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REVIEW ARTICLE

Strategies for oral health care for people with disabilities in Taiwan

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Oral health care for disabled patients is an important health issue in Taiwan. Disabled patients seeking dental care include those with mental retardation, cerebral palsy, epilepsy, Down syndrome, autism, xerostomia, AIDS, loss of function of major organs, and neurologic diseases. Current dental health care policies do not completely address this critical oral health issue. Most of these physically or mentally disabled patients cannot find suitable or qualified dental services in local dental clinics or even hospitals. Our current health care insurance system should provide greater benefits for dental practitioners who are willing to care for such disabled patients. The Department of Health (DOH) should legislate policies to provide greater financial support and equipment and encourage hospital dental clinics and dentists to join this special oral care program. Dental schools, hospitals, and the DOH can also provide curricula and special training programs for both dentists and undergraduate dental students so that they can learn about diseases and dental care of these patients. The government and DOH should cover the fees of lawsuits if dentists have medical legal problems while treating patients with disabilities. Questions on special care dentistry can possibly be included in the National Board Dental Examination. The government can establish some national oral health care centers to treat these disabled patients. Through the development of effective preventive and treatment strategies, the incidence of oral diseases in these patients can be reduced in the future.

Introduction

Special care dentistry is the field of dental practice that provides oral hygiene care and treatment needs for patients who have physical, mental or medical problems, or limitations. It encompasses preventive, diagnostic and treatment services. These patients often cannot adequately maintain their oral health by themselves. Their dental care is neglected not only because of insufficient motivation and appreciation of oral health, but also a lack of appropriate dental care providers and health policies.

The regulations of the Social Security Disability Program of the US Department of Labor, which prohibit discrimination against persons with disabilities,

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define that an individual has a disability if he/she has a physical or mental impairment that substantially limits major life activities.[1] In the US, around 20% of the non-institutionalized population has some extent of disability.[2] One major difficulty in estimating the number of people who have a disability is how *disability* is defined. It is often defined from a broader perspective, which provides a more accurate picture of the number of people with disabling conditions in the US who could benefit from physical activity.[3] Based on the 2007 data from the Ministry of the Interior (MOI), Taiwan, about 4.4% of the Taiwanese have some disabilities who fulfill the definition in the *Physically and Mentally Disabled Citizens Protection Law* (Table 1).[4] The number of disabled patients is increasing in the world because of higher disease survival rates after adequate medical care. During the aging progress, the likelihood of obtaining a disability is therefore increased. According to information from the MOI, the physically and mentally disabled population older than 65 years comprised 36.4% of the total population in 2007, which was greatest among the different age groups.

People with disabilities are divided into two major groups, according to the onset of the disease conditions: those who are born with congenital defects and those who acquire a disorder or injury later in life.[2] The former includes mental retardation, congenital developmental defects, cerebral palsy, and genetic diseases such as Down syndrome. The latter includes disabilities acquired because of traumatic injury, systemic diseases, psychologic disorders, and life-threatening illnesses like cancer and AIDS.

In order to fulfill social welfare policies for the medical and dental care of disabled patients, the purposes of this review were to determine the general conditions, disadvantaged status, and needs of special dental care for patients with disabilities. Since oral health is an integral part of general health, suitable dental treatments to establish preventive dental programs and oral health care policies should be promoted in Taiwan.

Table 1. Number and proportion of people who suffer from 16 major disabling conditions

Classification	*n*	%
Visual disabilities	54,615	5.3
Hearing disabilities	109,578	10.7
Balance disorders	2173	0.2
Limb disorders	401,826	39.2
Mental retardation	91,617	8.9
Multiple disabilities	99,563	9.7
Facial damage	4004	0.4
Voice or speech disabilities	12,986	1.3
Loss of functions of a primary organ	105,732	10.3
Chronic unconsciousness	4987	0.5
Senile dementia	24,852	2.4
Autism	7443	0.7
Chronic psychosis	97,738	9.5
Stubborn epilepsy	3279	0.3
Disability caused by infrequent diseases	1014	0.1
Others (chromosome defects, congenital defects, metabolic disorders, etc.)	3753	0.4

From statistics of the Ministry of Interior, Taiwan in 2008.[4]

Review of physically and mentally disabled populations

According to a report by the Department of Health, Executive Yuan, Taiwan, physically and mentally disabled conditions in Taiwan are divided into 16 major categories (Table 1).[4] Limb disability (i.e., being reliant on crutches or a wheelchair for mobility) comprised 39.2% of the total disabled population in Taiwan and is frequently found in the general population. Their oral hygiene care is compromised by decreased hand dexterity. Their dental and medical problems were also neglected in the past, because it was not convenient for them to receive dental treatment. This is why the government should create a barrier-free environment for these patients with disabilities and provide more services for them and access to dental clinics. The population with disabling conditions also includes those with hearing disabilities (10.7%), loss of function of primary organs (10.3%), multiple disabilities (9.7%), chronic psychosis (9.5%), and mental retardation (8.9%) (Table 1).[4] However, dental resources in different regions of Taiwan differ, and not every dental clinic or even every hospital dental department provides treatment for patients with disability (Table 2).[5] General dentists should be aware of their special needs for oral care and learn more basic techniques for their treatment in dental practice. While hearing disabilities, reduced hand dexterity, and multiple disabilities affect some people, they do not critically influence oral health care and are not further reviewed in detail in this study. Some other common disabling conditions which affect dental treatment and their clinical characteristics are summarized below.

Mental retardation

According to a survey in 2008, there were 91,617 persons with mental retardation comprising about 9% of the total population of disabled persons in

Table 2. Number of dental facilities and disabled citizens in different cities and counties of Taiwan

Districts	Hospital dental departments, *n*	Local dental clinics, *n*	Citizens with a disability, *n*
Taipei City	35	2794	113,981
Kaohsiung City	52	1599	60,803
Taipei County	57	2651	122,444
Ilan County	11	292	32,650
Taoyuan County	31	1286	65,711
Hsinchu County	8	293	17,662
Miaoli County	16	345	28,331
Taichung County	35	1284	62,470
Changhua County	35	951	60,249
Nantou County	10	401	31,364
Yunlin County	17	486	54,428
Chiayi County	6	248	36,415
Tainan County	24	725	54,983
Kaohsiung County	32	889	52,094
Pingtung County	27	597	47,094
Taitung County	6	146	18,713
Hualien County	9	266	25,715
Penghu County	3	80	5,725
Keelung City	8	278	17,216
Hsinchu City	8	371	13,633
Taichung City	31	1636	35,071
Chiayi City	11	370	12,261
Tainan City	15	855	26,658
Lienchiang County	1	5	331
Kinmen County	1	29	4727

From the Ministry of Interior, Taiwan, 2008,[4] and Bureau of National Health Insurance.[5]

Taiwan.[4] The severity of mental retardation ranges from minor to severe. These patients usually have poor attention and cooperation, which may affect the efficacy of routine outpatient dental treatment. People with mental retardation usually have more untreated caries, and higher prevalences of gingivitis and other periodontal diseases than the general population.[6,7] Keeping appointments short and postponing difficult procedures until the patient is familiar with the dentist and his/her staff are suggested.[7] In Taiwan, Choi et al.[8] examined oral conditions in 152 subjects with intellectual disabilities and found that dental caries and gingival inflammation were the most prevalent oral diseases. Kao and Chou[9] also reported that children with mental retardation are generally smarter than children with Down syndrome and their periodontal health care differs. Hu et al.[10] further showed that it is more difficult for mentally retarded children to find a dental clinic for oral health care. Therefore, a higher percentage of mentally retarded children never visited dental clinics in their survey. Interestingly, mentally retarded children and healthy children showed comparable decayed, missing, and filled primary teeth, and decayed, missing, and filled permanent teeth (DMFT) indices, but mentally retarded children had a higher percentage of tooth decay compared with the healthy group. Similarly, Yang[11] reported the DMFT index to be 8 and the caries prevalence rate to be 92–94% in patients with mental retardation in eastern Taiwan. This study indicates that crucial oral health care issues exist for these patients in Taiwan.

To treat dental problems in patients with severe mental retardation, sedation or general anesthesia is usually required because they cannot understand or follow instructions. However, there are few dentists with specialist training in anesthesiology in Taiwan, which means that current management of these patients is difficult. Regular oral hygiene care by their caregivers is important to prevent caries and periodontal diseases.

Cerebral palsy

Cerebral palsy is the most common disease which causes childhood disabilities and is due to hypoxic or traumatic brain damage during birth.[12] Few well-organized studies regarding the oral health care of cerebral palsy patients in Taiwan have been published. Hurng[13] examined the oral conditions of 632 adult cerebral palsy patients. Their mean DMFT

index was 7.8, and the prevalences of dental caries and gingivitis were 84% and 74–76%, respectively.

In these patients, motor activities are impaired and muscle tonicities are uncoordinated. They have problems in maintaining a proper position during dental treatment and have difficulty in daily oral hygiene maintenance. Some of them may have severe bruxism, which leads to excessive tooth wear.[7] During dental treatment, some form of physical restriction, sedation or general anesthesia is needed to maintain a proper position and prevent their uncoordinated muscle activities. If they are being medicated with phenytoin, different extents of drug-induced gingival hyperplasia are commonly noted, and daily oral hygiene care becomes very important.

Stubborn epilepsy

Epileptic patients usually take medication to control seizures throughout their life. If they take phenytoin, gingival hyperplasia may occur and oral hygiene care becomes difficult because of pseudopocket formation. Without proper dental care, gingivitis and eventually periodontal destruction are major problems.[7] Oral hygiene instruction for these patients and their caregivers should be emphasized and promoted. Regular dental examinations and maintenance prophylaxis to control periodontal health are also important.[14] In cases of seizures occurring during dental treatment, it is important to prevent tooth and soft tissue injuries due to muscle contractions and teeth clenching as well as to maintain a patent airway. Dentists should avoid placing any substance or their fingers into the mouth of the patients to prevent aspiration pneumonia and biting injury to their fingers. In some cases, the dentist may consult a medical doctor to check whether other drugs can be used instead of phenytoin and whether the condition of a patient is stable and suitable for dental treatment.

Down syndrome

Down syndrome is a genetic disease (trisomy 21) due to nondisjunction of the 21st chromosome. Persons born with this defect usually have congenital heart disease, developmental defects of the facial skeleton, and variable levels of mental retardation. Physical and orofacial characteristics of the teeth, gingiva, tongue, palate, and occlusion in patients with Down syndrome may show some variations with those of healthy persons.[15] Periodontal disease is a major oral health problem in people with Down syndrome. They may experience rapid, destructive periodontitis and lose their permanent teeth before adulthood.[15,16] The contributing factors include inadequate muscle (tongue) force, poor oral hygiene, malocclusion, and an abnormal host immune response.[15] If a dentist has questions about a patient's medical history, he/she can contact the patient's general physician. To avoid the risk of bacterial endocarditis, antibiotic prophylaxis before dental treatment is usually required, because these patients often have congenital heart disease. Most of them are cooperative during dental treatment, and techniques used in treating pediatric patients like "tell, show and do" can be helpful. Dentists need only be kindhearted and understanding when taking care of such patients. However, when general anesthesia or sedation is necessary for dental treatment, dentists should inform the patient's family about the potential life-threatening risk of anesthesia according to classification of their physical status by the American Society of Anesthesiologist system.[17] Sometimes, the dentist should refer a patient to a qualified medical facility with suitable equipment and anesthesiologists.

Autism

Autism is a complex disability that impairs the communication and social, behavioral and intellectual functioning of individuals.[18] Clinical symptoms and severities of autism vary widely, and some people have concomitant disabilities such as mental retardation or epilepsy. People with autism are sensitive to changes in the environment. It is very difficult in terms of behavioral management, because they are usually uncooperative. Physical restriction, sedation, or general anesthesia may be necessary to facilitate dental treatment. However, promoting cooperation is the best choice. Using a tell, show and do approach can encourage some patients to become more cooperative. Immobilization techniques should only be used when absolutely necessary to protect the patient and staff during dental treatment but not as a convenience.[7] Huang[19] reported an 89% prevalence rate of dental caries in 91 autistic patients in Kaohsiung City. The reasons these patients visit dental clinics include dental caries, pain, and mucosal swelling. Regular oral examinations, oral hygiene instruction, and design of preventive strategies are necessary to promote the oral health of patients.

Xerostomia

Xerostomia, also known as dry mouth, affects many people in different situations. It may be caused by side effects of medicine, such as tricyclic antidepressants, or by Sjögren syndrome, head

and neck radiotherapy, and aging. A common cause of xerostomia in Taiwan is radiotherapy in patients with head and neck cancers (e.g., nasopharyngeal carcinoma, oral cancer).[20] It can cause many dental problems. Dry mouth can cause difficulties in eating, swallowing, and taste sensation.[21] Without saliva lubrication, patients usually complain of a burning sensation in the mouth, and the risk of dental caries is increased.[22] Oral preventive treatments like fluoride varnish and prophylaxis are indicated before, during and after radiotherapy to reduce dental caries.[23] The risk of osteoradionecrosis is high in patients who receive over 65 Gy of radiation to the head and neck region.[24] Artificial saliva may be prescribed to relieve the discomfort. Regular dental examinations for early diagnosis and treatment are useful for maintaining long-term oral health. Only a few studies evaluated the prevalence of xerostomia in Taiwan. Lin[25] evaluated the salivary flow in patients before and after radiation therapy for head and neck cancer, and obvious xerostomia was reported by patients 2 weeks after radiation leading to difficulty with swallowing food, and the condition worsened after 4–8 weeks of radiation. Further studies are needed to evaluate the oral health condition of populations with xerostomia, Sjögren syndrome, and head/neck radiation in Taiwan.

AIDS

Oral problems are very common in people with HIV because of their weakened immune function. They may have mucosal lesions like hairy leukoplakia, candidiasis, and herpetic stomatitis which can affect eating.[26,27] Most of these problems can be treated or controlled. The extents of gingivitis and periodontitis are generally more severe than others,[28,29] probably because of embarrassment about their condition, so they seek dental treatment only when they are in pain. The most challenging situation is that there are few dentists who are willing to provide dental services for them. The Taiwanese government should establish a sound medical system including providing specialized institutions and equipment to give them privacy during treatment, and financially support the advanced training of dental professionals for better dental services. Standardized operative procedures for infection control in general dental clinics are important to prevent cross-infection during dental treatment. Although dentists are familiar with most dental treatments, they are often not familiar with this disease. Continuing education programs for general dentists to understand the nature of this disease and precautions to prevent infection during dental procedures would encourage more dentists to provide dental services for these patients.

Loss of functions of primary organs

Many patients suffer from dysfunction of a major organ due to diseases including cancer, autoimmune diseases, poisoning, and traumatic injury. While most of them survive these conditions, their general health is usually compromised. Impaired coagulating and immune functions may affect routine dental treatment.[30] In Taiwan, more than 100,000 citizens suffer from this problem (Table 2).[5] Dentists should pay attention to the general status of these patients before treatment. For example, patients who undergo organ transplantation always take immunosuppressants for a long period and thus impair their immune function. The immune status of these patients should be checked before tooth extraction or other invasive procedures. If necessary, medical consultation should be done to evaluate the systemic condition of the patient.

Neurologic diseases

Neurologic degenerative diseases like Alzheimer disease and Parkinson disease usually occur in the elderly. As human life expectancy is prolonged because of advanced medical technologies, patients with these kinds of disorders are accordingly increasing.

Parkinson disease shows characteristics of muscular rigidity, tremors, and bradykinesia. It is a pathologic condition caused by dysfunction of the brain's dopamine neuronal systems.[31] Patients may have problems with their oral hygiene care because of poor hand dexterity. Therefore, dental caries and periodontal disease can be severe if they cannot clean their mouth. Electric toothbrushes are helpful for patients with poor muscle control in their hands.

Persons with Alzheimer disease also have problems in maintaining good oral hygiene, because they forget what to do with the toothpaste or how to rinse and even where the toothbrush is. As the disease progresses, they may forget the importance of dental care and neglect taking care of their mouth.[32] Providing oral hygiene instruction to their caregivers is important, and regular dental follow-up and prophylaxis (every 3 months) should be arranged to maintain their oral health.

Other infrequent disorders

Fibrodysplasia ossificans progressiva, myositis ossificans progressiva, mucopolysaccharidosis, tuberous sclerosis complex, multiple sclerosis, osteogenesis

imperfecta, phenylketonuria, hereditary epidermolysis bullosa are infrequent disorders, because their prevalence rates are <1 per 10,000.[33] Dental treatments for these patients do not differ from the general population unless the physical condition is not stable. Some medications might affect their health condition, and consultation with their physician before prescribing any medicine would be the best policy.[33]

Oral health care strategies for persons with disabilities who need more oral health care

Recently, the Taiwanese government has dedicated itself to improving the social welfare of disabled people. These policies include daily allowances, employment policies, and medical insurance mitigation. However, the health care system should be further improved to become more accessible to patients with disabilities. According to the MOI's survey, 90% of disabled persons live at home.[4] Community-based medical services should be provided, because patients with disability are not ambulant and are dependent on their caregivers.

The result of a survey performed by Kaohsiung Medical University in 2004 showed that the caries rate of permanent tooth in people with disabilities was >90%, and only 32% of the teeth were filled.[34] According to data from Taiwan Dental Association, only 54% of the special service program budget was utilized in 2006. By examining the global budget of the dental payment system, we found that the amount was <0.2% of the total expenditures of dental payments.[33]

Disabled patients are grouped into four categories (minimal, mild, moderate, and severe) based on the severity of their disability in Taiwan.[4] Accordingly, medical institutions should be graded to provide suitable services to different categories of patients as is done in Japan.[35] In order to make medical institutions community-based, general practitioners in local dental clinics should be able to treat patients with minor and median grades of disability. This kind of ability or skill should be taught or trained in dental schools as a required curriculum. Educational programs and associated training for dental students seem insufficient in this field of dental service.[36]

Dental treatment for patients with severe disabilities or more complicated oral conditions should be handled in a specialized medical center with special equipment and in a multi-disciplinary approach. Thus, a dental specialist system for patients with disabilities should be established. Qualified institutions could provide postgraduate clinical training programs for the dental health care of disabled patients.

In addition to the above strategies, the most important and effective method, however, is to prevent oral diseases from occurring in disabled people. Oral health education like tooth cleaning methods, diet consultations, and regular dental examinations should be designed and provided to patients with disabilities or their caregivers to decrease the incidences of dental caries and periodontal disease. Prophylactic measures like ultrasonic scaling and fluoridated mouth rinsing or professional topical fluoride application should be encouraged in these patients.

Conclusion

In Taiwan, there are seven dental schools and about 400 dental graduates each year. However, only Chung-Shan Medical University provides the curriculum, Dentistry for Patients with Disabilities, for undergraduate students. The other six dental schools include this only as one of the topics in pedodontics for undergraduates and put it into more advanced training in the postgraduate program. In addition, not every teaching hospital provides dental services for patients with disabilities, and only a few general dental clinics treat such patients (Tables 2 and 3).[5,37]

In order to encourage more dentists to get involved in treating patients with disabilities, special training programs should be promoted for the dental care of these patients and educational courses should be designed in undergraduate schools. If dentists are more familiar with the physical and dental conditions of patients with disabilities, they will not be so reluctant to treat them.

The Department of Health should help establish sound and clear policies regarding various levels of suitable dental facilities for patients with different levels of severity of disabilities, provide more financial incentives for dentists willing to treat such patients, and pass regulations concerning those who refuse to treat or refer these patients to suitable facilities. Questions on special-care dentistry can possibly be included in the National Board Dental Examination. The government can also establish national oral health care centers for treating disabled patients in different regions of Taiwan and provide support for potential legal problems which may be confusing to dentists. It is not too late to emphasize this issue and begin to establish a sound health care system. Further development of effective preventive and treatment strategies, oral hygiene care, special dental management, and even multidisciplinary approaches for these disabled

Table 3. Dental facilities which offer special-care services in Taiwan in 2009

District	Service, *n*		
	Primary	Advanced	Total
Keelung City	2	9	11
Taipei City	37	91	128
Taipei County	41	32	73
Taoyuan City	1	0	1
Taoyuan County	14	113	127
Hsinchu City	19	4	23
Hsinchu County	4	0	4
Miaoli City	1	0	1
Nantou County	4	1	5
Taichung City	24	45	69
Taichung County	34	8	42
Changhua City	2	0	2
Changhua County	56	4	60
Yunlin County	20	1	21
Chiayi City	2	0	2
Chiayi County	5	6	11
Tainan City	18	9	27
Tainan County	21	0	21
Kaohsiung City	6	6	12
Kaohsiung County	4	6	10
Pingtung City	2	0	2
Pingtung County	18	0	18
Ilan County	21	0	21
Hualien County	14	4	18
Taitung County	4	2	6
Total	374	341	715

From the Bureau of National Health Insurance.[5]

patients would help meet their needs both in quality and quantity.

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ELSEVIER

ORIGINAL ARTICLE

Clinical efficacy of toothpaste containing potassium citrate in treating dentin hypersensitivity

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Background/purpose: Many adults suffer from sensitivity, and dentin hypersensitivity is most commonly the result of exposed dentin which can sometimes trigger pain or discomfort when eating, drinking or simply brushing. The purpose of this study was to evaluate the clinical efficacy of desensitizing toothpaste containing potassium citrate in treating dentin hypersensitivity.
Materials and methods: This study was performed at the Department of Periodontics, Chung Shan Medical University Hospital. In total, 172 volunteers who provided informed consent were included in the study. A visual analog scale (VAS) was used to indicate dentin sensitivity in response to an air stimulus. Participants who had a VAS score of 30–80 were qualified for the study and were provided with their assigned desensitizing toothpaste containing potassium citrate (Colgate Sensitive Multi Protection) and a soft-bristled adult toothbrush for home use. After 4 weeks, the hypersensitive teeth were examined again, and the VAS score was also recorded.
Results: In this study, 38% (65/172) participants were found to suffer from dentin hypersensitivity, 40 persons who reported VAS scores of 30–80 were qualified for the clinical trial. Among these, 34 (85%) participants reported reduced dentin hypersensitivity after using the desensitizing toothpaste containing potassium citrate for 4 weeks. The mean value of the VAS score prior to the use of the toothpaste was 50.72, while the mean value of the VAS score after use was 30.68. The use of Colgate Sensitive Multi Protection was found to significantly reduce dentin hypersensitivity after 4 weeks ($P<0.01$).
Conclusion: The prevalence of dentin hypersensitivity in this study was 38%. The use of desensitizing toothpaste containing potassium citrate with oral hygiene instruction can effectively reduce dentin hypersensitivity.

Introduction

Dentin hypersensitivity is one of the most commonly encountered dental problems, and estimates of its prevalence in the adult dentate population range from 8% to 57%.[1,2] It was shown to peak in 20–30 year olds and then rise again when people are in their 50s.[3] When thermal, tactile, osmotic and mechanical stimuli, such as tooth brushing, sweet and sour foods, and hot or cold water, are applied to the exposed dentin, patients feel a short sharp pain which is termed dentin hypersensitivity.[4,5] Most

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dentin hypersensitivity is a result of abrasion, attrition, erosion, abfraction, gingival recession, and improper brushing habits. Sites of predilection in descending order are the canines, first premolars, incisors, second premolars, and molars.[6]

Several theories have been put forward to explain the mechanisms involved in dentin hypersensitivity. Several hypotheses have been proposed to explain it:[7,8] (1) odontoblasts and their processes act as dentinal receptor mechanisms; and (2) pulp nerves are stimulated by a hydrodynamic mechanism, and nerve impulses in the pulp are modulated by the release of certain polypeptides during pulp injury. Of these, the most widely accepted theory is the so-called hydrodynamic theory of sensitivity. This theory postulates that rapid shifts, in either direction, of fluids within the dentinal tubules following stimulus application result in activation of sensory nerves in the pulp/inner dentin region of the tooth.[9]

Treatment for dentin hypersensitivity may include mucogingival surgery, a pulpectomy, and application of resin, lasers, topical desensitizing agents, and desensitizing toothpaste.[7] Desensitizing toothpaste is considered the simplest and most cost-effective treatment for most patients. Toothpastes with ingredients that include stannous fluoride, strontium chloride hexahydrate, aluminum ferric oxalates, potassium ferric oxalates, and fluorides are designed to reduce flow in the dentin tubules by occluding or sclerosing the tubules.[7] The most widely and simplest available desensitizing toothpaste ingredients are potassium compounds.[10] Potassium ions are thought to block the action potential generated in intradental nerves.

A clinical examination to measure levels of pain responses to dentin hypersensitivity stimuli might include a subjective assessment of tactile and cold stimuli. Whatever methods are used, they should be reproducible and quantifiable. The Yeaple probe and a new mechanical probe which run over the area of exposed dentin represent the state of the art for tactile sensitivity testing. A blast of cold air from a triple syringe offers the potential for a well-controlled thermal stimulus. Care should be taken to ensure, as much as possible, that neither stimulus interferes with the other stimulus used in the measuring procedure.[11]

The visual analog scale (VAS) offers the advantage of being a continuous scale, thus providing quantitative measurements. The validity and reliability of the VAS for measuring both experimental and clinical pain are well demonstrated.[11,12] The purpose of this study was to investigate the effects of a toothpaste containing potassium citrate (Colgate Sensitive Multi Protection; Colgate-Palmolive, Amphur Muang, Chonburi, Thailand) on dentin-hypersensitive teeth over a 4-week period to reduce hypersensitivity. Symptoms and signs of dentin hypersensitivity were measured using a VAS.

Materials and methods

Patient selection

This study was performed in the Department of Periodontics, Chung Shan Medical University Hospital. In total, 172 volunteers (99 males and 73 females) were recruited after informed consent was obtained. The inclusion criteria were: (1) patients must have good general health with no known allergies to commercial dental products; (2) patients must not have used desensitizing toothpaste within the last 3 months; (3) the study teeth must be decay-free and have no restorations; (4) the study teeth had to have clinical mobility of = grade 1; (5) the study teeth had to show signs of facial/cervical erosion, abrasion, and/or gingival recession; and (6) patients had to be able to read and understand the consent form and be willing to sign it.

Exclusion criteria included: (1) teeth which had undergone periodontal surgery within the past 6 months or been scaled/root planed within the past 3 months; (2) pregnant or lactating women; (3) individuals demonstrating gross oral neglect or requiring extensive dental therapy; (4) teeth used as abutments for fixed or removable partial dentures, or teeth with full crowns or obvious cracks in the enamel; (5) subjects who were unable to strictly adhere to product use; (6) subjects who had taken certain medications (anti-inflammatory drugs or antibiotics) on a daily basis during the previous 7 days; and (7) subjects suffering from a chronic medical disease or conditions which are associated with intermittent episodes or constant daily pain, such as arthritis.

Measurements

A VAS is commonly used to assess dentin hypersensitivity.[13,14] A VAS consists of a 100-mm line with *0* at one end indicating not painful at all and *100* at the other end indicating extremely painful. A participant was asked to draw a vertical line on the horizontal scale at a point that corresponded to his/her reaction to the air stimulus. Teeth identified by the subject were tested for thermal sensitivity using a 1-second air blast 1 cm from the study tooth after isolating the tooth from the adjacent teeth using cotton rolls. Volunteers were asked to place a vertical mark on the line that represented the intensity of their pain on the

100-mm scale. Volunteers who had VAS scores of 30–80 qualified for the clinical study. One tooth was tested in each subject.

Experimental procedures

This was a single-blinded design study. The brand name of the toothpaste (Colgate Sensitive Multi Protection) was hidden, so that participants did not know what brand of toothpaste they were given. Participants who met the criteria (VAS scores of 30–80) were provided with their assigned dentifrice and a soft-bristled adult toothbrush for home use after receiving some basic oral hygiene instruction. Toothpaste was applied to the entire length of the brush. Participants were asked to brush their teeth twice a day. Four weeks later, participants returned for the final examination of their pre-selected sensitive teeth as described above.

Statistical analysis

In order to determine the effect of Colgate Sensitive Multi Protection on hypersensitive teeth, VAS scores were analyzed using the Wilcoxon signed rank test. A level of significance of ≤ 0.05 was used for each comparison.

Results

As shown in Fig. 1, 107 participants (69 males and 38 females) did not respond to the air blast stimulus and had a VAS score of 0. Sixty-five (30 males and 35 females) participants reported VAS scores of >10. The prevalence of dentin hypersensitivity in this study was thus 38% (65/172).

Forty participants (20 males and 20 females) who met the criteria (VAS scores of 30–80) qualified for the clinical study. The VAS scores of each person at the baseline and 4 weeks later are shown in Table 1. Thirty-four of the 40 (85%) participants were found to have reduced dentin hypersensitivity after using Colgate Sensitive Multi Protection for 1 month (Table 2). The percentage of improvement was 90% in females and 80% in males. The sites examined in descending order were canines, first premolars, incisors, second premolars, and molars (Table 3). Table 4 illustrates changes in the mean VAS sensitivity scores for air stimuli after the 4-week period. Scores decreased after desensitizing toothpaste use, from 50.72 mm at the baseline to 30.68 mm at the end of the 4-week period. Desensitizing toothpaste containing potassium citrate was found to have significantly reduced dentin hypersensitivity ($P<0.01$).

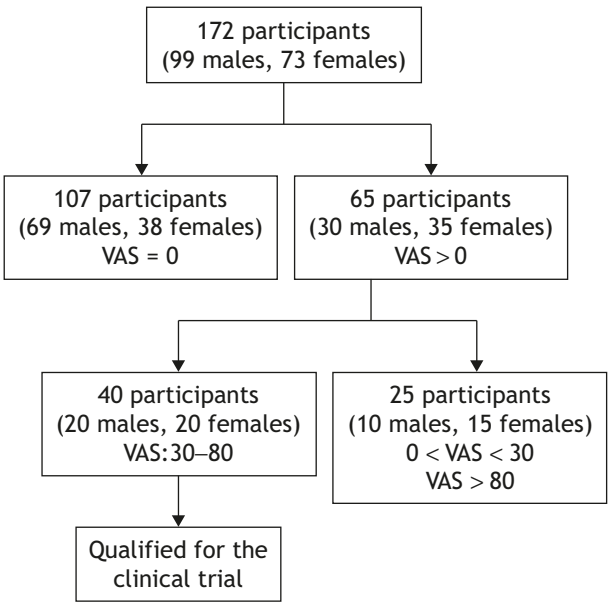

Fig. 1 Distribution of participants in this study.

Discussion

The prevalence of dentin hypersensitivity was widely reported previously. Graf and Galasse[15] reported a prevalence of 14.5%, while Irwin and McCusker[2] reported a prevalence of 57%. The study by Irwin and McCusker[2] was carried out using a sensitivity questionnaire with no subsequent clinical examination, so that it is likely to have overestimated the prevalence owing to inclusion of other causes of sensitivity. One report showed a prevalence of 32% in Taiwanese by evaluating the presence of cervical dentin hypersensitivity by means of a questionnaire and intraoral tests.[16] Consistently, our result showed that 38% of participants suffered from dentin hypersensitivity. This wide variation in prevalence may be due to certain factors, including different methods used to diagnose the condition, sensitivity questionnaire-based surveys probably exaggerating the true figure, and variations in the consumption of erosive drinks.

A clinical examination for dentin hypersensitivity includes objective assessments, such as a tactile or thermal stimulus.[11] Often individuals do not respond to all types of stimuli, but classically clinicians only use the air-blast stimulus.[17] Prolonged air blasts have an unknown and varying temperature effect, which can be avoided by using a short application time, usually 1 second. The 1-second air blast is easily controlled and is the best period for a dentin hypersensitivity assessment.[11,18] Those are the reasons why the teeth identified by subjects were tested using a 1-second air blast in the present study.

Table 1. Visual analog scale (VAS) scores at the baseline and after 4 weeks of using desensitizing toothpaste containing potassium citrate

Patient no.	Sex	VAS I (baseline)	VAS II (4 weeks)
1	F	71	53
2	F	37	26
3	F	44	29
4	F	32	16
5	M	52	29
6	M	78	37
7	M	57	26
8	M	42	23
9	M	43	28
10	F	55	43
11	F	49	11
12	F	36	39
13	F	43	18
14	M	41	18
15	M	59	26
16	M	44	29
17	M	73	75
18	M	26	12
19	F	61	41
20	M	74	29
21	F	42	12
22	F	38	0
23	F	26	27
24	M	26	29
25	F	60	34
26	M	76	80
27	M	64	36
28	F	58	37
29	F	32	16
30	F	54	32
31	M	44	47
32	M	51	23
33	F	30	15
34	F	49	33
35	M	63	47
36	F	57	39
37	F	58	27
38	M	55	23
39	M	66	24
40	M	63	38

F=female; M=male.

Table 2. Data on improvements after using the desensitizing toothpaste containing potassium citrate

Sex	*n*	Improvement, *n*	Improvement (%)
Male	20	16	80
Female	20	18	90
Total	40	34	85

Table 3. Sites examined for dentin hypersensitivity*

Site	Male (*n*=44)	Female (*n*=42)	Total (*n*=86)
Canine	14 (31.82)	15 (35.71)	29 (33.72)
First premolar	14 (31.82)	10 (23.81)	24 (27.91)
Lateral incisor	7 (15.91)	6 (14.29)	13 (15.12)
Incisor	5 (11.36)	4 (9.52)	9 (10.47)
Second premolar	3 (6.82)	4 (9.52)	7 (8.14)
Molar	1 (2.27)	3 (7.14)	4 (4.65)

*Data are presented as *n* (%).

Table 4. Summary of visual analog scale (VAS) scores at the baseline and after using desensitizing toothpaste containing potassium citrate for 4 weeks

	VAS		
	Median	Range	Mean±SD
Baseline	51.5	26–78	50.72±14.39*
4 weeks	29	0–80	30.68±15.50*

*Statistically significant between the baseline and 4 week, $P<0.01$. SD=standard deviation.

The VAS for dentin hypersensitivity has been measured using several methods, such as mechanical, thermal and air-blast stimuli.[12] VAS pain intensity can be shown either as a percent of the maximum or as an absolute score value.[11] Clark and Troullos[12] reported that the VAS is simple to understand and suitable for use in evaluating stimulus responses in dentin hypersensitivity studies. The validity and reliability of the VAS for measuring both experimental and clinical pain were demonstrated by several investigators.[11,12] Several studies compared the VAS with other pain scales, and the results indicated that the VAS correlated well with those methods and appeared to be more sensitive in discriminating between various treatments and changes in pain intensity.[11,19]

Toothpastes containing potassium ions are reported by several clinical studies to be effective in reducing dentin hypersensitivity, and the American Dental Association Council on Dental Therapeutics has granted a Seal of Acceptance to toothpastes containing 5% potassium nitrate.[20] Toothpastes which contain 3.75% potassium chloride[21,22] or 5.3% potassium citrate[23] are also reported to be clinically effective in treating dentin hypersensitivity. Potassium ions are thought to act by blocking synapses between nerve cells, thereby reducing nerve excitation and the associated pain.[24,25] Several clinical studies demonstrated that toothpastes containing potassium ions provide effective

desensitization. It was postulated that potassium ions released from toothpastes diffuse along the dentinal tubules to inactivate intradental nerves. A previous study reported that potassium citrate and potassium nitrate were more effective than other potassium salts in blocking nerve conduction and may be more effective dentinal desensitizing agents.[26] Similar results were found in this study: desensitizing toothpaste containing potassium citrate was effective in reducing dentin hypersensitivity after 4 weeks of use.

Taken together, the prevalence of dentin hypersensitivity in this study was 38%. It was also found that the desensitizing toothpaste which contains potassium citrate was effective in reducing dentin hypersensitivity with oral hygiene instruction. The improvement rate was as high as 85%.

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ORIGINAL ARTICLE

Evaluation of cytotoxicity of resin bonding materials toward human oral epithelial cells using three assay systems

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WST-1

Background/purpose: Gingival tissue around teeth can be contacted by flowable photocured resin agents during operative procedures. The cytotoxicity of resin bonding agents toward human oral epithelial cells cannot be neglected. This study evaluates the cytotoxicity of resin bonding agents toward human oral epithelial cells using three assay systems.
Materials and methods: Nine commercial resin bonding agents (Clearfil Protect Bond, Clearfil SE Bond, Prime & Bond NT, Prisma Universal Bond 3, UniFil Bond, 3M Single Bond, CharmBond, Compobond, and ExciTE) and two resin monomers (methyl methacrylate and triethylene glycol dimethacrylate) were used. The detection methods for the cell survival rate included: (1) the CellTiter 96 AQueous One Solution Cell Proliferation Assay System, (2) PreMix WST-1 Cell Proliferation Assay System, and (3) CellTiter-Glo Luminescent Cell Viability Assay System. One-way analysis of variance and Scheffé test were used for the statistical analyses.
Results: Most of the components of the uncured bonding agents had cytotoxicity at 0.1 vol% (survival rates, 21.4%±1.4% to 113.1%±19.1%), except for UniFil Bond Primer (survival rates, 101.8%±3.5% to 113.1%±19.1%). More severe cytotoxicity was found at the 1.0 vol% concentration (survival rates, 0.2%±0.02% to 147.3%±10.8%), except for the methyl methacrylate monomer (survival rates, 96.5%±1.3% to 147.3%±10.8%). In the post-cured group, most bonding agents had little or no cytotoxicity, except for 3M Single Bond (survival rates, 52.4%±7.9% to 97.4%±17.8%) and Compobond (survival rates, 29.1%±7.1% to 79.5%±6.7%).
Conclusion: The same material detected by different assay systems may give different cytotoxic effects, and examination by three methods may provide more-objective results. Furthermore, since most of the bonding agent components detected by the three assay systems showed cytotoxicity before curing (and some components even showed cytotoxicity after curing), the application of bonding materials in the oral cavity should be very carefully performed.

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Introduction

The use of dental materials to replace missing tooth structures is an important component of dental treatment in which the biocompatibility of the restorative materials with the human body cannot be neglected. Most dental materials, such as metal, rubber, resin and cement, cause some degree of irritation.[1,2] The most common tooth restoration materials, amalgam and composite resin, are of particular concern.[3,4] Composite resins that can mechanically bond to tooth structures have been used for cavity restoration for more than 40 years owing to superior aesthetic results. Bonding agents are crucial to ensure that the composite resin strongly bonds to the tooth structure.[5]

In clinical applications, bonding agents are directly painted onto the wall of the prepared cavity using a disposable brush; the liquid component may penetrate to the dental pulp or directly contact the oral mucosa tissue and cause tissue irritation.[6,7] Schedle et al.[8] used flow cytometry to evaluate the cytotoxicity of 19 types of materials (including various composite resins and cements) toward mouse fibroblasts (L-929 cells), and concluded that all of the test materials initially exhibited cytotoxicity but not 1 week later. Ozen et al.[7] used a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to analyze the cytotoxicity of four dentin bonding agents toward gingival fibroblasts, and found that, while none of them caused cytotoxicity after 24 hours, a cytotoxic effect was evident 72 hours later. Caughman et al.[9] used different methods to evaluate the cytotoxicity of glass ionomer cement; this cement appeared to exert no morphologic effects but greatly inhibited the macromolecular synthesis of fibroblasts. These results indicate that using different methods to evaluate biocompatibility may produce different results.[9] Vajrabhaya et al.[10] used an MTT assay to evaluate the cytotoxicity of single-component dentin bonding agents, and found that a total-etching bonding system was more cytotoxic than a self-etching bonding system. Grobler et al.[11] used an MTT assay to evaluate the cytotoxicity of a dentin bonding agent at two concentrations on a mouse lung tissue (3T3) and four human pulp fibroblast cell lines, and found that the bonding system tested at a higher concentration was more cytotoxic. The above studies showed that even when using the same method, types and concentrations of tested material are important factors in the test results. In general, using different methods and different conditions to determine cell cytotoxicities are necessary to obtain more-objective results.

The purpose of this study was to determine the cytotoxic effects of different concentrations of resin bonding agents in uncured and post-cured conditions toward oral epithelial cells using three assay systems.

Materials and methods

Nine commercial resin bonding agents were tested in this study. The product names, components, and manufacturers are listed in Table 1. The resin monomers were methyl methacrylate (MMA) (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), triethylene glycol dimethacrylate (TEGDMA) (Tokyo Chemical Industry Co., Ltd.), and urethane dimethacrylate (UDMA) (Shin-Nakamura Chemical Co., Ltd., Wakayama, Japan). The photoinitiator was camphorquinone (Tokyo Chemical Industry Co., Ltd.), and

Table 1. Components of the experimental materials

Material	Delivery system	Components	Manufacturer
Clearfil Protect Bond	Two bottles	Primer and bonding agent	Kuraray Co., Okayama, Japan
Clearfil SE Bond	Two bottles	Primer and bonding agent	Kuraray Co., Okayama, Japan
Prime & Bond NT	Two bottles	Conditioner and adhesive	Dentsply Co., Milford, DE, USA
Prisma Universal Bond 3	Two bottles	Primer and adhesive	Dentsply Co., Milford, DE, USA
UniFil Bond	Two bottles	Primer and bonding agent	GC Co., Tokyo, Japan
3M Single Bond	One bottle	Adhesive	3M Co., St. Paul, MN, USA
CharmBond	One bottle	Bonding agent	DentKist Co., Gyeonggi, Korea
Compobond	One bottle	Bonding agent	PROMEDICA Co., Nenmunster, Germany
ExciTE	One bottle	Adhesive	Ivoclar Co., Bromma, Sweden
MMA	One bottle	Resin monomer	GC Co., Tokyo, Japan
TEGDMA	One bottle	Resin monomer	Tokyo Chemical Industry Co., Ltd., Tokyo, Japan
UDMA	One bottle	Resin monomer	Shin-Nakamura Chemical Co., Ltd., Wakayama, Japan

MMA = methyl methacrylate; TEGDMA = triethylene glycol dimethacrylate; UDMA = urethane dimethacrylate.

the reducing agent was N,N-dimethylaminoethyl methacrylate (DAEMA) (Tokyo Chemical Industry Co., Ltd.). The cytotoxicity tests were performed on human oral epithelium (KB) cells purchased from the National Health Research Institutes Cell Bank at the Bioresource Collection and Research Center of the Food Industry Research and Development Institute (Hsinchu, Taiwan). The detecting agents included: (1) CellTiter 96 AQueous One Solution Cell Proliferation Assay System (MTS system; Promega, Madison, WI, USA), (2) PreMix WST-1 Cell Proliferation Assay System (WST-1 system; Takara Bio, Shiga, Japan), and (3) CellTiter-Glo Luminescent Cell Viability Assay System (CellTiter-Glo assay system; Promega).

Cell collection

KB cells were grown in 35-mm diameter Petri dishes containing 5 mL of α-minimum essential medium supplemented with 10% fetal bovine serum at 37°C in a humidified 5% CO_2 atmosphere. Cells were checked daily for growth, and the medium was changed twice weekly. The culture reached confluence within several days and was subcultured using 1:3 splits until the experiments began.

Cytotoxicity testing of bonding agent components before curing

When enough cells had been collected, 1×10^4 cells in 100 μL per well were prepared and seeded in a 96-well tissue culture plate, which was subsequently put into an incubator overnight for cell deposition. One-tenth microliter of each component of various uncured bonding agents was added to 9.9 mL of culture medium with a micropipette to a 15-mL plastic test tube to produce 1 vol% of test media. This was then vibrated (vortexed) for 2 minutes. One milliliter of the prepared mixture was pipetted into 9 mL of fresh medium in another plastic tube and vibrated again to produce 0.1 vol% test media. The medium of the cell culture plate was replaced with various prepared media, except the first and last columns of the 96-well test plate. These two columns were replaced with fresh culture medium without the bonding agent component to serve as untreated controls. Cells were incubated for a further 24 hours, and then the cell viability was detected by the following procedures:

1. MTS assay. MTS is a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt], which can be reduced by dehydrogenase enzymes of viable cells to form orange-colored formazan, which is then measured by an enzyme-linked immunosorbent assay (ELISA) reader under a 490-nm wavelength. The test procedures were performed as follows: 20 μL of CellTiter 96 AQueous One Solution was pipetted into each well. The plates were incubated for 4 hours; then the optical density was quantitated with a microtiter plate (ELISA) reader at a spectrophotometric absorbance of 490 nm.
2. WST-1 assay. This procedure measures the cell viability with a colorimetric assay, based on the cleavage of tetrazolium salts by mitochondrial dehydrogenase in viable cells. The test procedures were as follows: 10 μL of the PreMix WST-1 reagent was added to each well, incubated for 4 hours, and read under 450 nm with the same ELISA reader.
3. CellTiter-Glo assay. This is a method of measuring the number of viable cells in a culture based on quantitation of the adenosine triphosphate (ATP) present, which signals the presence of metabolically active cells. The assay system uses the properties of a proprietary thermostable luciferase to enable reaction conditions that generate a stable glow-type luminescent signal while simultaneously inhibiting endogenous enzymes released during lysis (such as adenosine triphosphatase). The release of adenosine triphosphatase usually interferes with the accurate measurement of ATP, but this assay has overcome this problem.

The test procedures were as follows: 100 μL of CellTiter-Glo reagent was added and mixed for 2 minutes on an orbital shaker to induce cell lyses. All plates were incubated at room temperature for 10 minutes to stabilize the luminescence signal. Then 100 μL of medium was pipetted into a test tube, and the luminescence was recorded with a luminometer. All detected values were calculated by comparison to data of the first column of the 96-well plate and were changed into percentages to obtain the survival rate. Data of the last column were compared to those of the first column as an internal control.

Cytotoxicity testing of bonding agent components after curing

Cells were prepared using the procedures described above; the test materials were prepared by the following procedures. First, the primer was painted on a transparent polyethylene sheet (5 × 5 mm) on one side using the tip of a disposable brush and left in place for 20 seconds, and then the volatile ingredients were evaporated with a mild oil-free air system. Second, the bonding agent was painted over the primer with another brush and light-cured for 10 seconds using a dental curing light (Visilux 2;

3M, St. Paul, USA) with an irradiation wave length of 420–500 nm). The products provided in the one-bottle system were prepared by the second procedure only. In addition to the nine commercial products, based on formerly published papers,[12–14] we prepared one experimental bonding agent in this study by mixing 70 wt% UDMA, 30 wt% TEGDMA, 1 wt% camphorquinone, and 1.5 wt% DAEMA, and used these like the one-bottle system. All prepared specimens were placed in 1 mL of medium in a 24-well plate for 24 hours, after which they were removed and used in the cytotoxicity test.

Statistical analysis

Eight samples of each test specimen were collected in a 96-well plate and tested. SAS version 9.1.3 software (SAS Institute, Cary, NC, USA) was used for the statistical analysis. One-way analysis of variance (ANOVA) was used to evaluate the effect of cytotoxicity of resin bonding materials on human oral epithelial cells. Scheffé test was then used to carry out *post hoc* pairwise comparisons when the ANOVA showed a significant main effect of the material.

Results

Cytotoxicity of the components of uncured bonding agents

The cytotoxicities of the various components of uncured bonding agents at different concentrations using different assay systems are listed in Tables 2 and 3. Table 2 displays the results of cytotoxicity of 0.1 vol% of the uncured bonding agents. By MTS assay, Clearfil SE Bond bonding agent (survival rate, 23.6%±5.0%), TEGDMA (survival rate, 51.9%±5.1%), and 3M Single Bond (survival rate, 54.2%±8.4%) were found to be relatively more cytotoxic. By the WST-1 assay, the most cytotoxic material was the UniFil Bond bonding agent (survival rate, 21.4%±1.4%), followed by 3M Single

Table 2. Cytotoxicity of 0.1 wt% pre-cured bonding agents components (survival rate, %) (n=8)

	MTS assay	WST-1 assay	CellTiter-Glo assay
Tested component			
Clearfil Protect Bond A	99.9±8.3	68.9±3.1	80.2±14.3
Clearfil Protect Bond B	92.0±7.7	56.8±19.3	76.9±5.4
Clearfil SE Bond A	106.8±12.4	32.7±5.4	92.3±6.5
Clearfil SE Bond B	23.6±5.0	56.1±6.9	79.8±4.7
Prime & Bond NT A	94.1±6.8	97.2±6.7	106.3±7.1
Prime & Bond NT B	95.6±3.4	52.3±2.7	99.1±9.6
Prisma Universal Bond 3A	99.5±4.7	60.9±7.7	70.0±8.4
Prisma Universal Bond 3B	80.6±8.2	50.3±4.3	56.9±6.9
UniFil Bond A	101.8±3.5	107.1±7.7	113.1±19.1
UniFil Bond B	90.12±4.3	21.4±1.4	59.0±16.3
3M Single Bond	54.2±8.4	31.8±0.8	69.7±4.9
CharmBond	92.8±4.9	37.8±3.8	87.2±6.3
Compobond	75.9±7.7	102.6±4.6	87.2±9.3
ExciTE	87.7±8.0	32.8±1.3	81.3±8.2
MMA	96.0±1.9	92.8±3.2	29.7±8.5
TEGDMA	51.9±5.1	46.9±5.2	58.1±3.3
Control*	100.6±4.9	106.5±11.9	96.6±10.9
P (F test)	<0.0001	<0.0001	<0.0001
Scheffé test	4<16,11,13,8,14,10,2,12,5,6,15,7,1,17,9,3 16<11,13,8,14,10,2,12,5,6,15,7,1,17,9,3 11<8,14,10,2,12,5,6,15,7,1,17,9,3 13<7,1,17,9,3 8<3	10,4<6,2,1,8,15,5,7,13,17,3,9 11,14,12<1,8,15,5,7,13,17,3,9 16,6<8,15,5,7,13,17,3,9 2<15,5,7,13,17,3,9 1<5,7,13,17,3,9 8<17,3,9	15<11,7,2,4,1,14,12,13,3,17,6,5,9 8,16,10<3,17,6,5,9 11,7<5,9 2,4<9

*Without test material. A = primer or conditioner; B = bonding agent or adhesive; MMA = methyl methacrylate; TEGDMA = triethylene glycol dimethacrylate.

Table 3. Cytotoxicity of 1 wt% pre-cured bonding agents components (survival rate, %) (n=8)

	MTS assay	WST-1 assay	CellTiter-Glo assay
Bonding agent			
Clearfil Protect Bond A	30.5±3.5	50.6±12.8	59.2±10.4
Clearfil Protect Bond B	50.8±4.6	24.6±6.4	51.2±10.2
Clearfil SE Bond A	37.9±3.1	53.0±12.9	11.8±3.1
Clearfil SE Bond B	30.9±1.4	30.1±9.2	6.3±5.7
Prime & Bond NT A	86.3±2.2	49.7±8.1	58.9±19.3
Prime & Bond NT B	25.7±2.0	29.2±2.3	256.6±50.6
Prisma Universal Bond 3A	17.7±0.9	36.6±8.6	0.2±0.02
Prisma Universal Bond 3B	26.8±1.4	22.3±2.8	50.2±9.5
UniFil Bond A	79.2±3.6	50.6±7.5	89.8±32.2
UniFil Bond B	18.1±1.3	18.4±1.9	9.7±4.3
3M Single Bond	19.6±1.1	34.2±3.7	0.4±0.4
CharmBond	17.6±1.0	32.7±4.6	1.7±0.4
Compobond	41.3±2.9	66.4±7.6	0.3±0.2
ExciTE	18.1±1.0	37.1±6.1	24.6±12.9
MMA	96.5±1.3	147.3±10.8	106.7±17.9
TEGDMA	16.5±0.9	34.8±6.73	0.2±0.3
Control*	100.6±5.2	106.2±11.1	102.2±13.4
P (F test)	<0.0001	<0.0001	<0.0001
Scheffé test	16<6,8,1,4,3,13, 2,9,5,15,17 12,7,14<8,1,4,3,13, 2,9,5,15,17 10,11<1,4,3,13,2, 9,5,15,17 6,8<3,13,2,9,5,15,17 1,4<13,2,9,5,15,17 3,13<2,9,5,15,17 2<9,5,15,17 9,5<15,17	10,8<5,9,1,3, 13,17,15 2<3,13,17,15 6,4,12,11,16,7,14,5, 9,1<13,17,15 3<17,15 13<17,15 17<15	7,16,13,11<5,1,9, 17,15,6 12,4,10,3,14<9, 17,15,6 8,2,5,1,9,17,15<6

*Without tested material. A = primer or conditioner; B = bonding agent or adhesive; MMA = methyl methacrylate; TEGDMA = triethylene glycol dimethacrylate.

Bond (survival rate, 31.8%±0.8%) and the primer of Clearfil SE Bond (survival rate, 32.7%±5.4%). By the CellTiter-Glo assay, the most cytotoxic material was MMA (survival rate, 29.7%±8.5%), followed by the Prisma Universal Bond 3 adhesive (survival rate, 56.9%±6.9%) and TEGDMA (survival rate, 58.1%±3.3%). Table 3 shows the results of cytotoxicity of 1.0 vol% uncured bonding agents. When tested by the MTS assay, the most cytotoxic material was TEGDMA (survival rate, 16.5%±0.9%), followed by CharmBond (survival rate, 17.6%±1.0%) and the Prisma Universal Bond 3 primer (survival rate, 17.7%±0.9%). By the WST-1 assay, the most cytotoxic material was the bonding agent of UniFil Bond (survival rate, 18.4%±1.9%), followed by the Prisma Universal Bond 3 adhesive (survival rate, 22.3%±2.8%) and the Clearfil Protect Bond bonding agent (survival rate, 24.6%±6.4%). By the CellTiter-Glo assay, the most cytotoxic material was the Prisma Universal Bond 3 adhesive (survival rate, 0.2%±0.02%), followed by TEGDMA (survival rate, 0.2%±0.3%) and Compobond (survival rate, 0.3%±0.2%). Because different assay systems showed different results for the same component, it seems less significant to rank the cytotoxicity of the tested commercial products using only one test method.

Cytotoxicity of components of post-cured bonding agents

The cytotoxicities of various bonding agents after curing are listed in Table 4. According to the test results, most bonding agents were non-cytotoxic. For the MTS assay, only 3M Single Bond (survival rate, 64.5%±5.8%) showed cytotoxicity. For the WST-1 assay, only Compobond (survival rate, 29.1%±7.1%) revealed remarkable cytotoxicity. For the CellTiter-Glo assay, the most cytotoxic material was 3M Single Bond (survival rate, 52.4%±7.9%). It appears that among the post-cured bonding agents, only 3M Single Bond and Compobond exhibited any cytotoxicity.

Table 4. Cytotoxicity of components of post-cured bonding agents (survival rate, %) (n=8)

	MTS assay	WST-1 assay	CellTiter-Glo assay
Tested materials			
Clearfil Protect Bond	112.8±9.0	112.9±17.3	99.1±8.9
Clearfil SE Bond	108.8±6.3	96.3±23.0	119.8±4.1
Prime & Bond NT	102.0±10.9	100.8±22.2	108.9±13.1
Prisma Universal Bond 3	86.2±9.7	101.1±9.3	70.6±14.2
UniFil Bond	94.1±5.8	80.5±4.1	111.5±8.1
3M Single Bond	64.5±5.8	97.4±17.8	52.4±7.9
CharmBond	106.5±9.5	100.6±9.4	77.2±6.3
Compobond	79.5±6.7	29.1±7.1	63.5±10.2
ExciTE	118.4±9.8	91.0±14.8	102.7±19.1
Experiment	99.7±9.9	108.3±22.6	76.6±4.9
Control	91.2±8.3	110.4±12.3	93.2±5.9
P (F test)	<0.0001	<0.0001	<0.0001
Scheffé test	6<11,5,10,3,7,2,1,9 8<7,2,1,9 4<1,9 11<9	8<5,9,2,6,7,3,4,10,11,1	6<11,1,9,3,5,2 8<11,1,9,3,5,2 4<9,3,5,2 10<3,5,2 7<3,5,2

Discussion

Dental bonding agents have greatly improved in recent years. To improve bonding strength, components have evolved from simple etching of and bonding with the enamel to the use of a so-called dentin conditioner (to etch the dentin surface), a dentin primer (to increase the surface energy and improving wetting), and an adhesive (to form a hybrid layer). Currently, many products have an option of total etching as part of their procedures. The commercial products include single- and multiple-bottle systems. The rinsing procedure after etching was omitted.[15,16] For these improvements, we thought that the bonding agent is nearly perfect in terms of both function and usage. But because these materials are used in the human body, attention to their biocompatibility requires a high level of concern. In this study, we detected the effects of some currently available commercial bonding products on cell viability by evaluating the cytotoxicity of these materials.

Many types of cells are used for cytotoxicity tests in dental research, most of which can be categorized into two broad groups: (1) those derived from animals (e.g., V79, L-929 and 3T3 cells[17–19]) and those obtained from human oral tissues (such as human gingival fibroblasts, dental pulpal fibroblasts, and oral epithelial cells[6,20,21]). Yamagata and Oshima[22] tested the cytotoxicity of glass ionomer, composite resin, and amalgam on cells obtained from human gingival tissue, pulp tissue, and mouse subcutaneous tissue. They found that the results differed with the materials and cells used, with cells from gingival tissue exhibiting the lowest sensitivity. Schweikl and Schmalz[23] used mouse fibroblasts (L-929 cells) and human gingival fibroblasts to evaluate the cytotoxicity of glass ionomer cement, composite resin, and zinc phosphate cement, and found that human gingival fibroblasts had a weaker response to cytotoxicity. Geurtsen et al.[19] used fibroblasts from mouse lung tissue (3T3 cells) and three fibroblasts from human oral tissues (gingiva, pulp, and periodontal ligament) to detect 35 monomers of dental composite resins. Their results indicated that cells from the pulp and periodontal ligament were more sensitive than those from gingival tissue or 3T3 cells. Since all previous studies demonstrated that gingival tissue is the least sensitive, we chose oral epithelial cells (KB cells) for this experiment. Because this is a cancerous cell line, its reaction to cytotoxic materials may differ from those of primary cells.[24] However, since it is easily maintained in culture, exhibits high reproducibility and can be used for oral epithelial cell tests,[25,26] we considered this cell line to be appropriate for use in the present study.

In order to obtain objective results, we used two detection systems (the MTS and WST-1 assay systems) to detect cell viability. Because the MTS formazan product is soluble in tissue culture medium, this assay system requires fewer steps than other kinds of measuring systems that use tetrazolium compounds such as MTT or *p*-iodonitrotetrazolium violet.[27] The WST-1 system includes other tetrazolium salts. The manufacturer declared it is more sensitive than the MTT assay system and can be used to detect a cytotoxic effect or inhibition of

cell growth induced by a chemical or drug.[28] The reason to choose two similar assay systems was not to compare which method is better but simply to obtain consistent results from the two methods. In addition, we also chose another assay system, the CellTiter-Glo system. It is a homogeneous method of determining the number of viable cells in culture based on quantitation of ATP, which signals the presence of metabolically active cells. On the basis of the manufacturer's claims, it is suitable for screening cell proliferation and cytotoxicity,[29] and it is also more sensitive than the other methods.[30]

Comparing the results obtained by the three methods, we found that the same components had different results with the different test methods, even though the MTS and WST-1 systems have similar reaction mechanisms (Tables 2 and 3). Among the data of the three assay systems, most of the uncured tested materials revealed that the higher the concentration of the test material, the fewer cells that survived. Two test materials, however, presented different patterns. The Prime & Bond NT bonding agent in CellTiter-Glo assay and the MMA monomer in the WST-1 and CellTiter-Glo assays showed inconsistency in the cytotoxicity results. Because some other studies on cell viability *in vitro* also produced inconsistent results,[31–33] we retained these peculiar data (survival rate of 256.6% for Prime & Bond NT bonding agent in the CellTiter-Glo assay) for reference. Compared with the control group, a phenomenon of MMA increasing cell growth in the WST-1 and CellTiter-Glo assays at a 1.0 vol% concentration was observed, as shown in Table 3. At the moment, we do not understand why this material had the ability to promote cell growth, but it was reported to have less cytotoxicity.[34]

Although these diverse data are certainly difficult to explain, the results of the internal control were in an acceptable range, so we think that most of the measured data are reliable in each assay. This also supports our previous scruple that evaluations of the cell viability of dental materials cannot be ranked by just a single testing method. Although we had no basis to judge which of the methods used in this study was the most accurate, we think these assays can indicate the degree of cytotoxicity. In addition, our statistical analysis had good power and was conservative. In the one-way ANOVA study, sample sizes of 8, 8, 8, 8, 8, 8, 8, 8, 8, and 8 were obtained from the 10 groups the means of which (supposed to be 100, 90, 90, 90, 90, 90, 90, 90, 90, and 90) were compared. The total sample of 80 subjects achieved 97% power to detect differences among the means versus the alternative of equal means using an F test with a 0.05 significance level (if we supposed the size of the variation in the means is represented by their standard deviation which is 3.00, and the common standard deviation within a group is assumed to be 5.00). When the F test was significant, we chose the Scheffé test to do the *post hoc* pairwise comparison, since it is a valid, fairly conservative test, and therefore, sufficiently general to be applicable to analyze our data which may have some unexpected extremes.

At a 0.1 vol% concentration, half of the tested materials (Clearfil Protect Bond primer, Clearfil SE Bond primer, Prime & Bond NT conditioner, UniFil Bond primer, CharmBond, Compobond, MMA) showed little effect on cell viability; and in the 1.0 vol% condition, 11 components revealed a greater cytotoxic effect on cell viability. Among the two-bottle systems, all bonding components appeared to have greater cytotoxic effects and were more viscous than the primer or conditioner components. It seems that, in addition to the chemical toxicity, the highly viscous liquid that covered the cell surface caused interference with cell metabolism and may be one of the factors in the cytotoxic effect. In this study, all single-bottle systems also showed greater cytotoxicity than the conditioners or primer components of the two-bottle systems. The TEGDMA was more viscous than the MMA monomer and showed greater cytotoxicity in this study. Ratanasathien et al.[2] investigated the cytotoxicity of four dentin bonding components on mouse fibroblasts by the MTT assay. They ranked the degree of cytotoxicity in descending order as: BisGMA, UDMA, TEGDMA, and HEMA. Yoshii[35] evaluated the cytotoxic effect on HeLa S3 cells by the MTT assay and obtained the following ranking in descending order: BisGMA, UDMA, TEGDMA, HEMA, and MMA. Both rankings also coincided with the degree of their viscosity. Whether or not the viscosities of materials influence cell viability will be the next step in our investigation.

Costa et al.[36] evaluated the cytotoxic effect of three dental adhesive systems on an immortalized mouse odontoblast cell line. They tested uncured fresh adhesives by counting the cell numbers with scanning electron microscope, and tested the cured adhesives by an inhibition zone test. The cell morphology was discerned under scanning electron microscope, and an MTT assay was performed to evaluate mitochondrial respiration. They found that fresh adhesives exhibited more toxic effects than polymerized adhesives, and in the polymerized group, some cells grew in the walls despite the persistent cytotoxic effects. Szep et al.[37] tested the cytotoxicity of six dentin adhesives on primary human gingival fibroblasts by applying the dentin adhesive on glass slides, and placed them in a Petri dish after curing, then added the culture

medium to obtain the eluate for the toxicity test. After 3 days of culture, they examined the cell morphology and counted the number of cells under a phase-contrast microscope, and found that all test materials had undergone different degrees of cytotoxic reactions. Huang and Chang[38] used an MTT assay to investigate the cytotoxicity of five different dentin bonding agents in a large number of human pulp cells, and found that elutes of post-cured test materials had significant potential for pulpal toxicity. Kaga et al.[18] investigated the relationship between the monomers eluted from dentin bonding systems and their cytotoxicity, and found that the primer and uncured adhesives exhibited variable cytotoxicities, but the cytotoxicities decreased as the photoactivation time increased. The amount of monomers eluted from the cured adhesives was almost undetectable and did not reach a sufficient concentration to suppress cell viability or cell growth. In our study, most of the uncured materials had remarkable cytotoxic effects, especially at higher concentrations (Table 3), and most of the post-cured specimens revealed no cytotoxicity except for the 3M Single Bond and Compobond (Table 4). On the whole, our results are in complete agreement with those from previous studies. The absence of cytotoxic effects for post-cured materials might have been due to most of the bonding agent components having been polymerized and so less was eluted. This can be verified using our prepared experiment bonding agent; although the resin matrixes UDMA and TEGDMA are cytotoxic materials, they showed the same level of safety as most commercial products after curing. However, if the curing is incomplete, the residual monomer might irritate the tissues, and hence the cytotoxicity of resin bonding agents needs to be considered when they are used in clinical applications.

According to the results of this study, all the uncured components of the tested resin bonding agents had cytotoxic effects on human oral epithelial cells. Some cured commercial products also had a cytotoxic effect. Because the same material tested in different assays may give different results, testing using only one method is not sufficient to judge the cytotoxic effect. It would be more reliable if the results of a tested material were similar in the different tests.

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ELSEVIER

ORIGINAL ARTICLE

Profile of nonsurgical root canal treatment under the National Health Insurance in Taiwan in 2006

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Background/purpose: Nonsurgical root canal treatment (NSRCT) is a valuable dental procedure, because it is reported to have a high 5-year tooth retention rates of >90% in Taiwan. The purpose of this study was to investigate the pattern of NSRCT by analyzing different age groups of patients and tooth types according to different age groups of patients in Taiwan in 2006.
Materials and methods: Data on the population were obtained from a database of the Ministry of Interior, Taiwan, and the data of the NSRCT were obtained from a database of the Taiwan Dental Association. The population older than 5 years was divided into seven age groups, and the treatment rate and number of permanent tooth which received NSRCT per 1000 patients in each age group were analyzed.
Results: The results found that 8,865,201 patients received dental treatment, and 1,907,325 permanent teeth received NSRCT in a total population of 21,783,585. This gave an overall dental treatment rate of 40.7%, and overall number of NSRCTs of 215.1 teeth per 1000 patients. The number of NSRCTs per 1000 patients gradually increased from 34.6 in the 5- to 14-year age group to a peak of 342.5 in the 55- to 64-year age group and then slightly declined to 322.5 in the oldest age group of ≥65 years. The most frequent tooth type which received NSRCT was first molars (21.0%), followed by second premolars (18.4%), second molars (16.7%), first premolars (13.0%), central incisors (10.6%), lateral incisors (9.7%), canines (9.0%), and third molars (1.9%).
Conclusion: The overall NSRCT rate per 1000 patients was 215.1 teeth, the incidence of NSRCT increased with the age of the patients, and the frequency of canine treatment increased with the age of patients.

Introduction

Root canal treatment (RCT) is a basic part of comprehensive quality dental care. The incidence of RCT increases with the age of the population group at 3–6% for younger adults to 18–20% for patients older than 60 years.[1] The relative proportions of tooth types that require RCT considerably vary by

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age cohort, and therapy for all teeth peaks in the 55- to 64-year age group.[2]

In Taiwan, the National Health Insurance (NHI) plan was implemented in March 1995. Almost 95% of the total population (>22 million) participate in this insurance plan. Because dentists have to claim each dental treatment to the Bureau of NHI (BNHI) through the Taiwan Dental Association in order to get their service fees, the computerized dental database for every insured individual has been kept since March 1995. The national records of the Taiwan Dental Association are valuable to assist the study of the frequency of nonsurgical RCT (NSRCT) of the permanent dentition in different age groups of patients in Taiwan.

Materials and methods

Data on the population in Taiwan were obtained from the Ministry of Interior, Taiwan, and dental treatment records including the total number of dental treatments and the total number of NSRCT were obtained from the database of the Taiwan Dental Association. The dental records are a computerized database that includes all dental treatments. The present study only investigated and analyzed dental treatments claimed in 2006.

In this study, in order to match the age groups posted by the Ministry of the Interior, Taiwan, patient data were divided into seven age groups (5–14, 15–24, 25–34, 35–44, 45–54, 55–64 and ≥65 years).

The dental treatment rate was analyzed in the seven age groups by the percentage of the population which received services of the NHI in 2006. Numbers of permanent teeth which underwent NSRCT per 1000 patients were analyzed in the seven age groups of patients and by eight tooth types (central incisors, lateral incisors, canines, first premolars, second premolars, first molars, second molars, and third molars) according to different age groups of patients in Taiwan.

In total, data on 1,907,325 permanent teeth which claimed NSRCT in 2006 were collected by searching the database for the specific BNHI procedure codes, which represent NSRCT performed on teeth with one (90001C), two (90002C), or three or more root canals (90003C).

Results

Dental treatment rates and numbers of NSRCT per 1000 patients in different age groups of patients in Taiwan in 2006 are shown in Table 1. In 2006, the total population of Taiwan was 22,876,527, among which 21,783,585 individuals were older than 5 years. Of the 9,090,766 patients who received dental treatment, 8,865,201 were older than 5 years. This gave an overall dental treatment rate of 40.7% for the total number of individuals older than 5 years of age. We found that individuals in the age group of 5–14 years had the highest dental treatment rate (58.4%), followed in descending order by individuals in the age groups of 25–34 (41.1%), 55–64 (38.2%), 45–54 (37.9%), 15–24 (37.4%), and 35–44 years (37.3%). The individuals in the oldest age group of ≥ 65 years had the lowest dental use rate (33.3%).

In total, 8,865,201 patients underwent different kinds of dental treatments, and 1,907,325 permanent teeth received NSRCT. Thus, the overall number of NSRCT per 1000 patients was 215.1. The number of NSRCT per 1000 patients gradually increased from 34.6 in the youngest age group of 5–14 years to a peak of 342.5 in the next older age group of 55–64 years and then declined slightly to 322.5 in the oldest age group of ≥ 65 years.

Of those 1,907,325 teeth that received NSRCT, 557,333 were anterior teeth (29.2%), 597,365 were premolars (31.3%), and 752,727 were molars (39.5%). Numbers of NSRCT per 1000 patients for the different tooth types according to different age groups of patients in Taiwan in 2006 are listed in Table 2. The present study found that the most frequent

Table 1. Dental use rates and number of nonsurgical root crown treatments (NSRCTs) in different age groups of patients in Taiwan in 2006

Age (yr)	Population, *n* (%)	Patients, *n* (%)	Dental use rate (%)	NSRCT, *n* (%)	No. of NSRCTs/ 1000 patients
5–14	3,052,689 (14.0)	1,782,359 (20.1)	58.4	61,740 (3.2)	34.6
15–24	3,369,155 (15.5)	1,260,737 (14.2)	37.4	254,697 (13.4)	202.0
25–34	3,834,785 (17.6)	1,574,775 (17.8)	41.1	336,103 (17.6)	213.4
35–44	3,777,807 (17.3)	1,410,685 (15.9)	37.3	356,976 (18.7)	253.1
45–54	3,478,994 (16.0)	1,317,717 (14.9)	37.9	392,816 (20.6)	298.1
55–64	1,983,126 (9.1)	757,249 (8.5)	38.2	259,383 (13.6)	342.5
≥65	2,287,029 (10.5)	761,679 (8.6)	33.3	245,610 (12.9)	322.5
All ages	21,783,585 (100)	8,865,201 (100)	40.7	1,907,325 (100)	215.1

Table 2. Number of nonsurgical root canal treatments (NSRCTs) per 1000 patients and frequency in different tooth types according to different age groups of patients in Taiwan in 2006*†

Age (yr)	Tooth type								
	Central incisors	Lateral incisors	Canines	First premolars	Second premolars	First molars	Second molars	Third molars	All
5–14	6.7 (19.4)	3.7 (10.7)	0.3 (0.9)	1.6 (4.6)	4.1 (11.8)	16.5 (47.7)	1.8 (5.2)	0.0 (0.0)	34.6
15–24	31.0 (15.3)	24.0 (11.9)	5.6 (2.8)	18.1 (9.0)	34.7 (17.2)	52.1 (25.8)	34.8 (17.2)	1.8 (0.9)	202.0
25–34	23.9 (11.2)	19.6 (9.2)	9.5 (4.5)	24.4 (11.4)	43.1 (20.2)	47.1 (22.1)	40.9 (19.2)	4.7 (2.2)	213.4
35–44	20.7 (8.2)	19.2 (7.6)	17.1 (6.8)	33.3 (13.2)	51.2 (20.2)	55.2 (21.8)	50.8 (20.1)	5.6 (2.2)	253.1
45–54	22.9 (7.7)	24.6 (8.3)	30.2 (10.1)	43.6 (14.6)	56.2 (18.9)	60.9 (20.4)	53.8 (18.0)	6.0 (2.0)	298.1
55–64	31.3 (9.1)	35.0 (10.2)	48.2 (14.1)	53.1 (15.5)	60.3 (17.6)	55.9 (16.3)	51.6 (15.1)	7.2 (2.1)	342.5
≥65	38.4 (11.9)	41.5 (12.9)	63.1 (19.6)	51.2 (15.9)	51.1 (15.8)	39.0 (12.1)	32.8 (10.2)	5.5 (1.7)	322.5
All ages	22.7 (10.6)	20.9 (9.7)	19.3 (9.0)	27.9 (13.0)	39.5 (18.4)	45.1 (21.0)	35.9 (16.7)	4.0 (1.9)	215.1

*Data are presented as *n* (%); †percentages in each age category may not add up to 100% because of rounding.

tooth type to receive NSRCT was first molars (21.0%), followed by second premolars (18.4%), second molars (16.7%), first premolars (13.0%), central incisors (10.6%), lateral incisors (9.7%), canine (9.0%), and third molars (1.9%). In addition, first molars had the highest number of NSRCT per 1000 patients (45.1), followed in descending order by second premolars (39.5), second molars (35.9), first premolars (27.9), central incisors (22.7), lateral incisors (20.9), canines (19.3), and third molars (4.0).

The frequency of anterior teeth that received NSRCT increased with the age of patients, and canines became the most frequent tooth type of NSRCT in oldest age group of ≥65 years.

Discussion

The NHI plan of Taiwan began in March 1995. More than 95% of the population is covered by this insurance plan. By accessing the database of the Taiwan Dental Association, we can study the epidemiology of NSRCT in different age groups of patients and for different tooth types in Taiwan. In this study, we found that the overall dental use rate of patients older than 5 years in Taiwan in 2006 was 40.7%; this dental use rate was lower than that (43.2%) reported in the US in 1996,[3] and that (70%) reported in Denmark in 2003.[4] Patients in the 5- to 14-year age group had the highest dental use rate of 58.4%; this rate was even higher than that (52.5%) of the 6- to 18-year age group reported in the US in 1996. This high dental use rate may be due to the comprehensive oral examination by dentists for every grade 1 and 4 students in elementary schools in Taiwan. Students with detected dental defects are asked to see their dentists for further dental treatments. The dental use rate of patients in the age group 65 years and older was the lowest at 33.3%, which was much lower than the 41.3% reported in the US in 1996;[3] this lower dental use rate in Taiwan may be due to fewer teeth retained in the oral cavity in elderly patients in Taiwan than in the US and the lower total income per person in Taiwan than in the US.

Factors causing the greatest dissatisfaction with RCT were cost, time consumption, and pain.[5] The overall number of NSRCTs per 1000 patients in Taiwan was 215.1, which was about three times that (approximately 66) of Denmark.[4] This high overall incidence may be attributed to fact that the NSRCT is covered by the NHI in Taiwan, and this may encourage patients to receive more RCT if needed, or due to oral health in Taiwan still being much poorer than that in Denmark.

RCT is covered by the NHI in Taiwan, but there are still some patients who ask endodontic specialists for higher-quality treatment which they pay for by themselves; therefore, the actual number may be a little bit higher.

Although previous studies reported a relatively lower frequency of high technical quality for root canal filling ranging from 14% to 65%,[6–8] the NSRCT yielded a high success rate of >90%.[9] Epidemiologic studies also showed that although 16.8–64.5% of root canal-treated teeth had apical periodontitis, most of them still retained their function after treatment.[10] Furthermore, three epidemiologic studies of large patient populations performed in the US[11,12] and Taiwan[13] showed that the long-term tooth retention rates after NSRCT were as high as 92.9–97%. Other studies also demonstrated that the chance of root canal-treated teeth remaining functional over time was 91–97%.[14] These results suggest that NSRCT is a valuable and effective treatment for saving teeth.

Previous studies showed that the incidence of RCT increases with age,[2,4] and the cutoff points

of different age groups in the present study differed from those of previous studies. Our results showed a comparable tendency in the incidence of RCT. A recent study from Taiwan[13] showed that the percentages of each tooth type that received NSRCT in 1998 were 23.7% for anterior teeth, 29.8% for premolars, and 46.5% for molars. The present study also showed that more molars (39.5%) received NSRCT than premolars (31.3%) or anterior teeth (29.2%).

The most frequent tooth type treated by NSRCT in the present study was first molars (21.0%), followed by second premolars (18.4%), second molars (16.7%), first premolars (13.0%), central incisors (10.6%), lateral incisors (9.7%), canines (9.0%), and third molars (1.9%); this distribution of NSRCT in different types was similar to that reported in a previous study.[2] However, when we analyzed the incidences of NSRCT in different tooth types in each age group, we found that first molars had the highest incidence of NSRCT in the age groups of 5–14, 15–24, 25–34, 35–44, and 45–54 years. This finding may have been due to the fact that the first molar is the first erupted tooth which has the highest incidence of dental caries. In the age group of 5–14 years, the incidence of NSRCT for central incisors was higher than that for the other tooth types except for first molars, and the high incidence of dental trauma of central incisors in this age group might explain this situation. Second premolars had the highest incidence of NSRCT in the age group of 55–64 years. Canines had the highest incidence of NSRCT in the age group of ≥65 years. These results may indicate the earlier loss of molars than second premolars and canines in elderly patients; also, canines are mostly used as abutment of overdentures and require NSRCT before making removable dentures for patients ≥65 years.

In conclusion, the present study showed that the incidence of NSRCT in Taiwan in 2006 increased with age. The most frequent tooth type that received NSRCT was first molars, followed in descending order by second premolars, second molars, first premolars, central incisors, lateral incisors, canines, and third molars. The frequency of treatment of anterior teeth increased with the age of patients.

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ELSEVIER

ORIGINAL ARTICLE

Increased oral lichen planus in a chronic hepatitis patient associated with elevated transaminase levels before and after interferon/ribavirin therapy

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Background/purpose: Oral lichen planus (OLP) is the most frequent oral lesion found in patients with hepatitis C virus (HCV) infection. The aims of this study were to investigate the prevalence of OLP among chronic hepatitis C patients, to clarify the role of HCV in the pathogenesis of OLP, and to assess its relationship to transaminase levels.
Materials and methods: Two groups of subjects were studied; 277 hepatitis C patients were examined for OLP (Group 1) and 5273 outpatients seeking dental care within 1 year were used as a control (Group 2) to determine the prevalence of OLP in the general population. The dental and hepatic records were collected and analyzed.
Results: The prevalences of OLP were 4.7% (n=13) in Group 1 and 2.0% (n=104) in Group 2 and significantly differed (P=0.002). All 13 OLP cases occurred in hepatitis C patients who had experienced elevated alanine transaminase levels of >80 IU/L within the 2 previous years, regardless of whether they were treated with interferon-ribavirin combination therapy or not. There was a strong association between elevated transaminase levels and the development of HCV-related OLP lesions (P=0.014). Of the 13 OLP patients, two were in the group with a sustained virologic response (SVR) to HCV therapy, two were in the group without an SVR, and nine were in the non-therapy group. The incidence of OLP in hepatitis C patients did not significantly differ between those who showed an SVR to HCV therapy and those who did not respond or did not receive therapy (P=0.560).
Conclusion: We concluded that: (1) elevation of transaminase levels is associated with the detection of HCV-related OLP, and (2) HCV-related OLP can remain unchanged for years after an SVR to HCV therapy. The findings revealed that the role of HCV in OLP pathogenesis is due to host factors induced by HCV rather than a direct cytopathic effect of HCV.

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Introduction

Oral lichen planus (OLP) is frequently seen in patients with chronic hepatitis C virus (HCV) infection and was reported to be an extrahepatic manifestation of HCV.[1–3] Many studies associated OLP with HCV, especially in HCV-endemic areas such as southern Europe and Japan, although the mechanisms of HCV in OLP pathogenesis are unclear.[1,4–9]

HCV RNA was detected in the saliva and oral mucosa of patients with chronic hepatitis C. Persistent HCV infection of the oral epithelium may trigger a host immune response and result in HCV-related OLP. However, the underlying mechanism remains unclear.[1,9–11] Chung et al.[12] found that elevation of transaminase levels significantly increased the risk of atrophic-erosive OLP, particularly in HCV patients. Ali and Suresh[13] reported a strong association between elevated transaminase levels and detection of erosive OLP, but no correlation between OLP and HCV infection was determined in their study. Nagao et al.[8,14] observed HCV-related OLP in patients with severe liver dysfunction and also in patients without it. Further, there were no significant differences in serum HCV RNA levels and HCV genotypes between the HCV with OLP group and the HCV without OLP group. Arrieta et al.[15] detected HCV replicates in epithelial cells of anti-HCV-positive patients with and without OLP. Those reports suggest that host factors induced by HCV infection are more important than viral factors in the pathogenesis of HCV-related OLP.

Combination therapy with interferon (IFN) and ribavirin is the current standard treatment for chronic HCV infections. Some reported the therapeutic effects of IFN on OLP lesions, but others reported that OLP can be aggravated or triggered by IFN.[16,17]

There is no report describing the therapeutic effect of IFN and ribavirin for HCV-related OLP in chronic hepatitis C patients. Also, controversy and uncertainty regarding the association of the activity of HCV in OLP led us to conduct this study. The aims of this study were to investigate the prevalence of OLP among patients with chronic hepatitis C in southern Taiwan, to clarify the role of HCV in the pathogenesis of OLP by investigating the progression and occurrence of OLP in HCV patients treated with IFN plus ribavirin, and to assess the possible association of transaminase levels in OLP. We conducted oral examinations of chronic hepatitis C patients with the investigators blinded to their HCV status.

Material and methods

Two hundred and seventy-seven consecutive patients (152 women and 125 men), aged 34–87 years (mean, 60.5±10.7 years) with a diagnosis of chronic HCV infection and who had been followed up for at least 2 years at the Division of Hepato-Gastroenterology of Kaohsiung Chang Gung Memorial Hospital, were examined for OLP (Group 1), and 5273 outpatients seeking dental treatment at the Department of Oral Pathology and Family Dentistry within 1 year were used as the control group (Group 2) to determine the prevalence of OLP in the general population. All patients enrolled in the study came from the same general population in southern Taiwan. With the investigators blinded to a patient's medical history including his/her HCV status and whether he/she had received HCV treatment, OLP was observed through a clinical examination by a well-trained family dentist, and then double-checked by an oral medicine specialist. The study was conducted from August 2007 to March 2009. A diagnosis of OLP was made on the basis of the recognized typical clinical features; in suspicious cases, a diagnosis was confirmed by a biopsy. Reticular OLP was defined by the presence of a lace-like network of grayish-white lines (Wickham's striae). Atrophic, erosive and bullous types of OLP lesions were accepted as a subtype only in the presence of reticular OLP elsewhere.[18–20] Participants were divided into a reticular OLP group and an atrophic-erosive OLP group according to the clinical type of lesions observed. Patients suspected of graft-to-host disease, or areca nut use or drug-induced lichenoid eruptions were excluded. Demographic data such as age, sex, past medical history, type of OLP lesion, and its duration were collected using a checklist after receiving a patient's consent.

HCV infection was diagnosed by detecting antibodies against HCV (anti-HCV, with microparticle enzyme immunoassay (AxSYM HCV version 3.0; Abbott Diagnostics, Chicago, IL, USA). HCV RNA by qualitative polymerase chain reaction (COBAS AMPLICOR HCV test version 2.0; Roche Diagnostics, Basel, Switzerland; with a lower limit of detection of 50 IU/mL) was detected when needed. Liver function tests including serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were measured, and serum AST and ALT levels of <40 IU/L were assumed to be normal. AST and ALT levels were estimated in all cases in Group 1 from several weeks to several months. A sustained virologic response (SVR) was defined by the absence of HCV RNA at 24 weeks after completion of HCV therapy. The goal of treatment for chronic HCV infection is an SVR accompanied by improvement in the liver damage. In this study, a therapeutic response was judged after IFN-ribavirin combination therapy to be a complete responder if there was an SVR with normalization of serum ALT

levels, and an incomplete responder if there was neither an SVR nor a return to normal transaminase levels. The incidences of OLP in the hepatitis C patients (Group 1) were compared between those with elevated transaminase levels of >80 IU/L (double the normal limits of the value of ALT) and those whose ALT levels never exceeded 80 IU/L and between those who showed an SVR to HCV therapy and those who did not respond (non-SVR) or did not receive therapy. An ultrasonographic scoring system consisting of the liver surface, parenchyma, vascular structure and splenic size was used to describe the severity of hepatic parenchymal damage.[21] In the scoring system, a score of 4 indicates a normal situation, scores of 5–7 indicate hepatic fibrosis, a score of 8 indicates early cirrhosis, and scores of 9 and higher indicate cirrhosis. The intraobserver reproducibility of our gastroenterologist was documented.[22] In this study, we defined scores of 4–6 as normal or mild hepatic fibrosis, while scores of 7 and higher were defined as definite and advanced hepatic fibrosis. The ultrasonographic evaluation of 277 HCV patients by this scoring system was used to investigate the correlation between the prevalence of OLP and the degree of liver abnormalities.

All collected data were analyzed by the computer package SPSS version 15.0 software (SPSS, Chicago, IL, USA). Chi-squared and Fisher's exact tests were used to analyze the association between qualitative variables. In all analyses, $P<0.05$ was considered statistically significant. The study was approved by the institutional review board of Chang Gung Memorial Hospital.

Results

OLP was the most prevalent oral lesion in hepatitis C patients (Group 1) with 13 cases (9 females and 4 males, 4.7%; Table 1). The remaining lesions consisted of leukoplakia in five cases (1.8%), aphthous stomatitis in three (1.1%), oral candidosis in two (0.7%), and cheilitis in two (0.7%) (Table 2). No abnormalities of the oral mucosa were found in the remaining 252 cases (91.0%). In the 13 HCV-related OLP patients, the painless reticular form of OLP was the most common oral lesion ($n=10$, 76.9%); only three showed atrophic-erosive OLP (23.1%), with the buccal mucosa being the most-often affected site. On the contrary, of the 104 OLP patients in Group 2, the most prevalent OLP lesion was the painful erosive type ($n=90$, 86.5%) and 14 patients (13.5%) showed reticular OLP. The clinical features of OLP between HCV patients (Group 1) and dental outpatients (Group 2) statistically differed ($P<0.0001$).

The prevalences of OLP were 4.7% in Group 1 and 2.0% in Group 2 with a significant difference ($P=0.002$). In total, 125 hepatitis C patients completed HCV therapy with IFN or peginterferon and ribavirin; 83 (66.4%) of them showed an SVR with a return to normal ALT levels, and 42 patients (33.6%) showed a non-SVR with elevated ALT levels. Because the hepatitis C status did not reach the therapeutic requirements of health insurance of Taiwan (a persistent ALT level of >80 IU/L and significant hepatic fibrosis), adverse effects from IFN, or financial problems, the other 152 hepatitis C patients did not receive HCV therapy or failed to complete therapy. Of the 13 HCV-related OLP patients, nine were in the non-therapy group (69.2%), two were in the non-SVR group (15.4%), and two were in the SVR group (15.4%). The OLP prevalence in the SVR group ($n=2$, 2.4%) was lower than that of the non-SVR group plus the non-therapy group ($n=11$, 5.7%). However, no significant difference was found ($P=0.240$; Table 3). The HCV-related OLP in two patients developed before HCV therapy and persisted after an SVR to therapy for several months to 1 year. All 13 OLP patients were hepatitis C patients who had experienced elevated ALT

Table 1. Age and sex distribution of 277 hepatitis C patients with oral lichen planus (OLP) lesions in Group 1

	Patients with OLP		
	Male	Female	Total
Age (yr)			
30–39	0/6	1/5	1/11
40–49	0/15	0/15	0/30
50–59	2/38	2/52	4/90
≥60	2/66	6/80	8/146
Total	4/125	9/152	13/277 (4.7%)

Table 2. Distribution of oral mucosal lesions in 277 hepatitis C patients (Group 1)

Oral mucosa condition	*n* (%)
Oral lichen planus	13 (4.7)
Leukoplakia	5 (1.8)
Aphthous ulcers	3 (1.1)
Oral candidosis	2 (0.7)
Cheilitis	2 (0.7)
Normal mucosa	252 (91.0)
Total	277 (100)

levels of >80 IU/L within the past 2 years, regardless of whether they were treated with HCV therapy or not. Elevated transaminase levels of >80 IU/L were significantly related to the development of OLP (P=0.014; Table 4).

Ultrasonographic scores of 7–9 indicating definite to advanced hepatic fibrosis were detected in eight of 13 HCV-related OLP patients, and scores of 4–6 indicating normal to mild hepatic fibrosis were found in the other five HCV-related OLP patients. When possible differences in OLP prevalence with respect to the degree of liver damage by the ultrasonographic scoring system between the advanced hepatic fibrosis group and the normal to mild hepatic fibrosis group were investigated, a significant difference between the groups was not found (P=0.566; Table 5).

Discussion

According to our study, the prevalence of OLP in southern Taiwanese patients with hepatitis C was 4.7%. This was significantly higher than the prevalence of OLP in the general population (2.0%), which was used as a control in our study (P=0.002). The prevalence of OLP in 277 hepatitis C patients (4.7%) was quite similar to the results of other authors, such as Figueiredo et al.[23] who reported a 4.7% incidence of OLP among 126 patients with HCV infection in Brazil, Nagao et al.[24] who reported a 4.8% incidence of OLP among 84 patients with HCV infection in Japan, and Pawlotsky et al.[25] who reported a 5% incidence of OLP among 61 patients with HCV infection in France. To avoid investigator bias, the two investigators who investigated all of the HCV patients were blinded to their hepatitis C activity. If patients with known HCV therapy or transaminase levels were more likely to be overdiagnosed or underdiagnosed with OLP, this would have resulted in arbitrary statistics. We did not find a higher OLP prevalence in hepatitis C patients treated with IFN and ribavirin, in contrast to reports of OLP being triggered or aggravated by IFN by other authors.[16,26–28] Furthermore, HCV-related OLP was observed to be more common in the non-therapy group (69.2%) than in the IFN-ribavirin combination therapy group (30.8%). However there was no significant difference (P=0.287). To our knowledge, this is the first report to investigate the prevalence of OLP among chronic hepatitis C patients in southern Taiwan, and it describes the effect of IFN-ribavirin combination therapy for chronic hepatitis C patients with HCV-related OLP.

Among 13 HCV-related OLP cases, the reticular form of OLP (76.9%) was the most prevalent oral

Table 3. Prevalence of oral lichen planus (OLP) in hepatitis C virus (HCV) patients (Group 1), stratified by a sustained virologic response (SVR) to HCV therapy with interferon and ribavirin and non-SVR to therapy plus the non-therapy group*†

	OLP (−)	OLP (+)‡	Total
SVR	81 (29.2)	2§ (0.7)	83 (30.0)
Others	183 (66.1)	11‖ (4.0)	194 (70.0)
Total	264 (95.3)	13 (4.7)	277 (100)

*Data are presented as *n* (%); †percentages may not add up to total because of rounding; ‡not significant (P=0.240); §2.4% (2/83) with an SVR; ‖5.7% (11/194) with a non-SVR and no therapy.

Table 4. Prevalence of oral lichen planus (OLP) in hepatitis C virus (HCV) patients (Group 1), stratified by alanine transaminase (ALT) levels of >80 IU/L within 2 years of follow-up, by whether they were treated with HCV therapy or not*

	OLP (−)	OLP (+)†	Total
ALT			
<80 IU/L	85 (30.7)	0‡ (0)	85 (30.7)
>80 IU/L	179 (64.6)	13§ (4.7)	192 (69.3)
Total	264 (95.3)	13§ (4.7)	277 (100)

*Data are presented as *n* (%); †significant (P=0.014); ‡0% (0/13) with OLP; §100% (13/13) with OLP.

Table 5. Prevalence of oral lichen planus (OLP) in hepatitis C virus (HCV) patients (Group 1), stratified by the ultrasonographic scoring system*

	OLP (−)	OLP (+)†	Total
Echo score			
4–6 (normal or mild hepatic fibrosis)	123 (44.4)	5‡ (1.8)	128 (46.2)
7–9 (definite or advanced hepatic fibrosis)	141 (50.9)	8§ (2.9)	149 (53.8)
Total	264 (95.3)	13 (4.7)	277 (100)

*Data are presented as *n* (%); †not significant (P=0.566); ‡38.5% (5/13) with OLP; §61.5% (8/13) with OLP.

lesion in hepatitis C patients. This is compatible with the finding of Mignogna et al.[29] that the reticular form is more frequent in HCV-positive patients than in HCV-negative patients. Most hepatitis C patients with reticular OLP do not seek treatment because of the asymptomatic nature of the disease. Variations in OLP symptoms between HCV patients (Group 1) and dental outpatients (Group 2) may have influenced the option of seeing doctors and resulted in the different manifestations of OLP between the two groups. All HCV-related OLP cases among the 13 hepatitis C patients were tolerable. No patient subjectively complained about gross or symptomatic changes, regardless of whether they were treated with IFN-ribavirin combination therapy or not. Three atrophic-erosive OLP and two reticular OLP patients were referred to the dental department of the same hospital for treatment, and showed dramatic remission of signs and symptoms with levamisole and low-dose prednisolone therapy within 2 weeks, and there was no evidence of erosive OLP after 4–6 weeks of treatment. The HCV-related OLP in two cases developed before HCV therapy and remained unchanged after an SVR to therapy. Nevertheless, HCV-related OLP responded to conventional therapy for OLP as well.[30] The beneficial effects of prednisolone plus levamisole on HCV-related OLP also suggest immunopathogenesis.[30] The etiology of OLP is thought to reflect a cell-mediated immune response, although the mechanisms remain to be addressed.[31–33] Leukoplakia was the second most common lesion in hepatitis C patients. Caution should be taken to regularly follow up on the leukoplakia, as with OLP, for possible malignant changes.[1]

IFN with antiviral, antiproliferative and immunomodulatory functions is effective in treating chronic hepatitis C. The rate of response to IFN is enhanced by increasing the IFN dose and extending the treatment duration. The addition of ribavirin to IFN therapy increases the SVR rate in Taiwanese HCV patients by up to approximately 60%.[34–37] In this study, the SVR rate to IFN-ribavirin combination therapy was 66.4%. The long-term benefits of SVR are decreased infectivity, lower risks of developing cirrhosis and hepatocellular carcinoma (HCC), and an improved quality of life. Therapeutic effects of IFN and ribavirin have been confirmed in extrahepatic lesions other than OLP.[38–40] We found that HCV-related OLP in chronic hepatitis C patients with SVR to IFN-ribavirin combination therapy can be persistent. As OLP neither disappeared nor improved but remained unchanged when HCV RNA became negative after HCV therapy, it is unlikely that HCV directly participates in the pathogenesis of OLP lesions. Our data suggest that HCV is not sufficient in itself to be a causative agent for the development of OLP lesions, and that host factors play important roles in the pathogenesis of HCV-related OLP. This was supported by other researchers who reported that the percentages of HCV-infected oral mucosa cells do not correlate with serum viremia levels or the intensity of inflammatory infiltrates in OLP lesions, together with the fact that HCV RNA levels or HCV genotypes in HCV patients with or without OLP did not significantly differ.[14,15,41] Furthermore, Femiano and Scully[42] found that an increase in immunomodulatory cytokines in HCV-positive OLP lesions induced cell-mediated cytotoxicity to basal layer cells and resulted in OLP lesions. Pilli et al.[43] demonstrated that HCV-specific $CD4^+$ and $CD8^+$ T cells were present within intralesional infiltrates in HCV-positive OLP lesions, thus providing strong evidence for the involvement of HCV in the pathogenesis of OLP. Although it is unclear how HCV affects the immune system of hosts, it is thought that the host immune system plays an important role in the development of HCV-related OLP. For further elucidation of the therapeutic effects of IFN-ribavirin combination therapy on HCV-related OLP, an accumulation of more cases and long-term follow-up are needed. Also in this study, nine of 13 HCV-related OLP patients were female; therefore, sex may be related to the occurrence of OLP.

Chronic hepatitis C has peaks and valleys of activity, so most hepatitis C patients have ALT levels that may go up and down or remain stable over time. No HCV-related OLP was found in hepatitis C patients whose ALT levels were always <80 IU/L during 2 years of follow-up, regardless of whether or not they were treated with HCV therapy. Such a finding is quite interesting and corresponds to some earlier reports that there is a close association between transaminase elevation and the development of HCV-related OLP lesions,[8,12,13] although no definite values of elevated ALTs were mentioned in those reports. As far as we know, these ALT numbers do not have a linear relationship with the stage of liver damage in HCV infection. A level of ALT of 160 IU/L is not twice as bad as 80 IU/L, and an ALT value of 95 IU/L and 80 IU/L are essentially the same to a liver specialist. It is not surprising to find that there were no significant differences in OLP prevalence between the advanced hepatic fibrosis group and the normal to mild hepatic fibrosis group according to the ultrasonographic evaluation ($P=0.566$) in the study. However, the prevalence of OLP in the advanced hepatic fibrosis group was higher than that of the normal to mild hepatic fibrosis group. Therefore, further studies are needed to clarify the mechanism of this association between ALT levels of >80 IU/L and the detection of HCV-related OLP.

In conclusion, our data show that among HCV patients, the rate of OLP is twofold higher (4.7% *vs*. 2.0% in the general population); and among OLP patients the rate of HCV infection is 11-fold higher (22.1% *vs*. 2.0% in control subjects of our previous study). We concluded that at least in southern Taiwan, there is a positive association between HCV and OLP and a strong association between elevated transaminase levels of >80 IU/L and the detection of HCV-related OLP. Physicians should be aware that OLP can occur or persist even when serum HCV RNA is negative after IFN-ribavirin combination therapy for hepatitis C patients. These findings contradict the notion that HCV is the direct causal agent of OLP. In other words, HCV together with other factors (either viral or host-related immune responses) may be responsible for some cases of HCV-related OLP.

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CASE REPORT

Maxillary first molar with six canals

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In this case report, we present a maxillary first molar with six canals. A 40-year-old male patient was referred for non-surgical root canal therapy of tooth 16. Under magnification of a surgical operating microscope, a unique morphology with double canal systems in each root was identified. The morphology was characterized by a single palatal root with two canals joining in the apical third, two mesiobuccal canals, and two distobuccal canals with one orifice and two separate foramina.

Introduction

To ensure the long-term success of root canal treatment, it is essential to access, clean, and fill all of the canal spaces. However, the anatomic complexities and variations are constant challenges for successful endodontic therapy.[1]

The morphology of the maxillary first molar has been extensively studied and reported in the literature. Studies have revealed a high incidence of double canal systems in the mesiobuccal root. It is generally accepted that four canals in the maxillary first molar should be regarded as the rule rather than the exception.[2] However, there are reports showing maxillary first molars with more than four canals. Pineda and Kuttler[2] studied the radiographs of 262 maxillary first molars. The authors found a 3.6% incidence of two distobuccal canals, with no incidence of two canals in the palatal root.

In addition to morphologic studies, the presence of a double canal system in either the distobuccal or palatal root in maxillary first molars was documented in several case reports.[3–8] Molars with two palatal canals with separate foramina were presented by Bond et al.[3] and Harris[6]. Cecic et al.[4] reported a case with a bifurcated canal system in the palatal root. Anatomic variations with two palatal roots were also reported.[5,9] A double canal system in the distobuccal roots was reported by Martinez-Berna and Ruiz-Badanelli[8] and Hulsmann.[7]

This report presents endodontic treatment of an unusual maxillary first molar. The anatomic variation consisted of a double canal system in all three roots, which resulted in a total of six canals in a single tooth. There were two separate canal systems in the mesiobuccal root, and the distobuccal canal was bifurcated in the mid-root portion. In addition, the palatal root canal system was

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unique in that it contained two orifices and the canals joined in the mid-root portion.

Case presentation

A 40-year-old Chinese male was referred for root canal treatment of the right maxillary first molar. An emergency pulpectomy was carried out owing to symptoms of irreversible pulpitis. The patient had complained of pain of his right maxillary first molar for several days. The pain was intensified by thermal stimulation. The patient's medical history was noncontributory.

A clinical examination revealed no swelling or sinus tract, and tooth 16 was the only one that was sensitive to percussion in the right upper quadrant. A preoperative radiographic examination showed the residual root of tooth 17, a deep composite restoration on tooth 16, distal caries on tooth 15, and sinus proximity of all three teeth. There were no periapical or furcal radiolucencies but great curvatures in the apical portions of the buccal roots of tooth 16 (Fig. 1). A diagnosis of irreversible pulpitis with acute apical periodontitis was made on tooth 16. Since tooth 15 responded normally to the electric pulp test, reversible pulpitis was diagnosed. After the diagnosis, the treatment plan was discussed with the patient. Endodontic treatment was initiated on tooth 16. Tooth 15 was referred for restoration, and tooth 17 was referred for extraction.

Local anesthesia was administered using 2% lidocaine with epinephrine (1:70,000). A rubber dam was applied. Upon access opening, four well-defined root canal orifices were located using a DG-16 explorer (Hu-Friedy, Chicago, IL, USA) on the pulpal floor, one orifice for each of the buccal roots and two separate orifices for the palatal root. The radiograph taken to determine the working lengths showed two independent palatal canals joining in the mid-root portion, and the distobuccal file was not in the center of the canal (Fig. 2A). After removing the dentinal lips around the mesiobuccal root, the mesiopalatal canal orifice was revealed under magnification of a dental operating microscope. In addition, a small hemorrhagic point was noted over the distal wall that was about 2 mm from the distobuccal canal orifice, and this was then confirmed as a distopalatal canal on the radiograph (Fig. 2B). Severe curvature in the apical third was identified when size 10 K-files were used to negotiate the canals. The length was determined by both a radiograph and an electronic apex locator (Root ZX; Morita, Tokyo, Japan). The working lengths were: 21 mm for the mesiobuccal canal; 20.5 mm for the mesiopalatal canal; 20 mm for the distobuccal canal; 18 mm for the distopalatal canal; and 24 mm and 23.5 mm for the palatal canals. Biomechanical preparation was performed using the passive step-back technique with sizes 2 and 3 Gates Glidden burs (Mani Inc., Japan) in the cervical third of the root canals. The middle and apical thirds were instrumented with nickel-titanium (NiTi) rotary instruments (with a 0.04 tapered profile; Dentsply Maillefer, Ballaigues, Switzerland) and stainless steel K- and H-files. Sodium hypochlorite (2.5%) was used for irrigation. Calcium hydroxide was used as an inter-appointment dressing. After 2 months, tooth 16 became asymptomatic. The canals were dried with paper points and obturated with gutta-percha cones and Roth's 801 sealer (Roth International, Chicago, IL, USA) using a lateral compaction technique (Fig. 3). The master cone sizes were 35 for the mesiobuccal, mesiopalatal and distobuccal canals, 30 for the distopalatal canal, and 40 for both palatal canals.

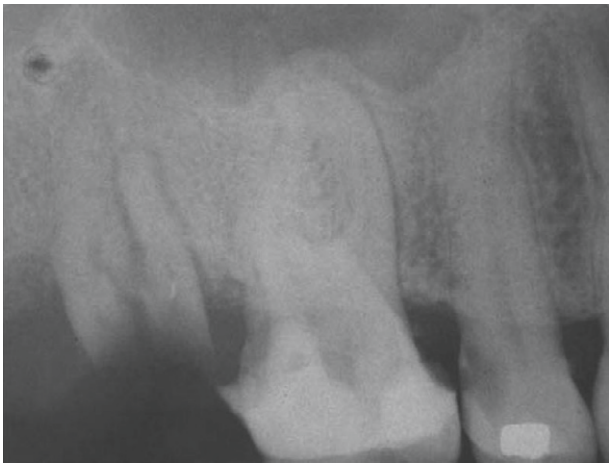

Fig. 1 Preoperative radiograph of tooth 16.

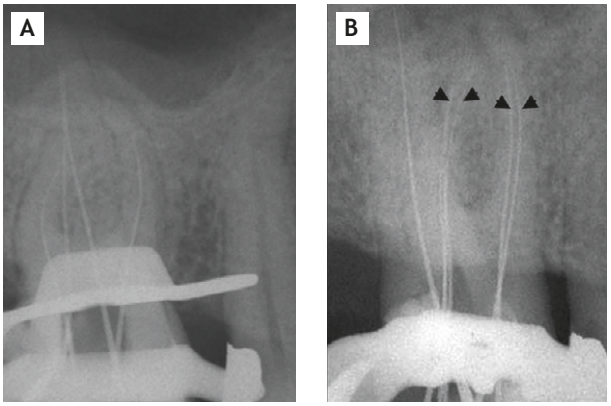

Fig. 2 (A) First working length radiograph showing two canals in the palatal root. (B) Second working length radiograph showing that both the mesiobuccal and distobuccal roots have two canals (arrowheads).

After canal preparation and enlargement, parts of the distobuccal and distopalatal canals were found to be connected. Tooth 16 was referred for crown restoration. The patient was asymptomatic at the 6-year recall. Tooth 16 had a metal crown restoration. Clinical examinations revealed no tenderness to percussion or palpation tests, and the periodontal probing depths of teeth 16 and 15 were within 3 mm. The recall radiographs showed no periapical radiolucency on tooth 16 (Fig. 4).

Discussion

The feasibility of negotiating cases with an unusual morphology depends upon a thorough knowledge of the normal anatomy and an awareness of the existence of anomalies. Proper access opening is key to the success of identifying and negotiating root canals. The most common design of access preparation for maxillary molars is a triangle formed by the orifices of the two buccal canals and the palatal canal. However, in the present case, a more trapezoid form of access preparation was necessary for the inclusion of all of the canal orifices.

Another difficulty confronted in this case was the curvature presented in the apical portion of the root canals. Since two canals were contained in all roots, the thin canal walls of the mesiobuccal and distobuccal roots made root canal treatment even more difficult. Hand files, Gates Glidden burs, and rotary Ni-Ti instruments were used in combination to facilitate cleaning and shaping of the curved canals in this case.

The simultaneous occurrence of double canal systems in all roots of a maxillary molar is an unusual finding. However, it is important for clinicians to use all the armamentaria available to locate and treat the entire root canal system. If such anatomic variations are suspected, they may be recognized on radiographs taken at different angles, or by examining the pulp chamber floor with a sharp explorer, staining the chamber floor with 1% methylene blue dye, performing the sodium hypochlorite "champagne bubble" test, and visualizing canal bleeding points. All are important aids in locating root canal orifices.[10] In addition, if the endodontic files are not well centered in the canal on the radiograph, the possibility of additional canals should always be considered. The use of magnification is also an aid in verifying the presence of morphologic variations. Although the occurrence of a second canal in the distobuccal and palatal roots is infrequent, it is important for clinicians to be aware of all possible anatomic variations for the success of the root canal therapy.

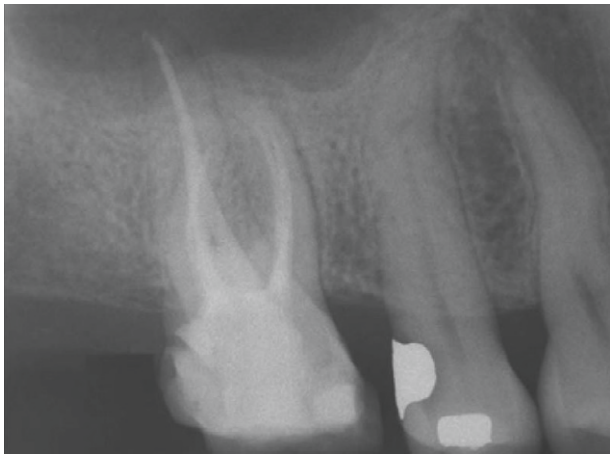

Fig. 3 Postoperative radiograph.

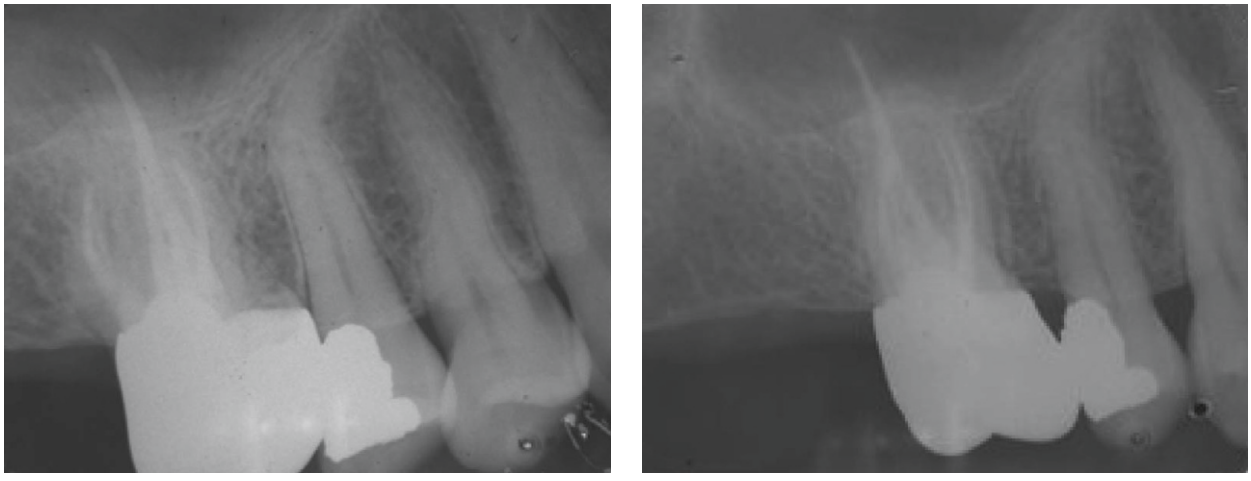

Fig. 4 Radiographs taken at two different angles at the 6-year recall.

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CASE REPORT

Buccal granulocytic sarcoma (chloroma)

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chloroma;
granulocytic sarcoma;
oral cavity

A granulocytic sarcoma (GS) is an extramedullary myeloid tumor which can occur in multiple sites around the body. GS rarely occurs in the oral cavity, and most cases are found in pediatric patients. In this report, we describe the case of a 56-year-old male who suffered from right buccal swelling for 1 week. Facial computed tomography revealed a low-density mass over the right buccal area. He was also a victim of acute myeloid leukemia; he had received chemotherapy and experienced remission 6 months later. An intraoral biopsy was performed and proved the mass to be a GS. He was referred to the oncology department for radiotherapy and chemotherapy. We report on this particular case because of its unusual clinical and histologic presence and its occurrence in a patient with a history of acute myeloid leukemia.

Introduction

A granulocytic sarcoma (GS) is an extramedullary myeloid tumor first described by Burns in 1811.[1] The tumor has also been referred to by a variety of names including *myeloblastoma*, *chloroleukemia*, and *chloroma*. The latter two designations indicate the green color of the gross tumor, secondary to the presence of myeloperoxidase within the cells.[2] GS has a rare occurrence, with an estimated incidence of 5% of myeloid leukemias in adults and 13% in children.[3] It occurs most commonly in bone, periosteum, soft tissues, lymph nodes, and skin, but can occur virtually anywhere.[4]

In the oral cavity, a GS is rare; only 33 cases have been described to date in the English literature (Table 1). Among these, most cases were associated with acute myeloid leukemia (AML). Oral GS usually presents as a localized mass located in the jaw bones, gingiva, palate, tongue, and buccal mucosa.[1,9,10,15,18,20–25,27–35] Almost all patients had GS in the jaw bones; only two patients had it in their cheeks. Oral leukemic infiltrates may also present as diffuse gingival enlargements and oral swelling, which make the differential diagnosis of oral GS more difficult. Because of the low incidence of involvement and the difficulty differentiating it, we present a case of a right buccal GS after chemotherapy for AML.

Case presentation

Case history

A 56-year-old male was diagnosed with AML and received chemotherapy. He came to our outpatient department for help in June 2006 with the chief

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Table 1. Intraoral granulocytic sarcoma patients

Author	Year	Age (yr)	Sex	Location	Malignancy	Outcome	
Wiernik and Serpick[5]	1970	35	F	Cheek R	AML	D	10 mo
Brook et al.[6]	1974	8	M	Maxilla R	AML	NR	NR
Neiman et al.[7]	1981	NR	NR	Palate soft	NR	NR	NR
Hansen et al.[8]	1982	83	F	Maxilla R	AML	NR	NR
Conran et al.[9]	1982	23	F	Mandible R	NR	S	16 mo
Takagi et al.[10]	1983	25	F	Mandible L	AML	NR	NR
Reichart et al.[11]	1984	35	F	Mandible R	APML	D	8 mo
Castella et al.[12]	1984	89	F	Palate hard	NR	D	2 mo
Timmis et al.[13]	1986	52	M	Mandible L	LL	D	13 d
Welch et al.[14]	1986	3	F	Maxilla L	NR	D	42 mo
Ficarra et al.[15]	1987	67	F	Maxilla L	AML	D	36 mo
Saleh et al.[16]	1987	62	F	Mandible	AML-M7	D	NR
Barker and Sloan[17]	1988	4	F	Maxilla L	MML	D	48 mo
Rodriguez et al.[18]	1990	56	M	Mandible L	AML	D	NR
Cho et al.[19]	1990	3	M	Mandible R	APML	S	12 mo
Eisenberg et al.[20]	1991	33	M	Maxilla, mandible, R	AMML	S	12 mo
Stack and Ridley[21]	1994	70	M	Mandible R	CML	D	1 mo
Tuset et al.[22]	1995	NR	NR	Mandible R	AML-M7	D	NR
Tong et al.[23]	2000	76	F	Maxilla R	AML	D	17 mo
Tomas Carmona et al.[24]	2000	60	F	Mandible R	CML	NR	NR
Lee et al.[25]	2001	43	F	Maxilla L	NR	S	NR
Jordan et al.[1]	2002	62	F	Mandible	MML	D	10 mo
Amin et al.[26]	2002	58	M	Hard palate	AML-M0	D	1 mo
Asna et al.[27]	2003	NR	NR	Tongue	AML	NR	NR
Sood et al.[4]	2003	27	M	Parotid	AML	NR	NR
Stoopler et al.[28]	2004	50	M	Cheek	AML	NR	NR
Colella et al.[29]	2005	62	F	Maxilla	AML	D	1 mo
Goteri et al.[30]	2006	84	F	Hard palate	Not developed	S	7 mo
Yinjun et al.[31]	2006	44	F	Maxilla, R	Not developed	S	NR
Yoon et al.[32]	2006	63	M	Maxilla	Not developed	D	4 mo
Xie et al.[33]	2007	32	F	Maxilla, mandible, L	CML	S	6 mo
Srinivasan et al.[34]	2008	77	M	Lip	AML	D	7 mo
Kim et al.[35]	2009	4	F	Mandible L	AML	S	4 mo
Present case	2009	56	M	Cheek	AML-M2	D	11 mo

F=female; R=right; AML=acute myeloid leukemia; D=died of disease; M=male; NR=not recorded; S=survived; L=left; APML=acute promyelocytic leukemia; LL=lymphoid leukemia; AML-M7=acute myeloid leukemia—megakaryoblastic; MML=myelomonocytic leukemia; CML=chronic myeloid leukemia; AML-M0=acute myeloid leukemia—undifferentiated; AML-M2=acute myeloid leukemia—myeloblastic with maturation.

complaint of right buccal swelling over the last 2 weeks. Tracing back his past medical history, he had had a dry cough and shortness of breath for 1 month, but he had paid no attention to those until he had a tarry stool and severe dyspnea. He was sent to our emergency room for further evaluation, and a bone marrow biopsy diagnosed AML (subtype M2) in January 2006. He subsequently received induction and consolidation chemotherapy. Unfortunately, his right buccal area progressively swelled with tenderness beginning in June 2006. A physical examination showed a firm, painful mass over his right buccal area extending to the infratemporal area. Facial asymmetry was found, and right submandibular lymphadenopathy was significantly palpable (Fig. 1). An open mouth limitation of a 25-mm maximum interincisal distance was noted. An intraoral examination showed that there was no odontogenic infection source regardless of percussion or palpation. Furthermore, no mucosal ulceration was found. Panoramic film revealed no radiolucent or radiopaque lesion on the jaw bones except for tooth 16 which had chronic periodontal disease with alveolar bone destruction. Aspiration was performed and revealed a solid mass without purulent discharge. A complete blood count revealed a white blood cell count of 4.6×10^9/L without blast cells, hemoglobin of 14.3 g/dL, and a platelet count of 128×10^9/L. A computed tomographic scan was taken and revealed a low-density mass occupying the right buccal mucosa (Fig. 2). Under the impression that the patient had a buccal

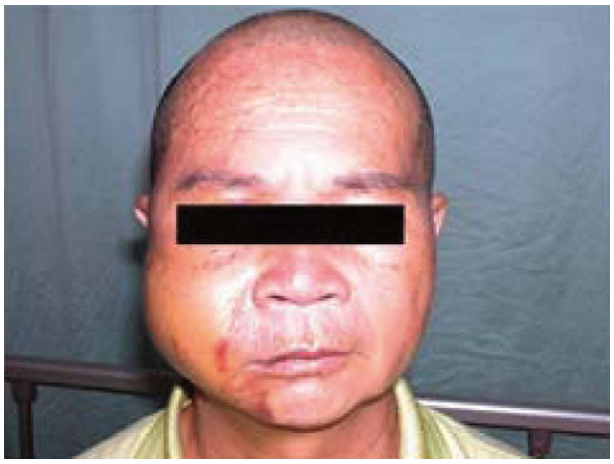

Fig. 1 Clinical photograph showing firm swelling of the right buccal area and facial asymmetry.

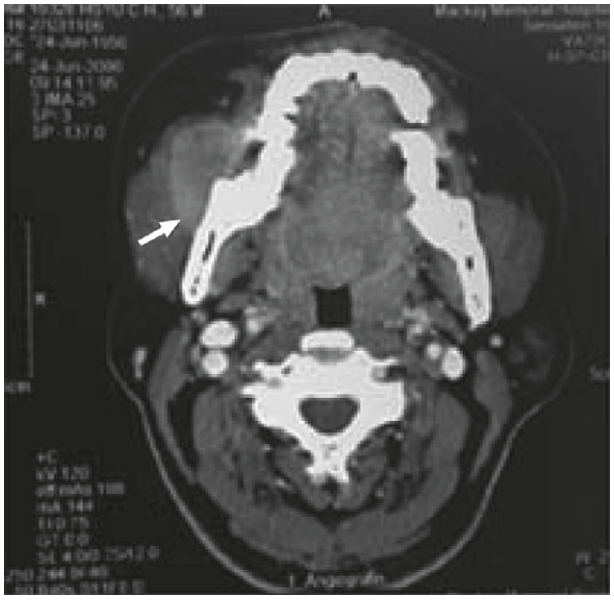

Fig. 2 Computed tomography revealing a low-density mass occupying the right buccal mucosa (arrow). The lesion shows homogenous enhancement.

tumor with infection, a biopsy was performed under general anesthesia and a frozen section revealed cellular infiltration, suggesting a malignancy.

Histopathology

Microscopic examination of the oral tissue showed a diffuse monotonous infiltrate of medium-sized or large neoplastic cells. The tumor cells had round to oval or irregularly folded vesicular nuclei with fine chromatin. The cytoplasm was pale and eosinophilic with fine granularity (Figs. 3A and 3B). Immunohistochemically, the neoplastic cells were positive for CD43 (Fig. 3C) but negative for CD3, CD20, and CD15. Immunostaining for myeloperoxidase was positive (Fig. 3D). The histologic features along with the immunoprofile led to a diagnosis of GS.

Treatment

The right buccal lesion of this patient was treated with chemotherapy (ara-C, idarubicin) and local radiotherapy (3900 cGy). After localized radiotherapy was given to the tumor mass, the lesion gradually diminished. The buccal swelling regressed after seven sessions of chemotherapy. In January 2007, his peripheral blood count showed a drop in the number of white blood cells and platelets. The results prompted him to get further chemotherapy. Unfortunately, neutropenia and thrombocytopenia still persisted, causing a spiking fever and general weakness. The patient was unresponsive to treatment and died of widespread disease 11 months later.

Discussion

GS is a localized, solid, extramedullary myeloid tumor that is often associated with malignant hematopoietic disease. GS occurs in 2–14% of patients with AML. Among patients diagnosed with GS, 87% subsequently develop AML in a mean time period of 10.5 months.[36] Stack et al.[21] also showed that most GS patients had no previous history of associated myeloid disease; however, evidence of acute leukemia subsequently emerged in an average period of 10 months. When there are no signs of malignancy (primary GS), the differential diagnosis should include benign tumors as well as granulomatous epulis, pyogenic granuloma, and peripheral giant granuloma.[37] GS cannot be readily distinguished by means of hematoxylin and eosin staining alone.[25] A definitive diagnosis of GS can be established by both immunostaining and a histologic study. Auer bodies (crystalline, rod-like, azurophilic intracytoplasmic structures representing clumped primary lysosomal granules of myeloid cell precursors) are considered to be a consistent marker of myeloid cells.[24] Immunohistochemical staining of GS is usually positive for myeloperoxidase and CD43. In addition, Lee et al.[25] found that magnetic resonance imaging is helpful for diagnosing GS with its lower signal intensity lesions on both the T1- and T2-weighted images.

GS is difficult to diagnose from its clinical pattern. Rapid swelling of the buccal space also indicates an infectious process. Laboratory data can help rule out cellulitis or some pathogenic microbes. In the present case, even though computed tomography revealed a tumor-like lesion, acute cellulitis of the buccal space still could not be

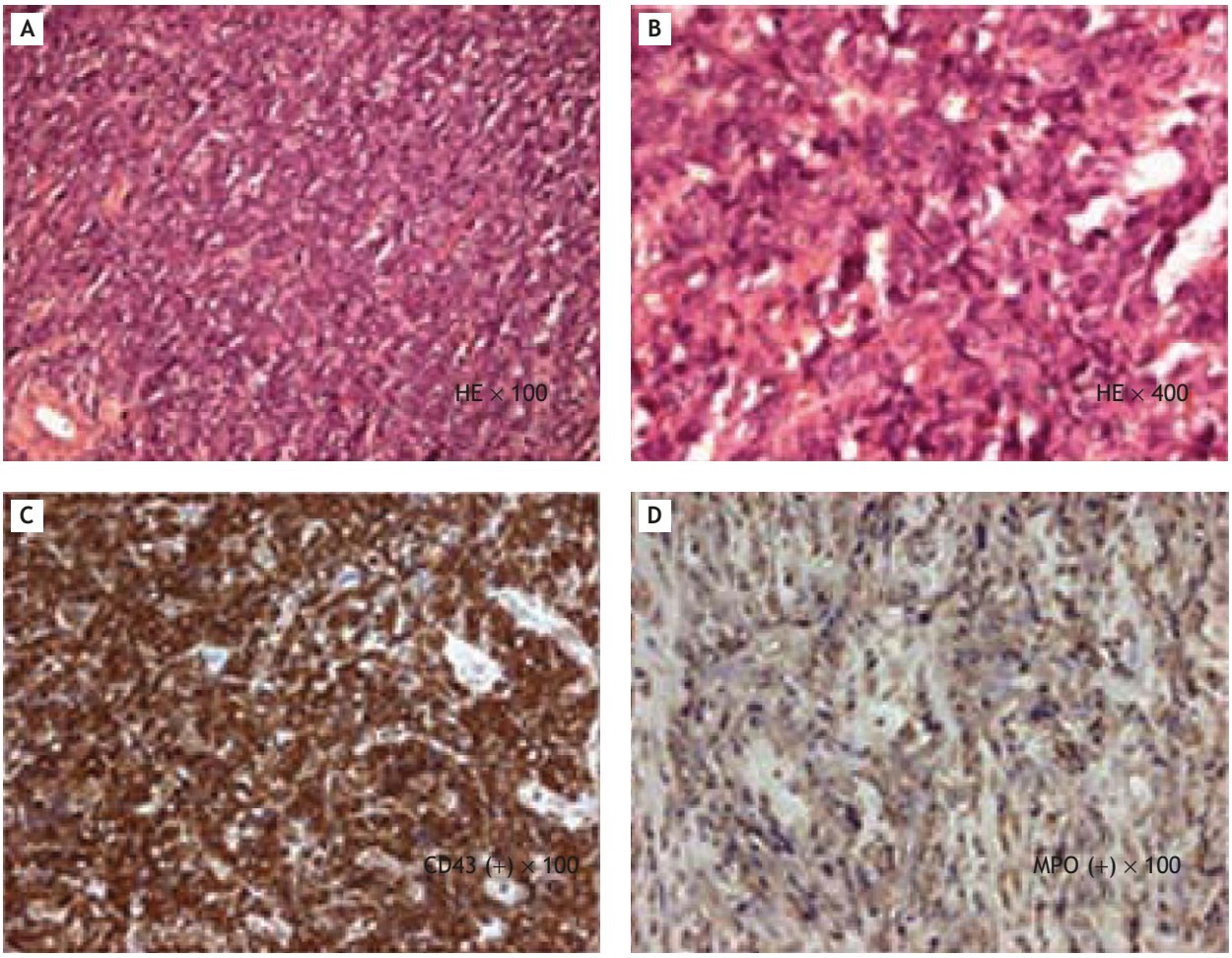

Fig. 3 (A, B) Tumor cells growing in a sheet-like pattern and showing irregular nuclear contours with vesicular chromatin in low- and high-power fields (A: hematoxylin and eosin, 100×; B: hematoxylin and eosin, 400×). Cells showed immunoreactivity for (C) CD43 and (D) myeloperoxidase.

completely ruled out. A malignant tumor was suspected until easy bleeding and a green-colored tumor were found. It is hard to diagnose GS because of the extremely few cases which have presented in the oral cavity.

Thirty-four cases (including the present case) of intraoral GS have been reported and are listed in Table 1. It occurs more often in women. The age predilection ranges are wide (mean age, 46.8 years; range, 3–89 years). The most commonly involved locations in the oral cavity are the maxilla and mandible (24/34, 70.6%). Other sites included the cheek, tongue, parotid, hard palate, soft palate, and lip. Since the survival rate is only 30.8% (8/26), the prognosis is poor; the survival rate of a primary GS (2/4, 50.0%) seems higher than GS associated with malignancy (4/18, 22.2%). However, the outcomes of eight patients and the associated malignancies of five patients were not described. Follow-up times ranged from 15 days to 48 months. Most GS patients were diagnosed with a known myeloproliferative disorder, of which more than half were AML (19/25). Among them, only four cases were primary GS patients. Tuset et al.[22] found that GS in a patient with AML did not affect the prognosis. But with non-leukemic patients or those with chronic myeloid leukemia, a poor prognosis was indicated. Our study cannot delineate a clear difference between these two groups, because many articles did not describe final outcomes. Rodriguez et al.[18] recommended treating GS with chemotherapy applied with a protocol similar to that used for AML. Xie et al.[33] presented a case of intraoral GS associated with the complete remission of CML after treatment with imatinib mesylate (Gleevec; Novartis, Basel, Switzerland). In our case, boost radiotherapy was suggested to control the local tumor. This was also used in other studies.[3,9,10,15,19,20,25,30]

In conclusion, although oral GS is a rare malignancy, it must be considered when an AML patient has buccal swelling without an infectious source. GS is difficult to diagnose clinically. Its acute onset is usually confused with facial cellulitis or another

infectious disease. Specific immunohistochemistry, magnetic resonance imaging, or an associated malignancy is helpful for the differential diagnosis. Even though the prognosis is poor, it is strictly related to a correct diagnosis and prompt treatment.

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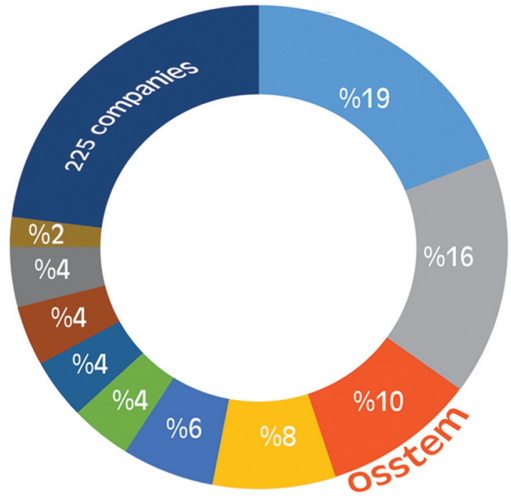

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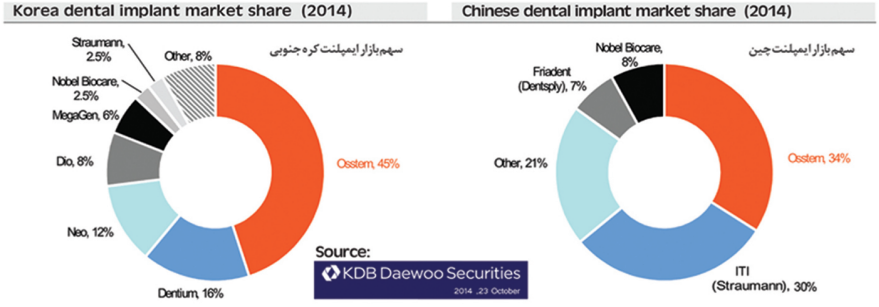

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