

Research Notes

Evaluation of Epidermal barrier function in the Skin of Japanese Obese Women: a Pilot Study

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肥満者の皮膚は表皮バリア機能が低下し乾燥した状態にあり、臨床で痒みを訴える患者が多いが、それらの関連因子に関する検討は極めて少ない。そこで本研究は、肥満者の皮膚の組織学的所見、表皮保湿因子およびヒスタミン関連因子の検討を目的とした。乳房再建術を施行する日本人女性患者を対象とし、手術時に腹部の余剰皮膚を採取した。対象者はBMIにより非肥満群、肥満群に分類した。組織学的解析の結果、非肥満群と比較し肥満群では真皮表層で炎症性細胞の浸潤が認められた。また、非肥満群と比較し肥満群では表皮バリア機能の指標である保湿因子フィラグリンのmRNA発現量の減少の可能性、痒みのメディエーターであるヒスタミン、およびヒスタミンH1レセプターの発現量の増加の可能性が示唆された。

Abstract

The purpose of this study was to test the hypothesis that obese patients have dry skin caused by a decrease in moisturizing factors in the stratum corneum and increased histamine and histamine H1 receptor (H1R) in the skin. Japanese women who had undergone breast reconstruction surgery were recruited and sorted into control and obese groups. Histological observation revealed that inflammatory cells infiltrated into the dermis in the obese group but not in the controls. The possibility that histamine concentration had a tendency to be increased in the obese group has been suggested. Furthermore, the possibility the expression of mRNAs for Filaggrin and H1R had a tendency to be increased in the obese group compared with the controls has been suggested. These results could be suggested that obese patients have dry skin caused by increased histamine and histamine H1R in the skin and decreased Filaggrin, the most common moisturizing factor protein.

Introduction

Obesity is one of the most common health problems worldwide. The Japan Society for the Study of Obesity defines "obesity" as a body mass index (BMI) ≥ 25 ¹⁾ In Japan, the prevalence of obesity has been increasing, and is currently 30.4% in men aged ≥ 20 years and 21.1% in women aged ≥ 20 years, respectively²⁾.

Obesity is not only associated with visceral diseases but also with skin pathophysiology, particularly epidermal barrier functions involved in maintaining stratum corneum (SC) hydration^{3,4)}. In normal skin, tightly connected keratinocytes in the stratum granulosum of the SC form an efficient barrier that inhibits extensive water loss while preventing the penetration of harmful irritants and allergens through the skin. Dry skin is a skin-barrier defect and is mainly attributed to a reduction in

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sebum secretion and the consequent loss of water from the SC, thus, skin is more likely to be exposed to irritants⁵⁾.

A previous study reported that obese individuals had significantly increased transepidermal water loss (TEWL) 4), which is a major factor of impaired skin barrier function and dry skin^{6,7)}. Furthermore, we found that obese outpatients had dry skin and suffered from pruritus (unpublished data). However, among the many related factors in the skin, it was not clear what factors actually associated with impaired skin barrier function in obese skin.

A previous study reported that dry skin can enhance susceptibility to pruritus⁸⁾. The mechanism of dry skin-associated pruritus was previously described as follows.

Penetration of harmful irritants lead to the infiltration of inflammatory cells into the dermis, which in conjunction with mast cells release histamine, a chemical mediator that induces pruritus⁹⁾. Histamine released from inflammatory cells and mast cells' signals through the histamine H1 receptor (H1R) expressed on nerve C-fibers, transmit itch sensations to the central nerves¹⁰⁾.

Thus, this study investigated the hypothesis that obese patients have dry skin, caused by decreased moisturizing factors in the SC and increased histamine and histamine H1R in the skin.

Methods

1. Design

This was a cross-sectional study. The survey was conducted from April to September 2014.

2. Subjects

The study population included Japanese females who had breast reconstruction surgery with rectus abdominis musculocutaneous flap at Yokohama City University Medical Center. Because aging disrupts skin barrier function and induces senile xerosis accompanied by pruritus^{11,12)}, so this study was included subjects who ranged in age from 20 to 64 years. Subjects with systemic skin disorders or those who had received chemotherapy before surgery were excluded. Subjects with a BMI of <25 kg/m² were sorted into the control group and those with a BMI of ≥ 25 kg/m² formed the obese group.

3. Skin sample collection

Abdominal skin samples were collected at the time of breast reconstruction surgery, which cut around the skin periumbilical area, and the center area used for breast reconstruction, the residual skin is usually discarded. In this study, we used the residual skin as a sample.

. Skin samples were divided into three pieces: i) fixed in formalin for histological analysis; ii) frozen at -80°C for enzyme-linked immunosorbent assay (ELISA); iii) soaked in RNA later for Total RNA extraction.

4. Ethical considerations

This study was approved by the Ethics Committee of the Yokohama City University School of Medicine (A140522018). In order to avoid compulsory participation, informed consent process was performed by the doctor of a third party other than the attending physician and no disadvantage was incurred by refusing to participate. Data obtained from subjects was anonymized and to not identified individuals. The data was strictly kept in a place where can be locked.

Measurements

1. Histological Analysis

A 5×10 mm full-thickness abdominal skin specimen was fixed in formalin (Wako Pure Chemical Industries, Tokyo, Japan), embedded in paraffin blocks, and sectioned (5- μm thick). Each section was stained with hematoxylin and eosin (H&E) to evaluate basic skin morphology and the presence of inflammatory cells.

2. ELISA

A total of 50 mg of abdominal skin sample was homogenized in 0.01 M HCl and histamine concentration measured by Histamine Research ELISA (LDN, Nordhorn, Germany). The experiment was performed according to the manufacturer's instructions.

3. Gene expression analysis by real-time reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was used to determine the mRNA expression of Filaggrin and Loricrin, which are the most common moisturizing factor proteins that facilitate terminal differentiation of the epidermis and formation of the skin barrier¹¹⁾ and histamine H1 receptor (H1R), which is related to the itch sensation of the skin¹²⁾. Total RNA was extracted from the skin using an RNeasy Tissue Mini Kit (Qiagen, Hilden, Germany). Reverse Transcription was performed using a MJ Mini thermal cycler (Bio-Rad, Richmond, CA, USA) and Quanti Tect Reverse Transcription Kit (Qiagen). For quantitative PCR, amplification of the target specific region of cDNA was performed using THUNDERBIRD™ SYBR® qPCR Mix (Toyobo, Osaka, Japan) with 40 cycles of 95°C for 60 s, 95°C for 15 s, and 60°C for 45 s, monitored using a real-time PCR system (CFX96/384, Bio Rad). Gene expression levels were quantified by the comparative Ct

method and normalized to the expression of an internal control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The primer sequences are shown in Table 1.

Statistical analysis

Descriptive data were expressed as the mean ± SD for continuous variables and n (%) for categorical variables. The subjects were classified into two groups according to BMI <25 and ≥ 25 representing the control and obese groups, respectively. These two groups were compared using independent t-test, Mann-Whitney U-test, and Fisher's exact test. IBM SPSS Statistics 22.0 software (IBM, NY, USA) was used for all statistical analyses. Values of p<0.05 (two-sided test) were considered statistically significant.

Table 1. Primer sequences for real-time RT-PCR

| Gene Name | Orientation | Sequences (5'-3') |
|------------------|-------------|------------------------|
| <i>Gapdh</i> | Forward | TGCACCACCAACTGCTTAGC |
| | Reverse | GGCATGGACTGTGGTCATGAG |
| <i>Filaggrin</i> | Forward | AAGGTTACATTATTGCCAAA |
| | Reverse | GGATTGCCGAAATTCCTTT |
| <i>Loricrin</i> | Forward | GGAGTTGGAGGTGTTTTCCA |
| | Reverse | ACTGGGGTTGGGAGGTAGTT |
| <i>H1R</i> | Forward | GGTCTAAGACAGAGTTGGTGCT |
| | Reverse | ATCCTCTTCTGCAACCTGGTCA |

Results

1. Demographics and characteristics of the study population

A total of 16 females were recruited (Table 2). The mean BMI of the 11 control subjects was 22.1 ± 1.8 kg/m² and that of the five obese subjects was 27.6 ± 1.9 kg/m². There were no significant differences between the other basic characteristics including age and current therapy.

Table 2. Demographics of subjects

| | Control (n = 11) | obese (n = 5) |
|--|------------------|---------------|
| Age ^{a)} (years) | 55.3 (8.3) | 49.5 (4.2) |
| BMI ^{b)} (kg/m ²) | 22.1 (1.8) | 27.6 (1.9) |
| Hormonal therapy ^{b)} | 3 (27.2) | 2 (40.0) |
| Radiation therapy ^{b)} | 0 (0.0) | 1 (20.0) |

a) Mean (SD), b) Number of subjects (%), c) Independent t-test, d) Fisher's exact test

2. Histological analysis

Histological examination revealed that inflammatory cells had infiltrated into the reticular layer of the dermis in the obese

group (arrowheads), but not in the control group. We also noted increased sweat glands in the obese group (arrow), compared with the control group (Figure 1).

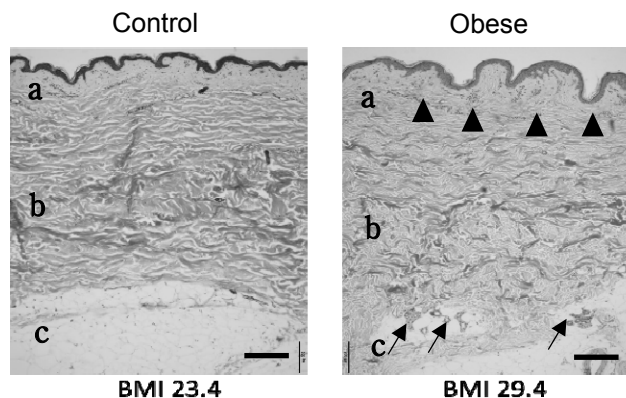


Figure 1. Histological analysis of the skin. a: epidermis, b: dermis, c: subcutaneous adipose tissue. Arrows: sweat glands, arrowheads: Immune cell infiltration. Bar = 200 µm.

3. Filaggrin and Loricrin mRNA expression

The possibility that the mRNA expression of Filaggrin had a tendency to be increased in the obese group compared with the control group has been suggested (Figure 2). However, Loricrin mRNA expression was similar between the two groups (Figure 3).

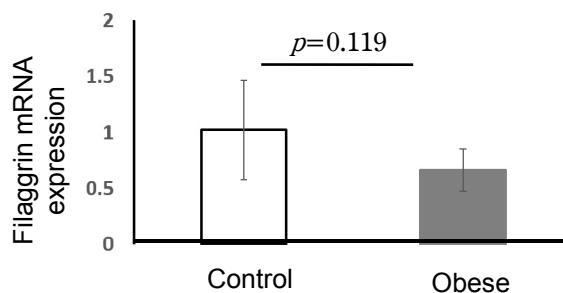


Figure 2. mRNA expression levels of Filaggrin in the control and obese groups. Means ± SD.

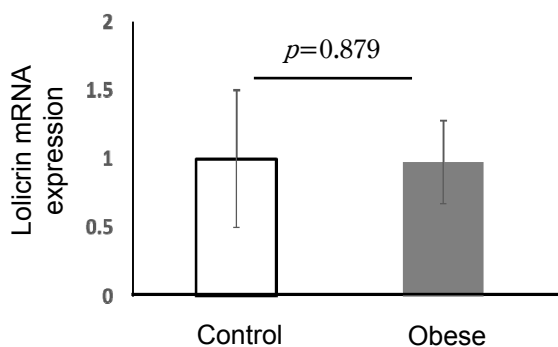


Figure 3. mRNA expression levels of Loricrin in the control and obese groups. Means ± SD.

4. Histamine concentration

Histamine is a chemical mediator released from mast cells that induces pruritus. The possibility that histamine concentrations had a tendency to be increased in the obese group compared with the control group has been suggested (Figure 4).

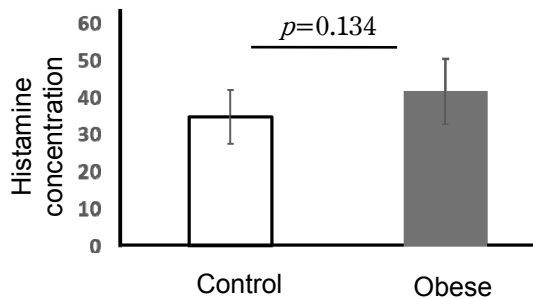


Figure 4. Histamine concentration in the control and obese groups. Means ± SD.

5. Histamine H1 receptor mRNA expression

The possibility that the mRNA expression of H1R had a tendency to be increased in the obese group compared with the control group has been suggested (Figure 5).

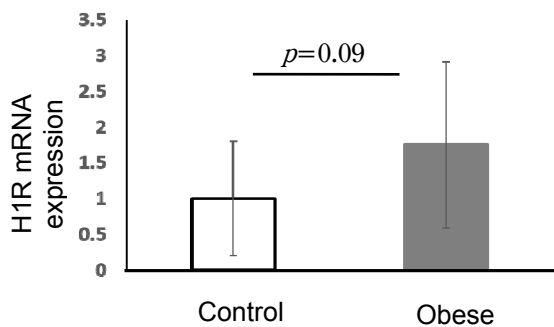


Figure 5. mRNA expression levels of H1R in the control and obese groups. Means ± SD.

Discussion

This study is the first attempt to measure factors related to epidermal barrier function in human skin from obese patients in a clinical setting.

Histological observations in this study demonstrated that inflammatory cells infiltrated the reticular layer of the dermis in the obese group compared with the controls. Akase et al.¹³⁾ demonstrated that gene expression of tumor necrosis factor (TNF) α , a marker of inflammation status^{14, 15)}, in the skin was

higher in obese mice compared with mice in the normal weight range. This suggests obese patients may have continual latent inflammation in the skin. Furthermore, we noted increased numbers of sweat glands in the obese group. A previous study reported that obese patients sweat more profusely than individuals of normal weight because of the thick layers of subcutaneous adipose tissue¹⁶⁾. However, the mechanisms involved in increased numbers of sweat glands is unclear in obese skin. Further studies are required to clarify this point.

The possibility that the mRNA expression of filaggrin, a moisturizing factor of SC, had a tendency to be decreased in the skin of the obese group has been suggested. Some studies reported that keratohyalin granules, which store the filaggrin, was diminished and decreased the amount of filaggrin in the skin of elderly^{17, 18)}. Since this study was excluded the elderly over the age of 65 years, the effect of age on the present results is unlikely to have a significant impact.

A previous study reported that Filaggrin gene expression was significantly decreased in lesional psoriasis skin and atopic dermatitis, which are common chronic inflammatory skin diseases characterized by impaired skin barrier function^{19, 20)}. Filaggrin is a major protein that aggregates keratin filaments to form the cytoskeleton and lipid envelope²¹⁾. Thus, this study could be suggested that obese skin is exposed to irritants and allergens and that impaired skin barrier function might lead to inflammation and pruritus. Because this study was analyzed only collected skin tissue, whether obese patients in this study had actually feel pruritus is unknown. Therefore, we should be measured the pruritus before surgery in the future. Furthermore, this study only measured mRNA expression levels, therefore protein assays such as immunostaining or western blotting should be performed in the future. The possibility that H1R mRNA expression levels and concentrations of histamine, a mediator of pruritus, had a tendency to be increased in the skin of obese patients has been suggested. Thus, histamine, released by mast cells, might affect H1R expression on nerve C-fibers that transmits pruritus to central nerves.

Although this was a pilot study with a small number of subjects, the results may provide important evidence regarding the elucidation of obese skin pathophysiology.

Conclusions

This pilot study made the following important observations:

1. Inflammatory cells infiltrated into the reticular layer of the dermis in the obese group.
2. The possibility that mRNA expression of filaggrin, an SC moisturizing factor tended to be decreased in the skin of the

obese group has been suggested.

3. The possibility that the concentration of histamine, a mediator of pruritus, tended to be increased in the skin of the obese group. Furthermore, mRNA expression levels of H1R tended to be increased in obese skin has been suggested.

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