

# The Cleaning Effect of Shampooing Care by Adenosine Triphosphate Bioluminescence System

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## Abstract

This paper discusses the cleaning effect of shampooing care by using Adenosine Triphosphate (ATP) bioluminescence system. Shampooing care is an important nursing one for patients who are difficult to care oneself. It is necessary to perform effective and comfortable care. So, we need to evaluate these effects quantitatively and qualitatively. We measured ATP, blood pressure, heart rate, heart rate variability, peripheral skin temperature and Visual Analog Scale (VAS) before and after shampooing. When we analyzed ATP adhering to head as "contamination" to evaluate that effect quantitatively, it found that the contamination of the scalp after shampooing decreased by 31%, and that of the hair decreased by 22%. The correlation between ATP and VAS showed that the more ATP decreased, the more subjects felt refresh. The physiological index was stable, and it found that the shampoo method in this study had little load on the body. We were able to clarify the cleaning effect of shampooing using rapid and simple method. These results showed can be the basic data that evaluated the effect by shampooing comprehensively.

**Keywords:** Shampooing; Nursing care; Adenosine triphosphate (ATP); Cleaning effect

## Introduction

Shampooing care is an important nursing one for patients who are difficult to care oneself. It shows various effects, such as keeping one's scalp and hair clean, facilitating the flow of blood, improving the function of scalp and activating mental state. If patients cannot wash their hair for a long time, they come to feel itchiness, clammy and uncomfortable gradually. This care is essential to improve quality of life. In fact, the frequency of shampoo is limited (once or twice a week in Japan), because it has a heavy burden for patients and nurses [1]. In order to wash one's hair more comfortable and effective on the few occasions, many studies about shampooing method were performed. Some studies examined physiological and psychological response in a standardized procedure [2,3]. They suggested the effect of warming, circulation-enhancing and relaxation. Other studies examined effective way of rinsing [4,5], the amount of water and its temperature [6,7], shampooing position [8-13], nursing skill [14,15] and supporting tool [16-19]. They measured hemodynamics, metabolism, muscle load and subjective assessment and recommended some methods which were able to reduce burden on body. However, there are few studies about cleaning effect which has great significance for shampooing care. It is important that it can improve metabolism and keep scalp healthy by removing dead skin cell, sebum and bacteria. In previous study, they examined the amount of sebum, resident microbiota, and scaling of the scalp [20-28]. They require special facilities, apparatus and a specialized technique. Moreover, it will take two or three days pending the outcome of the resident microbiota. Thus, the effective method for evaluating the cleaning effect of shampooing is not clarified.

In recent years, the Adenosine Triphosphate (ATP) method is used for examination of the cleaning effect in a nursing field. For example, it is often used for management of contamination on medical equipment [29-33], evaluation and education of hand-washing [34-40]. These studies analyzed ATP adhering to each object as "contamination" and suggested the utility of that method as a rapid and simple monitoring system. ATP is a chemical substance that acts as an energy source for all living organisms on the planet. The presence of ATP can be considered proof of the presence of a living organism, or of a substance produced by a living organism. This method measures the amount

of luminescence which is produced by the enzyme reaction of ATP and luciferase. The amount of luminescence is proportional to that of ATP and it is calculated as Relative Light Units (RLU) [41]. Anyone can measure ATP level simply for a short time by using a dedicated kit. When a rapid and simple measuring method becomes clear, we can propose new shampooing method whose cleanliness level is high.

In this study, we assumed ATP adhering to one's head as "contamination" and evaluated the cleaning effect of shampooing care by using ATP bioluminescence system. In addition, it is necessary to take into consideration not only the cleaning effect but other effects. So, we examined the physiological and psychological responses to assess the shampooing effect comprehensively.

## Method

### Experimental condition

**Subjects:** All subjects provided written informed consent, and the protocol was approved by Ethics Committee at Osaka University Division of Health Sciences (No. 215). Subjects were comprised of 9 healthy people, 6 men and 3 women, with a mean age of  $25.0 \pm 5.43$  years. Hairstyle was targeted from short hair to medium length hair, less than 400 mm. Their hair length was an average of  $246 \pm 108$  mm. They were instructed as follows: 1) to avoid shampooing the day before experiment 2) to avoid excessive use of alcohol and caffeine, sleep deprivation and excessive exercise the day before experiment 3) no hair dressing and nail polish 4) to forbid having meal from 3 hours before the experiment. Since it has been pointed out an estradiol cycle affects

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the autonomic nervous system, we checked that female subjects were a cryogenic period on the experiment day.

**Environment:** It experimented in the stable environment of  $24 \pm 2$  degrees C and  $50 \pm 10\%$  humidity.

**Procedure of shampooing:** Nurses with a clinical experience performed shampooing at the sink. They were provided the same amount of shampoo formulation (10 ml per one person) and shampoo time (1 minutes rinse, 2 minutes wash and 1.5 minutes rinse). Subjects were in the Fowler position during shampooing.

### Method of measurement

**Index of cleanliness level:** The cleanliness level was measured using the ATP + AMP swab test kit (Kikkoman Co., Japan). Adenosine Monophosphate (AMP) is derived from ATP during the processing, such as heat treatment and fermentation [41]. This kit can have a high sensitivity for detecting ATP + AMP compared with other kits which detect only ATP. It consists of LuciPac Pen as a reagent and Lumitester PD-20 as an instrument. Subjects were measured the level of ATP + AMP of head by wiping measurement areas before and after shampooing using LuciPac Pen. Measurement areas were top side, left side, right side and back side of their scalp and hair. The level of ATP + AMP was measured as RLU. Each value before and after shampooing was called "ATP-BS" and "ATP-AS" (Figure 1). ATP + AMP were abbreviated to ATP. Measurement was performed by the same person to minimize the effects of its procedure.

**Index of physiological response:** Blood Pressure (BP), Heart Rate (HR), Heart Rate Variability (HRV) and peripheral skin temperature (PST) were measured as physiological response. Subjects wore the wrist digital manometer (EW-BW30, Panasonic Co., Japan) on their right wrist, wore the sphygmograph (SA-3000P, Tokyo Iken Co., Japan) on one's left index finger and took the dermatherm sensor (DS101, Tateyama Kagaku Industry Co., Japan) between one's left middle finger and left thumb. A measurement procedure is shown in figure 1. They were measured 4 times with their eyes closed as follow: resting supine position after resting on a bed for 10 min "Rest Before Shampooing (RBS)", sitting position after moving from bed to chair and resting on a chair for 5 min "Before Shampooing (BS)", sitting position after shampooing in the Fowler position and drying their hair with a towel "After Shampooing (AS)", resting supine position after drying their hair with a dryer and resting on a bed for 10 min "Rest After Shampooing (RAS)". HRV was measured for 3 min.

**Index of psychological response:** Subjective assessment was assessed using VAS (Figure 1). It investigated four subjective scales of "Comfort", "Fatigue", "Refreshing" and "Itching". Subjects were asked to each feeling before and after shampooing by placing a mark on a VAS line. Each value before and after shampooing was called "VAS-BS" and "VAS-AS". The VAS was horizontally positioned with the extremes labeled "extremely discomfort, extremely fatigue, extremely unrefreshing, no itching" and "extremely comfort, no fatigue, extremely refreshing, extremely itching".

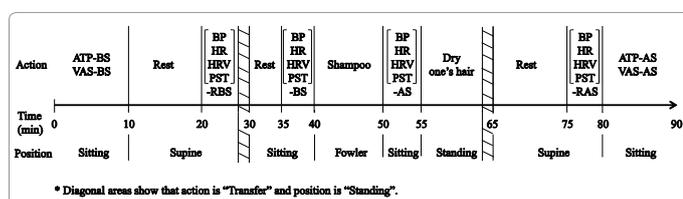


Figure 1: Measurement procedure.

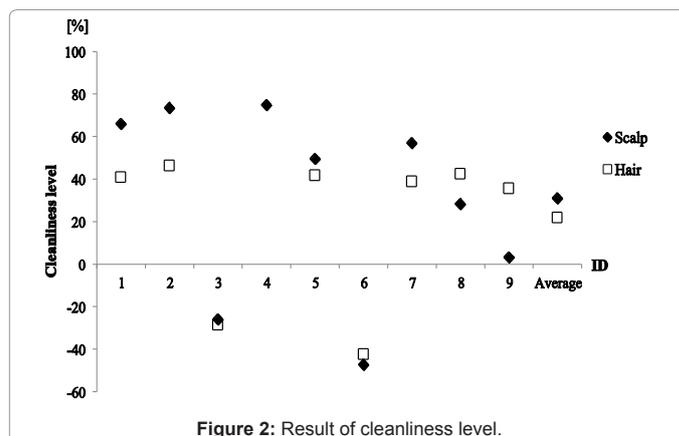


Figure 2: Result of cleanliness level.

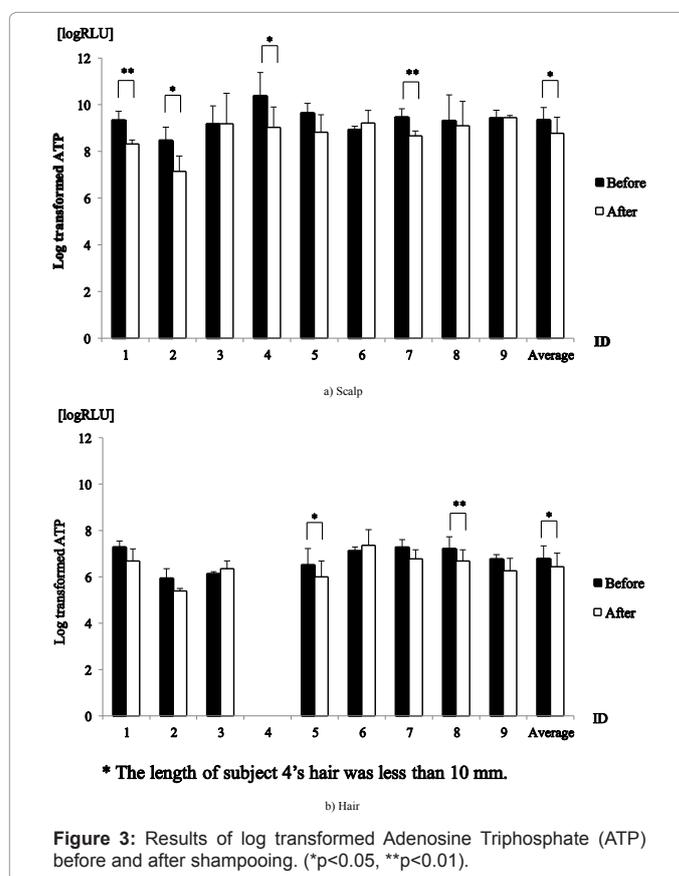


Figure 3: Results of log transformed Adenosine Triphosphate (ATP) before and after shampooing. (\*p<0.05, \*\*p<0.01).

### Method of analysis

**Index of cleanliness level:** The average of ATP of four areas was used as each subject's data. In order to clarify the rate of ATP which decreased by shampooing, the observed value of ATP-AS was normalized with that of ATP-BS, and it was defined as cleanliness level. Secondly, to assess the significance of change before and after, log-transformed ATP-AS was compared with log-transformed ATP-BS for every subject. All results are presented as means  $\pm$  SD. For statistical analysis, the paired *t*-test P values <0.05 were considered statistically significant.

**Index of physiological response:** HRV analyzed Standard Deviation of the RR Interval (SDNN) as the index of an autonomic nerve activity, High Frequency (HF: 0.15-0.4Hz) as that of parasympathetic nerve

activity, Low Frequency (LF: 0.04-0.15Hz) as that of sympathetic and parasympathetic nerve activity and LF/HF as that of balance of autonomic nerve. The difference between before and after shampooing was analyzed by Wilcoxon matched-pairs signed-rank test, and P values <0.05 were considered statistically significant.

**Index of psychological response:** VAS score put into numbers (where 100 = “extremely comfort, no fatigue, extremely refreshing, extremely itching” and 0 = “extremely discomfort, extremely fatigue, extremely unrefreshing, no itching”) and was analyzed. The difference between before and after shampooing was analyzed by Wilcoxon matched-pairs signed-rank test. Secondly, VAS-AS score was normalized with VAS-BS score. The correlation between VAS scales, and the index of cleanliness and that of physiological response were examined using Spearman’s rank correlation coefficient. P values <0.05 were considered statistically significant.

## Results

### Index of cleanliness level

The cleanliness level is shown in figure 2. Subject 4 couldn’t measure ATP on hair, because length of his hair was less than 10 mm. The average of cleanliness of scalp was  $31.0 \pm 44.9\%$ , and that of hair was  $21.9 \pm 35.7\%$ . The values of ATP before and after shampooing are shown in figure 3. The average of log-transformed ATP-BS on scalp was  $9.36 \pm 0.52$ , log-transformed ATP-AS on scalp was  $8.76 \pm 0.69$  log-transformed ATP-BS of hair was  $6.79 \pm 0.53$ , and log-transformed ATP-AS on hair was  $6.43 \pm 0.58$ . ATP-AS on both scalp and hair significantly decreased compared with ATP-BS ( $p < 0.05$ ). In terms of each subject, there were four subjects whose ATP-AS on scalp significantly decreased ( $p < 0.05$ ) and other three subjects whose ATP-AS on scalp were tend to decrease. And there were two subjects whose ATP-AS on hair significantly decreased ( $p < 0.05$ ) and other four subjects whose ATP-AS on hair were tend to decrease.

### Index of physiological response

**Blood pressure:** Systolic Blood Pressure (SBP) and Diastolic Blood Pressure (DBP) before and after shampooing are shown in figure 4. The average of SBP was  $108 \pm 10.2$  mmHg (RBS),  $116 \pm 11.5$  mmHg (BS),  $116 \pm 11.7$  mmHg (AS),  $106 \pm 9.96$  mmHg (RAS). The average of DBP was  $67.8 \pm 8.57$  mmHg (RBS),  $74.2 \pm 9.16$  mmHg (BS),  $76.3 \pm 11.5$  mmHg (AS),  $69.7 \pm 11.4$  mmHg (RAS). Both SBP and DBP of BS significantly increased than that of RBS ( $p < 0.05$ ). There was no difference between RBS and RAS, and BS and AS.

**Heart rate:** HR before and after shampooing is shown in figure 4.

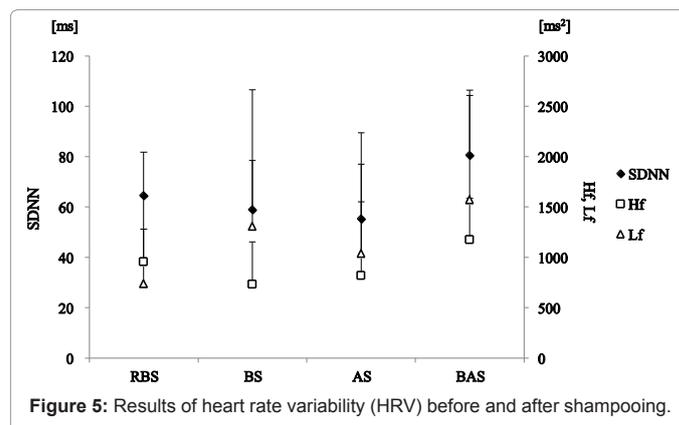
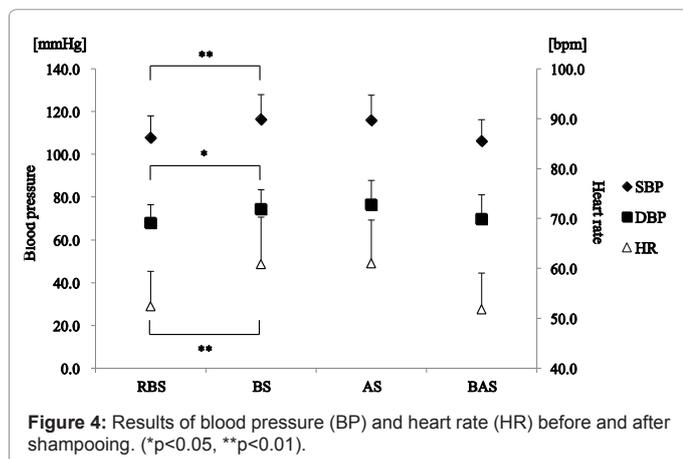


Figure 5: Results of heart rate variability (HRV) before and after shampooing.

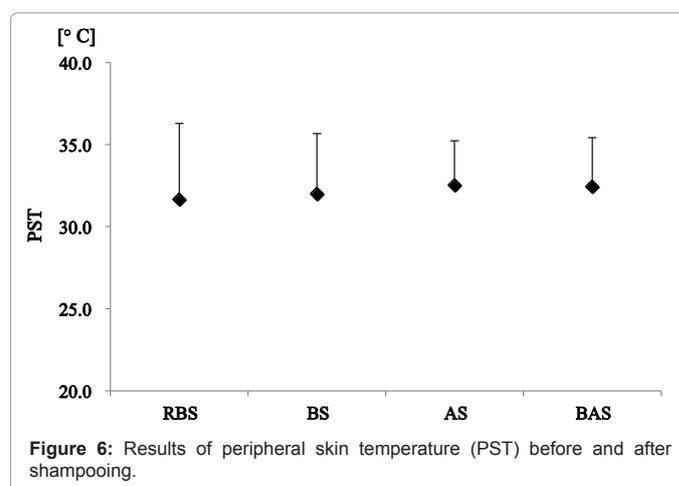


Figure 6: Results of peripheral skin temperature (PST) before and after shampooing.

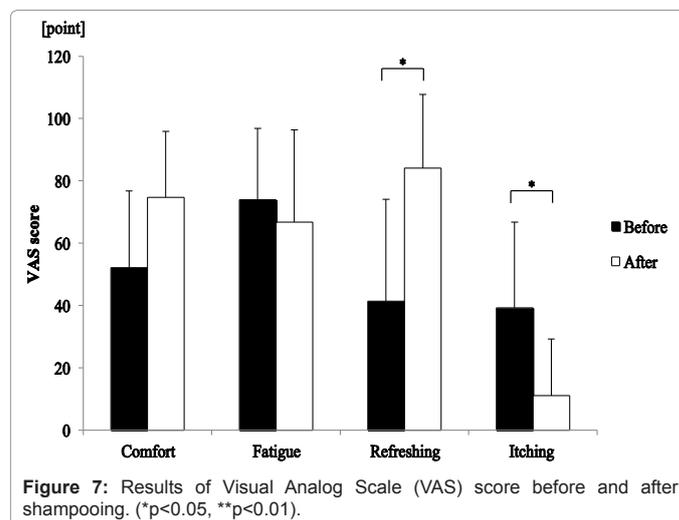


Figure 7: Results of Visual Analog Scale (VAS) score before and after shampooing. (\* $p < 0.05$ , \*\* $p < 0.01$ ).

The average of HR was  $52.3 \pm 7.11$  bpm (RBS),  $60.8 \pm 9.51$  bpm (BS),  $61.0 \pm 8.66$  bpm (AS),  $51.8 \pm 7.26$  bpm (RAS). BS significantly increased than that of RBS ( $p < 0.01$ ). There was no difference between RBS and RAS, and BS and AS.

**Heart rate variability:** HRV before and after shampooing is shown in figure 5. The average of SDNN was  $64.6 \pm 17.2$  ms (RBS),  $59.0 \pm 19.4$  ms (BS),  $55.3 \pm 21.7$  ms (AS),  $80.6 \pm 25.8$  ms (RAS). The average of HF was  $(9.55 \pm 6.49) \times 10^2$  ms<sup>2</sup> (RBS),  $(7.33 \pm 4.21) \times 10^2$  ms<sup>2</sup> (BS),  $(8.20 \pm 7.32) \times 10^2$  ms<sup>2</sup> (AS),  $(11.7 \pm 4.12) \times 10^2$  ms<sup>2</sup> (RAS), and that of LF was

$(7.39 \pm 5.42) \times 10^2$  ms<sup>2</sup> (RBS),  $(13.1 \pm 13.6) \times 10^2$  ms<sup>2</sup> (BS),  $(10.4 \pm 12.0) \times 10^2$  ms<sup>2</sup> (AS),  $(15.7 \pm 10.4) \times 10^2$  ms<sup>2</sup> (RAS). The average of LF/HF was  $1.07 \pm 0.91$  (RBS),  $1.51 \pm 0.85$  (BS),  $1.32 \pm 1.04$  (AS),  $1.52 \pm 0.96$  (RAS). There was no difference in all points.

**Peripheral skin temperature:** PST before and after shampooing is shown in figure 6. The average of PST was  $31.7 \pm 4.64^\circ\text{C}$  (RBS),  $32.0 \pm 3.68^\circ\text{C}$  (BS),  $32.5 \pm 2.73^\circ\text{C}$  (AS),  $32.4 \pm 2.98^\circ\text{C}$  (RAS). There was no difference in all points.

### Index of psychological response

VAS before and after shampooing is shown in figure 7. The average of "Comfort" score was  $52.1 \pm 24.6$  points (BS),  $74.7 \pm 21.2$  points (AS), "Fatigue" score was  $74.0 \pm 22.9$  points (BS),  $66.7 \pm 29.7$  points (AS), "Refreshing" score was  $41.3 \pm 32.7$  points (BS),  $84.1 \pm 23.6$  points (AS), and "Itching" score was  $39.2 \pm 27.5$  points (BS),  $11.0 \pm 18.3$  points (AS). "Refreshing" significantly increased ( $p < 0.05$ ), and "Itching" significantly decreased ( $p < 0.05$ ). There was a strong correlation between "Comfort (BS)" and "Refreshing (BS)" ( $r = 0.83$ ), "Refreshing (BS)" and "Itching (BS)" ( $r = -0.72$ ), "Fatigue (AS)" and "Refreshing (AS)" ( $r = 0.74$ ), "Fatigue (AS)" and "Itching (AS)" ( $r = -0.73$ ), "Refreshing (AS)" and "Itching (AS)" ( $r = -0.91$ ) ( $p < 0.05$ ). The normalized VAS score was 2.08 (Comfort), 1.05 (Fatigue), 5.85 (Refreshing), 0.50 (Itching). There was no correlation between the obtained ATP-BS and VAS-BS, ATP-AS and VAS-AS. There was strong correlation between the cleanliness level and normalized VAS "Refreshing" ( $r = -0.76$ ) ( $p < 0.05$ ).

### Discussion

This study aimed to evaluate the cleaning effect of shampooing care by using ATP bioluminescence system and to assess the physiological and psychological responses comprehensively.

When we analyzed ATP adhering to head as "contamination" to evaluate that effect quantitatively, it found that the contamination of the scalp after shampooing decreased by 31%, and that of the hair decreased by 22%. In order to compare the result to previous works, the cleanliness level was calculated from papers. Triglyceride on the scalp decreased by about 45%, free fatty acid on the scalp decreased by about 85% [20,21,23] and bacteria on hair decreased by about 45% [26]. Although our cleanliness level of the hair was lower than that, it was considered to be the cause that there were many opportunities for ATP to shift from other things to the hair, such as a towel and hand at the time of hairdressing and a sheet at the time of supine position after shampooing. In addition, since the ATP system measures the all things together adhering to head, such as sebum, bacteria, scaling of the scalp and dust, the cleanliness level together may be lower than the result examined alone. It is said that it is appropriate to evaluate the cleaning effect using the ATP system because shampooing care aims at removing contamination of head comprehensively. We were able to clarify the cleanliness level using rapid, simple and invasive method.

The results by subjects showed that there were no statistically significant differences between ATP-BS and ATP-AS for more than half of subject, however their ATP-AS numerical decreased compared to their ATP-BS. One of the major causes is that the variation in four areas was large to use the average of ATP of four areas as each subject's data. We need to compare ATP between areas, and normalize it with a specific area, as required. 23.5% of all ATP-AS increased in this study and previous studies showed similar results. Nakano et al. [42] showed ATP level of three of nine subjects increased after shampooing and suggested that the contamination which had gotten into pore or

wrinkles came from within to surface of skin. Kudo et al. [26] showed bacteria on hair of 37.5% of all subjects increased. As these results, it concluded that the contamination might increase after shampooing. However it remains presumptive, it is necessary to clarify the reason in the future.

The results of VAS showed "Refreshing" increased, and "Itching" decreased. The correlation between "Refreshing" and "Itching" before and after shampooing suggested that subjects used "Refreshing" as a synonym for "No or little itching". Compared the observed ATP as quantitative contamination to VAS as qualitative contamination, there was no correlation between them. It turned out that the degree of actual contamination differed from the degree of contamination which subjects feel. It has been reported that there was wide individual variation in subjective assessment, and it suggested that it is difficult to guess them from ATP level. There was a strong correlation between the cleanliness level and the normalized VAS of "Refreshing". It considered that the more ATP decrease, the more subjects feel refresh. So, it can be said that new shampooing method whose cleanliness level is high provides patients with comfort.

There was little or no effect of physiological responses. SBP, DBP and HR of BS significantly increased than that of RBS. It is thought that subjects changed body position from supine to sitting or the Fowler position. In fact, that of BS or AS in sitting or the Fowler position significantly decreased than that of RAS in supine position. Some studies showed the relaxing effect by shampooing [2,3,6,7]. It is reported that a relaxation will decrease BP, HR, muscle tone and sympathetic nerve activity, and it will increase parasympathetic nerve activity and body temperature and so on. However, such change was not seen in this study. Since VAS of "Comfort" and "Fatigue" before shampooing had a high score, it turns out that subjects' condition before shampooing was comparatively good and there was not much relaxation effect. The physiological index was stable. It found that the shampoo method in this study had little load on the body.

### Conclusion

This study evaluated the cleaning effect by using ATP method and the physiological and psychological responses comprehensively. When we analyzed ATP adhering to head as "contamination", the result showed that the contamination of the scalp after shampooing decreased by 31%, and that of the hair decreased by 22%. The degree of actual contamination differed from the degree of contamination which subjects feel, however it considered that the more ATP decrease, the more subjects feel refresh. It showed the cleaning effect of shampooing by rapid, simple and invasive method as objective and subjective index. There was little or no effect of physiological responses. We were able to produce basic data that evaluated the shampooing effect.

In the future, we hope to develop new shampooing method or supporting tool that consider the cleansing effect and comfort of patient.

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