－原著－

腫瘍壊死因子標的療法中の関節リウマチ患者におけるアミノ酸・歯周プロファイル

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Amino acid and periodontal profiles in rheumatoid arthritis patients with tumor necrosis factor targeted therapy

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Abstract:

Tumor necrosis factor-alpha (TNF-α) has been suggested to regulate expression of enzymes that metabolize amino acids associated with rheumatoid arthritis (RA) and periodontitis. TNF-α inhibition has been reported to influence periodontal condition in patients with RA. The aim of the present study is to compare amino acid and periodontal profiles between RA patients with and without TNF targeted therapy. The study participants consisted of 52 RA patients whose informed consents were obtained with and without anti-TNF therapy (n＝26 for RA-TNF group, and n＝26 for RA-C group) and 29 healthy controls (H group). Clinical periodontal and rheumatologic parameter values and plasma levels of amino acids and immunoglobulin G to periodontopathic bacteria were evaluated. No differences were observed between the RA-TNF and RA-C groups in any parameter values, except for a significantly lower % sites with bleeding on probing (BOP) in the RA-TNF group ($P<0.05$). Of 21 amino acids, glutamic acid, tryptophan, and ornithine were significantly different in the concentrations between the RA-TNF and RA-C groups ($P<0.05$). A significantly negative correlation was found between tryptophan level and % of sites with BOP ($P=0.03$). No associations were obtained between other amino acid levels and periodontal conditions in patients with RA. These results suggest a difference in amino acid and periodontal profiles between RA patients with and without anti-TNF therapy.

抄録：

腫瘍壊死因子 -alpha (TNF-α) はアミノ酸代謝酵素の発現を制御し，関節リウマチ(RA)と歯周炎に関与することが示唆されている。TNF-α阻害は RA 患者の歯周状態に影響を及ぼすことが報告されている。本研究の目的は，RA 患者の TNF 標的療法の有無でアミノ酸・歯周プロファイルを比較することである。インフォームドコンセントが得られた RA 患者 52 名のうち，TNF 標的療法を受けた RA 患者 26 名（RA-TNF 阻害群）と受けてない RA 患者 26 名（RA- 対照群），健常者 29 名を対象に，RA 検査，歯周検査，血液採取を行った。血漿中のアミノ酸濃度と歯周病原細菌に対する免疫グロブリン G 抗体価を測定した。RA-TNF 阻害群では RA- 対照群と比べ，プロービング時の出血（BOP）陽性部位の割合が有意に低く（$P < 0.05$），それ以外の評価項目では有意差を認めなかった。TNF 標的療法の有無で有意差が認められたのは glutamic acid, tryptophan, ornithine（$P < 0.05$）で，tryptophan レベルと BOP 陽性部位の割合との間に有意な負の相関が認められた（$P = 0.03$）。以上の結果から，RA 患者では TNF 標的療法の有無でアミノ酸および歯周プロファイルが異なることが示唆された。

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease characterized by inflammation of synovium and destruction of bone and cartilage in the joints, which leads to functional disability. Prevalence of RA is estimated to be approximately 0.5% in the world, and RA affects women about three times as often as it affects men[1]. Similarities in the clinical and pathological features have been suggested between RA and periodontitis[2,3]. RA patients were more likely to exhibit periodontitis than subjects without RA[4-6]. Individuals with periodontitis also had a higher prevalence of RA than those without periodontitis[6-8].

There is evidence to suggest that high levels of circulating pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) play a role in the pathogenesis of RA and periodontitis[9-11]. The authors previously showed that serum TNF-α levels were positively correlated with disease activity score including 28 joints using C-reactive protein (DAS28-CRP) and bleeding on probing (BOP) in RA patients[12]. Other clinical trials indicated that TNF-α inhibition therapy had a beneficial effect on RA and periodontal condition in patients with RA[13,14]. These observations suggest that circulating TNF-α may influence the onset and development of RA and periodontitis.

Recently, multiple metabolites have been shown as a potentially useful marker for diagnosis of inflammatory diseases such as RA and periodontitis[15-20]. It has been reported that patients with RA were diagnosed with high sensitivity and specificity by detection of 52 metabolites[15]. Certain amino acid profiles were also related to periodontal condition in patients with periodontitis[18-20]. Additionally, serum levels of metabolites were linked to RA activity during anti-TNF therapy[16], while urine metabolic profiles were associated with response to anti-TNF therapy[17]. Moreover, TNF-α has been shown to mediate expression of indoleamine 2,3-dioxygenase (IDO) that metabolizes the amino acid tryptophan[21]. These observations have led us to the hypothesis that amino acid and periodontal profiles may be different between RA patients with and without TNF-α inhibition, which has not been studied yet. To elucidate this hypothesis, the authors examined plasma amino acid levels and periodontal parameter values in RA patients with and without anti-TNF therapy. A possible association was also evaluated between amino acid levels and periodontal conditions in patients with RA.

Materials and methods

Participants

Fifty-two Japanese adults with RA (41 females and 11 males; mean ± SE age 60.5 ± 1.6 years), who attended the Niigata Rheumatic Center, Shibata, Japan, were recruited between July 2010 and August 2011 in the present study. The study was approved by the Institutional Review Board of the Niigata University Faculty of Dentistry (No. 22-R9-10-06, on June 16, 2010) and Niigata Rheumatic Center (No. 1, on April 26, 2010). Signed informed consent was obtained before inclusion from all participants who were confirmed to fulfill the 1987 revised classification criteria of American Rheumatism Association[22]: 1) morning stiffness in and around joints lasting at least 1 hour before maximal improvement; 2) soft tissue swelling of 3 or more joint areas; 3) swelling of the proximal interphalangeal, metacarpophalangeal, or wrist joints; 4) symmetric swelling; 5) rheumatoid nodules; 6) the presence of rheumatoid factor; and 7) radiographic erosions and/or periarticular osteopenia in hand or wrist joints. Criteria 1) through 4) must have been present for at least 6 weeks. RA was defined by the presence of four and more criteria. All patients fulfilled the following three exclusion criteria: 1) the presence of diabetes mellitus and pregnancy, 2) having antibiotic treatment within the previous 3 months, 3) a history or the presence of any periodontal therapy and mouth rinse usage within the previous 3 months.

RA patients were divided into two groups. The RA-TNF group consisted of 26 patients (20 females and 6 males; mean ± SE age 60.3 ± 2.3 years) who had received medication of corticosteroids, disease-modifying antirheumatic drugs (DMARDs), and non-steroidal anti-inflammatory drugs (NSAIDs), as well as TNF-inhibitors including infliximab (6 cases), etanercept (18 cases), and adalimumab (2 cases) for 20.7 ± 2.7 (mean ± SE) month. The dosages of these medications were determined by treating rheumatologists based on the patients' needs. As a RA control, the RA-C group included other 26 patients (21 females and 5 males; mean ± SE age 60.7 ± 2.3 years) whose activity of RA had been well controlled only with medication of corticoste-

roids, DMARDs, and NSAIDs. As an another control group (H group), 29 age- and gender-balanced healthy individuals with no signs of systemic diseases and periodontitis (24 females and 5 males; mean ± SE age 54.5 ± 2.6 years) referred to the Niigata University Medical and Dental Hospital were included from July 2010 and February 2011 in the present study.

Clinical assessments

Clinical periodontal assessments were performed by two calibrated examiners (T.K. and M.O.) who were masked from the group assignments and the rheumatologic data. The calibration was performed before the study with 5 volunteer subjects in Niigata University Faculty of Dentistry. Reproducibility of the clinical measurements was calculated by means of the κ index, and a value of 0.857 was obtained for clinical attachment level (CAL) with a difference of ± 1 mm. All participants were evaluated clinically in the following measurements: number of teeth present, probing depth (PD), CAL, supragingival plaque accumulation, and bleeding on probing (BOP). The presence or absence of supragingival plaque and BOP were recorded at four and six sites around each tooth, respectively. Measurements of PD and CAL were conducted with a Williams probe at six sites around each tooth, recorded to the nearest millimeter, and every observation close to 0.5 mm was rounded to the lower whole number. The averaged score for whole-mouth PD, CAL, and the number of sites with plaque and BOP divided by the total number of sites per mouth and multiplied by 100 were calculated for each subject. The presence of periodontitis was defined as having at least one site with CAL $\geq$ 4 mm[23].

The disease activity of RA was determined with Disease Activity Score including 28 joints using CRP (DAS28-CRP), constituting four categories: remission (DAS28-CRP < 2.3), low (2.3 $\leq$ DAS28-CRP < 2.7), moderate (2.7 $\leq$ DAS28-CRP < 4.1), and high disease activity (4.1 $\leq$ DAS28-CRP), which underestimates disease activity compared with DAS28 using erythrocyte sedimentation rate (DAS28-ESR) in Japan[24]. Smoking status of the participants was classified as current-smokers, former-smokers, or never-smokers, according to information provided on a standard questionnaire.

Measurements of inflammatory markers

Peripheral venous blood samples were obtained from all participants. Plasma was isolated from the blood by centrifugation at 1,500 g for 20 min, and stored at -70℃ until use. Concentrations of RF and high-sensitive CRP were determined with a latex particle-enhanced and a simple nephelometric method (SRL, Tokyo, Japan). Anti-CCP antibody levels were determined by sensitive enzyme-linked immunosorbent assay (ELISA) (anti-CCP: Medical & Biological Laboratories, Aichi, Japan), according to the manufacturer's instructions. The microtiter plates were read at a wavelength of 450 nm with an automated microplate reader (Bio-Rad Japan Laboratories, Tokyo, Japan). The lower limits of detection for these measurements were as follows: RF, 1.25 IU/ml; CRP, 0.004 mg/dL; anti-CCP antibodies, 0.4 U/ml. Positivity of RF and anti-CCP antibodies was defined as showing more than15 IU/ml and 4.5 U/ml, respectively. Levels of measurements below the lower limit of detection were recorded as being not determined.

Measurements of IgG against periodontopathic bacteria

Levels of IgG antibodies against periodontopathic bacteria were determined by ELISA. Briefly, each of the 96-wells microtiter plates was coated with 50 μl of sonicated extracts of *Aggregatibacter actinomycetemcomitans* ATCC29523, *Eikenella corrodens* FDC 1073, *Porphyromonas gingivalis* FDC381, or *Prevotella intermedia* ATCC25611 (10 μg/ml) in 50 mM sodium carbonate coating buffer (pH 9.6) for overnight at 4℃. After washing thrice with phosphate-buffered saline (PBS) containing 0.1% Tween 20 (PBST, pH 7.4), 50 μl of the serum (1/3,100 dilution) in PBST was added to each well, and incubated for 2 hours at 37℃. Each well was washed thrice with PBST, and incubated with 50 μl (1/5,000 dilution) of alkaline phosphatase-conjugated goat anti-human IgG (H+L) antibodies (Jackson ImmunoResearch Laboratories, Baltimore, MD, USA) in PBST 2 hours at 37℃. After washing thrice with PBST, color development was performed with 50 μl of *p*-nitrophenylphosphate (Wako Pure Chemical Industries, Osaka, Japan) in 10 % diethanolamin buffer (pH 9.8) for 10 minutes at 15℃, and stopped by addition of 50 μl of 3M N_aOH. Optical density at 405 nm (OD 405 nm) was measured with a microplate reader. ELISA units were defined as the ratio of serum IgG titer specific for each antigen in each individual to that of the control serum that was pooled from ten periodontally healthy donors.

Measurements of amino acid

Plasma levels of amino acids were determined with precolumn derivatization high performance liquid chromatography/electrospray mass spectrometry[25]. The following 21 amino acids and related molecules were measured: Glycine (Gly), Alanine (Ala), Serine (Ser), Proline (Pro), Valine (Val), Threonine (Thr), Isoleucine (Ile), Leucine (Leu), Aspragine (Asn), Glutamine (Gln), Glutamic Acid (Glu), Methionine (Met), Histidine (His), Phenylalanine (Phe), Arginine (Arg), Citrulline (Cit), Tyrosine (Tyr), Tryptophan (Trp), Ornithine (Orn), Lysine (Lys), alpha-aminobutyric acid (α -ABA).

Statistical Analyses

After evaluating the normality of distribution by Kolmogorov-Smirnov test, differences in clinical and laboratory parameter values were assessed among the three groups by Kruskal-Wallis test, and when significant differences were detected, further assessment of differences between the two groups were done by Scheffe's test. Differences in rheumatologic parameter values between the patients with and without anti-TNF therapy were evaluated by Mann-Whitney *U* test. The Spearman's rank correlation coefficient was used to determine the relationship between levels of amino acids and CRP and periodontal conditions. Statistical significance was accepted at 5% ($P < 0.05$).

Results

Demographic and rheumatologic parameters and periodontal infection levels were first compared among the three (RA-TNF, RA-C, and H groups). Both the RA-TNF and RA-C groups showed significantly higher CRP levels and significantly lower number of teeth present and % of never-smokers than the H group ($P < 0.05$) (Table 1). No significant differences were observed between the RA-TNF and RA-C groups in de-

Table 1. Characteristics of patients with rheumatoid arthritis (RA) with and without anti-tumor necrosis factor (TNF) therapy and healthy controls

Parameters	RA-TNF group (n = 26)	RA-C group (n = 26)	H group (n = 29)
Age (years; mean ± SE)	60.3 ± 2.3	60.7 ± 2.3	54.5 ± 2.6
Female (n [%])	20 (76.9)	21 (80.8)	24 (82.8)
Smoker of current/former/never (%)	0/27/73*	0/35/65*	0/0/100
Number of teeth present (mean ± SE)	23.9 ± 1.0*	21.3 ± 1.1*	28.3 ± 0.3
Duration of RA (years; mean ± SE)	11.2 ± 1.3	12.4 ± 2.5	NA
DAS28-CRP (mean ± SE)	2.32 ± 0.20	2.41 ± 0.15	NA
DAS28-CRP category			
Remission/Low-/Moderate-/High-activity (%)	69/19/12/0	50/12/38/0	NA
RA medication			
Corticosteroids (n [%])	13 (50.0)	12 (46.2)	NA
DMARDs (n [%])	18 (69.2)	16 (61.5)	NA
NSAIDs (n [%])	7 (26.9)	7 (26.9)	NA
TNF-inhibitor (n [%])	26 (100.0)**	0 (0.0)	NA
RF levels (IU/ml; mean ± SE)	122.6 ± 57.2	80.4 ± 22.8	NA
RF positive (n [%])	19 (73.1)	17 (65.4)	NA
Anti-CCP titer (U/mL; mean ± SE)	130.1 ± 21.4	171.1 ± 30.3	NA
Anti-CCP antibody positive (n [%])	20 (77.0)	17 (65.4)	NA
CRP levels (mg/dL; mean ± SE)	0.37 ± 0.12*	0.43 ± 0.09*	0.04 ± 0.01
Anti-*A. actinomycetemcomitans* IgG (mean ± SE)	0.36 ± 0.08	0.36 ± 0.11	NA
Anti-*E. corrodens* IgG (mean ± SE)	0.58 ± 0.16	0.40 ± 0.23	NA
Anti-*P. gingivalis* IgG (mean ± SE)	15.72 ± 3.72	11.31 ± 3.90	NA
Anti-*P. intermedia* IgG (mean ± SE)	0.33 ± 0.09	0.23 ± 0.06	NA

RA-TNF, RA patients with anti-TNF therapy; RA-C, RA patients without anti-TNF therapy; H, healthy;

DAS28-CRP, disease activity score including 28 joints using C-reactive protein;

DMARDs, disease-modifying antirheumatic drugs; NSAIDs, non-steroidal anti-inflammatory drugs;

RF, rheumatoid factor; Anti-CCP, anti-cyclic citrullinated peptide; IgG, immunoglobulin G; NA, not applicable.

* Significantly different from the H group (Kruskal-Wallis and Scheffe's tests, $P < 0.05$) .

** Significantly different between the RA-TNF and RA-C groups (Mann-Whitney *U* test, $P < 0.05$) .

mographic profile (age and gender), rheumatologic parameter values (RA duration, DAS28-CRP, RA medication except for anti-TNF therapy) and levels of RF, anti-CCP antibodies, CRP, and IgG against four periodontopathic bacteria ($P > 0.05$) (Table 1). No adverse events were observed in gingival, oral mucosa, and rheumatologic condition of all patients with RA during the study period.

Next, periodontal conditions were compared among the three groups. Both the RA-TNF and RA-C groups exhibited significantly higher % of sites with plaque, PD, CAL, and % of sites with CAL ≥ 4 mm than the H group ($P < 0.05$) (Figure 1 A, C, D, F). Notably, the RA-TNF group showed significantly lower % sites with BOP than the RA-C group ($P < 0.05$), and similar % sites with BOP compared with the H group ($P > 0.05$) (Figure 1 B). % of sites with PD ≥ 4 mm were significantly different between the RA-C and H groups ($P < 0.05$), but not between the RA-TNF and RA-C groups and between the RA-TNF and H groups ($P > 0.05$) (Figure 1 E).

Both the RA-TNF and RA-C groups exhibited significantly lower levels of three amino acids (glycine, glutamine, and histidine) than the H group ($P < 0.05$) (Table 2). Notably, levels of three amino acids (glutamic acid, tryptophan, and ornithine) were significantly different between the RA-TNF and RA-C groups ($P < 0.05$) (Table 2). A significantly higher level of glutamic acid in the RA-TNF group, as well as, a significantly lower and higher level of tryptophan and ornithine in the RA-C group, respectively, was found compared with the H group ($P < 0.05$) (Table 2).

Of these three amino acids, the patients with RA exhibited a significantly negative correlation between tryptophan level and % of sites with BOP ($P = 0.03$) (Figure 2 B). No associations were obtained between levels of other amino acids and CRP and % of sites with BOP ($P > 0.05$) (Figure 2 A, C, and D).

Table 2. Amino acid concentrations in patients with rheumatoid arthritis (RA) with and without anti-tumor necrosis factor (TNF) therapy and healthy controls

Amino acid	RA-TNF group (n = 26)	RA-C group (n = 26)	H group (n = 29)
Non-essential amino acid			
Glycine (Gly)	211.4 ± 10.5*	218.5 ± 8.6*	262.7 ± 13.9
Alanine (Ala)	404.0 ± 17.1	374.0 ± 19.5	404.1 ± 15.5
Serine (Ser)	115.7 ± 8.0	102.2 ± 4.7	121.0 ± 4.0
Proline (Pro)	187.6 ± 11.7	151.2 ± 7.4	185.6 ± 12.7
Aspragine (Asn)	51.7 ± 2.4	48.5 ± 2.1	52.7 ± 2.2
Glutamine (Gln)	542.7 ± 17.1*	544.9 ± 14.4*	600.6 ± 14.3
Glutamic Acid (Glu)	41.5 ± 2.8*	36.3 ± 3.2	28.0 ± 1.7
Arginine (Arg)	88.7 ± 4.7	86.6 ± 3.7	97.1 ± 5.0
Tyrosine (Tyr)	70.6 ± 3.7	67.5 ± 3.3	70.0 ± 3.7
Essential amino acid			
Valine (Val)	228.1 ± 10.9	207.9 ± 8.4	234.3 ± 9.0
Threonine (Thr)	130.0 ± 6.6	117.6 ± 4.7	128.9 ± 5.3
Isoleucine (Ile)	68.1 ± 4.0	61.3 ± 3.7	72.0 ± 3.9
Leucine (Leu)	117.3 ± 6.6	105.2 ± 6.2	124.4 ± 5.2
Methionine (Met)	26.9 ± 1.9	21.9 ± 1.1	24.2 ± 1.2
Histidine (His)	74.2 ± 2.0*	68.0 ± 2.2*	85.8 ± 2.1
Phenylalanine (Phe)	71.4 ± 3.3	77.9 ± 5.7	66.8 ± 2.4
Tryptophan (Trp)	55.9 ± 2.2	50.7 ± 1.9*	59.9 ± 1.8
Lysine (Lys)	178.8 ± 7.9	173.8 ± 5.7	185.8 ± 7.4
Other			
Citrulline (Cit)	29.3 ± 1.8	30.6 ± 1.5	30.9 ± 2.2
Ornithine (Orn)	69.5 ± 3.2	74.5 ± 2.8*	59.9 ± 3.1
alpha-aminobutyric acid (α-ABA)	15.5 ± 1.2	14.3 ± 0.8	14.2 ± 0.8
Sum of non-essential amino acid	190.4 ± 11.3	181.1 ± 11.1	202.4 ± 11.6
Sum of essential amino acid	105.6 ± 4.4	98.3 ± 4.1	109.1 ± 4.2

RA-TNF, RA patients with anti-TNF therapy; RA-C, RA patients without anti-TNF therapy; H, healthy;

Values represent the mean ± SE (μmol/L).

* Significantly different from the H group (Kruskal-Wallis and Scheffe's tests, $P < 0.05$) .

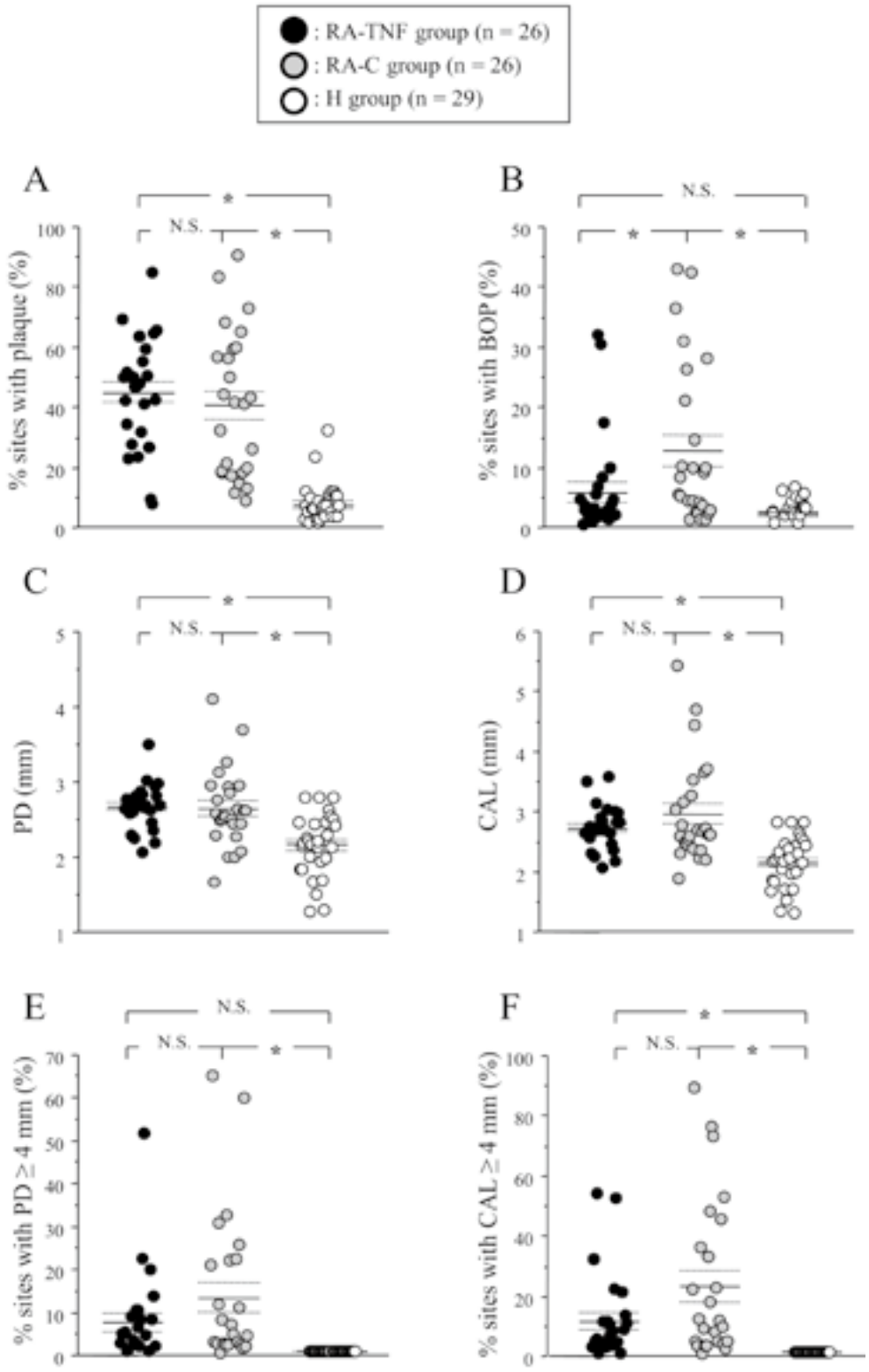

Figure 1. Periodontal profiles in patients with rheumatoid arthritis (RA) with and without anti-tumor necrosis factor (TNF) therapy and healthy controls. The mean and standard error are indicated for % sites with plaque (A), % sites with BOP (bleeding on probing) (B), PD (probing depth) (C), CAL (clinical attachment level) (D), % sites with PD ≥ 4 mm (E), % sites with CAL ≥ 4 mm (F) in the three groups. RA-TNF, RA patients with anti-TNF therapy; RA-C, RA patients without anti-TNF therapy; H, healthy.

*: A significant difference was observed between the two groups by Scheffe's test, following a significant difference was obtained among the three groups by Kruskal-Wallis test ($P < 0.05$).

N.S.: No significant difference was observed between the two groups.

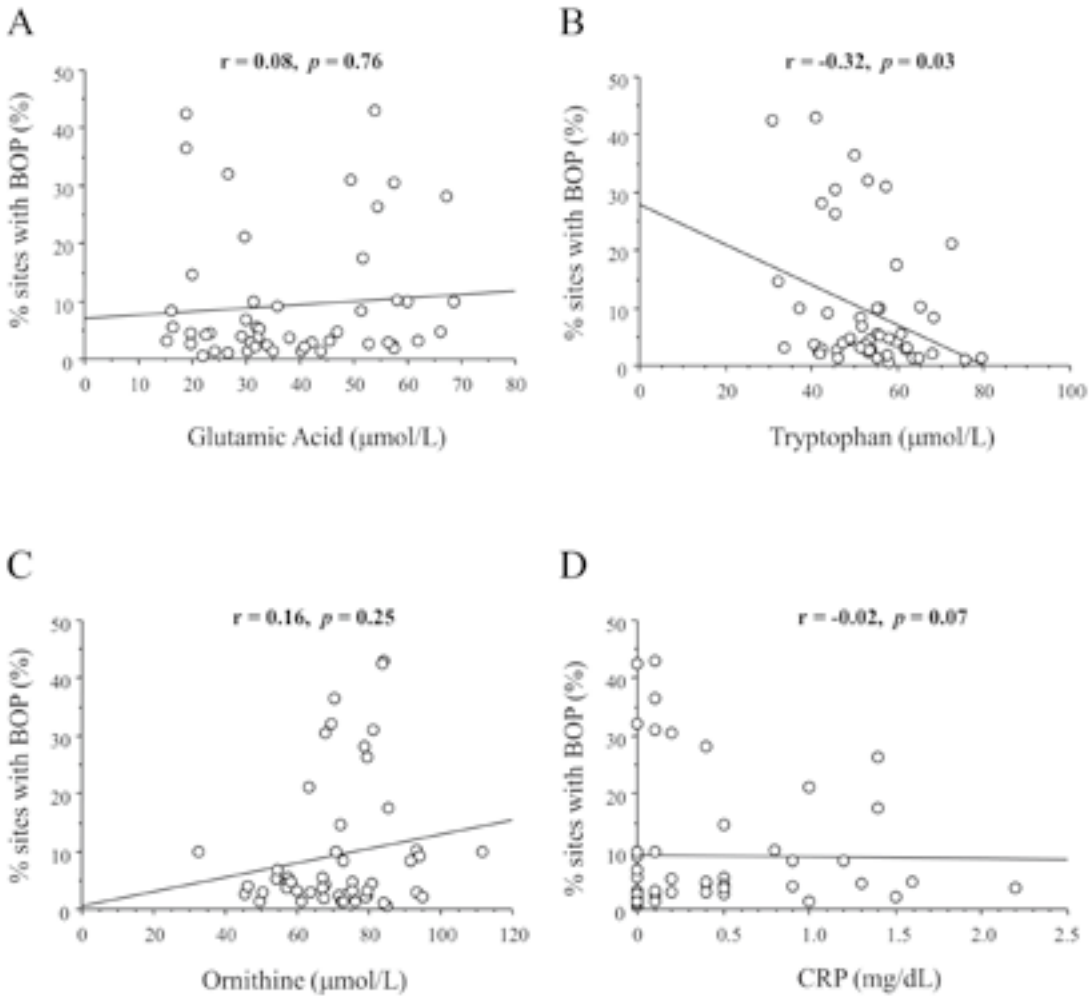

Figure 2. Relationship between levels of amino acids (A: glutamic acid; B: tryptophan; C: ornithine) and CRP (D) and % sites with bleeding on probing (BOP) in 52 patients with rheumatoid arthritis. A significantly negative correlation was found between tryptophan levels and % sites with BOP (P = 0.03), as assessed by the Spearman's rank correlation coefficient.

Discussion

The results of the present study showed that the percentage of females was 78.8%, the mean age was 60.5 years, and the mean RA duration was 11.8 years in the RA group. These observations are almost in accordance with the results of a race-matched large-cohort (7512 patients) IORRA study showing a marked female predominance (82.36 to 84.09 %), the mean age of 57.08 to 58.05 years, and 9.96 to 12.03 years of RA duration[26]. Additionally, the data showed no difference in rheumatologic condition such as DAS28-CRP between the patients with and without anti-TNF therapy. The lack of difference in RA activity might be partially explained by the results of the present study showing that more than 50 % of patients in both RA groups displayed remission. These findings may reflect the well-controlled clinical condition in patients with RA, regardless of TNF inhibition.

As expected, the patients with anti-TNF therapy exhibited significantly lower BOP scores than those without TNF inhibition, which is consistent with the results of other studies[13,14]. A possible confounding factor including oral hygiene condition, demographic, periodontal, and rheumatologic profiles, and levels of CRP and IgG against periodontopathic bacteria proved comparable between the patients with and without anti-TNF therapy. It has been demonstrated that a decreased level of TNF-α was observed during anti-TNF thera-

py[27]. Therefore, it is conceivable that improvement of BOP score might be partially explained by a decrease in TNF-α level. It would be necessary to monitor TNF-α levels from the start of anti-TNF therapy, which is the major limitation of the present study.

The patients with RA showed decreased levels of glycine, glutamine, and histidine, regardless of TNF inhibition. These observations are supported by the results of other studies[15,17,28-30]. It has been documented that lower levels of histidine in individuals with RA might be partially due to increased degradation of histidine through the histidine decarboxylase[15,17,28,29]. It has also been reported that plasma glycine levels were decreased in patients with RA than those in healthy individuals[30].

Notably, the patients without anti-TNF therapy exhibited a significantly lower level of tryptophan than the healthy controls, while the patients with TNF inhibition showed the intermediate level. Additionally, a significantly negative correlation was found between tryptophan level and BOP score. These observations suggest an inhibitory effect of TNF-α on tryptophan metabolism, which is supported by the results of other study demonstrating TNF-α-mediated up-regulation of IDO, the tryptophan-degrading enzyme[21]. It has also been reported that IDO expressions were higher in the periodontitis lesions than those in the healthy gingival tissue[31], implicating a decreased level of tryptophan in inflammation. To best of our knowledge, this is the first study demonstrating an association between plasma tryptophan level and periodontal inflammatory condition in patients with RA. However, the authors cannot conclude from these findings alone whether tryptophan plays a role in the pathogenesis of RA and periodontitis. In order to confirm and extend the observations obtained from the present study, further studies would be required to evaluate the effects of tryptophan metabolism on the periodontal condition in patients with RA in a longitudinal clinical study.

Other amino acid results indicated a significant increase in level of glutamic acid in the patients with anti-TNF therapy compared with healthy controls, which is in agreement with the findings of other studies[30,32]. The results also showed that the ornithine level proved significantly increased in the control patients with RA and a relatively increased in the patients with TNF inhibition compared with healthy controls. These observation are different from the results of other study documenting a similar level of ornithine between individuals with RA and healthy control[30].

In summary, these results suggest that the amino acid and periodontal profiles may be different between RA patients with and without anti-TNF therapy. A possible association is also implicated between tryptophan level and periodontal inflammation in patients with RA.

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