Minimally Invasive Laser-Assisted Biopsy of the Oral Lesions for Oral Graft-Versus-Host Disease after Hematopoietic Stem-Cell Transplantation

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Hematopoietic stem-cell transplantation (HSCT) is a treatment for immune deficiency, autoimmune diseases, and hematopoietic malignancies. The main complication of allogenic HSCT is graft-versus-host disease (GVHD). Oral mucosal biopsy is needed for a definitive diagnosis and treatment planning of GVHD, but this procedure causes bleeding and bacteremia in a poor general condition. We evaluated the efficacy of laser-assisted biopsy as a minimally invasive treatment. Three cases were described in this article. All patients' medical records, clinical photographs, and histopathologic findings were reviewed. All patients felt comfortable and no severe complications occurred. The quality of the obtained biopsy material was adequate for a definitive diagnosis of GVHD. Laser-assisted, minimally invasive biopsy of the oral mucosa does not cause bleeding, and it reduces the chances of infection, bacteremia, and postoperative scarring compared to the usual histopathologic biopsy procedure. It would thus be advantageous to use this procedure to biopsy GVHD patients.

Key words : Allogenic bone marrow transplantation, Biopsy, Graft vs Host Disease, Er,Cr:YSGG lasers

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I. INTRODUCTION

Hematopoietic stem-cell transplantation (HSCT) is a definitive treatment for immune deficiency, autoimmune diseases, and hematopoietic malignancies such as leukemia, lymphoma, and malignant anemia.[1)] The success and survival rates of HSCT are increasing, with over 40,000 individuals currently receiving HSCT annually, of which 15,000 are performed with cells from an allogenic donor.[1)] It is well known that autologous HSTC has fewer complications than its allogenic counterpart.[2)] However, if autologous HSTC is impossible, allogenic HSCT is the only method available to treat malignant hematopoietic disease.

The complications of HSCT include infection, graft failure, relapse, hemorrhage, and graft-versus-host disease (GVHD).[1] An estimated 40~70% of grafted patients develop chronic GVHD,[3] which is a major cause of death.[4] GVHD arises when immunocompetent cells in the donor recognize the host protein as a foreign antigen.[3,5] Donor T cells migrate to the target organ and react against proteins such as human leukocyte antigens (HLAs) on host cells, injuring the host tissue. These processes induce immune deficiency and frequently lead to opportunistic infections.

GVHD can be categorized as either acute or chronic depending on the duration, with 100 days being a common cutoff. However, the most recent National Institutes of Health (NIH) consensus criteria recommend classification based on the presence of certain signs and symptoms.[6] GVHD should be avoided, but if it does occur it should be detected early in order to reduce the associated major complications.[1] The main target organs of GVHD contain epithelial cells, and include the skin, liver, and gastrointestinal (GI) tract; most symptoms develop on the skin, followed by the liver and the GI tract. Because of these epithelial characteristics, oral signs and symptoms are very useful for the early detection of GVHD. Acute GVHD symptoms include gingivitis, mucositis, oral erythema, and pain. Chronic GVHD symptoms include distinct features such as xerostomia, lichen-type features, mucosal atrophy, hyperkeratoic plaques, and restriction of mouth opening due to sclerosis or scarring at the corners of the mouth.[3] Biopsy of the oral mucosa is recommended for the definitive diagnosis of GVHD, but since the general condition of the patient is poor due to malnutrition or chemotherapy, the biopsy procedure can cause bleeding and bacteremia due to destruction of the oral integumentary system.[7]

The use of lasers in dental clinics is increasing because they facilitate minimally invasive, bloodless, and painless procedures, which are desired by most patients.[8-10] In particular, the Er,Cr:YSGG laser, which is the latest product used in minimally invasive oral laser applications,[11-13] uses water molecules as the chromophore (the group of atoms in a molecule in which the light induces electron transitions).[14-16] This type of laser causes less soft-tissue damage than a conventional CO_2 laser, and has been used in various oral surgical procedures.[16]

Here we present a case series involving patients in a poor general condition who submitted to a minimally invasive oral mucosal biopsy to obtain a definitive diagnosis of GVHD without any complications such as bleeding, pain, or postoperative infection.

Ⅱ. CASE 1

A 24-year-old female was referred from the Department of Internal Medicine (Division of Hematology, Yonsei University) for a biopsy to rule out oral GVHD on May 12, 2009. She was diagnosed with acute lymphocytic leukemia in January 2007, and had received three cycles of chemotherapy (one cycle of hyper CVAD (central venous access device) chemotherapy, and two cycles of combined vincristine, prednisolone, and daunorubicin chemotherapy), and eight bouts of total body irradiation (150 cGy/fraction over 4 days, totaling 10 MV) to destroy and suppress her immune system in order to prevent immunologic rejection of the transplanted donor bone marrow or blood stem cells. Cyclophosphamide was prescribed for the prevention of GVHD before the operation.

In May 2007 she received a allogenic peripheral-blood stem-cell transplantation (PBSCT) from her sister (serology matched, with HLA fully matched by genotyping). She unfortunately experienced oral mucositis, nausea, and vomiting on the day after surgery, with these symptoms worsening on the 4th day. On the 128th day, she was referred to the Department of Dermatology for a skin biopsy to rule out GVHD due to the presence of multiple erythematous macules on her trunk and arms. The biopsy revealed grade II skin GVHD based on the presence of superficial interface dermatitis with focal spongiosis and occasional infiltrates of dyskeratotic

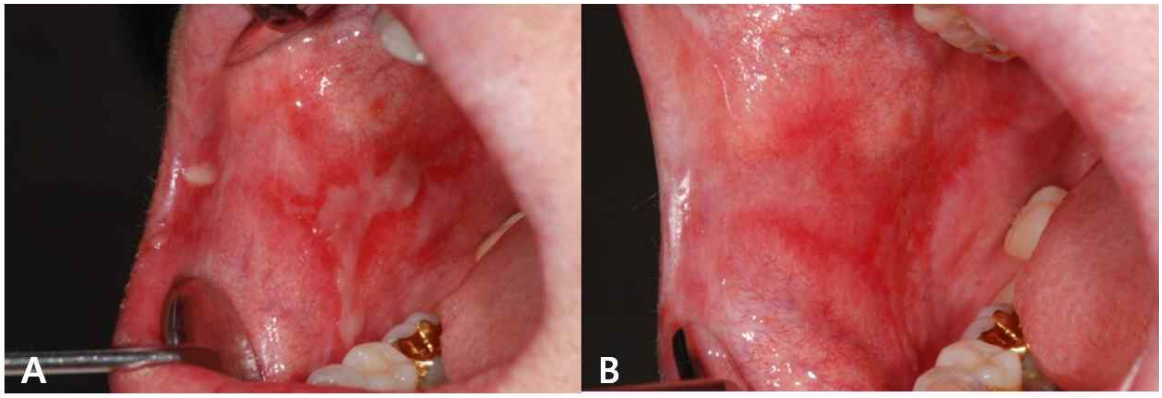

Fig. 1. Case 1: preoperative and postoperative photographs after laser-assisted mucosal biopsy. a: Right buccal mucosa with white striae. b: Right buccal mucosa, 1 week after biopsy.

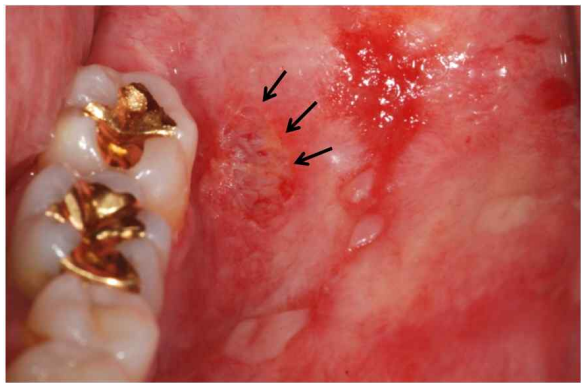

Fig. 2. Case 1: immediately after the biopsy, there was little bleeding or injury in the adjacent tissue.

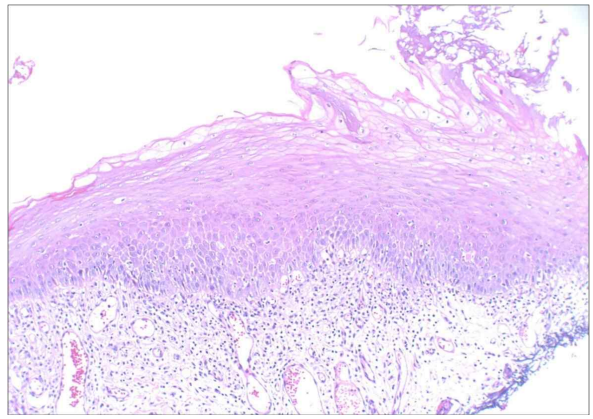

Fig. 3. Case 1: histopathologic findings of the specimen showing distinct vacuolization of the basal cells (hematoxylin and eosin stain, x100).

keratinocytes, which were associated with lymphocytic exocytosis. However, this was not a definitive diagnosis, since these findings could have been manifestations of a drug eruption. A liver-function test was performed on the 182nd day, and her condition was diagnosed as grade II hepatic GVHD based on a lactate dehydrogenase level of 715 IU/l. After 2 years, due to complaints of burning sensation and painful mouth, she was referred to the Department of Advanced General Dentistry for examination to rule out oral GVHD.

There were no specific findings on evaluation of a dental panoramic radiograph and dental structures. A clinical examination revealed white striae, a vesicle, and an ulcer on her buccal mucosa, and she complained of pain in the right buccal mucosa (Fig. 1). We performed laser-assisted, minimally invasive biopsy with an Er,Cr:YSGG laser (Waterlaser, BIOLASE Technology, USA) under local anesthesia. The laser settings were 2.25 W, 25 pps, 15% water, and 15% air. There was little bleeding during the incisional biopsy, and the operation field was sufficient clear to discriminate the submucosal tissues (Figs. 2 and 3). No sutures were needed after excision of the tissues. One day after the biopsy there was no swelling, postoperative

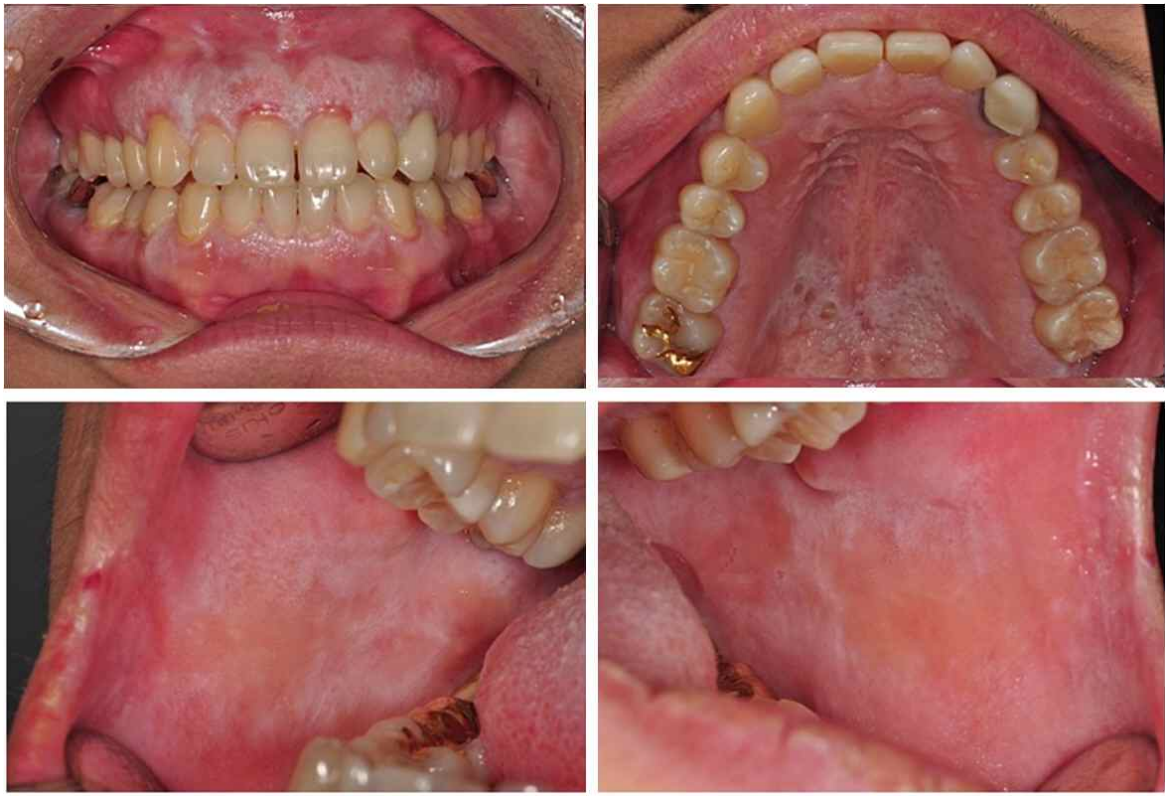

Fig. 4. Case 2: intraoral photograph showing atrophy of the labial and palatal surfaces of the upper and lower anterior teeth.

bleeding, or pain.

Histologically, the lesion exhibited ulcerations and superficial stomatitis with diffuse vacuolization of the basal cells. These should be distinguished from lichen planus or other lichenoid lesions, but we suspected grade I oral GVHD based on the history of PBSCT 2 years previously for acute lymphatic leukemia.

Ⅲ. CASE 2

In May 2009, a 34-year-old male patient was referred from the Department of Internal Medicine for an oral mucosal biopsy for the definitive diagnosis of GVHD. After being diagnosed with acute myeloid leukemia in April 2008, he had received four cycles of chemotherapy times, cyclosporine, and short-course methotrexate for GVHD prophylaxis. In October 2008, he had received peripheral HSCT from his brother (HLA fully matched by genotyping). GVHD developed after transplantation, at which time he was diagnosed with grade III skin GVHD and grade II ocular GVHD.

During an oral examination this patient complained of discomfort of the oral mucosa, with hypersensitivity, a stretching sensation, and pain. There were whitish, plaque-like lesions on both buccal cheeks, and hyposalivation with foamy saliva (Fig. 4). Biopsy of the buccal cheek and soft palate using an Er,Cr:YSGG laser revealed ulcerations and superficial interface stomatitis with diffuse vacuolization of the basal cells, which confirmed the presence of grade I oral GVHD. Figure 5 shows the patient's immediate postoperative status after this biopsy; there was no bleeding, and this method produces only a very small wound that does not need suturing.

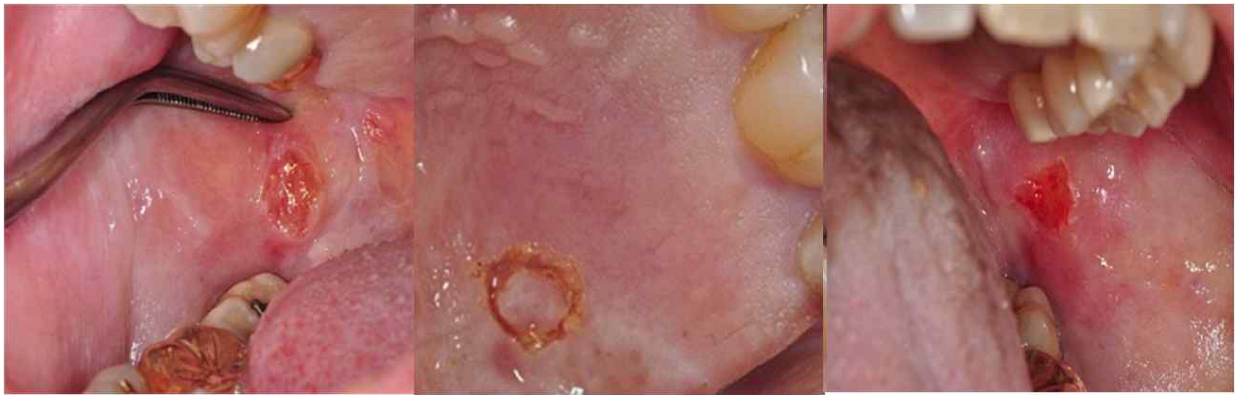

Fig. 5. Case 2: mucosal thinning with white striae due to inflammation in both buccal mucosae. Hyperkeratotic plaque-like changes are evident on the tongue. This photograph was taken immediately after a biopsy procedure performed using an Er,Cr:YSGG laser, showing that the technique is good for bleeding control and produces only a small wound that does not need suturing.

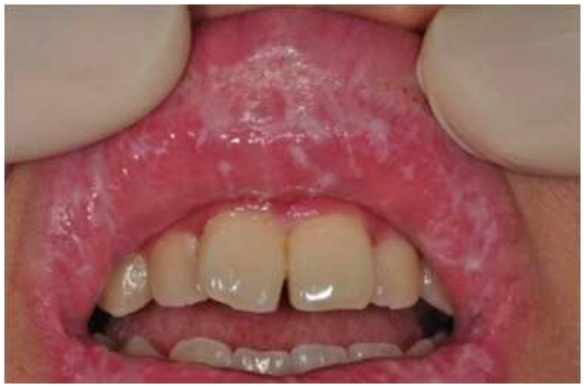

Fig. 6. Case 3: white striae evident on the upper and lower lips.

Ⅳ. CASE 3

A 21-year-old female patient was referred from the Department of Internal Medicine to rule out GVHD in June 2009. After being diagnosed with acute myelogenous leukemia on February 19, 2008, she received five cycles of chemotherapy, and Tacrolimus was administered on the day before surgery, along with methotrexate for GVHD prophylaxis. In October 2008, this patient underwent allogenic PBSCT (serology matched, with HLA two-locus mismatched by genotyping) from an unrelated donor. She complained of a tingling sensation in both fingertips at 14 days after transplantation, and after 19 days skin rashes were evident on both hands and feet, and she was diagnosed as skin GVHD. After 243 days, she was diagnosed with grade II ocular GVHD based on the results of an ophthalmic examination. She also developed white striae on the upper and lower lips. Microstomia was observed due to constriction of the corners of the mouth. There were also erythematous lesions with white striae on both buccal mucosae (Fig. 6).

Minimally invasive, laser-assisted oral mucosal biopsy was performed, as for cases 1 and 2, without any complications. Histopathologic findings of a left buccal mucosa biopsy sample reported lichenoid inflammation with vacuolar changes of the basal cells, consistent with chronic GVHD.

Ⅴ. DISCUSSION

According to NIH consensus, in GVHD, individual organs are scored clinically according to the severity of the condition. A method for scoring was proposed that made it impossible to evaluate chronic GVHD severity relative to the prognosis. Each organ is scored on a scale of 03, with a larger number corresponding to a more-severe condition. Global scoring of chronic GVHD, which is proposed

for the global evaluation of chronic GVHD, evaluates the severity of the condition and the number of affected organs.[6] Global scoring may be considered to establish the diagnosis and plan treatment; however, for acute GVHD, the most reliable grading method was proposed by Ball.[17] Acute GVHD is classified into five grades. The site and severity of the manifestation determine the grading. With the exception of cases of drug reaction and other reasons, each organ has specific and sufficient manifestations for a diagnosis of chronic GVHD. The most common histopathologic findings of acute GVHD in the oral mucosa are lymphocytic infiltration, apoptotic bodies, while in chronic GVHD, additional findings of hydrophilic degeneration of the basal cells, lichenoid lesion, and exocytosis in the oral mucosa are also documented.[18,19] Chronic GVHD also has distinctive sclerotic changes on the mucosa, including hyperkeratotic plaques and limitation of mouth opening caused by skin sclerosis.

A definitive diagnosis of chronic GVHD is useful for predicting the outcome of the disease and the patient's prognosis [20], especially if the signs are relatively nonspecific. Moreover, it is certain that biopsy of the oral mucosa is helpful for the early detection and diagnosis of this condition, due to its convenience.[2] Invasive mucosal biopsy is inevitable in order to define the grade of oral GVHD. The correct diagnosis requires biopsy of both pathologic and normal tissues of the oral mucosa, and a reasonable depth of oral tissue is important for the evaluation of infiltrative features. Destruction of the integumentary system is unavoidable when obtaining these tissues, so that the biopsy procedure will inevitably produce bleeding, which carries with it the risk of bacteremia. The specific problems of performing a biopsy in patients with suspected GVHD are (i) they are in a very poor general condition and may not cope well with a minor operation, (ii) there is chance of bleeding due to a low platelet count, and (iii) they are very vulnerable to infections, and so the bacteremia caused by a mucosal biopsy could prove dangerous. Cautious surgical skill and patient care could prevent some of the complications associated with the mucosal biopsy procedure, and use of the laser could be a good tool for these medically compromised patients.

Lasers were first developed for use in dentistry in the 1960s. They have been used in the broad field of oral procedures such as tooth extractions, periodontal treatment, implant placement, and root-canal therapy.[10-13,14,15] The advantages of using lasers in the dental office are well documented. First of all, a laser makes it easy to control hemorrhaging and can be used on the oral mucosa with the ablation of only a small amount of tissue. During the ablation of soft tissues using a laser, the small vessels are coagulated, and so hemostasis is achieved. The size and depth of the ablation differ according to several parameters, including the type of laser, wavelength, power density, exposure time, and tissue characteristics. The following settings are recommended for the excision of soft tissues during use of an Er,Cr:YSGG laser: 2.25 W, 25 pps, 15% water, and 15% air. However, these settings can be changed according to the user's preference.

The other advantages of using lasers are the reduced postoperative sequence, the short operation time, improvement of tissue healing, reduction of the chance of bacteremia, and decreased postoperative pain.[14] It may also improve patient cooperation and lessen the risk of secondary infection because of improved tissue healing. These characteristics of lasers are advantageous for GVHD patients, and especially those taking immunosuppressants such as cyclosporine to control GVHD. Although the shortcomings of using lasers are the high costs, longer operative time, and capacity to damage the eyes, taking appropriate measures and care will increase possibility of obtaining a good result.

In conclusion, based on our case reviews, we believe that the laser represents a good treatment tool for minimally invasive oral mucosal biopsy in chronic GVHD patients.

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국문초록

조혈줄기세포이식후 발생한 이식편대숙주병의 구강병소에 대한 최소침습적 레이저조직생검 증례

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김윤미[1] · 윤희정[1] · 김현실[2] · 김기덕[1] · 정복영[1] · 방난심[1] · 박원서[1]

조혈줄기세포이식은 면역 결핍 질환, 자가 면역 질환, 악성 조혈 종양의 주된 치료법이다. 이종 조혈줄기세포이식의 주요한 합병증은 이식편대 숙주질환이다. 구강 점막 생검은 이식편대 숙주 질환의 확진과 치료계획 수립을 위해 필요하나 영양 결핍과 화학 요법으로 인해 전신상태가 불량한 환자에게 출혈과 균혈증을 일으킨다. 본 논문에서는 레이저를 이용한 최소 침습적 조직생검의 효능을 평가하였다. 모두 3개의 증례가 소개되었고 모든 환자의 의료 기록, 임상 사진, 조직병리학적 결과가 레이저를 이용한 조직생검의 효능을 평가하기 위해 검토되었다. 모든 환자는 생검 후 불편감이 없었고 심각한 합병증이 발생하지 않았다. 생검 조직의 질이 이식편대 숙주 질환을 확진하는 데에 적당했다. 구강 점막의 레이저를 이용한 최소 침습적 생검은 통상의 조직병리학적 생검에 비해 출혈을 일으키지 않아 감염과 균혈증, 술 후 흉터 생성을 줄이므로 이식편대 숙주 질환의 확진에 이로울 것이다.

주제어: 동종 골수이식, 조직 생검, 이식편대 숙주질환, Er,Cr:YSGG 레이저