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RESEARCH ARTICLE

# GATA-binding protein 3 and gross cystic disease fluid protein 15 as a potential diagnostic marker for extramammary Paget's disease

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## Abstract

**Objectives:** The aim of this study was to evaluate the expression of GCDFP15 and GATA-binding protein 3 (GATA-3) in extramammary Paget's disease (EMPD) skin and serum samples and to assess their availability as tumor markers for the diagnosis and assessment of disease severity in primary EMPD.

**Methods:** Skin samples and serum samples were obtained from 16 patients with primary EMPD (10 cases from male, six cases from female; stage IA six cases, stage IB seven cases, stage III one case, stage IV two cases). By immunohistochemistry, the expression of GCDFP15 and GATA3 was examined in skin specimens. The serum levels of GCDFP15 and GATA3 were quantified by ELISA.

**Results:** In our study, eight out of 16 patients showed positive staining for GCDFP15. In contrast, all 16 patients showed positive staining for GATA-3. Immunohistochemical staining of EMPD skin samples showed that GATA-3 had a higher positivity rate than GCDFP15. However, there was no correlation between serum levels of GCDFP15 or GATA-3 and the disease stage.

**Conclusion:** Our results indicate that GCDFP15 and GATA-3 are useful for the diagnosis of primary EMPD, but not for monitoring disease progression, and suggest that GATA-3 is a more reliable marker than GCDFP15 for the diagnosis of primary EMPD.

**KEYWORDS**
dermatology, diagnosis, immunohistochemistry, Paget disease, extramammary, sensitivity and specificity

## 1 | INTRODUCTION

Extramammary Paget's disease (EMPD) is a malignant neoplasm that arises in areas rich in apocrine glands.[1] Mammary Paget's disease was first described by James Paget in 1874.[2] Radcliffe Crocker reported EMPD involving the scrotum and penis in 1889,[3] and William Dubreuilh described vulvar EMPD in 1901.[4] As of now, it has been known that EMPD occurs more frequently in Asians (10 cases per million) than in Westerners (0.9 cases per million), and the most frequently affected site is the vulva, followed by perianal, scrotal, and penile skin.[5] In the clinical diagnosis of EMPD, it is often misdiagnosed as many other benign inflammatory skin diseases such as contact dermatitis, seborrheic dermatitis, eczema, and superficial fungal infections due to

the clinical appearance. The definitive diagnosis is made by not only histopathological findings but also immunohistochemical findings. Immunohistochemistry shows that gross cystic disease fluid protein 15 (GCDFP15) has relatively high specificity for EMPD, but its sensitivity is 60%–85% at the highest.[6] In addition, there is no serum tumor marker specific for EMPD. Serum carcinoembryonic antigen (CEA) and cytokeratin 19 fragment (CYFRA) levels are useful for predicting the disease severity and metastasis of EMPD, but elevated levels of these tumor markers may also be observed in patients with digestive and gynecologic cancers.[7] GATA-binding protein 3 (GATA-3), which has been identified as a highly sensitive nuclear marker for breast carcinoma and is more sensitive than GCDFP15 in this context, is highly expressed in apocrine glands and adnexal tumors.[8] Given the origin of EMPD and the similarity between EMPD and breast carcinoma, we hypothesized that GATA-3 could be a sensitive marker for EMPD. In this study, we analyzed the expression of GCDFP15 and GATA-3 in skin and serum samples and assessed their availability as tumor markers for the diagnosis and assessment of disease severity in primary EMPD.

## 2 | MATERIALS AND METHODS

### 2.1 | Patients

Skin and serum samples were obtained from 16 patients with primary EMPD (10 male, six female) who underwent biopsy or radical surgery. All patients were classified into six stages (stage 0, stage I, stage II, stage IIIa, stage IIIb, and stage IV) based on the TNM staging system proposed by Ohara et al.[9] The study was approved by the Ethical Committee of Jichi Medical University. All experimental protocols were carried out in accordance with the Declaration of Helsinki, and written informed consent was obtained from all patients.

### 2.2 | Expression of GCDFP15 and GATA-3 in lesions

The expression of GCDFP15 and GATA-3 was examined by immunohistochemistry, using formalin-fixed paraffin-embedded skin tissue samples. The expression of cytokeratin 7 (CK7) and CK20 was also examined. The sections were immunohistochemically stained with anti-GCDFP15 mouse monoclonal antibody (Abcam), anti-GATA-3 rabbit polyclonal antibody (Abcam), anti-CK7 mouse monoclonal antibody (Dako), and anti-CK20 mouse monoclonal antibody (Dako), according to the manufacturer's protocol. Antigen expression was assessed as positive or negative.

### 2.3 | Measurement of serum GCDFP15 and GATA-3

Serum levels of GCDFP15 and GATA-3 were evaluated using the Human Prolactin-Inducible Protein ELISA Kit (Cusabio) and the Human GATA-3 ELISA kit (LifeSpan BioSciences) according to their respective assay procedures, respectively. Serum levels of CEA and CYFRA were also evaluated at our in-facility laboratory. The normal range of CEA and CYFRA was 0–4.5 and 0–3.5 ng/ml, respectively.

## 3 | RESULTS

### 3.1 | Expression of GCDFP15 and GATA-3 in EMPD skin samples

The clinical characteristics of all patients are summarized in Table 1. To evaluate the expression of GCDFP15 and GATA-3 in primary EMPD, we immunohistochemically analyzed skin samples obtained from 16 patients with primary EMPD. Typical immunohistochemical findings are shown in Figure 1. Positive GCDFP15 staining was observed in only eight out of the 16 patients (five males, three females; five cases at stage 0, one case at stage I, one case at stage IIIb, one case at stage IV; Table 1), with no correlation observed between the positivity rate of GCDFP15 and the disease stage. In contrast, positive GATA-3 staining was observed in all 16 patients (10 males, six females; eight cases at stage 0, five cases at stage I, one case at stage IIIb, two cases at stage IV). These results suggest that GATA-3 is more sensitive than GCDFP15 as a diagnostic marker for EMPD.

### 3.2 | Expression of GCDFP15 and GATA-3 in EMPD serum samples

To assess the availability of GCDFP15 and GATA-3 as tumor markers in primary EMPD, we analyzed their levels in patient sera using ELISA. All of the patients' results are summarized in Table 1. Although serum levels of CEA and CYFRA were elevated in advanced cases such as cases 15 and 16, there was no correlation between serum GCDFP15 or GATA-3 levels and the disease stage.

## 4 | DISCUSSION

Extramammary Paget's disease lesions present as well- or ill-demarcated red or brown plaques and are associated with subjective symptoms including itching, burning, and pain, and the lesions may become erosive or ulcerated with nodule formation at a later stage.[5] EMPD may clinically mimic many other benign inflammatory skin diseases such as contact dermatitis, seborrheic dermatitis, eczema, and superficial fungal infections, leading to delayed diagnosis. A retrospective review of 246 Chinese male EMPD patients found a significant delay in diagnosis for almost all patients, with a mean delay in diagnosis of 43.2 months after onset of symptoms.[10] Similarly, another retrospective review of 145 cases of EMPD in Japan found the average time to diagnosis was 39.7 months.[11] Histopathological findings are necessary to confirm a definitive diagnosis of EMPD. The tumor cells of EMPD

**TABLE 1** Summary of the results of this study, which enrolled 16 patients with extramammary Paget's disease

| | | | | IHC | | | | Serum level | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Age (years) | Sex | Stage | CK7 | CK20 | GCDFP15 | GATA-3 | CEA (ng/ml) | CYFRA (ng/ml) | GCDFP15 (ng/ml) | GATA-3 (pg/ml) |
| 1 | 87 | F | 0 | + | − | + | + | 3.6 | 2.2 | 3.12 | 1047.9 |
| 2 | 69 | M | 0 | + | − | − | + | 4.6 | 2.4 | 3.37 | 1644.0 |
| 3 | 72 | M | 0 | + | − | − | + | 3.1 | 3.0 | 2.84 | 2124.9 |
| 4 | 74 | M | 0 | − | − | + | + | 1.6 | 2.5 | 2.95 | 733.9 |
| 5 | 66 | M | 0 | + | − | − | + | 2.7 | 2.4 | 1.47 | 932.6 |
| 6 | 90 | F | 0 | + | − | + | + | 2.2 | 2.5 | 1.86 | 1226.7 |
| 7 | 57 | M | 0 | + | − | + | + | 1.5 | 1.4 | 0.66 | 789.5 |
| 8 | 80 | F | 0 | + | − | + | + | 5.0 | 1.3 | 9.70 | 1179.0 |
| 9 | 55 | F | I | + | − | − | + | 0.6 | 0.9 | 3.96 | 1453.2 |
| 10 | 62 | M | I | + | − | − | + | 3.6 | 1.0 | 0.60 | 586.8 |
| 11 | 70 | M | I | + | − | − | + | 4.6 | 1.7 | 2.04 | 376.8 |
| 12 | 74 | M | I | + | − | + | + | 3.3 | 2.1 | 1.29 | 3011.2 |
| 13 | 84 | F | I | + | − | − | + | 2.7 | 1.2 | 6.52 | 892.9 |
| 14 | 69 | M | IIIb | + | + | + | + | 6.4 | 1.9 | 2.32 | 367.0 |
| 15 | 83 | F | IV | − | − | − | + | 27.5 | 13.8 | 1.70 | 262.3 |
| 16 | 83 | M | IV | + | − | + | + | 6.6 | 32.8 | 1.16 | 996.6 |

Abbreviations: CEA, carcinoembryonic antigen (normal range, 0–4.5 ng/ml); CK20, cytokeratin 20; CK7, cytokeratin 7; CYFRA, cytokeratin 19 fragment (normal range, 0–3.5 ng/ml); F, female; GATA-3, GATA-binding protein 3; GCDFP15, gross cystic disease fluid protein 15; IHC, immunohistochemistry; M, male.

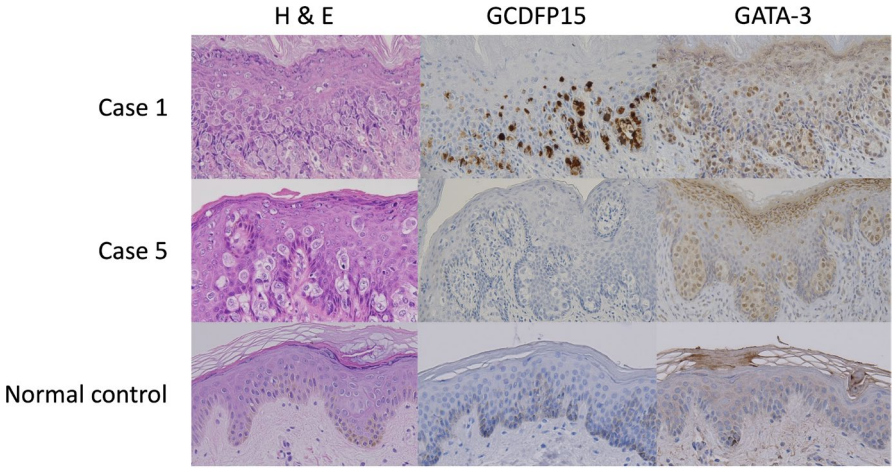


**FIGURE 1** Typical results of hematoxylin and eosin staining and immunohistochemical staining for gross cystic disease fluid protein 15 (GCDFP15) and GATA-binding protein 3 (GATA-3) in extramammary Paget's disease

(Paget's cells) have abundant pale cytoplasm and large nuclei with a prominent, vesicular nucleus.[12] Special staining and immunohistochemical staining facilitate an accurate diagnosis. EMPD stains positively for periodic acid-Schiff (PAS) and Alcian blue, reflecting abundant mucin production in the cytoplasm. For immunohistochemistry, EMPD is typically positive for eccrine and apocrine markers such as CEA, CK7, CAM5.2 (reacting with CK7 and CK8), and GCDFP15, whereas it is negative for S100 protein and melanocytic markers (melan-A, MITF, HMB45, etc.).[5] These immunohistochemical stainings are useful when it is difficult to rule out other skin malignant tumors that might mimic EMPD, such as malignant melanoma (MM), Bowen's disease, and sebaceous carcinoma.[5]

One of the most important differential diagnosis of EMPD is secondary EMPD, a rare form of the disease resulting from secondary intraepithelial spread of an associated regional carcinoma, typically colorectal or urothelial carcinoma. Immunohistochemical staining for GCDFP15 and CK20 is especially useful in distinguishing primary from secondary EMPD, as Paget's cells are usually GCDFP15+/CK20- in primary EMPD,[13] and GCDFP15$^{-}$/CK20$^{+}$ in secondary EMPD.[14] However, false-positive and false-negative staining could occur in some cases. GCDFP15 is a useful marker for primary EMPDs, but its sensitivity was 60%–85%.[6] In contrast, the frequency of positivity for CK20 for secondary EMPD was 50% and for primary EMPD was 22%,[15] although CK20 is generally positive in

secondary EMPD and negative in primary EMPD. Taken together, in some cases, Paget's cells could be GCDFP15$^-$ or CK20$^+$ that makes the diagnosis difficult.

In our study, eight out of 16 patients (50%) showed positive staining for GCDFP15, and 15 out of 16 (94%) patients showed negative staining for CK20 (Table 1). These results mean that eight cases showed false-negative staining for GCDFP15, and one case showed false positive staining for CK20. In contrast, all 16 patients (100%) showed positive staining for GATA-3 in tumor cells, while GATA-3 could be observed in normal epidermal cells.[6,8] These results suggest that GATA-3 is more sensitive than GCDFP15 as a diagnostic marker for EMPD. Although our study was retrospective and the sample size was small, the result was consistent with that of a previous study.[6] In this study, Zhao et al. investigated immunohistochemical expression of GCDFP15 and GATA-3 in 72 primary EMPDs and concluded that GATA-3 is more sensitive than GCDFP15. Therefore, in the cases that are suspicious for false negative staining for GCDFP15, additional staining for GATA-3 could be useful. In addition, staining for GATA-3 could also be useful in the cases that are difficult to differentiate from colorectal carcinoma and suspicious for false positive staining for CK20. We observed the expression of GATA-3 in two patients with secondary EMPD with colorectal carcinoma, and both patients showed negative staining for GATA-3 (data not shown). This finding was consistent with that of previous study.[8] Miettinen et al. reported that the frequency of GATA-3 positivity in carcinomas of colon is only 1%. Therefore, in addition to GCDFP15 and CK20, GATA-3 staining could be useful in distinguishing primary from secondary EMPD.

We next analyzed their levels in patient sera to assess the availability of GCDFP15 and GATA-3 as tumor markers in primary EMPD. However, in contrast to CEA and CYFRA, there was no correlation between their serum levels and disease stage. We also analyzed the serum levels of GCDFP15 and GATA-3 in healthy controls and patients with MM. It has been known that GCDFP15 and GATA-3 are not expressed in the lesion of MM.[8] Thus, we speculated that serum levels of GCDFP15 and GATA-3 were not elevated in patients with MM as well as healthy individuals. Serum samples were obtained from 16 healthy individuals and 24 patients with MM (16 males, eight females; stage IA two cases, stage IB three cases, stage IIA three cases, stage IIB seven cases, stage IIC four cases, stage IIIB two cases, stage IV one case, unknown two cases). The mean ± standard deviation (SD) values of GCDFP15 in normal control, EMPD, and MM were 4.41 ± 4.07, 2.85 ± 2.27, and 2.88 ± 2.31 ng/ml, respectively. Serum levels of GCDFP15 in EMPD and MM were lower than those in normal control. In contrast, the mean ± SD values of GATA-3 in EMPD and MM were 1101.6 ± 684.0 and 939.7 ± 361.1 ng/ml, while GATA-3 was not detected in the normal control sera. The significance of serum levels of GCDFP15 and GATA-3 in the clinical setting was unclear, and we concluded that GCDFP15 and GATA-3 were not useful for serum tumor marker.

Our results indicate that GCDFP15 and GATA-3 are useful for immunohistochemical diagnosis of primary EMPD, but not for monitoring disease progression as serum tumor marker, and suggest that GATA-3 is a more reliable marker than GCDFP15 for immunohistochemical diagnosis of primary EMPD. Our findings are limited by the fact that our study was retrospective and the sample size was small. Further research involving prospective studies and larger sample sizes will be required to confirm the usefulness of GCDFP15 and GATA-3 as EMPD tumor markers.

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## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

## DECLARATION SECTION

Approval of the research protocol: No human participant was involved in this study. The study was approved by the Ethical Committee of Jichi Medical University.
Informed consent: N/A.
Registry and the Registration No.: N/A.
Animal Studies: N/A.

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