Proteomic analysis identified salivary immunoglobulin gamma-3 chain C as a potential biomarker for systemic lupus erythematosus

Ju-Yang Jung

Ajou University School of Medicine and Graduate School of Medicine

Jin-Young Nam

Ajou University School of Medicine and Graduate School of Medicine

Keun-Sil Ryu

Ajou University School of Medicine and Graduate School of Medicine

Joo-Ho Shin

Sungkyunkwan University - Suwon Campus

Sung- Min Lee

Ajou University School of Medicine and Graduate School of Medicine

Wook-Young Baek

Ajou University School of Medicine and Graduate School of Medicine

Hyoun-Ah Kim

Ajou University School of Medicine and Graduate School of Medicine

Chang-Hee Suh (✉ chsuh@ajou.ac.kr)

Ajou University School of Medicine <https://orcid.org/0000-0001-6156-393X>

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Abstract

Background

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of autoantibodies and systemic inflammatory response. We aimed to characterize the salivary protein components and find biomarkers in patients with SLE.

Methods

The pooled salivary proteins of patients with SLE and healthy controls were subjected to 2-dimensional gel electrophoresis. The spots exhibiting > 2-fold intensity change between SLE and healthy controls were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry analysis.

Results

The proteomic analysis using 2-dimensional gel electrophoresis and mass spectrometry revealed 10 differentially expressed protein spots, which included immunoglobulin gamma-3 chain C region (IGHG3), immunoglobulin alpha-1 chain C region (IGHA1), protein S100, lactoferrin, leukemia-associated protein 7, and 8-oxoguanine deoxyribonucleic acid glycosylase. The patients with SLE exhibited enhanced salivary IGHG3 (3.9 ± 2.15 pg/mL) and lactoferrin (4.7 ± 1.8 pg/mL) levels than patients with rheumatoid arthritis (1.8 ± 1.01 pg/mL and 3.2 ± 1.6 pg/mL, respectively, $p < 0.001$ for both) or healthy controls (2.2 ± 1.64 pg/mL and 2.2 ± 1.7 pg/mL, respectively, $p < 0.001$ for both). The salivary IGHG3 levels correlated with erythrocyte sedimentation rate ($r = 0.26$, $p = 0.01$), anti-double-strand deoxyribonucleic acid antibody levels ($r = 0.25$, $p = 0.01$), and nephritis ($r = 0.28$, $p = 0.01$).

Conclusions

Patients with SLE exhibited elevated salivary IGHG3 and lactoferrin levels, and the salivary IGHG3 levels correlated with disease activity markers of SLE. Salivary IGHG3 may be a promising non-invasive biomarker in SLE.

Background

Systemic lupus erythematosus (SLE) is a heterogeneous autoimmune disease characterized by the production of pathogenic autoantibodies and aberrant inflammatory response leading to diverse clinical manifestations.[1]. The disease status of SLE, including clinical manifestations and disease activity varies with the progression of the disease. However, there are a lack of indicators to represent changes of disease in SLE. Currently, anti-double-stranded deoxyribonucleic acid (dsDNA) antibody and complement protein levels are used as the markers for diagnosis or monitoring of SLE [2, 3]. The anti-dsDNA

antibodies target the intracellular DNA and induce apoptosis. The anti-dsDNA antibodies cross-react with the α -actin present in the glomeruli of patients with SLE exhibiting renal disease [4]. Although anti-dsDNA antibody is detected in 60–83% of patients with SLE, contradictory results have been reported by the studies investigating the association of anti-dsDNA antibody with disease flares within a few weeks or months [5]. Deficiencies of complement proteins involved in the classical complement pathway are reported to confer susceptibility to SLE. The activation of complement system leads to the complement protein deficiency during SLE disease flares [6]. However, complement protein is not a reliable indicator of active SLE status as its concentration varies widely. Additionally, the complement protein level does not indicate the consumption in tissue or the presence of anti-complement autoantibody [7]. The changes in the expression levels of these biomarkers are often non-specific and thus are not a reliable indicator of the progression of SLE. Therefore, several studies are focused on identifying new biomarkers for SLE. Several candidates, such as cytokines, immune cells, autoantibodies, or genetic markers have been identified as potential biomarkers for SLE.

Proteomics is used to detect the protein or peptide in the body fluids or tissues. Two-dimensional gel electrophoresis (2-DE) with mass spectrometry (MS) is a reliable method for identification and characterization of protein constituents in the tissues or body fluids including blood, urine, or saliva. The 2-DE with MS method can detect the protein constituents with high sensitivity and specificity [8, 9]. The 2-DE with MS method has been used to identify novel biomarkers for the diagnosis or disease monitoring of rheumatic diseases [10].

Saliva is a body fluid that can be collected repeatedly using a non-invasive and risk-free procedure [11]. The salivary proteins are derived from the salivary glands and blood. As the composition of saliva is similar to that of blood, saliva is used to identify the disease biomarkers for not only oral disorders, but also for systemic diseases [12–14]. Some studies have suggested that salivary cytokines secreted by the B cells are potential biomarkers for Sjogren's syndrome (SS) [15]. Salivary biomarkers are promising candidates for the diagnosis of SS, which is an autoimmune disorder involving salivary gland and shares several common characteristics with SLE [16, 17]. Proteomic analysis revealed a differential salivary protein composition between primary and secondary SS [18]. The levels of salivary α -enolase, β -2 microglobulin, and immunoglobulin kappa light chain are different between patients with SS and those with other autoimmune diseases exhibiting sicca symptoms.

Currently, there are limited studies that have analyzed the saliva samples of patients with SLE. In this study, we analyzed the composition and concentration of salivary proteins in patients with SLE by 2-DE with MS. The clinical relevance of the differentially expressed proteins was analyzed in patients with SLE.

Methods

Participants

The analysis was performed in two steps (additional file 1). In the first step, the differential salivary protein composition between patients with SLE and healthy controls (HCs) were analyzed by 2-DE with

MS. In the next step, the differentially expressed proteins identified in the 2-DE with MS analysis were validated by western blotting and enzyme-linked immunosorbent assay (ELISA).

There were two participant groups in this study: The first group included 11 patients with SLE and 11 HCs, whose salivary samples (the samples were pooled for each group) were subjected to 2-DE with MS proteomic analysis. The second participant group included 94 patients with SLE, 57 patients with RA, and 62 HCs (Table 1). The concentration of proteins in the saliva samples of HCs and patients with SLE or RA was validated by western blotting and ELISA.

All enrolled patients with SLE met the revised American College of Rheumatology classification criteria [19]. Additionally, the age-matched and sex-matched patients with RA and HCs who had no history of autoimmune or inflammatory disorders were enrolled in the study. This study was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines. All subjects provided their informed consents for participating in the study. The study protocol was approved by the institutional review of board of our hospital (BMR-SMP-13-199).

The information on medical history and clinical manifestations was collected from a chart review and blood test results, such as complete blood count, erythrocyte sedimentation rate (ESR) and the levels of anti-nuclear antibody, complement 3 and 4, and anti-dsDNA antibody. The patients with RA were enrolled as a disease control to analyze the differential expression of specific proteins between SLE and RA, which are both chronic autoimmune diseases.

Basic characteristics of second participant group is here: the mean age of patients with SLE, patients with RA, and HCs was 39.8 ± 9.8 , 41 ± 7.9 , and 39.5 ± 6.9 years, respectively. The mean age was not different between the three groups. Among the patients with SLE, 41 patients (43.6%) tested positive for anti-dsDNA antibody, 28 patients (29.8%) had mucocutaneous symptoms, 31 patients (33.0%) had arthritis, and 29 patients (30.9%) had nephritis. The mean SLE disease activity index (SLEDAI) was 3.8 ± 4.2 . Among the 57 patients with RA, 45 patients (77.6%) tested positive for rheumatoid factors and their mean disease activity score including 28 joints (DAS28) was 3.3 ± 1.15 .

Table 1 Clinical characteristics of the subjects

	SLE	RA	HC
Number	94	57	62
Age, years	39.8 ± 9.8	41 ± 7.9	39.5 ± 6.9
Sex (F/M)	87/7	50/7	58/4
Leukocyte, / μ L	5165 ± 2365		
Hemoglobin, / μ L	12.2 ± 2.4		
Platelet, x 10 ³ / μ L	221.8 ± 76.1		
Lymphocyte, / μ L	1454 ± 654		
ESR, mm/h	16.4 ± 18		
Complement 3, mg/dL	85.2 ± 26.9		
Complement 4, mg/dL	18.6 ± 8.9		
Anti-dsDNA Ab (+), n (%)	41 (43.6)		
Rheumatoid factor (+), n (%)		45 (77.6)	
Mucocutaneous involvement, n (%)	28 (29.8)		
Arthritis, n (%)	31 (33)		
Nephritis, n (%)	29 (30.9)		
Serositis, n (%)	4 (3.8)		
Hematologic involvement, n (%)	35 (37.6)		
SLEDAI	3.8 ± 4.2		
DAS-28		3.3 ± 1.2	

All values presented as number (%) or mean ± standard deviation

Ab, antibody; DAS28, disease activity score including 28 joints; dsDNA, double-strand deoxyribonucleic acid; ESR, erythrocyte sedimentation rate;

HC, healthy control; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SLEDAI, systemic lupus erythematosus disease activity index

Saliva sample collection

As salivary proteins exhibit diurnal variations, the saliva samples were collected from all participants between 9:00 and 11:00 am. The subjects were not allowed to eat, drink, smoke, or perform oral hygiene procedures for at least 1 h prior to the sample collection. The saliva samples were collected for 5 min after the subjects rinsed their mouth with water [20]. The saliva secretion was not stimulated in the study

subjects. The subjects were asked to keep their mouths closed and expectorate the saliva into a tube once per minute. Each saliva sample was immediately treated with the protease inhibitors to preserve the integrity of the protein constituents. The saliva samples were centrifuged at 3,000 rpm for 15 min at 4°C. After removing the clear supernatant, the samples were aliquoted and stored at -20°C until further use.

Two-dimensional gel electrophoresis (2-DE)

The samples from 11 patients with SLE or 11 HCs were pooled equally to avoid intra-class variations that were detected between the patients in 2-DE analyses. A 1 mL aliquot of the sample was concentrated 10 times using the Amicon-3K centrifugal filters at 14,000 g and 4°C for 20 min. The proteins in the salivary samples were precipitated using 500 µL of trichloroacetic acid/acetone (90%; v/v)-dithiothreitol mixture overnight at -20°C. The samples were centrifuged at 10,000 rpm and 10°C for 10 min. The supernatant was collected and the samples were pretreated with 250 µL of rehydration buffer. Next, the samples were centrifuged at 10,000 rpm and 10°C for 10 min to remove any insoluble material. The protein concentration of the samples was estimated by Bradford protein assay (Bio-Rad, Hercules, CA, USA).

Liquid chromatography tandem mass spectrometry (LC-MS)

With 2-DE proteomic analysis of the saliva samples of 11 patients with SLE and 11 HCs, the proteins separated into numerous spots with different concentrations. The proteins in 20 spots were subjected to liquid chromatography tandem-mass spectrometry (LC-MS) to analyze the proteins with high specificity [21]. The gel pieces containing the protein spots were destained, reduced, alkylated, and digested with modified sequencing grade trypsin (Sigma, MO, US A), as previously described [22]. Peptide mixtures were lyophilized and stored at -80°C for further LC-MS analysis.

Each sample was resuspended in 0.1% trifluoroacetic acid and injected into a Zorbox 300SB-C18 75 µm × 15 cm column (Agilent, Santa Clara, CA, USA) via the trap column. The peptides were separated in an acetonitrile gradient at a flow rate of 200 nL/min in an UltiMate 3000 nano HPLC system (Dionex, Sunnyvale, CA, USA). The peptides were then applied on-line to an LTQ (Thermo Fisher, Waltham, MA, USA) ion-trap mass spectrometer. The mobile phase gradient was initiated with an increase from 5 to 40% buffer within 110 min. Next, the gradient was increased to 80% buffer in 1 min. The gradient was maintained at isocratic conditions of 80% buffer for 15 min. The main working liquid-junction electrospray ionization source parameters were as follows: ion spray voltage, 1.6 kV; capillary voltage, 24 V; and capillary temperature, 200°C. The spectra were obtained in full scan mode using the dynamic exclusion criteria. LC-MS runs were analyzed using the DeCyder MS (version 2.0; GE Healthcare, Uppsala, Sweden) software.²³ The peptide peaks were detected with an average peak width of 1 min and matched with a mass accuracy of at least 0.6 Da and a maximum time window of 4 min. The abundance of individual peptides in the respective gradient fraction was calculated by peak integration.

The data were manually examined, and the overlapping peaks were discarded (additional file 2). The threshold level for differentially expressed proteins was defined as at least 2-fold increase or decrease in spot intensity that was statistically significant. The MS spectra of the peptide peaks were searched

against the Uniprot Human database using the Mascot version 2.3 (Matrix Science, London, UK). For quantitative protein profiling, only the proteins identified by multiple peptides with significant MASCOT score ($p < 0.05$) were considered.

Western blotting analysis

The immunoglobulin gamma-3 chain C region (IGHG3), immunoglobulin alpha-1 chain C region (IGHA1), protein S100-A8 (S100A8), lactoferrin, and 8-oxoguanine DNA glycosylase (OGG1) were analyzed by western blotting using the rabbit anti-human IGHG3 polyclonal (MBS248789, MyBiosource, San Diego, CA, USA), rabbit anti-human IGHA1 polyclonal (MBS9206028, MyBiosource), rabbit anti-human rat S100A8 polyclonal (MBS127619, MyBiosource), mouse anti-human lactoferrin monoclonal (ab101110, Abcam, Cambridge, UK), and rabbit anti-human OGG1 polyclonal (NB100-106, Novusbio, Centennial, CO, USA) antibodies, respectively. The proteins were subjected polyacrylamide gel electrophoresis using 10% (for IGHG3) or 15% (for IGHA1, S100/A8, lactoferrin, and OGG1) gel. The resolved proteins were transferred to a polyvinylidene fluoride membrane. The membranes were incubated with secondary antibody (goat anti-rabbit antibody A120-101P for IGHG3, IGHA1, S100A8, and OGG1, and goat anti-mouse antibody for lactoferrin, Bethyl Laboratories, Montgomery, TX, USA) diluted at 1:10,000 (IGHG3 and S100/A8), and 1:2,000 (IGHA1, lactoferrin, and OGG). All analyses were performed in triplicates. The protein concentration was determined by the optical density of specific immunoreactive bands, and the optical density of each bands was measured using image J software (NIH, Bethesda, MD, USA).

Enzyme-linked immunosorbent assay (ELISA)

The levels of salivary IGHG3 and lactoferrin were measured in patients with SLE or RA and HC by ELISA using the human IGHG3 ELISA kit (ab137981, Abcam) and human lactoferrin ELISA kit (ab108882, Abcam), respectively, following the manufacturer's instructions. All measurements were performed in duplicates.

Statistical analysis

The difference in the expression of salivary IGHG3, IGHA1, S100A8, lactoferrin, and OGG1 determined by western blotting and the concentrations of salivary IGHG3 and lactoferrin measured by ELISA in patients with SLE or RA and HCs were compared using the two-sample Wilcoxon rank-sum (Mann-Whitney) test. As the data were not normally distributed and the variance was not homogeneous among the groups, one-way analysis of variance (ANOVA) was not used. Hence, post-hoc test with Bonferroni correction was used. The correlations between the levels of salivary IGHG3 or lactoferrin and clinical features in patients with SLE were determined using the Spearman's rank correlation coefficient. On the receiver operating characteristic (ROC) curve analysis of salivary proteins, area under curve (AUC), sensitivity, and specificity were calculated. The difference was considered statistically significant when the p value was less than 0.05. All statistical analyses were performed in the Statistical Package for the Social Sciences version 22.0 (IBS Corp, Armonk, NY, USA) and SAS9.4 (SAS institute Inc, Cary, NC, USA).

Results

Salivary protein identification

The 2-DE analysis revealed a differential salivary protein expression pattern between patients with SLE and HCs (Fig. 1). The protein identity, fold change, and peptide sequence between the two groups were determined by LC-MS analysis and quantitative protein profiling. Among the 10 spots exhibiting fold change values higher than 2, two spots were identified as alpha-amylases, protein S100, and OGG1 (Table 2). The other spots were identified as IGHG3, IGHA1, lactoferrin, and leukemia-associated protein 7.

Table 2 List of 10 salivary peptides with different concentration on 2-dimensional electrophoresis analysis between patients with systemic lupus erythematosus and health controls

Spot	Increased protein name	Fold of variation SLE vs HC	Anova, <i>p</i> value
2	Immunoglobulin gamma-3 chain C region	4.101	0.000158
3	Protein S100	3.894	0.0045
6	8-oxoguanine DNA glycosylase	3.516	0.00637
7	Protein S100	2.947	2.48E-08
8	Lactotransferrin	2.827	4.06E-07
9	8-oxoguanine DNA glycosylase	2.794	0.00653
Spot	Decreased protein name	Fold of variation SLE vs HC	Anova, <i>p</i> value
1	Alpha-amylase 1	-4.228	0.000691
4	Alpha-amylase 1	-3.676	0.0372
5	Immunoglobulin alpha-1 chain C region	-3.532	0.00511
10	Leukemia-associated protein 7	-2.176	0.0178

Anova, analysis of variance; DNA, deoxyribonucleic acid; HC, healthy control; SLE, systemic lupus erythematosus

Expressions of salivary proteins in patients with SLE

Western blot analysis was performed on age-matched 14 saliva samples of patients with SLE, 12 saliva samples of patients with RA, and 8 saliva samples of HCs (Fig. 2). Among the patients with SLE, the disease duration was 6.6 ± 5.7 years, systemic lupus erythematosus disease activity index (SLEDAI) was 8.5 ± 4.6 , complement 3 (C3) level was 59.3 ± 25.8 mg/dL, C4 level was 9.4 ± 4.5 mg/dL, and ESR was 31.4 ± 24.5 mm/h. In addition, 10 patients tested positive for anti-dsDNA antibody, 5 patients had mucocutaneous symptoms, 5 patients had arthritis, 6 patients had active lupus nephritis (LN), and 5

patients had hematologic symptoms. Among the 8 patients with RA, the mean disease duration was 5.8 ± 7.6 years and mean disease activity score-28 (DAS28) was 2.49 ± 1.23 .

Western blotting analysis revealed that the patients with SLE exhibited enhanced expression of salivary IGHG3 and lactoferrin when compared to patients with RA and HCs (both $p < 0.01$). The expression levels of salivary IGHA1 in patients with SLE were not significantly different when compared to those in patients with RA and HCs (both $p = 0.09$). Similarly, the expression levels of salivary S100A8 and OGG1 in patients with SLE were not different.

Salivary IGHG3 and lactoferrin in SLE

The salivary levels of IGHG3 and lactoferrin were measured using ELISA (Fig. 3). The salivary levels of IGHG3 were elevated (3.9 ± 2.15 pg/mL) in patients with SLE when compared to those in patients with RA (1.8 ± 1.01 pg/mL, $p < 0.001$) and HCs (2.2 ± 1.64 pg/mL, $p < 0.001$). The salivary levels of lactoferrin in patients with SLE were elevated (4.7 ± 1.8 pg/mL) when compared to those in patients with RA (3.2 ± 1.6 pg/mL, $p < 0.001$) and HCs (2.2 ± 1.7 pg/mL, $p < 0.001$).

The ROC curve analysis of salivary IGHG3 and lactoferrin revealed that the AUC was 0.78 (95% confidence interval 0.72-0.84) and 0.79 (95% confidence interval 0.73-0.85), respectively (Fig. 4). The sensitivity and specificity of salivary IGHG3 were 67.3% and 76.8% with a cut-off value of 2.37 pg/mL for the diagnosis of SLE, respectively. The sensitivity and specificity of salivary lactoferrin were 77.3% and 73.9% with a cut-off value of 4.36 pg/mL for the diagnosis of SLE, respectively.

Correlation of salivary proteins and clinical features in SLE

The concentrations of salivary IGHG3 correlated with the ESR ($r = 0.26$, $p = 0.01$), anti-dsDNA antibody levels ($r = 0.25$, $p = 0.01$), and nephritis ($r = 0.28$, $p = 0.01$), whereas the levels of salivary lactoferrin were not correlated. Additionally, the concentration of salivary IGHG3 in patients with LN (4.66 ± 1.87 pg/mL) was significantly higher than that in patients without LN (3.57 ± 2.2 pg/mL, $p = 0.006$) (Fig. 5).

Table 3 Correlation of salivary proteins and clinical characteristics of systemic lupus erythematosus

	IGHG3		Lactoferrin	
	r	p value	r	p value
ESR	0.26	0.01	-0.09	0.4
Complement 3	-0.02	0.89	0.21	0.05
Complement 4	-0.02	0.88	0.2	0.06
Anti-dsDNA Ab (+)	0.25	0.01	-0.05	0.63
Mucocutaneous involvement	0.15	0.14	0.08	0.48
Arthritis	-0.1	0.36	0.01	0.9
Nephritis	0.28	0.01	-0.01	0.94
Serositis	0.04	0.74	-0.15	0.16
Hematologic disease	-0.02	0.83	-0.08	0.45
SLEDAI	0.03	0.74	-0.09	0.42

Ab, antibody; dsDNA, double-strand deoxyribonucleic acid; ESR, erythrocyte sedimentation rate; IGHG3, immunoglobulin gamma-3 chain C

region; SLEDAI, systemic lupus erythematosus disease activity index

Discussion

The 2-DE with MS proteomic analysis of saliva revealed that the densities of ten spots were significantly different between patients with SLE and HCs. These spots were identified as IGHG3, IGHA1, protein S100, lactoferrin, OGG1, and leukemia-associated protein 7. Immunoblotting analysis revealed that the expression levels of salivary IGHG3 and lactoferrin in patients with SLE were significantly higher than those in patients with RA and HCs. Additionally, the salivary IGHG3 levels correlated with the ESR, anti-dsDNA antibody, and nephritis in patients with SLE.

The role of IgG in the pathogenesis of SLE is well known. The interaction between anti-dsDNA IgG antibody and pleural mesothelial cells induces the synthesis of proinflammatory cytokines, which is the pathogenic mechanism underlying serositis in SLE [23]. Elevated level of serum IgG is a poor prognostic factor for autoimmune hepatitis associated with SLE. Additionally, the deposition of IgG is reported to cause tissue damage [24, 25]. The serum IgG levels correlated with the serologic activity and can predict disease flares in patients with LN. Additionally, IgG induces the expression of calcium/calmodulin-dependent protein kinase IV, which is highly expressed in the podocytes of patients with LN causing renal damage [26, 27]. IGHG3 is a constant region of immunoglobulin heavy chains. IGHG3 enables the binding of IgG to the Fcγ receptor (FcγR) of neutrophilic granulocyte and macrophages. FcγRI on the surface of dendritic cells promotes the antigen presentation of dendritic cells to the T cells [28]. Aberrant expression of FcγR for IgG was observed in patients with arthritis and SLE. FcγR is involved in the antigen

presentation and immune-complex-mediated maturation of dendritic cells, regulation of B-cell activation, and plasma cell survival in SLE [29]. The role of FcγR and the effect of its gene polymorphisms on the susceptibility or manifestations of SLE has been previously studied [30–32]. A study on patients with autoimmune hemolytic anemia demonstrated that the levels of IGHG3 in the red blood cells (RBC) were associated with the frequency of RBC transfusion after diagnosis [33]. The enhanced expression of IGHG3, an immunoprotein, in RBC may result in severe hemolysis. Our ROC analysis suggested that the salivary IGHG3 had reliable specificity and sensitivity to differentiate the patients with SLE from patients with RA and HCs. The mechanism underlying elevated salivary IGHG3 levels in patients with SLE must be investigated further. However, our results suggest that salivary IGHG3 may be a differential diagnostic biomarker for SLE. Moreover, salivary IGHG3 levels correlated with the disease activity markers, such as the ESR and anti-dsDNA antibody levels. Additionally, the levels of IGHG3 were significantly elevated in patients with LN.

Lactoferrin, also known as lactotransferrin, is a multifunctional glycoprotein that belongs to the transferrin family. Lactoferrin expression is detected in the mucosal secretions and secondary granules of polymorphonuclear leukocytes [34]. Lactoferrin not only plays an important role in protection against microorganisms, but also in immunomodulation, inflammation, and anticancer activity through its interactions with the host immune system [35, 36]. Lactoferrin-specific IgG autoantibodies were detected in the serum of patients with SLE or RA [37]. The release of surface-expressed lactoferrin from the polymorphonuclear neutrophils modulates the cytokine production in the T helper cell type 1 (Th1). The decreased expression of lactoferrin in patients with SLE is associated with abnormal Th1/Th2 production [38]. Lactoferrin-containing immune complexes induce the production of pro-inflammatory cytokines in the monocytes and monocyte-derived macrophages and can promote the pro-inflammatory M1-like phenotype of human macrophages [39, 40]. Inflammatory M1 macrophages are known to mediate severe and non-resolving inflammation in SLE [41]. Salivary lactoferrin has a role in antimicrobial activity against bacteria, viruses and fungi. Patients with periodontal disease exhibit elevated levels of salivary lactoferrin [42]. In this study, the levels of salivary lactoferrin were elevated only in patients with SLE, and not in patients with RA and HCs.

One of the limitations of this study was that most of the patients were administered with standard drugs for the management of SLE at the time of sample collection. Hence, the difference in their disease activity was not large. Additionally, the administration of drugs may have affected the protein components in the samples. Furthermore, the number of samples was not sufficient to analyze the difference in protein components based on clinical features.

Conclusions

This work revealed the differential salivary protein composition in patients with SLE and RA and HCs. The levels of salivary IGHG3 and lactoferrin in patients with SLE were significantly higher than those in patients with RA and HCs. The concentrations of salivary IGHG3 and lactoferrin can be used as a differential biomarker for diagnosing SLE. Additionally, salivary IGHG3 may be a non-invasive biomarker

of disease activity because that level correlated with the ESR and anti-dsDNA antibody and were higher in patients with LN.

Abbreviations

AUC, area under curve; DAS28, disease activity score including 28 joints; 2-DE, 2-dimensional gel electrophoresis; dsDNA, double-strand deoxyribonucleic acid; ELISA, enzyme-linked immunosorbent assay; ESR, erythrocyte sedimentation rate; HCs, healthy controls; IGHA1, immunoglobulin alpha-1 chain C region; IGHG3, immunoglobulin gamma-3 chain C region; LTF, lactoferrin; LN, lupus nephritis; MS, mass spectrometry; OGG1, 8-oxoguanine DNA glycosylase; RA, rheumatoid arthritis; RBC, red blood cells; ROC, receiver operating characteristic; S100A8, protein S100-A8; SLE, systemic lupus erythematosus; SLEDAI, SLE disease activity index, SS, Sjogren's syndrome.

Declarations

Ethics approval and consent to participate

This study was conducted according to the Declaration of Helsinki and Good Clinical Practice guidelines. All subjects provided their informed consent for participating in the study. The study protocol was approved by the institutional review of board of our hospital (BMR-SMP-13-199).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author Contributions

JYJ analyzed and interpreted the data and drafted the work. JYN, KSR, SML, WYB, and HAK analyzed and interpreted the data. JHS, HK interpreted the data. CHS designed of the work, interpreted the data, and

revised the work substantively. All authors agreed and approved the submitted version.

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Additional Files

Additional file 1. Supplementary Fig. 1 Brief process to find salivary biomarker for systemic lupus erythematosus

Twenty spots were selected as candidates through 2 dimensional-electrophoresis, and the concentrations of 5 proteins were compared through western blot (WB). Finally, the levels of salivary immunoglobulin gamma-3 chain C region (sIGHG3) and lactoferrin (sLTF) were analyzed among patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and healthy controls (HC).

Additional file 2. Supplementary Table 1 List of 20 salivary peptides with different concentration on 2D electrophoresis analysis between patients with systemic lupus erythematosus and health controls

The raw data of mass spectrometry analysis

Figures

Figure 1

Representative 2-dimensional gel electrophoresis protein map The salivary samples from 11 patients with systemic lupus erythematosus (SLE) or 11 healthy controls (HCs) were pooled equally and isoelectric focusing was conducted with immobilized pH gradient strip and isoelectric point of 3-10 nonlinear. The concentration of 10 separated spots (marked as arrow) was different between the two groups. The experiments were performed in triplicate.

Figure 2

Expressions levels of salivary proteins Western blot analysis was performed on age-matched 14 saliva samples of patients with systemic lupus erythematosus (SLE), 8 saliva samples of patients with rheumatoid arthritis (RA), and 8 saliva samples of healthy controls (HCs). Patients with SLE exhibited enhanced expression of salivary IGHG3 and lactoferrin compared to patients with RA and HCs

Figure 3

Concentrations of salivary immunoglobulin gamma-3 chain C region and lactoferrin The levels of salivary immunoglobulin gamma-3 chain C region (sIGHG3) and salivary lactoferrin (sLTF) were measured in patients with systemic lupus erythematosus (SLE) or rheumatoid arthritis (RA) and healthy controls (HCs) by enzyme-linked immunosorbent analysis. The salivary levels of IGHG3 and lactoferrin were elevated in

patients with SLE compared to those in patients with RA and HCs. All measurements were performed in duplicates.

Figure 4

The receiver operating characteristics curves for salivary immunoglobulin gamma-3 chain C region and lactoferrin The area under curve (AUC), sensitivity and specificity of salivary immunoglobulin gamma-3 chain C region (IGHG3) were 0.78, 67.3%, and 76.8% with a cut-off value of 2.37 pg/mL for the diagnosis

of systemic lupus erythematosus (SLE) compared to patients with rheumatoid arthritis (RA) and healthy controls (HC), respectively. The AUC, sensitivity, and specificity of salivary lactoferrin (LTF) were 0.79, 77.3%, and 73.9% with a cut-off value of 4.36 pg/mL for the diagnosis of SLE, respectively.

Figure 5

Concentrations of salivary immunoglobulin gamma-3 chain C region in patients with lupus nephritis The levels of salivary immunoglobulin gamma-3 chain C region (sIGHG3) patients with lupus nephritis was significantly higher than that in patients without lupus nephritis. All measurements were performed in duplicates.

Supplementary Files

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