

ORIGINAL RESEARCH

Effects of oral administration of equine placental extract supplement on the facial skin of healthy adult women: A randomized, double-blind, placebo-controlled study

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Abstract

Introduction: Placenta extract is used as an ingredient in ointments for treating dermatological diseases, skin dryness, and for skin beautification. However, the clinical effects of the equine placenta on humans and the underlying mechanism of action are unclear. This randomized, controlled, double-blind study aimed to clinically evaluate the effect of oral intake of equine placental extract on human skin quality.

Methods: Healthy women volunteers between the ages of 30 and 59 years ($n = 29$) were randomly assigned to receive 220 mg of equine placental extract–placebo orally, once daily for 4 weeks. Skin quality parameters such as skin hydration, skin barrier function (transepidermal water loss [TEWL]), and melanin index were assessed at baseline and after 4 weeks of administration.

Results: The melanin index was significantly increased in the placebo group, whereas it remained unchanged in the equine placenta group. The pattern of melanin index change was significantly different due to intake or no intake of equine placenta supplements over 4 weeks. No significant difference was found in skin hydration and TEWL between the two groups at 4 weeks of postadministration. It was shown that the intake of the equine placenta was more effective in protecting the skin condition against the change of ultraviolet (UV) sensitively than the change in temperature and humidity.

Conclusions: Effect of equine placental extract intake was evident on the cheek skin of the equine placenta group where participants were protected from UV-induced pigmentation. Equine placental extract is useful for decreasing melanin synthesis and melanin content in the human skin and can be used as an effective food supplement to maintain human skin quality.

KEYWORDS

clinical trial, integrative medicine, melanin, skin barrier function, skin hydration, transepidermal water loss

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1 | INTRODUCTION

The placenta is a temporary organ in women during gestation. Fetal liver and placenta form an integrated organ system in early development, interorgan exchange of glutamine and glutamate play important roles during fetal development, and fetuses use large amounts of glutamine derived from the circulation.^{1,2} The placenta is considered a reservoir of cytokines, hormones, bioactive peptides, enzymes, growth factors, vitamins, and minerals.³ The human placenta has been traditionally used and is now widely used for improvement of fatigue, skin whitening, and antiaging in Asian countries.⁴ However, its effects on human body are unclear and inconsistent, human placental lipid induces melanogenesis by increasing the expression of tyrosinase and related proteins^{5,6}; human placental proteins-peptides stimulate melanin synthesis by increasing the expression of tyrosinase gene.⁷ In contrast, porcine placental extracts have been reported to induce the expression of antioxidant enzyme genes and suppress melanogenesis in B16 melanoma cells.⁸ Several studies have reported the immunomodulatory and antioxidant activities of placenta extract, including equine extracts.⁹⁻¹¹ As equine is classified as perissodactyl, it does not have a risk of diseases such as bovine spongiform encephalopathy (BSE) and foot-and-mouth disease; therefore, equine placenta is safe and noninvasive and being expected to use for complementary and alternative medicine (CAM). Regarding previous studies of CAM, Alyasin et al reported that significant efficacy of oral supplementation of whey protein in patients with contact dermatitis to avoid side effects of medication,¹² Li et al evaluated one traditional Chinese medical extract, Panax ginseng phenolic acid extract to protect skin against ultraviolet-B (UVB)-induced photoaging in a mouse experiment,¹³ Law et al reviewed a traditional Chinese herbal, dandelion and its applications on skincare and Integrative Medicine. CAM for dermatological diseases and cosmetic purposes has received much interest for its medical use with no side effects, and placenta is one of the important traditional animal materials.¹⁴ Various hormones and growth factors secreted by equine placenta³ can be used medically as an antioxidant or to improve menopausal symptoms.^{4,8} However, the clinical effects of equine placental in humans have not been adequately studied.

Therefore, in this study, we clinically explore the effects of oral intake of equine placental extract on skin quality parameters such as skin hydration, skin barrier function or transepidermal water loss (TEWL), and skin melanin content in middle-aged (40-59 years) women.

2 | METHODS

2.1 | Study design

This randomized double-blind placebo-controlled clinical study was performed between March 25, 2019, and April 26, 2019, in the Laboratory of Systematic Forest and Forest Products, Science Faculty of Agriculture, Kyushu University, Japan. The study

included two groups with a 1:1 allocation ratio of placebo capsules or placenta capsules. Block randomization of participants was done to reduce bias.

2.2 | Intervention

Equine placenta extract capsules were obtained from Dr. PROLABO JAPAN Co., Ltd. (Toyo, Japan). Equine meat that met the strict safety criteria of EU was imported from Argentina and used to obtain equine placenta. It was sorted, irrigated, decomposed with an enzyme for 10 hours, pressured, and packaged aseptically; lastly, the extract (100%) was collected (Product code: Dr.pro UMA PLACENTA FD-100). Based on presampling in-house survey, 220 mg of placenta extract powder was found to be the best physical well-being dose. A placenta capsule (650 mg) was prepared using 220 mg of placenta extract powder and 430 of beeswax, and natural coloring agent. A placebo capsule (650 mg) was made of safflower oil and beeswax, and natural coloring agent. The safety of the product has been guaranteed by safety of raw materials and manufacturing methods, and sales performance over 4 years with no adverse events or health hazards. Two staff members (not the investigator) confirmed that both (placebo and placental extract) capsules were indistinguishable in appearance. After the preparation of samples, they were kept safely in the laboratory where temperature and humidity were controlled until the study started.

2.3 | Participants and setting

We adopted repeated measures analysis of variance (ANOVA) to analyze the data; priori analysis revealed that at least 24 participants in total are required at the power of 80%, the effect size of 0.4, and the alpha level of 0.05. Healthy female volunteers between the ages of 30 and 59 years were invited via laboratory network ($n = 30$), and their eligibility was checked based on the inclusion and exclusion criteria (Table 1) by a staff member (not the investigator). Selected women volunteers signed an informed consent that stated the purpose, method, compensation, confidentiality, and right to withdrawal from the study. In collaboration with two clinics, we consulted medical doctors in the case of adverse events.

2.4 | Randomization

Randomization was centralized and performed on the basis of a computer-generated list of random numbers by a staff member, without involving any of the investigators. Since the number of participants in each group was low, there was a possibility of a difference in the background of two groups. We adopted block randomization, which is assigned in consideration of the difference in the factors that affect the results between the groups. Since age and health condition are factors that affect the skin, the randomization was performed by creating blocks of age and body mass index (BMI) factors. It was performed using block

TABLE 1 Inclusion and exclusion criteria

Inclusion criteria
<ul style="list-style-type: none"> Persons who are generally judged as healthy Persons who give voluntary written consent to participate in the present trial
Exclusion criteria
<ul style="list-style-type: none"> Persons who take any dietary supplements, quasi-drugs, or medicines, which cause the same or similar effects as the supplements evaluated in this study Persons who have changed their habits in respect to supplements or cosmetics use within the past 4 weeks Persons who work in night shift or in day and night shift Persons who work outdoors for a long-time Persons who have been treated for their condition or prevention in a clinic with their informed consent Persons with the following medical histories: skin disease or atopic dermatitis, serious diseases of sugar metabolism, lipid metabolism, hepatic function, renal function, heart, circulatory, respiratory, endocrine, or immune system, or mental illness of the nervous system Persons with a medical history of alcoholism or drug addiction Persons who may develop an allergic reaction to food Persons who are pregnant, breast-feeding, or hope to be pregnant during the study period Persons who are participating in or will participate in any other clinical trial (on the use of foods–medicines–quasi medicines–medical devices) Persons who are not judged suitable for participation by the investigator

random sampling based on the mean age and BMI (age < 45 years and BMI < 22; age < 45 years and BMI ≥ 22; age ≥ 45 years and BMI < 22; age ≥ 45 years and BMI ≥ 22). None of the investigators were aware of the group assignments or involved in the allocation.

2.5 | Study schedule

One capsule of either placebo or equine placental extract was orally administered to all the participants as per their respective study group, once daily. To minimize errors in the study, all the participants were asked to refrain from taking any similar dietary supplements, quasi-drugs, or medicines. The participants were also prohibited from using any skincare treatments such as face masks, packs, massages, sunscreens, or from changing their daily skincare cosmetics from the start to the end of the study. Each participant visited the research laboratory for the assessment twice for efficacy measurements: before intake of the study formulation (baseline, 0 W) and 4 weeks (4 W) after the intake of study formulation. The participants were requested to apply daily skincare products on their face in the mornings of visit days and to remove the products before each visit. The skin region of interest was the left cheek (inner position, 5 cm from the lower end of the left earlobe). It was cleaned using a cleansing sheet (Bifesta Cleansing Sheet,

Mandom Corporation, Osaka, Japan), wiped using cotton containing the cleansing liquid (Bifesta Face Wash, Mandom Corporation), rinsed with warm water, wiped, and allowed to dry for 20 minutes at a stable temperature ($21 \pm 1^\circ\text{C}$) and humidity ($42\% \pm 10\%$).

2.6 | Measurement of skin quality parameters

Skin hydration (arbitrary units; a.u.), skin barrier-formation or TEWL ($\text{g}/\text{h}/\text{m}^2$), and melanin index were measured using Corneometer CM 825, TEWAMETER TM 300, and Mexameter MX18, respectively (both instruments from Courage and Khazaka, Cologne, Germany).¹⁵ Each measurement was completed in 1 to 3 minutes, and a series of five values were obtained. The three middle values were used to calculate the mean values.

2.7 | Outcome

The primary outcome was the change in skin melanin index within groups and among the groups toward the oral administration of the equine placental extract capsule. The secondary outcomes included changes in skin hydration and TEWL.

2.8 | Safety evaluation

All participants were asked to complete a questionnaire of their health conditions at each examination. In addition, participants were asked to keep a daily record of the food and medications consumed and general health conditions.

2.9 | Statistical analysis

SPSS (version 25.0, Chicago, Illinois) was used for data analysis. The independent sample *t*-test was used to determine the statistical significance of differences found between the two groups. Means and SDs were calculated. The differences before and after the treatment within groups and among the groups were analyzed by repeated measure ANOVA. The significant difference in the changed pattern between the two groups with longitudinal data was analyzed as a significant interaction between two factors (group × time). A *P* value of <.05 was considered statistically significant. The effect size Wilks' λ was also calculated as the representative of the effect size of interaction for group × time.

3 | RESULTS

3.1 | Baseline demographic information

Between March 2019 and April 2019, after 35 adult females were assessed for eligibility, one participant dropped out of the study

because of personal reasons, and the results of 29 healthy adult females were recorded (Figure 1). The background characteristics of participants in each group are presented in Table 2. No significant difference was observed in the age or BMI of participants between the two groups. Regarding the skin quality parameters, hydration (a.u.) and melanin index were not significantly different, whereas TEWL was significantly different between the two groups (Table 2).

3.2 | Effects of equine placental extract on skin hydration and TEWL and Melanin index

No significant difference was found in skin hydration and TEWL between the two groups at 4 weeks of postadministration. Conversely, although the skin melanin index within the test group did not change significantly ($P = .329$) that within the placebo group increased

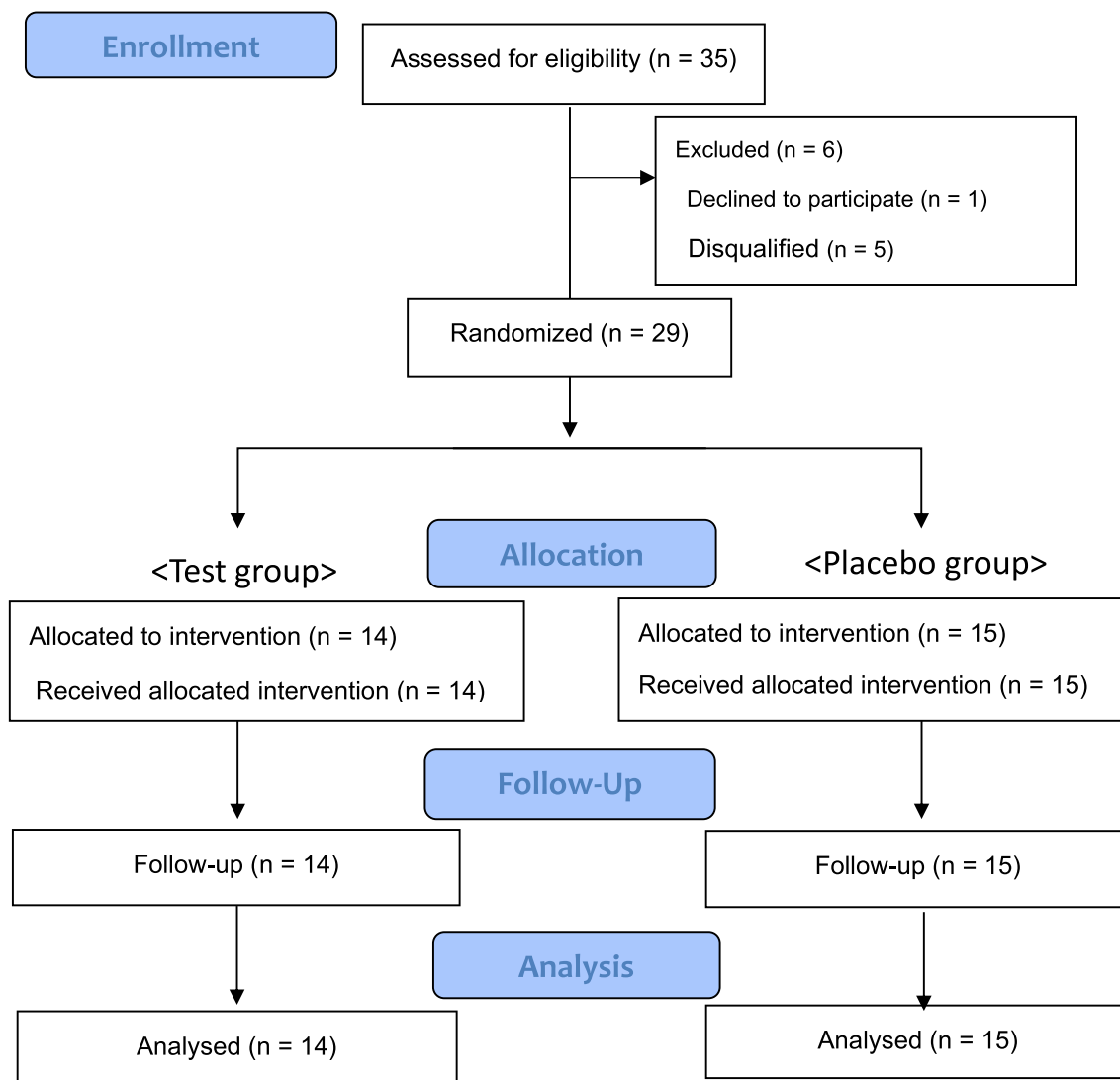


FIGURE 1 CONSORT flow diagram

TABLE 2 Background characteristics

Baseline characteristics	Test group (placenta) (N = 14)	Placebo group (N = 15)	P value
Age (years)	44.8 ± 5.7	45.1 ± 6.1	.902
BMI (kg/m ²)	22.1 ± 3.6	22.0 ± 2.5	.933
Hydration at the cheek (a.u.)	68.9 ± 10.5	67.4 ± 7.2	.640
TEWL (g/h/m ²)	10.7 ± 6.2	16.2 ± 5.4	.017
Melanin index at the cheek	130.6 ± 27.2	139.1 ± 26.8	.405

Note: Each value is expressed as Mean ± SD, P values were determined by independent test. Abbreviations: a.u., arbitrary unit.; BMI, Body mass index.

significantly ($P = .006$) during 4 weeks of administration. Moreover, the interaction between the groups and time was significant. ($P = .009$, effect size Wilks' $\lambda = 0.233$). The pattern of melanin index change was significantly different due to intake or no intake of equine placenta supplements over 4 weeks. (Table 3, Figure 2).

3.3 | Safety assessment

No side effects or adverse events were observed under the conditions in this study throughout the study period.

4 | DISCUSSION

In the present study, we explored the effects of oral intake of equine placental extract on human skin parameters, including skin hydration, skin barrier function or TEWL, and skin melanin index. Skin hydration and skin barrier function or TEWL in the test and placebo groups did not change significantly from the baseline. However, the melanin index of the placebo group increased significantly, whereas that of the test group did not change. This could have happened because of the following reasons.

Skin hydration and TEWL showed moisturizing and barrier-formation ability of the skin. This study was performed at the beginning of spring, and participants did not change skincare cosmetics or use sunscreen and avoid sun exposure for a long-time for the 4 weeks within the study. The mean temperature and humidity increased from 5 days of the preintervention measurement [March end: $14.1 \pm 2.8^\circ\text{C}$ (mean \pm SD); $61.8 \pm 4.9\%$, respectively] to 5 days of the post-intervention measurement (April end: $18.4 \pm 2.0^\circ\text{C}$; $80.8 \pm 5.1\%$, respectively) significantly ($P = .022$ for temperature, $P < .001$ for humidity). Although both temperature and humidity increased significantly during the 4 weeks. Since such environmental changes positively affected moisture and barrier-formation of human skin,¹⁶ the hydration of the stratum corneum and the consequent skin barrier function were thought to have been maintained. Conversely, according to the Japan Meteorological Agency UV index (analysis

level), the mean UV index of 3.8 in March 2019 increased to 4.9 on April 17, 2019.¹⁷ Melanin pigments are produced in the specialized group of cells known as melanocytes and essentially play a role in protecting skin melanocytes and keratinocytes from DNA damage caused by hydrogen peroxide.¹⁸ Since UV irradiation results in an increase in melanin content in the human skin,^{18,19} increased UV exposure might have induced a higher risk of pigment formation. Since the equine placental extract might have some agent that inhibited melanin synthesis, melanin index of the cheek skin of the placebo group increased significantly, whereas that of the test group did not. Cumulative UV-radiation exposure contributes to UV-induced DNA damage, oxidative stress, and skin inflammation.²⁰ Moreover, overproduction of melanin pigments in the localized areas of skin has no beautification, in the worst case, has skin problems such as melasma.²¹ Wakame et al reported enzymatically treated horse placenta and obtained placental peptides with molecular weights of <3000 Da, and the presence of the epidermal growth factor (EGF) and fibroblast growth factor (FGF) in the placenta.²² The placental peptide was demonstrated to have strong antioxidant activity and is expected to confer UV protective and anti-inflammatory effects for skin. The intake of placental extract is expected to protect the skin from oxidative stress^{8,22,23} and regulate melanin synthesis.²⁴

Several of the reports published regarding the effect of placental extracts in humans and mammals are inconsistent. A porcine placental extract has been reported to inhibit melanogenesis,⁸ whereas human placental protein-peptides and lipids have been shown to induce melanogenesis.⁷ Yoshimoto et al examined porcine placental effect regarding melanin synthesis on normal human melanocytes in vitro.²¹ A whole porcine placental extract decreased melanin synthesis; in contrast, an extract containing exudates and insoluble materials increased melanin synthesis. Such ambiguity in results indicates that not only different placental sources but also biologically different agents affect melanin synthesis differently. Although their mechanism of action in the body has not been clarified in detail, mitochondrial function may be associated with the regulation of melanogenesis.^{24,25} When melanin synthesis was inhibited by treatment with a whole porcine placental extract, an upregulation of MnSOD, reduction in mitochondrial respiration, and induction of glycolysis was simultaneously observed. Thus, placental

TABLE 3 Comparison of skin item values pre and postintervention

Item (unit)	Group	n	Time		P value (pre vs post) ^a	P value (Interaction) ^b	Partial η^2 (Interaction) ^c
			Preintervention	Postintervention			
Hydration (a.u.)	Placebo	15	68.94 ± 2.70	73.92 ± 2.29	.130	.933	.000
	Test	14	67.35 ± 1.92	72.50 ± 3.10	.104		
TEWL (g/h/m ²)	Placebo	14	12.20 ± 0.64	12.46 ± 0.75	.784	.423	.025
	Test	14	16.25 ± 1.45	17.62 ± 1.30	.165		
Melanin index	Placebo	15	130.64 ± 7.03	141.60 ± 9.37	.006	.009	.233
	Test	14	139.14 ± 7.15	135.05 ± 7.29	.329		

Note: Each value is expressed as Mean \pm SE. P value for a repeated Measures analysis of variance (ANOVA).

^aP value for within group.

^bP value, partial η^2 .

^cFor group \times time.

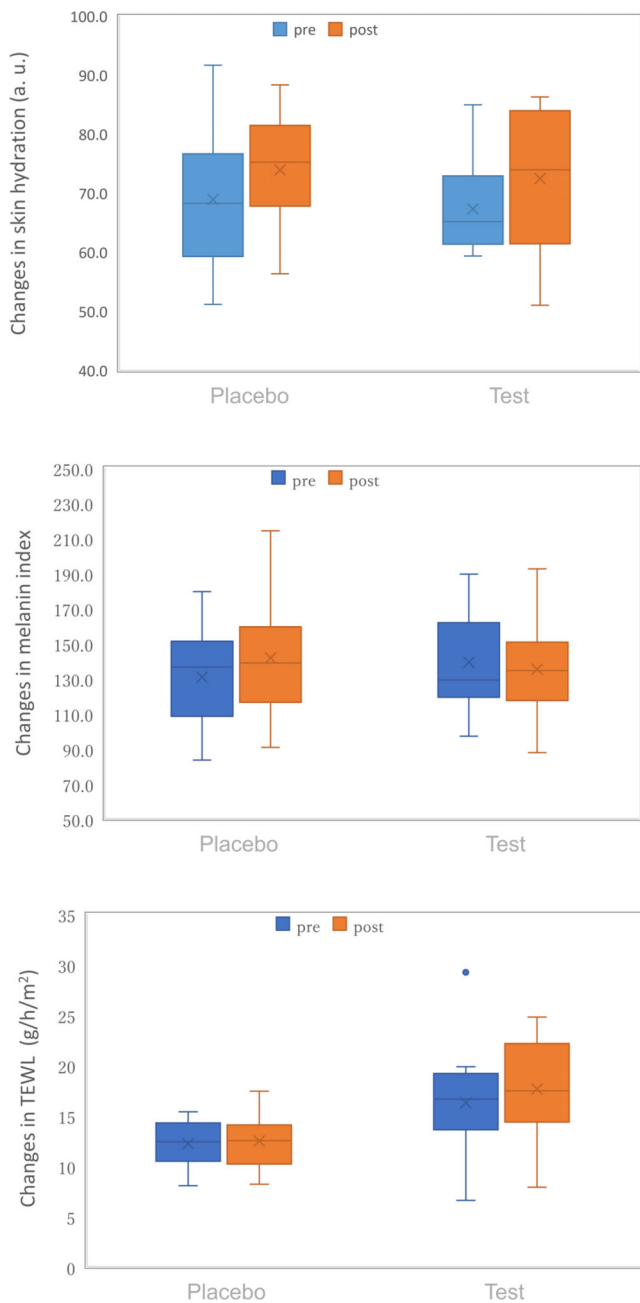


FIGURE 2 Changes in cheek skin parameters: skin hydration, melanin index, TEWL: transepidermal water loss, a. u.: arbitrary unit

extracts might regulate melanogenesis via a possible association with mitochondrial and/or nonmitochondrial respiration.

5 | CONCLUSION

The present study showed that the oral intake of equine placental extract is useful for decreasing melanin synthesis and melanin content in the human skin. The test supplement in this study was an extract from whole equine placenta, in this regard, the result of present study was accorded with those of previous studies in

which whole placental extract regulate melanogenesis.²¹ Equine placenta is relatively safe, noninvasive, and includes several useful ingredients that can be used as cosmetic agents. As the antigenicity and allergenicity of equine proteins are low, the risk of allergy by eating equine flesh is minimum. To the best of our knowledge, this study is the first to clinically evaluate the effect of oral intake of equine placental extract on skin quality parameters. However, this study has some limitations. This study investigated a limited age group of women, and the number of participants was low, and the intake period was relatively short, and the period of measurement was not enough long. We should evaluate the effect of remote changes on skin. Furthermore, measurement of skin quality was performed with instruments from Courage and Khazaka quantitatively, but image evaluation of pictures was not conducted. These results should be verified before applying them to clinical practice. Further studies including both male and female participants of different ages with different doses and intake periods and additional evaluation by pictures are needed. Moreover, the bioactive compounds present in equine placenta should be isolated to elucidate the underlying mechanisms of its medicinal potential.

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CONFLICT OF INTEREST

Y.N. is the CEO of Dr. PROLABO JAPAN Co., Ltd, Inc. Except for supplement contribution; the funding sources had no role in the design, conduct, or analysis of the study or the decision to submit the manuscript for publication. All the other authors declare no competing interest.

AUTHOR CONTRIBUTIONS

Data Curation: Masumi Nagae.

Formal Analysis: Masumi Nagae.

Funding Acquisition: Tomoe Nishio.

Investigation: Masumi Nagae.

Methodology: Koichiro Ohnuki.

Project Administration: Koichiro Ohnuki, Kuniyoshi Shimizu.

Resources: Kuniyoshi Shimizu.

Software: Masumi Nagae.

Supervision: Koichiro Ohnuki, Kuniyoshi Shimizu.

Writing Original Draft Preparation: Masumi Nagae.

Writing, Review & Editing: Masumi Nagae.

All authors have read and approved the final version of the manuscript.

Masumi Nagae had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

TRANSPARENCY STATEMENT

Masumi Nagae affirms that this manuscript is an honest, accurate, and transparent account of the study being reported that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

INFORMED CONSENT STATEMENT

Participants signed an informed consent form stating the purpose, method, compensation, confidentiality, and right of withdrawal from this study. Participants signed an informed consent form stating the purpose, method, compensation, confidentiality, and right of withdrawal from this study, and involved in the study. Informed consent was obtained from all participants.

DATA AVAILABILITY STATEMENT

The authors confirm that the data supporting the findings of this study are available within the article.

ETHICS STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Kindai University Faculty of Humanity-Oriented Science and Engineering Ethics Committee (March 4, 2017) and was registered in the University Hospital Medical Information Network Clinical Trials registry (UMIN-CTR) with ID: 000036273.

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