The Role of an Uncommon Type of Oral *Streptococcus Sanguis* in the Etiology of Behcet's Disease

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Abstract

The relationships of Behcet's disease (BD) with oral diseases and the prevalence of an uncommon type of oral *Streptococcus sanguis* (*Str. sanguis*) in the oral cavity were investigated in a case-control study. BD patients were compared to patient controls (collagen disease) and healthy controls.

An interview questionnaire survey of BD and oral diseases showed that during the pre-onset, onset, and post-onset periods, the incidences of tonsillitis and dental caries, or the history of dental treatment, were greater in BD cases. Typological analysis showed a higher prevalence of an uncommon type of *Str. sanguis*, differing from the common type, among BD cases compared to control groups. These results, showing a higher incidence of tonsillitis and dental caries during the presymptomatic period, a greater frequency of dental treatments during the symptomatic period, and the presence of an uncommon type of *Str. sanguis*, indicate that *Str. sanguis* of an uncommon type is related to increased risk of BD, and the possibility of a causal role is suggested.

Key words: Behcet's disease, Oral disease, Oral *Str. sanguis* uncommon type, Causal role

Introduction

The pathological mechanism of Behcet's disease (BD) presently remains unclear. The disease appears to have a genetic association that is strongly affected by some environmental factors. HLA-B₅₁ has been identified as an important genetic factor contributing to it¹⁾. Significant environmental factors have been hypothesized based on the occurrence of intra-family disease clusters²⁾, and also because of the very low incidence among Japanese-Americans³⁾. Viral infections and the effects of trace chemical substances have been investigated^{4,5)}. Conclusive evidence, however, has not yet been found.

In recent years, oral bacteria, especially *Streptococcus*, have been suggested to be involved in the disease's etiology⁶⁾. Support for this view includes evidence of significantly higher incidences

of tonsillitis, dental caries, and periodontitis, all thought to be associated with *Streptococcus* infection, in BD cases in comparison with those in either healthy or non-collagen-disease patients⁷⁻⁹⁾. Similarly, BD cases display elevated lymphocytic activity and delayed dermal sensitivity to *Streptococcus* antigen^{10,11)}. In addition, BD cases show an increase in *Streptococcus*-derivative antigens¹²⁾.

Barile et al.¹³⁾ and others¹⁴⁾, in pathogenetic studies, have isolated *Streptococcus sanguis* (*Str. sanguis*) from infectious foci of oral ulcers in BD cases. Recently, Okonogi and Tsurumizu¹⁵⁾ isolated an uncommon type of *Streptococcus sanguis*, biologically distinguishable from the heretofore common type and proposed that it is involved in the pathogenesis of BD. They speculated that this new uncommon type has high pathogenicity, although this has not been adequately confirmed.

Our case-control study compared BD cases to controls with diseases closely related to BD, collagen diseases, a scheme heretofore not attempted. In addition, while others have studied separate aspects of important environmental factors related to BD, we attempted a combined overall study of oral manifestations of BD

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and the prevalence of the uncommon type of *Str. sanguis* to elucidate the relationship.

Materials and Methods

BD patients diagnosed at the outpatient clinic of T University, located in Tokyo, were studied. All cases (n=45) were residents of the Tokyo metropolitan area and had either the complete type or incomplete type of BD, as diagnosed by the criteria of the Japanese Committee on Behcet's Disease (1987).

Forty-five patients were selected from the outpatient clinic of S Medical University, and 45 healthy hospital workers or their relatives were selected from residents of the Tokyo metropolitan area and were matched by gender and age (± 3 years) to the BD cases. For patient controls, patients with collagen diseases were used. Collagen diseases comprise a broad group of autoimmune disorders that share similar clinical features, principally systemic connective tissue inflammation, but show wide dissimilarities in location and response to treatment. BD, due to the similarity in characteristics, is considered a closely related disorder, and use of this comparison group was expected to increase the possibility of elucidating the characteristics peculiar to BD. All subjects were

surveyed by questionnaire, and, in addition, samples of their dental plaques were obtained and examined for the uncommon type of *Str. sanguis*. Based on these data, relationships between BD incidence and these factors were analyzed.

The questionnaire surveyed the conditions during the pre-onset stage, at onset, and in the post-onset stage. These stages were defined as follows. Onset was the estimated date of the inception of abnormalities derived from the date when diagnosis was confirmed and the stage of the disease. Pre-onset was defined as a short period prior to manifestation of the disease, and post-onset as the period from diagnosis to the present. The subjects were interviewed regarding past history of tonsillitis, dental caries, and periodontitis during those periods. For dental caries in the pre-onset stage, in addition to the responses of subjects, two trained investigators examined the oral cavity to judge dental decay, mottling, and fillings (DMF). A subject who reported having had frequent dental caries and was actually estimated to have a DMF score of 15 or more by the investigators, was regarded as having had frequent dental caries before onset.

To isolate *Str. sanguis* in the oral cavity, a sterilized cotton swab was used to remove plaque from the tooth surface. Samples were placed in a preservative (10% glycerine, 3% peptone) in

Table 1 Result of interviews on oral manifestation history

Item	BD patients	Patient controls	Healthy controls	Relative risk	
	(n=45)	(n=45)	(n=45)	(95% Confidence Interval)	
	No (%)	No (%)	No (%)	Patient controls	Healthy controls
<i>Pre-onset</i>					
Frequent swelling of tonsils	24 (53)	17 (38)	9 (20)	1.77 (0.80-3.94)	4.00 (1.56-10.3)**
Frequent dental caries (DMF 15 or more)	29 (64)	26 (58)	10 (22)	1.38 (0.57-3.32)	5.75 (2.08-15.9)***
Treatment of dental caries	32 (71)	44 (98)	41 (91)	0.08 (0.01-0.24)	0.10 (0.02-0.61)
Tooth extraction	30 (67)	39 (87)	35 (78)	0.35 (0.13-0.95)	0.54 (0.21-1.42)
Periodontitis	13 (29)	13 (29)	9 (20)	1.14 (0.43-3.03)	1.67 (0.63-4.41)
Treatment of periodontitis	8 (18)	6 (13)	3 (7)	1.33 (0.48-3.68)	3.50 (0.83-14.8)
<i>Onset</i>					
Tonsillitis	21 (47)	4 (9)		18.0 (3.06-105.9)***	
Dental caries under treatment	22 (49)	7 (16)		8.5 (2.19-33.1)**	
Tooth extraction	14 (31)	3 (7)		7.0 (1.78-27.6)**	
Periodontitis under treatment	7 (16)	3 (7)		2.3 (0.61-8.28)	
<i>Post-onset</i>					
Aggravation of symptoms after tonsillitis, dental treatment, tooth extraction	12 (27)	1 (2)		12.0 (2.00-71.9)**	

No: number of subjects who answered 'yes', (%): percentage of the subjects,

** $p < 0.01$, *** $p < 0.001$.

Table 2 Prevalence of uncommon types of oral *Str. sanguis* and their serotypes

Strain [ATCC registration number]	BD patients	Patient controls	Healthy controls	Relative risk	
	(n=45)	(n=45)	(n=45)	(95% Confidence Interval)	
	No (%)	No (%)	No (%)	Patient controls	Healthy controls
Uncommon types	31 (69)	11 (24)	10 (22)	11.0 (2.87-42.2)***	5.20 (2.06-13.1)***
<i>Serotype</i>					
KTH-1 [ATCC 49298]	17	7	7		
KTH-2 [ATCC 49296]	5	1	2		
KTH-3 [ATCC 49295]	7	3	1		
KTH-4 [ATCC 49297]	2	N.D.	N.D.		

No: number of subjects from whom uncommon types were isolated, (%): percentage of 45 subjects,

N.D.: not detected, *** $p < 0.001$.

tubes and stored at -20°C . Samples were cultured in TYC (*Streptococcus* mutation confirmation) agar plate medium (Kokuto Co., Japan) at 37°C under anaerobic conditions (Aneropack System, Mitsubishi Gas Co., Japan) for 48 hours. Cultures were then observed under a microscope, and characteristic colonies of *Str. sanguis* were isolated¹⁶⁾.

The biological properties of the isolated strains were classified using Carlsson's method¹⁷⁾ according to the ability to ferment sorbit, mannitol, salicin, inulin, cellobiose, esculin, raffinose, and melibiose into the common and uncommon types of *Str. sanguis*¹⁸⁾. Unlike the common type, strains that degraded only raffinose and melibiose were classified as the uncommon type. Serological properties were evaluated by the gel precipitation test using antigens extracted by the method of Kholy et al¹⁹⁾. Four uncommon types that differ from ATCC 10556 (type I), ATCC 10557 (type II), ATCC 10558 (type III), and ST-7 (type IV) as standard serological strains were termed KTH-1 (ATCC 49298), KTH-2 (ATCC 49296), KTH-3 (ATCC 49295) and KTH-4 (ATCC 49297), respectively, and rabbit immune sera for these 4 types were prepared.

The data were analyzed and relative risks and 95% confidence intervals were calculated. Gender- and age-matched data, shown in Table 1 and Table 2, were analyzed by McNemar test.

Results

The 45 BD patients consisted of 33 with complete BD and 12 with incomplete BD; there were 19 males and 26 females. Their mean ages and standard deviations were 48.4 ± 10.0 years for males, and 52.5 ± 7.1 for females. The estimated age (mean $\pm$ SD) of onset of BD was 34.6 ± 8.8 for males and 34.8 ± 6.8 for females. The patient controls with collagen disease consisted of 31 with rheumatoid arthritis, 8 with systemic lupus erythematosus, 2 with progressive systemic sclerosis, 2 with polyarteritis

nodosa, 1 with polymyositis, and 1 with mixed connective tissue disease.

Oral manifestations reported in the pre-onset period are shown in Table 1. Prior to the onset of BD, 50-60% of BD patients reported frequent swelling of the tonsils as well as frequent dental caries. There was no significant difference compared to patient controls, whose reported incidence was approximately 50% for a history of these diseases. However, compared to healthy controls, of whom only 20% reported a history of these diseases, a significantly increased relative risk was seen for these two items in BD patients. For all other reported items, there were no increased relative risks for BD patients.

At the time of onset, BD patients reported significantly increased rates of treatments for tonsillitis, dental caries, or tooth extraction. However, patient controls did not report such high incidences of these conditions, and in the post-onset period, 27% of BD patients reported tonsillitis, dental treatment, or complications after tooth extraction, resulting in a significantly increased relative risk compared to patient controls. Symptoms such as erythema nodosum, arthritis, and stomatitis were experienced by BD patients.

The *Str. sanguis* strains isolated from the oral cavities of subjects were identified as shown in Table 2. An uncommon type of *Str. sanguis* was isolated from 69% of BD patients. In contrast, the uncommon *Str. sanguis* was only found in roughly 20% of the patient controls and healthy controls. The relative risk for the uncommon type of *Str. sanguis* was significantly higher in BD patients than for either of the two control populations. The main serotype of the uncommon *Str. sanguis* isolated from the majority of BD patients was KTH-1, followed by KTH-3 and KTH-2, while a few had KTH-4.

The results analyzed by pre-onset history of frequent swelling of the tonsils or having many dental caries, and the presence or absence of the uncommon type of oral *Str. sanguis* are

Table 3 Association between the development of BD and the presence or absence of a history* of oral manifestations prior to onset and the uncommon type of oral *Str. sanguis*

Presence or absence of history and uncommon types in the same patients	BD patients	Patient controls	Healthy controls	Relative risk (95% Confidence Interval)	
	(n=45)	(n=45)	(n=45)	Patient controls	Healthy controls
	No (%)	No (%)	No (%)		
History (-) and Uncommon type (-)	1 (2)	10 (22)	22 (49)	1.00	1.00
History (+) and Uncommon type (-)	13 (29)	24 (53)	13 (29)	5.42 (0.73-40.3)	22.0 (9.88- 124.2)***
History (-) and Uncommon type (+)	7 (16)	4 (9)	8 (18)	17.5 (2.05-149.7)*	19.3 (2.99- 124.1)**
History (+) and Uncommon type (+)	24 (53)	7 (16)	2 (4)	34.3 (5.86-200.8)***	264.0 (44.3-1572.2)***

*History: frequent swelling of the tonsils or frequent dental caries at prior to onset, No: number of subjects, (%): percentage of 45 subjects, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Table 4 Association between the development of BD and presence or absence of a history* of oral manifestations at onset and the uncommon type of oral *Str. sanguis*

Presence or absence of history and uncommon types in the same patients	BD patients	Patient controls	Relative risk (95% Confidence Interval)
	(n=45)	(n=45)	
	No (%)	No (%)	
History (-) and Uncommon type (-)	2 (4)	24 (53)	1.00
History (+) and Uncommon type (-)	11 (24)	10 (22)	13.2 (2.96- 58.9)**
History (-) and Uncommon type (+)	15 (33)	10 (22)	18.0 (1.98- 76.4)***
History (+) and Uncommon type (+)	17 (38)	1 (2)	204.0 (31.7-1312.2)***

*History: Tonsillitis or dental caries under treatment, or tooth extraction at onset, No: number of subjects, (%): percentage of 45 subjects, ** $p<0.01$, *** $p<0.001$.

shown in Table 3. Based on relative risk analysis of patient controls, the presence of oral *Str. sanguis* was found to be significantly related to the onset of BD. Similar results were found in healthy controls. Moreover, in subjects with a history of oral symptoms with the presence of the uncommon type of *Str. sanguis* there was a marked increase in relative risk compared to both either healthy controls and patient controls.

A history of tonsillitis, treatment for dental caries, tooth extractions at the onset of BD and the presence or absence of the uncommon type of oral *Str. sanguis*, were analyzed to determine the relationships to the onset of BD. Results of the analysis are shown in Table 4. Relative to patient controls, having had tonsillitis, or dental caries, and tooth extractions, in conjunction with the presence of oral *Str. sanguis*, were strongly associated with BD onset and significantly increased the degree of relative risk.

Discussion

Epidemiological studies of BD conducted in Japan have indicated that there is a possible relationship between BD and oral diseases. Prior to the onset of BD, Aoki et al.²⁰, Terao et al.²¹, and Tsuchida et al.⁷ performed case-control studies using healthy controls and reported that BD patients have a significantly greater incidences of tonsillitis and dental caries. Similarly, Nakae et al.²² and Seki et al.⁹ reported that a history of oral diseases such as tonsillitis and dental caries was significantly more frequent in BD patients before the onset of BD in comparison to that in non-collagen-disease patients. At BD onset, Hashimoto²³ showed that more than 60% had tonsillitis or dental caries. In case-control studies using healthy controls, Terao et al.²¹ obtained results showing that the relative risk for tonsillitis, dental caries, or periodontitis was significantly greater in BD patients at onset. Nakae et al.²⁴ and Hashimoto²³ reported that BD symptoms became aggravated after tonsillitis or dental treatment in 30% and 5.9% of BD patients, respectively.

In this study, BD patients, prior to the onset of disease, had significantly greater frequencies of tonsillitis, dental caries, and other diseases in the oral cavity compared to healthy controls. At the time of disease onset, treatment for oral diseases and dental diseases was required significantly more often in BD patients than in patient controls. Our study used a patient control group with collagen diseases, which are similar to BD. As a result, characteristics peculiar to BD at onset were more clearly elucidated. These results were consistent with those of previous reports and suggested a close direct or indirect relationship between the development of BD and oral diseases or their treatments before and at the onset of BD. In addition, the exacerbation of symptoms following tonsillitis, dental treatment, and tooth extractions observed in BD patients and the similarity with results reported by others appear to support a causal relationship. In this study, subjects were interviewed retrospectively regarding past oral health and dental caries, and the possibility existed that subjects' reports overestimated actual conditions, or were biased by greater recall due to the survey. These possible effects were not investigated, but should be considered in formulating conclusions.

From the relationship with tonsillitis, *Str. sanguis* is surmised to be involved in the pathogenesis of BD. Recently, an

uncommon type of *Str. sanguis* has received attention. The uncommon type of *Str. sanguis* has been reported to cause much greater stimulation of neutrophilic activity and platelet clotting as well as showing greater cross antigenicity with human tissue than the common type of *Str. sanguis*^{25~27}. Furthermore, BD patients demonstrate a significantly higher level of agglutinating antibody titers against the uncommon strain²⁸, and the skin reaction to the uncommon-type of antigen resulted in a markedly greater degree of erythema¹¹. Isogai et al.²⁸ reported that the flora of BD patients showed a greater proportion of *Str. sanguis*. In addition, they found that the uncommon type of *Str. sanguis* was found in all BD cases, compared to only 20% of healthy controls. A similar case-control study by Hoshi et al.²⁹ reported that tooth surfaces yielded the uncommon strain of *Str. sanguis* in 43% of BD cases, and in 36% of healthy controls. In the present study, 69% of BD cases, but only about 20% of either healthy controls or patient controls, yielded the uncommon type of *Str. sanguis*. The high prevalence of *Str. sanguis* clearly appears to be a unique characteristic distinguishing BD patients from patient controls as well as healthy controls. The uncommon type of *Str. sanguis* has been implicated as a highly possible pathogen in BD.

Serotyping done by Okonogi and Tsurumizu³⁰ showed that the uncommon type of *Str. sanguis* was isolated from 60% of 192 BD patients. The most common serotype was KTH-1 (40%), followed by KTH-4 (10%), KTH-3 (8%), and KTH-2 (2%). In Kawasaki disease, in which the uncommon type of *Str. sanguis* is also suspected to be the cause, the most common serotype isolated from patients is reported to be KTH-2³¹. The distribution in the present study was very similar to the finding of Okonogi et al. Serotype distributions in BD patients, patient controls, and healthy controls were all similar, and a distinct serotype characteristic of BD was not confirmed.

The analysis of this study showed a strong relationship between disease history and the uncommon type of *Str. sanguis*. Existence of both these factors increased the risk of BD. The uncommon type of *Str. sanguis*, which causes reported bouts of oral diseases, may have induced BD through some mechanism. The hypothesis advocated by Mizushima³² proposes that the interaction of HLA-B₅₁ or other precipitating factors and the uncommon type of *Str. sanguis*, acts as a potent environmental co-factor that can repeatedly enter the body through oral diseases. Sensitization is established, and T-cells and macrophages are activated. A subsequent systemic or localized exposure to streptococcus antigen then induces the onset of BD. This is supported by our results. In our study, repeated bouts of oral diseases involving persistent infection with the uncommon type of *Str. sanguis* were observed followed by subsequent reexposure to the uncommon type of *Str. sanguis* through tonsillitis, dental caries, or dental treatment, all of which strongly suggest a causal relationship to BD onset. It appears that some cases of BD may be induced by this mechanism.

It is suggested that *Str. sanguis* of an uncommon type is related to increased risk of BD, and plays a causal role. However, further detailed studies on the relationship between the uncommon type of *Str. sanguis* and analysis of host factors, are required to further elucidate the etiology of BD.

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