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General Summary

Clinical studies of temporomandibular disorders
We have continued our studies of screening questionnaires and the evaluation of quality of life for patients with temporomandibular disorders (TMDs). We studied clinical questions for drafting guidelines for TMDs.

Morphological and histological studies of the temporomandibular joint
We continue to study anatomical and histological examination of temporomandibular joint and articular disk in Mammalia.

Basic studies of oral mucosal keratinocytes
We examined the expression and function of trefoil factor 3 in normal oral mucosal keratinocytes *in vitro*. *In-situ* immunohistological expression of nerve growth factor and its receptors TrkA and p75NTR in keratinocytes from normal oral mucosa and oral lichen planus was also studied.

Research Activities

Clinical studies of TMDs
1. Epidemiological study of TMDs in working persons in Tokyo
We have developed a 4-item questionnaire for the screening of patients for TMDs, which showed a sensitivity of 0.746 and a specificity of 0.811.
Purpose: Using this 4-item questionnaire, we screened persons working in the Tokyo metropolitan area for TMDs in 2005 and 2006, to determine the prevalence of TMDs and contributing factors.
Methods: We used the secondary data of the Tokyo Dental Association for both 2006 (412 people, 396 effective analysis subject [96.1%]) and 2007 (795 people, 679 effective analysis subjects [85.4%]).
Results: The prevalence of TMDs in men was 19.5% for those in their 20's, 35.1% for those in their 30's, 27.3% for those in their 40's, 14.3% for those in their 50's, and 3.9% for those in their 60's. The prevalence among women was 32.2% for patients in their 20's, 38.3% for those in their 30's, 23.5% for those in their 40's, and 6.1% for those in their 50's. Multivariate logistic regression analysis revealed that significant factors among persons with TMDs were fatigue (odds ratio = 1.55) in men and depression (odds ratio = 1.37) and fatigue (odds ratio = 1.30) in women.
Conclusions: These results were developed from secondary data; therefore, while direct relations cannot be confirmed, the need to investigate work-time sleep and its association

with the onset of TMD was revealed.

2. Comparison of epidemiologic studies of TMDs using the same screening question

Some epidemiological surveys of TMDs have used different questionnaires whose validity has rarely been reviewed; therefore, comparative study is difficult.

Purpose: To study epidemiological surveys of TMDs which have used the TMD screening questionnaire that was acquired by validity examination.

Methods: Subjects were asked the following question: "Do you have jaw pain when you widely open and close your mouth?"

Results: A 2005 survey of dental diseases by the Ministry of Health, Labour and Welfare showed that the percentages of persons replying "yes" were 3.5% (139 of 3,969) among persons aged 15 to 85 years; 20.0% (130 of 649) among persons aged 18 to 70 years who were employed in Tokyo; 5.8% (29 of 501) among persons aged 40 to 55 years living in Yokote-shi, Akita; and 20.5% (258 of 1,261) among persons aged 12 to 93 years who were patients of general clinics in urban and suburban areas of Tokyo.

Conclusions: This study revealed local differences and subject differences in epidemiological survey findings of disease with the same questionnaire. The prevalence of TMDs was particularly high among persons working in Tokyo. Therefore, these results indicate the need to investigate working conditions.

3. Questionnaire analysis of "clinical questions" for treating TMDs collected from dental care providers: A preliminary investigation of participants in the 20th annual meeting of the Japanese Society for the Temporomandibular Joint

The committee drafting guidelines for the primary care of TMDs of the Japanese Society for the Temporomandibular Joint performed a questionnaire survey of participants of the 20th annual meeting held on July 14 and 15, 2007. The purpose of the survey was to consider strategies for collecting clinical questions from health care providers. The questionnaire included the position in the society and social stage, the length of time treating TMDs, the format for clinical questions, and opinions regarding guidelines. Efforts to publicize the next investigation were considered necessary because we were able to collect completed surveys from only 61 respondents. Of these respondents, 54 (89%) were society members and 24 (39%) were authorized specialists. There were 31 dentists (51%) who had treated TMDs for more than 11 years. Of the symptoms described in the clinical question format, pain was the most frequent and was followed by joint noise and limited mouth opening. Splint therapy was the most common treatment and was followed by mouth-opening exercises and a pharmacotherapy. For several answers the responders seemed unable to recognize the format style for clinical questions. These findings suggest that more-detailed methods should be considered for collecting clinical questions.

4. Questionnaire analysis of general practice dentistry for the systematic understanding of clinical questions for clinical guidelines for TMDs

When drafting clinical guidelines, we should adopt the acronym "PICO": patient (or disease), intervention (a drug or test), comparison (another drug, placebo or test), and outcome of clinical questions.

Purpose: We performed a questionnaire survey of dentists in general practice to collate clinical questions for the treatment of TMDs.

Subjects and Methods: The Japan Dental Association (JDA) collected questionnaires, and we analyzed the secondary data, which eliminated personal information. All subjects were general members of the JDA or were members or nonmembers of the JDA working in participating clinics. In principle, 10% of the general members were extracted from every age group. To unify terms, one author compiled a list of similar terms using the text-mining method.
Results: We sent questionnaires to 5,999 dentists and received responses from 1,412 (response rate, 23.8%). Inadequate and incomplete clinical questions (353) were excluded from the total analysis set of 4,423, leaving an effective analysis set of 4,070. The main therapies (more than 5%) chosen for main symptoms (more than 3%) were 32 kinds of clinical questions.
Conclusion: These data and/or combination should be considered when drafting clinical guidelines for TMD.

Morphological and histological studies of the temporomandibular joint

1. Absence of an articular disk in the Tasmanian devil temporomandibular joint

Background: The articular disk of the temporomandibular joint is a constant structure in the Mammalia. According to Parson's report in 1900, however, the articular disk is absent in 4 mammals: the armadillo, 2 kinds of monotreme (the echidna and the platypus), and the Tasmanian devil. Since 1900, however, no research has been done to confirm this observation. The aim of this study was to determine by means of anatomical and histological examination whether the Tasmanian devil has an articular disk in its temporomandibular joint.
Methods: Fresh corpses of 8 Tasmanian devils were obtained from the School of Zoology, University of Tasmania. They were dissected, and the structure of the temporomandibular joint was carefully observed anatomically. Then, the temporomandibular joint was removed, immersed in 10% buffered formaldehyde solution, decalcified in 10% ethylenediaminetetraacetic acid solution, and embedded in paraffin. Serial sagittal sections were cut and stained with hematoxylin and eosin for histological examination.
Results: In all cases, gross observation and dissection revealed the absence of an articular disk. Histological examination showed that the surface layers of both the condyle and the glenoid fossa consisted of fibrous tissue thicker than that in other mammals. A synovial membrane-like structure was observed in the anterior and posterior parts of the fibrous structure of the condyle.
Conclusion: We confirmed the absence of an articular disk in the Tasmanian devil's temporomandibular joint. Furthermore, thick fibrous layers on the surfaces of both the condyle and the glenoid fossa might play a role as a buffer against hard jaw movement instead of articular disk.

2. Observation of the condyle using micro-computed tomography in the Tasmanian devil temporomandibular joint

Purpose: The aim of this study was to examine the structures of cancellous and cortical bone of the Tasmanian devil's condyle.
Methods: Fresh carcasses of 6 Tasmanian devils (5 male and 1 female; body weight: 4.3 to 10.4 kg) were obtained from the School of Zoology, University of Tasmania. One

of these dry skulls was obtained and used for micro-computed tomography (CT) examination. The condyle on the left side of the dry skull was examined with micro-CT (HMX-225 Actis 4, Tesco, Tokyo, Japan). Imaging was performed with a tube voltage of 140 kV, a tube current of 120 μA, a magnification of 6.0, and a slice width of 50 μm. Three-dimensional images were created with the volume-rendering method using Vgstudio (Nihon Visual Science Inc., Tokyo Japan). The findings were compared with those of a beagle.
Results and Conclusion: Sagittal and coronal micro-CT scans revealed dense and fine cancellous bone and thinner covering cortical bone in the condyle of the Tasmanian devil as compared with those of the beagle. These findings might be due to rapid turnover/renewal of bone as a result of powerful mastication and heavy loading on the condyle.

Basic studies of oral mucosal keratinocytes

1. Salivary trefoil factor 3 enhances migration of oral keratinocytes

Purpose: Trefoil factor (TFF) 3 is a member of the mammalian TFF family. TFFs are secreted onto mucosal surfaces of the entire body and exert different effects according to the tissue location. TFFs may enhance mucosal healing by modulating motogenic activity, inhibiting apoptosis, and promoting angiogenesis. TFF3 is secreted from the submandibular gland and is present in whole saliva. The aim of this study was to assess the migratory and proliferative effects of TFF3 on primary oral human keratinocytes and oral cancer cell lines.
Results: The addition of TFF3 increased the migration of both normal oral keratinocytes and the cancer cell line D12, as evaluated with a 2-dimensional scratch assay. In contrast, no increase in proliferation or energy metabolism was observed after stimulation with TFF3. The TFF3-enhanced migration was found to be driven partly by the extracellular signal—related kinase pathway, as shown by addition of the mitogen-activated protein kinase inhibitor PD 98059. Conclusion: All previous functional studies of trefoil peptides have been based on cells from monolayered epithelium, such as the intestinal mucosa; this is the first report to show that normal and cancerous keratinocytes from stratified epithelium respond to TFF stimuli. These findings suggest that salivary TFF3 contributes to oral wound healing.

2. Nerve growth factor and its receptors TrkA and p75NTR in the epithelium of oral lichen

Background: Nerve growth factor (NGF) can, through its receptors TrkA and p75NTR, convey signals for cell survival, differentiation, and death. The aim of this study was to examine whether NGF plays a role in the pathology of oral lichen.
Methods: Sections of biopsies from patients with erythematous oral lichen and from volunteers with normal oral mucosa were immunostained with antibodies against NGF, proNGF, TrkA, phosphorylated Trk, p75NTR, and phosphorylated Akt, and the expression of RNA coding for proNGF/NGF was investigated with *in situ* hybridization.
Results: Both in erythematous oral lichen and normal oral mucosa, cytoplasmic staining for NGF was seen in granular and upper spinous cell layers of the epithelium, whereas

proNGF staining was seen in all epithelial cell layers. In situ hybridization showed that the proNGF protein was produced in the same cell layers. In oral lichen, strong cytoplasmic staining for TrkA and phosphorylated Trk was observed in all epithelial cell layers, but staining was weak in normal oral mucosa. Basal keratinocytes in oral lichen showed no or only weak cytoplasmic staining for p75NTR, but in normal oral mucosa there was clear cell-membrane staining. In oral lichen, strong cytoplasmic and intermittent nuclear staining for phosphorylated Akt was observed in spinous, granular, and superficial layers, whereas basal and parabasal keratinocytes showed no staining. This staining was weak or absent in the entire epithelium of normal oral mucosa.
Conclusion: TrkA upregulation and activation in oral lichen is a pathway that can activate phosphorylated Akt and thereby rescue epithelial cells from untimely cell death.

Publications

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