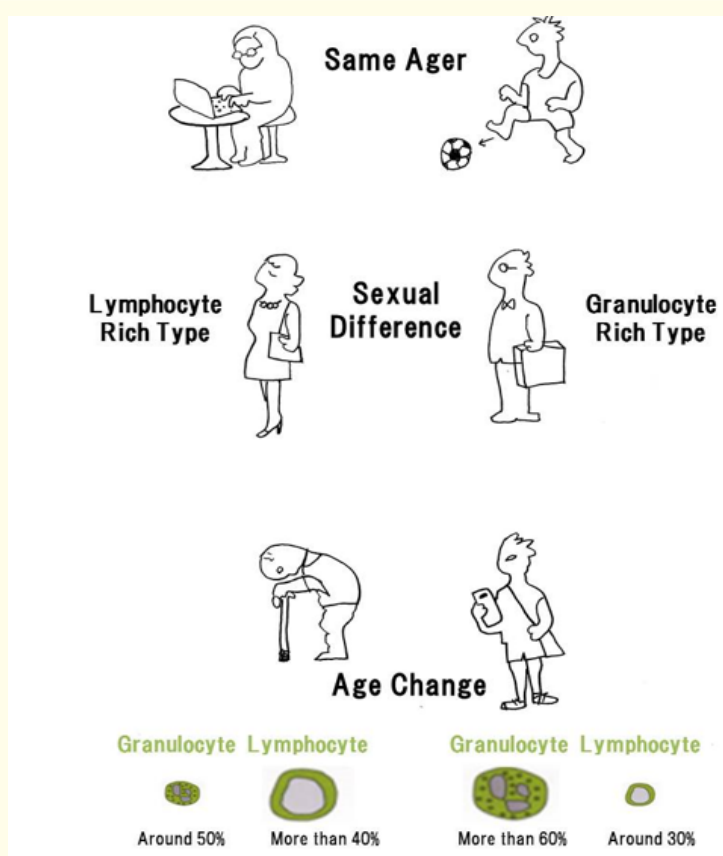


Figure 1: Conceptual Representation of Human Constitution in Digitally. There were two type of constitution, according to lymphocyte/Granulocyte ratio, namely Lymphocyte-rich type and Granulocyte rich type. Lymphocyte rich type contains over 40 % of lymphocyte and Granulocyte rich type has over 60% of granulocyte [2,3].

Subjects and Methods

Subjects

We recruited 14 healthy volunteers (mean age, 19.5 ± 10.2 years, ranging 9 ~ 45 years old in both sexualities) and informed consented according to The Ethics Committee of Kanazawa Medical University. The percentage of sexuality were 45.3% of female and 54.7% for male. They were the pre-graduated member of Medical University and the staff and their offspring of the School of Medicine. The attendant were divided in to two group and each group was started after informed consent. The blood sample were prepared at the same time zone of the first sampling. 14 normal volunteers (age, 19.5 ± 10.2 years) were participated in each report. We collected peripheral blood from fore arm vein of them before and after hot-spring hydrotherapy, at the same time zone on the day, in adjusting of circadian rhythm of leukocyte and emotional hormone [12]. Estimation of the total adrenalin, nor-adrenaline and dopamine levels performed double in the peripheral blood in the same time zone, adjusting circadian rhythm. We asked and charged on the laboratory of Ishikawa Prefecture Preventive Medicine Association for authorized and precise and reliable assessment. The total and differential leukocyte counts were measured by the automated hematology analyzer XE-2100 (Sysmex, Inc., Kobe, Japan). The levels of adrenalin, nor-adrenaline, dopamine and other hormone were estimated by high performance liquid chromatography (HPLC) system (Tosoh Co. and Hitachi High-Technologies Co., Japan).

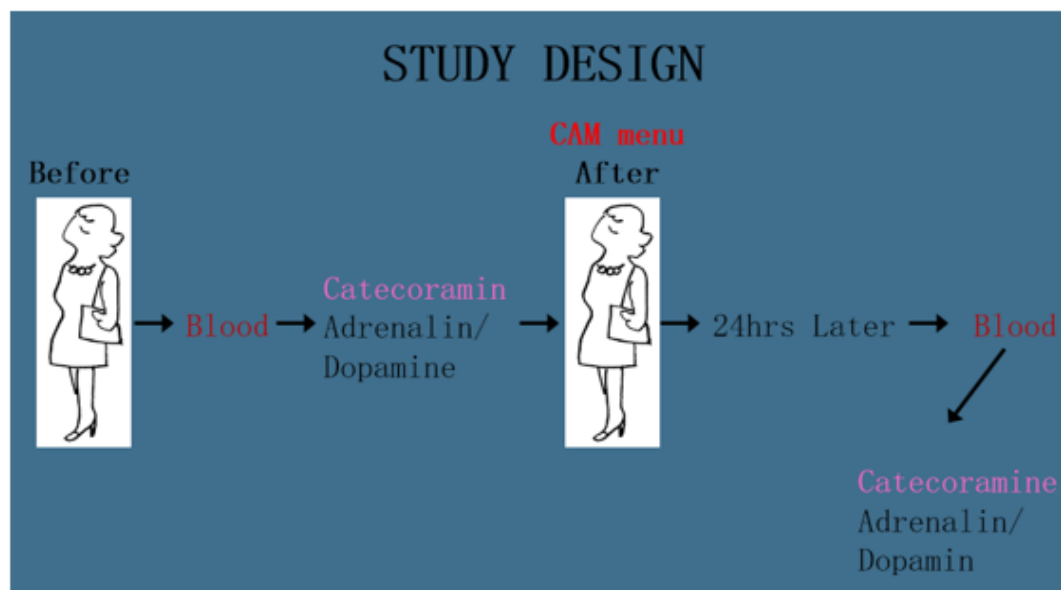


Figure 2: Experimental Schedule.

General experimental design to evaluate each CAM therapy. Time interval of blood sampling between before and after the trial was approximately 24 hours, adjusting circadian rhythm.

Analysis of the β₂-AR Positive Lymphocyte Subsets by FCM

The blood withdrawn from the volunteer by blood collection tube containing an anticoagulant EDTA-2K. 100 µl of whole blood were added the anti β₂-AR antibody (Santa Cruz Biotechnology, Inc. U.S.A.) of the primary antibody and were reacted for 30 minutes at 4°C. M In order to estimate a CD+ cell, the blood was collected from the subjects by blood test tube containing an anticoagulant EDTA-2K. 100 µl of whole blood were mixed with each corresponding antibody. In order to estimate a CD+ cell, the whole blood was collected from the attendants by blood collection tube containing an anticoagulant EDTA-2K. 100µl of whole blood were mixed with each corresponding antibody. After washing out excessive antibody with PBS, the suspensions were mixed with phycoerythrin (PE)-conjugated streptavidin (Beckman Coulter Inc. France) and fluorescence-activated monoclonal ABs: peridinin chlorophyll protein-cyanin 5.5 (PerCP-Cy5.5)+CD2,

fluorescein isothiocyanate (FITC)+CD4, FITC+CD8, FITC+CD16, FITC+CD19, FITC+CD57 (Becton Dickinson Co. U.S.A.), allo-phycoyanin (APC)+CD8, and APC+CD57 (Beckman Coulter). After washing out with Phosphate Buffered Saline, the cell suspensions were fixed employing a X10 diluted Cell FIX (Becton Dickinson) and analyzed by flow cytometer system, FACS Caliber (Becton Dickinson). The negative controls were prepared PE+streptavidin and the isotype control antibodies to the CD antibodies. After incubating for 0.5 hr at 4°C, these samples were hemolyzed abundant RBC with a 10-times dilution FACS Lysing Solution (Becton Dickinson).

Statistical Analysis

The statistical analysis along with the groups (before and after trial) for the test of significant difference were calculated by paired t-test and wilcoxon signed-ranks test. As for the examination of the correlation was found a spearman's correlation coefficient by rank test. Data are expressed as means \pm standard error of mean (SE). A P value < 0.05 was recorded to be statistically significant. The Kendall tau Rank Correlation and the two-sided p-value were also analyzed.

Results

Hot Spring Hydrotherapy

Catecholamine levels in the peripheral blood

So as to confirm the regulatory effect for leukocyte subset in simultaneous report, we tried to check the emotional hormones for systematic regulation to each constitution or not. We set up to access the emotional hormone adrenaline, or-adrenaline, dopamine and thyroid hormones. The recruited 14 healthy attendants (mean age, 19.5 ± 10.2 years, ranging 9 ~ 45 years old in both sexualities) were informed and consented according to The Ethics Committee of Kanazawa Medical University. The serum level of adrenalin in the peripheral blood of 14 subjects tended to down-regulated after hot-spring hydrotherapy especially in younger ager, on the contrary to up-regulated in older ager, showing negative coefficientcy ($p < 0.05$) (Figure 3 and 4). With reproducible result were obtained in the figure, the turning point of vector was 40 years old, both female and male. We did not directed precisely how they enjoy the hydrotherapy but left participants free as they were. Summarizing from the interview, they tried to hydrotherapy 2 - 3 times for hot spring hydrotherapy and about 30-40 minutes per one hydrotherapy within 24 hrs. On the other hand, the dopamine levels significantly up-regulated after hot-spring hydrotherapy ($p < 0.05$) [10-14]. The results indicate that the hot-spring hydrotherapy may influence the secretion of adrenaline from the adrenal medulla. The manner of regulation was correlated with value day before for controversial vector according to each constitution.

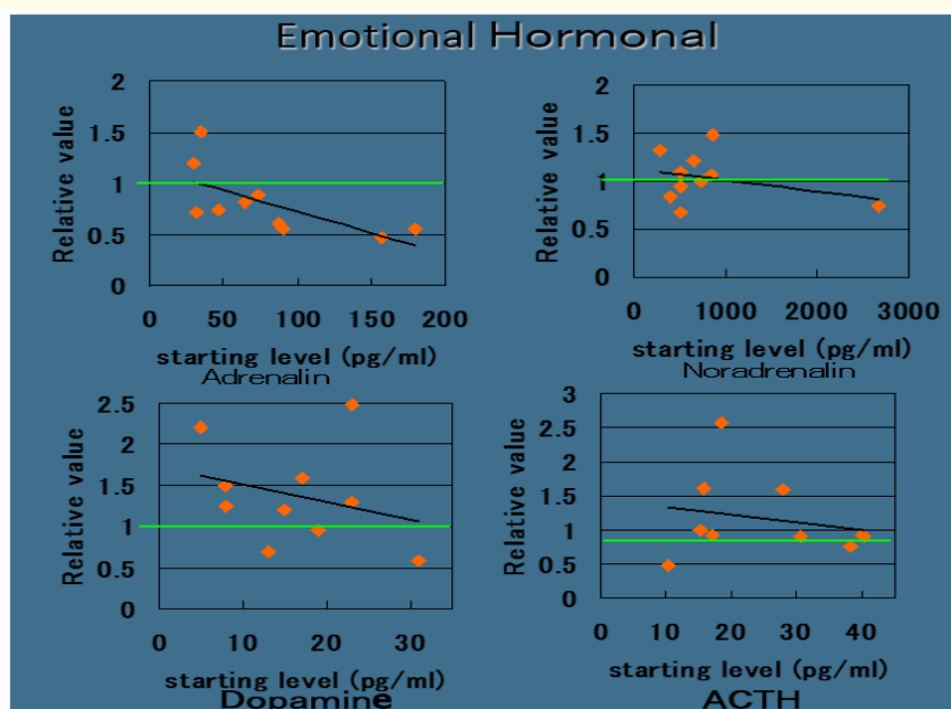


Figure 3: Hot Spring Hydrotherapy, Trace for Emotional Hormone.

These subjects participated in this study after giving their informed consent. We conducted the experiment at Chugu Onsen Spa (Oguchi Village, Ishikawa Pref., Japan) in one night of the day. The hot-spring quality is a weak sodium chloride with sodium carbohydrate of the water temperature $41 \pm 1^\circ\text{C}$. During the night and in the morning of the next day, they had a bath in the hot spring two or three times, for 20 - 30 minutes each time. Time interval of blood sampling between before and after hot-spring hydrotherapy was approximately 24 hours. Measurements of the total and differential leukocyte counts and 3 catecholamine levels in the peripheral blood. We charged 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 subjects. 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., Japan).

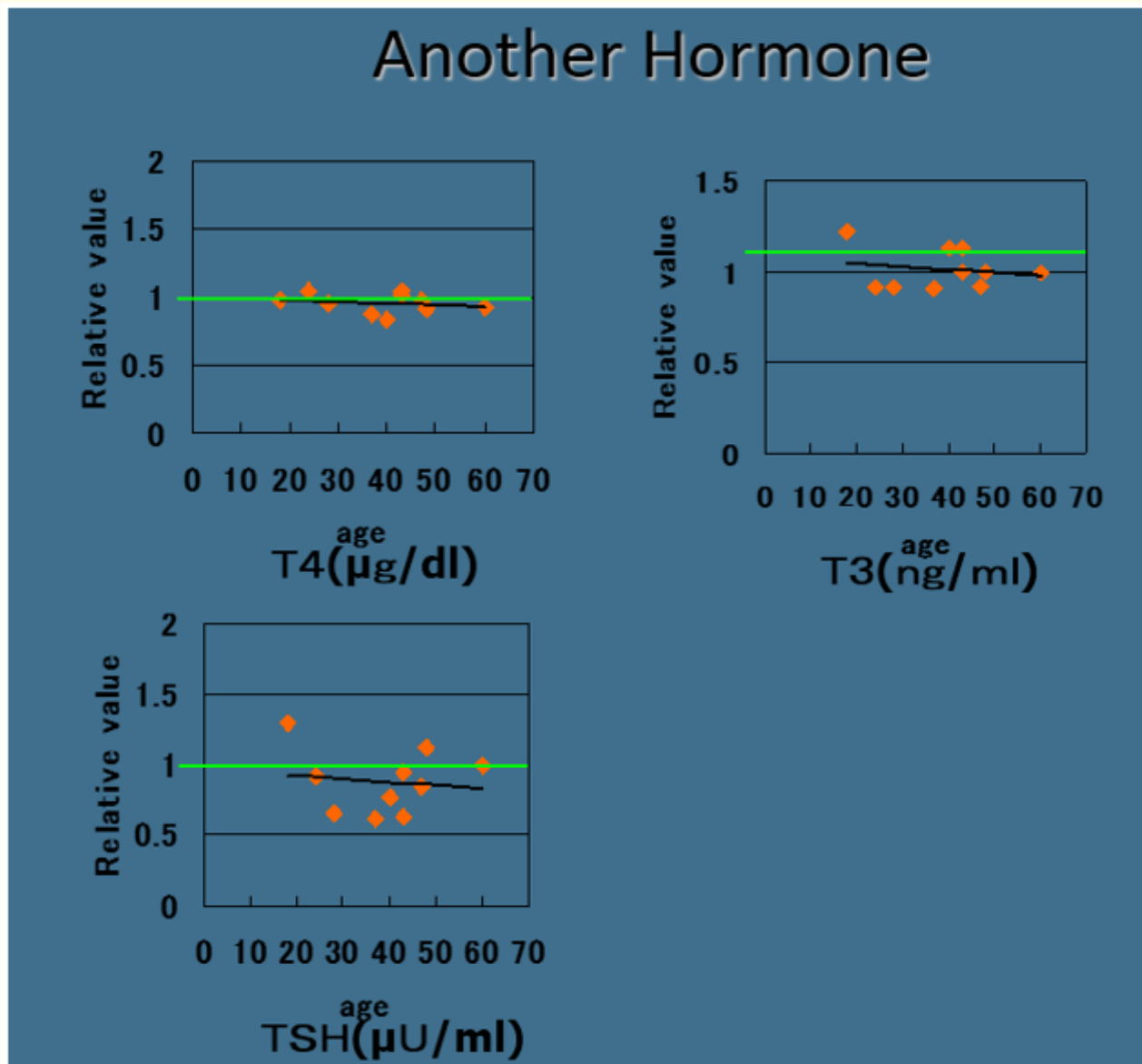


Figure 4: Hot Spring Hydrotherapy, Trace for Another Hormone.

In this section, another hormone, thyroid hormone was traced after the menu. The volunteers were the students of Medical University and the staff and their children for the school of medicine. The volunteers were divided in to two group and each group was started. The blood sample were prepared at the same time of the first sampling. 14 healthy volunteers (age, 19.5 ± 10.2 years) were tested. We sampled peripheral blood from them before and after hot-spring hydrotherapy, at the same time on each day, in consideration of circadian rhythm of leukocyte and emotional hormone.

FCM Analysis of β_2 -AR Expression for Emotional Hormone

In order to confirm the change in emotional hormone was systematic regulation for each constitution or not, we set up to access receptor positive cell for emotional hormone. The analysis of β_2 -AR positive cells and CD+ cells by FCM was counted by gating in the lymphocytes area on the scattered gram. Nonspecific reaction of the PE fluorescence was found in the isotype control. So, the actual values of the AR positive cell numbers were estimated by subtracting the control one. The CD19 positive cells were observed nonspecific reactions which seems to response of the second antibody. The comparison of each lymphocyte subpopulations before and after hot spring hydrotherapy showed that the CD8 positive cell and CD56 positive cell counts tended to increase (Figure 5). However, the comparison of β_2 -AR expressing cells was not seen the significant variation around hot-spring hydrotherapy. The mean % of β_2 -AR expressing cells in the lymphocyte subsets were 18 - 19% in CD3 positive cells, 5% in CD4 positive cells, 57 - 63% in CD8 positive cells, and 93 - 95% in CD56 positive cells. We confirmed the correlation with the degree of change in adrenaline levels and that of change in β_2 -AR positive cell counts of each subset or that in each CD-positive cell counts around hot-spring hydrotherapy. In the CD-positive cells, the relative value of change in adrenaline value was a positive co-relation with which in that of CD56 positive cells, CD8 positive cells, and CD3 positive cells; in particular a correlation with CD56 positive and CD8 positive cells was higher than another. In β_2 -AR positive cells, the percentage of change in adrenaline levels was a positive correlation with the rate of change in the levels of β_2 -AR positive and CD56 positive cells. These results showed that the variation in adrenaline levels is correlation with CD56 positive cells regulation together with host constitution.

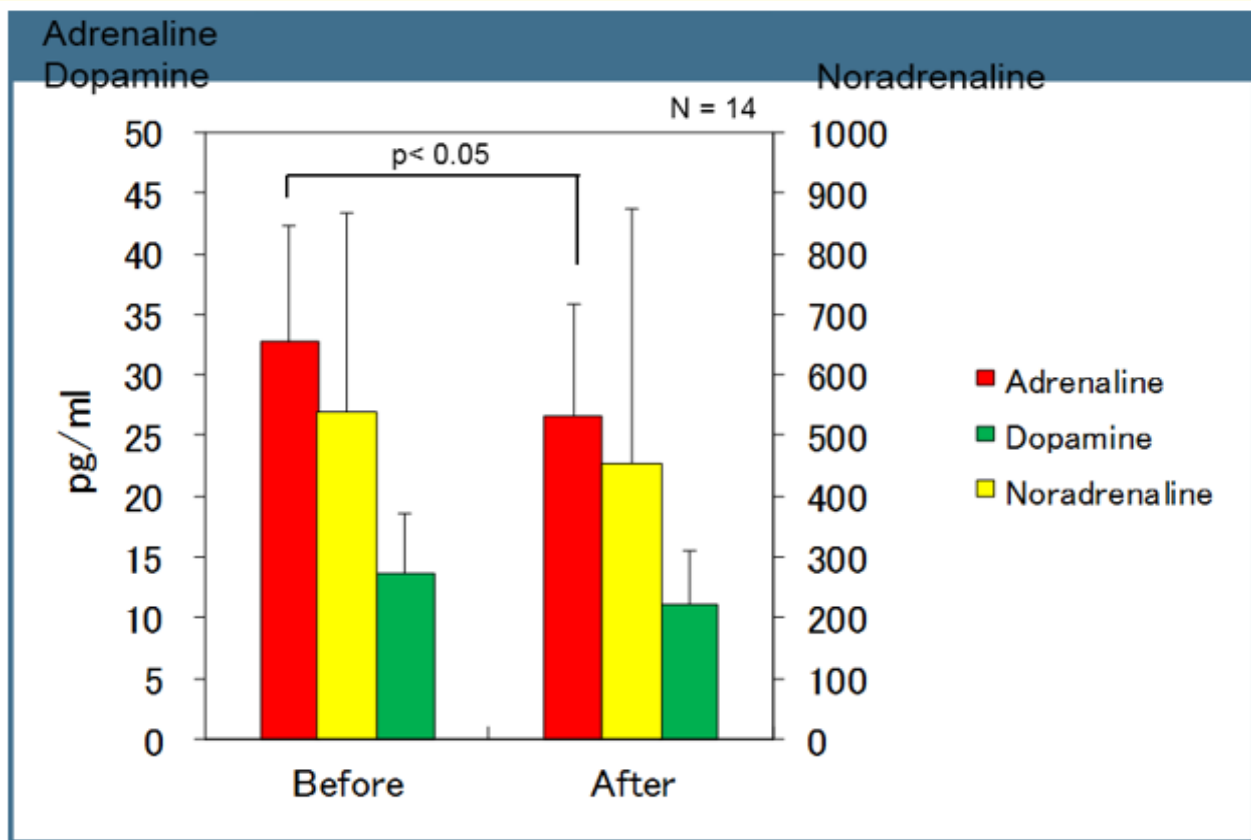


Figure 5: Hot spring, leukocyte receptor and emotional hormone.

FACS analyses of β_2 -AR+ cells in lymphocyte subpopulations before and after hot spring hydrotherapy. The correlation between adrenalin receptor positive lymphocyte and the serum emotional hormone were estimated by the FACS calibration.

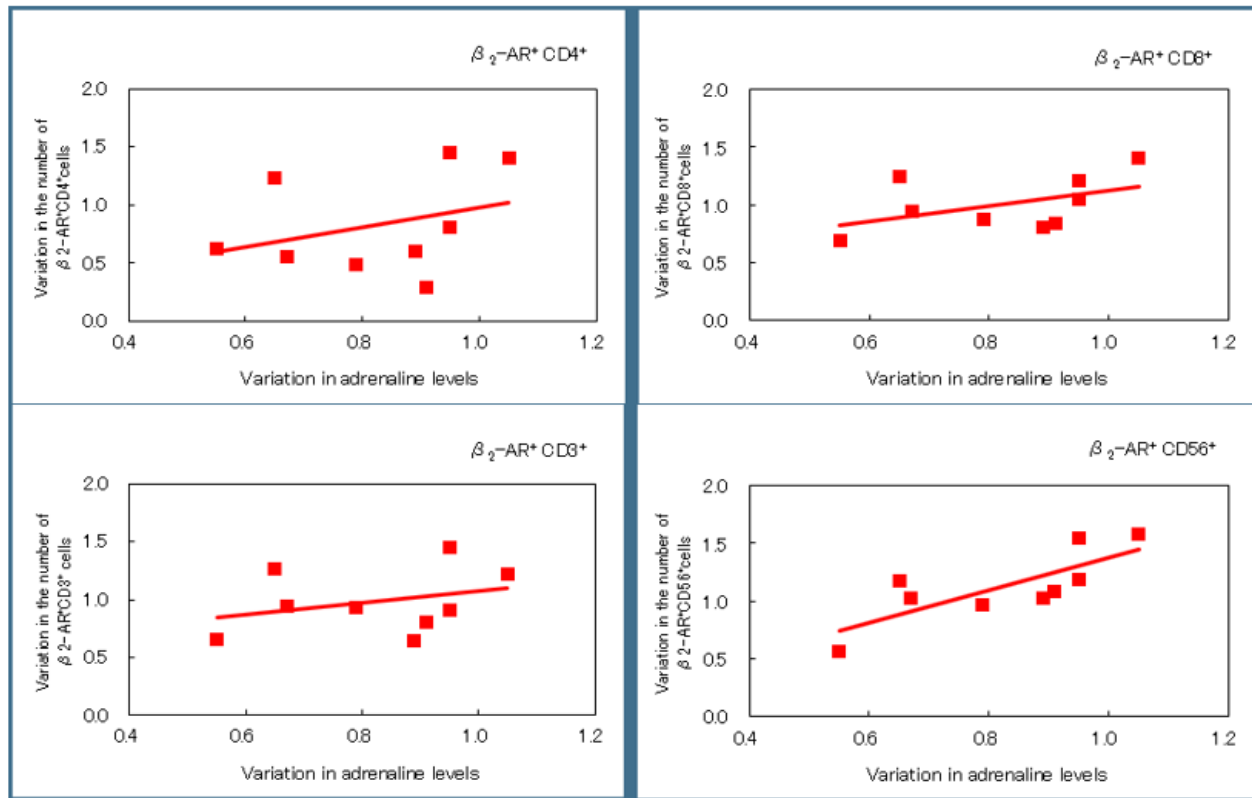


Figure 6: Hot spring, CD receptor and emotional hormone.

Variations in the number of β_2 -AR+ cells in lymphocyte subpopulations in the peripheral blood before and after hot spring hydrotherapy. The correlation between adrenalin receptor positive CD+ cell and the serum emotional hormone were estimated by the FACS calibration.

Light Excessive/Walking by 4 km/60 min and 4 km/30 min

The recruited 14 healthy attendants (mean age, 19.5 ± 10.2 years, ranging 9 ~ 45 years old in both sexualities) were informed and consented according to the Ethics Committee of Kanazawa Medical University. We planned first for two exercises, 4 Meta and 8 Mets. The two menu were only different in time limitation on the same course, one is for 4 km/60 min (4 Mets) and other was 4 km/30 min (8 Mets), Twenty-one days after cooling down. We collected peripheral blood from the same 14 volunteers before and after the exercise, at the same time zone on each day, in matching of circadian rhythm of leukocyte. The data from 4mets and 8mets exercise was brought an ideal regulation of the leukocyte and emotional hormones. But, the regulatory effect by more intense 8 Mets showed less efficiency than that of 4 Mets, more intense exercise suggesting suitable impact of leukocyte regulation. The course of exercise located at 40 meter in high and 1015 mb of atmospheric pressure on the day. We collected peripheral blood from the forearm vein from 14 volunteers before and after exercise, at the same time on each day, in consideration of circadian rhythm of leukocyte. In this light walking, the hormonal regulation was good by 4 Mets compared than 8mets, paralleling to the emotional hormone and constitutional regulation to lymphocyte.

Acupuncture Non-transcutaneous System

In practical consideration, wide variation of diagnosis and needling system are used such as meridian, reaction point, trigger point, skin impedance, anatomy, physiology, and so on. These subtype of Japanese acupuncture may due to its position of the medical systems in Japan. It has developed for the adjustment of the physiological status of the host especially in older ager. Recently, more advanced acupuncture based on physiology and anatomy of the body has been develop traditionally, each heat therapy has its own character and efficacy for various syndrome. Through the years, local acupuncture was evaluated for its specific properties and with the advent of better transportation in our separated island, even remote springs in the mountains were visited for their specific medicinal effect. An ontogenical and phylogenetical consideration of constitution, for the best condition. Even in non-transcutaneous type of Japanese acupuncture could regulated serum adrenaline level.

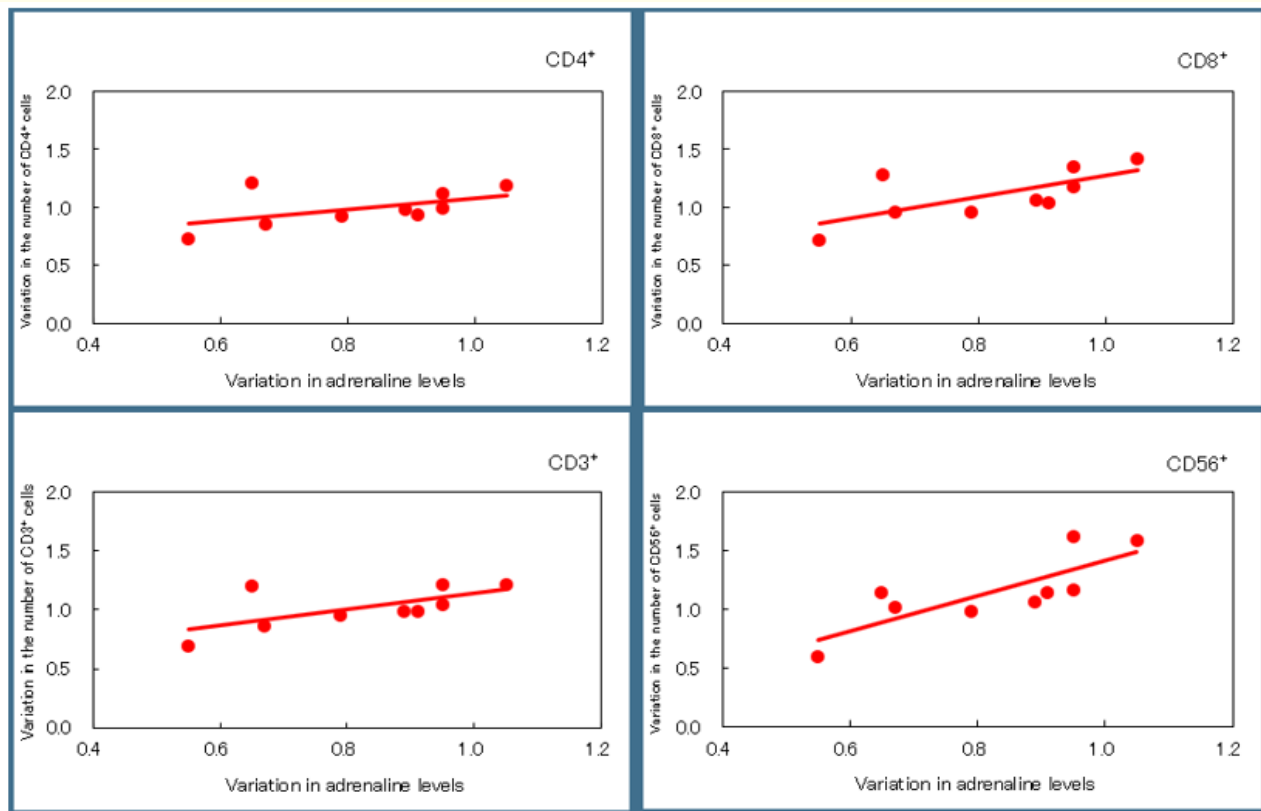


Figure 7: Acupuncture Non-transcutaneous System.

Traditionally, each CAM therapy has its own character and efficacy for various complaints. Non-transcutaneous Japanese type of acupuncture was tried to regulate the emotional hormone as well.

Floor Heating

Leukocyte Regulation after Working in Room, the Room Heating by Floor Heater

Each attendant was collected their blood before start the trial, after written informed consented to the experimental purpose by Ethics of the Committee in Kanazawa Medical University. After lodging the housing, attendants enjoyed for the aim of trial. The trial for leukocyte regulation written by digital words had been first for floor heating in this report. The system had been developed in North-East

part in China and Korean peninsula. The two major subsets, granulocyte and lymphocytes as digital scale for indicating their constitution in the floor heating system as a regulatory menu. As shown in figure 8, the groups was separated out for three, up-regulated individuals, down-regulated one and stood still. The correlation of change was demonstrated as a linear function. Figure 8 showed the best inclination also by floor heating, especially in cooling head and leg heating system. For example, in an individual with a low adrenaline level, the serum level were increased after the trial, while it decreased in another individual which was a higher adrenalin level. The correlation of regulation was expressed as a linear function and significant reverse correlation, suggesting ideal value of correlative index -0.5. The data obtained from floor heating was brought ideal regulation. As is in the leukocyte subset regulation, the ideal regulation of adrenaline and dopamine were found by floor heating system, cooling head and leg heating system but not in cooling leg and head heating system.

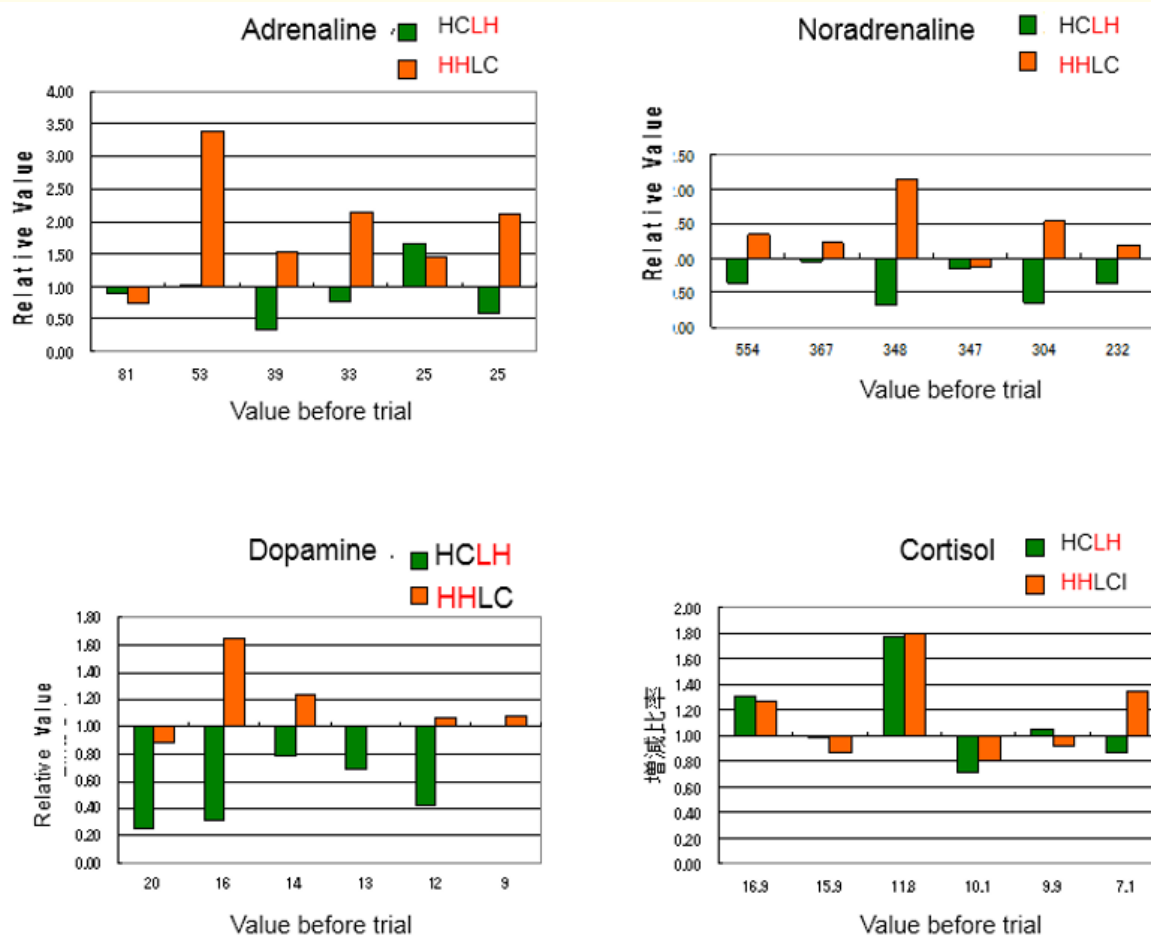


Figure 8: Floor heating.

Variations in the levels of 3 catecholamines (adrenaline, noradrenaline, and dopamine) in the peripheral blood before and after the floor heating system. We tried to express the effect of serum hormone by individual level (HCLH: Head Cooling and Leg Heating System, LCHH: Leg Cooling and Head Heating System).

Discussion

The data in this report exhibited that within 24 hours after each CAM, the emotional hormone, but not other thyroid one was not. The peripheral blood could controlled emotional hormone significantly, not only in total serum level but also dopamine ratio. The results indicated that these subsets could also reflect the immuno-competent cells in quantity and quality [10]. For example, in an individual with a low granulocyte in number, such number up-regulated after the menu, while it down-regulated in another individual with a higher cell counts. Our results also led us to believe that leukocyte subsets could be an interesting indicator for the efficacy of alternative medicinal therapy. Many ethnic medicine are in place to evaluate Western therapies that aim at healing the symptoms of the illness include complains. Abo reported that according to the lymphocyte subset content, there were two major type according to the number of the leucocyte subsets, namely, lymphocyte-rich type and granulocyte rich type [15-17]. There are many kinds of medicine including ethnic medicine in the world, therefore it is not many who can receive benefit from the Western medicine like us in Japan. It required to assess the efficacy of each traditional medicine on the same standard and digitally. The possible scale for them was proposed as immunological factor [14,18]. On the other hand, if these traditional medicines are practiced in the West, it is classified into the alternative medicine. The medicine practiced in today's Japan is called the Japanese medicine, and it is thought as one of the Far Eastern medicine like Chinese medicine, Tibetan medicine and Korean medicine other than Uyghur medicine [5-13]. Moreover, the regulational effect was obtained in peripheral leukocyte that we propose that this digital presentation of CAM is the best scale for access the physiological condition not by analog one. So as to compare the each traditional medicine in the each country, a common digital evaluation system is necessary. We propose that the best way for the scale is an immunological factor and as constitution dependent manner [19-23]. As a results of CAM, sample exhibiting efficacy through immunological factor by hot spring hydro therapy, light exercise, floor heating and TCM etc. We expect that our work will attract more attention to the mechanisms of which hot spring hydrotherapy regulates the human immune system. The contents are the result from the data showing the too much number individual tend to downy regulate, on the other hand fewer valued one is up-regulated and went to the suitable number. This type of regulation evident at least within a 24 hours, for the leukocyte subsets, granulocyte and lymphocyte are changed even under circadian rhythm as a constitution dependent manner. Converge to the neutral/healthy value.

Conclusions

We have measured the serum level of 3 catecholamine (adrenalin, dopamine, nor-adrenaline) after different mode of CAM. The conclusions were as following:

- 1) The qualitative regulation of total hormonal level could be assessed by down-regulated, namely tailored scale for each constitution.
- 2) With each CAM proved to regulate the total level of emotional hormone, granulocyte and lymphocyte.
- 3) With each CAM proved down-regulated for adrenalin level, but up-regulated for dopamine in adult and older ager.
- 4) With each CAM proved down-regulated for adrenalin as well as dopamine to neutral level, especially in young ager.
- 5) This regulation by CAM depend on the constitution by the status in day before.
- 6) It is important to know the constitution in each individual by the digital value.

Conflict of Interests

No conflict of interest hit in this trial.

Acknowledgement

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