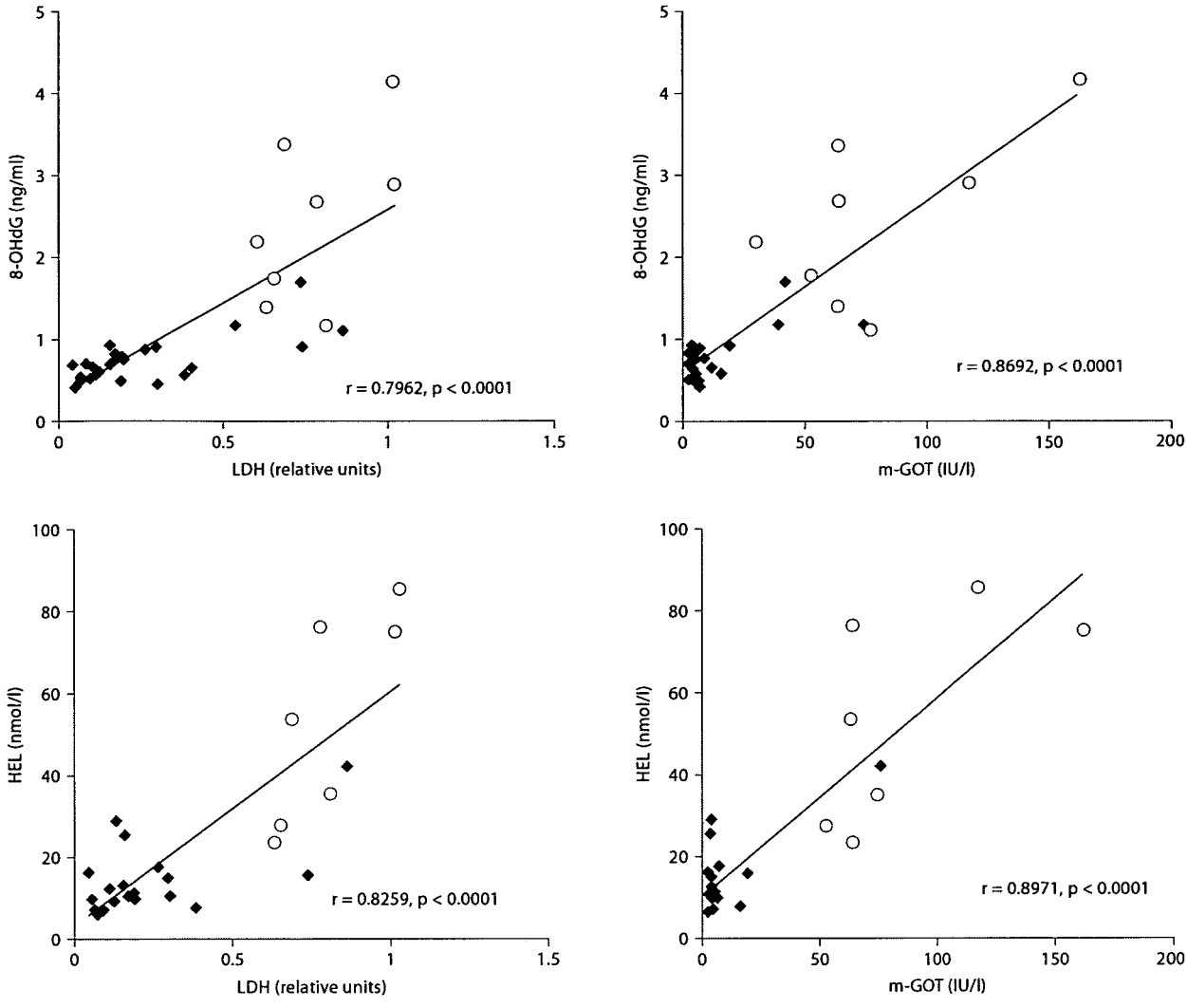

Fig. 6. Comparison of saliva concentrations of 8-OHdG, HEL, LDH, and m-GOT in all the groups. A significant positive correlation was observed. ○ = SS group; ◆ = G1, G2, control groups.

Discussion

Many autoimmune diseases are associated with multiple factors for their development. It has been said that for their onset, environmental and genetic factors are intertwined in a complex manner and that viral or bacterial infections and exposure to ultraviolet rays are known exacerbating factors. It is also known that these infections and activation of infiltrating monocytes or neutrophils in inflammation are responsible for generating a large quantity of ROS. In fact, it has been reported that the 8-OHdG level increases in infections with *Helicobacter pylori* [38] or papilloma virus [39]. It is possible that environmental factors were responsible for the oxidative stress detected in the current study.

Evidence for an association between EBV infection and SS has been accumulating. EBV antigens and increased levels of EBV DNA have been found in infiltrating B lymphocytes and a few salivary gland epithelial cells of SS patients [21, 40]. Infectious EBV is present in

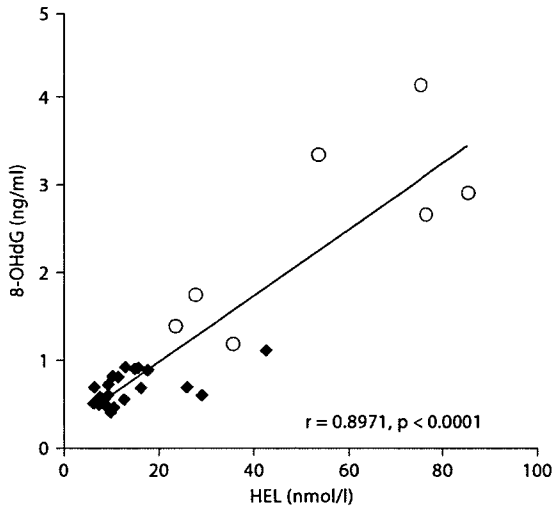

Fig. 7. Comparison of saliva concentrations of 8-OHdG and HEL in all the groups. A significant positive correlation was observed. ○ = SS group; ◆ = G1, G2, control groups.

both the saliva of SS patients [41–43] and culture supernatants of B cell lines established from SS patients [44]. Other defined manifestations of an active EBV infection are the presence of infected B cells that can transform into B cell lymphomas in the circulation [45]. Mariette et al. [40] previously used in situ hybridization to detect EBV DNA in a substantial proportion of lymphoid cells and epithelial cells in salivary glands of patients with SS. It has also been shown that antibodies against EBV antigens are elevated in the sera of SS patients [46, 47]. Since EBV is known to induce strong immune responses [48, 49] and increase levels of ROS [50], these reports suggest that a reactivated EBV infection may play a role in SS, contributing to the initiation or perpetuation of this disease in the target organs. However, factors responsible for triggering oxidative stress in this condition or the details of the etiological mechanism are still unexplained. Based on the results obtained thus far, studies are continuing on the relationship with EBV.

SS is an autoimmune disease where autoantibodies, such as SS-A/Ro and SS-B/La antibodies, are detected very frequently [1]. The anti-SS-A antibody has been attracting attention because it is detected not only in cases of SS but also in subacute dermal lupus, in which a specific skin eruption is caused by ultraviolet rays, annular erythema and neonatal lupus associated with a complete atrioventricular block [51]. The antigen corresponding to the anti-SS-A antibody is expressed when keratinocytes are exposed to ultraviolet rays and its induction is blocked by N-acetylcysteine, a reductant in oxidative stress [52]. Thus the involvement of oxidative stress through a certain mechanism is suggested in the development of the physiopathology of SS.

In the present study, we found a significantly increased salivary concentration of 8-OHdG and HEL in the patients with SS compared with the corresponding controls. These results are consistent with those for the patients with type 1 and type 2 diabetes [28, 53]. Of note, LDH and m-GOT were correlated with these oxidative stress markers. This result suggests a connection between salivary gland destruction and oxidative damage in the SS salivary gland. In contrast, we did not find a significant correlation between the histological grade of SS salivary glands and the levels of 8-OHdG and HEL in the saliva of SS patients. This may be explained by limited evaluation of tissue destruction in the glands since the histological grade is scored only by focal lymphocytic infiltration. Studies of a larger scale are needed to confirm the results of the present study.

These biomarkers for oxidative stress are conventionally detected in blood or urine [12, 54–56]. Saliva, in comparison with these fluids, can be collected more easily and noninvasively. The analysis of 8-OHdG and HEL in saliva samples directly reflects the damage to the salivary gland; thus, it is considered to be contributory to the diagnosis of SS.

In conclusion, the present results support the contention that increased oxidative stress and glandular destruction may start in the course of SS and that determination of salivary 8-OHdG and HEL may be helpful for the assessment of oxidative stress, and also for the decision of the optimal intervention time for dry mouth in patients with SS.

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References

1 Fox RI: Sjogren's syndrome. Lancet 2005; 366:321–331.
2 Bell DA, Maddison PJ: Serologic subsets in systemic lupus erythematosus: an examination of autoantibodies in relationship to clinical features of disease and HLA antigens. Arthritis Rheum 1980;23:1268–1273.
3 Mann DL, Moutsopoulos HM: HLA DR alloantigens in different subsets of patients with Sjogren's syndrome and in family members. Ann Rheum Dis 1983;42:533–536.
4 Manthorpe R, Teppo AM, Bendixen G, Wegelius O: Antibodies to SS-B in chronic inflammatory connective tissue diseases. Relationship with HLA-Dw2 and HLA-Dw3 antigens in primary Sjogren's syndrome. Arthritis Rheum 1982;25:662–667.
5 Buttke TM, Sandstrom PA: Oxidative stress as a mediator of apoptosis. Immunol Today 1994;15:7–10.
6 Jacobson MD: Reactive oxygen species and programmed cell death. Trends Biochem Sci 1996;21:83–86.
7 Holmgren A: Thioredoxin. Annu Rev Biochem 1985;54:237–271.
8 Nakamura H, Nakamura K, Yodoi J: Redox regulation of cellular activation. Annu Rev Immunol 1997;15:351–369.
9 Mayer M, Noble M: N-Acetyl-L-cysteine is a pluripotent protector against cell death and enhancer of trophic factor-mediated cell survival in vitro. Proc Natl Acad Sci USA 1994;91:7496–7500.
10 Cossarizza A, Franceschi C, Monti D, Salvioli S, Bellesia E, Rivabene R, Biondo L, Rainaldi G, Tinari A, Malorni W: Protective effect of N-acetylcysteine in tumor necrosis factor-alpha-induced apoptosis in U937 cells: the role of mitochondria. Exp Cell Res 1995;220:232–240.
11 Saito I, Shimuta M, Terauchi K, Tsubota K, Yodoi J, Miyasaka N: Increased expression of human thioredoxin/adult T cell leukemia-derived factor in Sjogren's syndrome. Arthritis Rheum 1996;39:773–782.
12 Bashir S, Harris G, Denman MA, Blake DR, Winyard PG: Oxidative DNA damage and cellular sensitivity to oxidative stress in human autoimmune diseases. Ann Rheum Dis 1993;52:659–666.
13 Miyata T, Ishiguro N, Yasuda Y, Ito T, Nangaku M, Iwata H, Kurokawa K: Increased pentosidine, an advanced glycation end product, in plasma and synovial fluid from patients with rheumatoid arthritis and its relation with inflammatory markers. Biochem Biophys Res Commun 1998;244:45–49.
14 Mantle D, Falkous G, Walker D: Quantification of protease activities in synovial fluid from rheumatoid and osteoarthritis cases: comparison with antioxidant and free radical damage markers. Clin Chim Acta 1999; 284:45–58.
15 Ozkan Y, Yardym-Akaydyn S, Sepici A, Keskin E, Sepici V, Simsek B: Oxidative status in rheumatoid arthritis. Clin Rheumatol, in press.
16 Baskol G, Demir H, Baskol M, Kilic E, Ates F, Karakukcu C, Ustdal M: Investigation of protein oxidation and lipid peroxidation in patients with rheumatoid arthritis. Cell Biochem Funct 2006;24:307–311.
17 Jikimoto T, Nishikubo Y, Koshiba M, Kanagawa S, Morinobu S, Morinobu A, Saura R, Mizuno K, Kondo S, Toyokuni S, Nakamura H, Yodoi J, Kumagai S: Thioredoxin as a biomarker for oxidative stress in patients with rheumatoid arthritis. Mol Immunol 2002; 38:765–772.
18 Rall LC, Roubenoff R, Meydani SN, Han SN, Meydani M: Urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a marker of oxidative stress in rheumatoid arthritis and aging: effect of progressive resistance training. J Nutr Biochem 2000;11:581–584.
19 Reznick AZ, Shehadeh N, Shafir Y, Nagler RM: Free radicals related effects and antioxidants in saliva and serum of adolescents with type 1 diabetes mellitus. Arch Oral Biol 2006;51:640–648.
20 Hata I, Kaji M, Hirano S, Shigematsu Y, Tsukahara H, Mayumi M: Urinary oxidative stress markers in young patients with type 1 diabetes. Pediatr Int 2006;48:58–61.
21 Saito I, Servenius B, Compton T, Fox RI: Detection of Epstein-Barr virus DNA by polymerase chain reaction in blood and tissue biopsies from patients with Sjogren's syndrome. J Exp Med 1989;169:2191–2198.
22 Inoue H, Tsubota K, Ono M, Kizu Y, Mizuno F, Takada K, Yamada K, Yanagi K, Hayashi Y, Saito I: Possible involvement of EBV-mediated alpha-fodrin cleavage for organ-specific autoantigen in Sjogren's syndrome. J Immunol 2001;166:5801–5809.
23 Haneji N, Nakamura T, Takio K, Yanagi K, Higashiyama H, Saito I, Noji S, Sugino H, Hayashi Y: Identification of alpha-fodrin as a candidate autoantigen in primary Sjogren's syndrome. Science 1997;276:604–607.
24 Halliwell B, Aruoma OI: DNA damage by oxygen-derived species. Its mechanism and measurement in mammalian systems. FEBS Lett 1991;281:9–19.
25 Kasai H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, Tanooka H: Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. Carcinogenesis 1986;7: 1849–1851.
26 Cooke MS, Evans MD, Lunec J: DNA repair: insights from urinary lesion analysis. Free Radic Res 2002;36:929–932.
27 Shigenaga MK, Gimeno CJ, Ames BN: Urinary 8-hydroxy-2′-deoxyguanosine as a biological marker of in vivo oxidative DNA damage. Proc Natl Acad Sci USA 1989;86: 9697–9701.
28 Dandona P, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D, Nicotera T: Oxidative damage to DNA in diabetes mellitus. Lancet 1996;347:444–445.
29 Takane M, Sugano N, Iwasaki H, Iwano Y, Shimizu N, Ito K: New biomarker evidence of oxidative DNA damage in whole saliva from clinically healthy and periodontally diseased individuals. J Periodontol 2002;73: 551–554.
30 Tsukahara H, Sekine K, Uchiyama M, Kawakami H, Hata I, Todoroki Y, Hiraoka M, Kaji M, Yorifuji T, Momoi T, Yoshihara K, Beppu M, Mayumi M: Formation of advanced glycosylation end products and oxidative stress in young patients with type 1 diabetes. Pediatr Res 2003;54:419–424.
31 Kato Y, Mori Y, Makino Y, Morimitsu Y, Hiroi S, Ishikawa T, Osawa T: Formation of Nepsilon-(hexanonyl)lysine in protein exposed to lipid hydroperoxide. A plausible marker for lipid hydroperoxide-derived protein modification. J Biol Chem 1999;274: 20406–20414.
32 Fox RI, Saito I: Criteria for diagnosis of Sjogren's syndrome. Rheum Dis Clin North Am 1994;20:391–407.
33 Toyokuni S, Tanaka T, Hattori Y, Nishiyama Y, Yoshida A, Uchida K, Hiai H, Ochi H, Osawa T: Quantitative immunohistochemical determination of 8-hydroxy-2′-deoxyguanosine by a monoclonal antibody N45.1: its application to ferric nitrilotriacetate-induced renal carcinogenesis model. Lab Invest 1997; 76:365–374.
34 Saito S, Yamauchi H, Hasui Y, Kurashige J, Ochi H, Yoshida K: Quantitative determination of urinary 8-hydroxydeoxyguanosine (8-OH-dg) by using ELISA. Res Commun Mol Pathol Pharmacol 2000;107:39–44.
35 Greenspan JS, Daniels TE, Talal N, Sylvester RA: The histopathology of Sjogren's syndrome in labial salivary gland biopsies. Oral Surg Oral Med Oral Pathol 1974;37:217–229.
36 Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, Daniels TE, Fox PC, Fox RI, Kassan SS, Pillemer SR, Talal N, Weisman MH: Classification criteria for Sjogren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002;61:554–558.
37 Kregel KC, Zhang HJ: An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J Physiol Regul Integr Comp Physiol 2007;292:R18–R36.

38 Hahm KB, Lee KJ, Kim JH, Cho SW, Chung MH: *Helicobacter pylori* infection, oxidative DNA damage, gastric carcinogenesis, and reversibility by rebamipide. Dig Dis Sci 1998; 43:72S–77S.
39 Romano G, Sgambato A, Mancini R, Capelli G, Giovagnoli MR, Flamini G, Boninsegna A, Vecchione A, Cittadini A: 8-Hydroxy-2′-deoxyguanosine in cervical cells: correlation with grade of dysplasia and human papillomavirus infection. Carcinogenesis 2000;21: 1143–1147.
40 Mariette X, Gozlan J, Clerc D, Bisson M, Morinet F: Detection of Epstein-Barr virus DNA by in situ hybridization and polymerase chain reaction in salivary gland biopsy specimens from patients with Sjogren's syndrome. Am J Med 1991;90:286–294.
41 Saito I, Nishimura S, Kudo I, Fox RI, Moro I: Detection of Epstein-Barr virus and human herpes virus type 6 in saliva from patients with lymphoproliferative diseases by the polymerase chain reaction. Arch Oral Biol 1991;36:779–784.
42 Fox RI, Pearson G, Vaughan JH: Detection of Epstein-Barr virus-associated antigens and DNA in salivary gland biopsies from patients with Sjogren's syndrome. J Immunol 1986; 137:3162–3168.
43 Fox RI, Bumol T, Fantozzi R, Bone R, Schreiber R: Expression of histocompatibility antigen HLA-DR by salivary gland epithelial cells in Sjogren's syndrome. Arthritis Rheum 1986;29:1105–1111.
44 Tateishi M, Saito I, Yamamoto K, Miyasaka N: Spontaneous production of Epstein-Barr virus by B lymphoblastoid cell lines obtained from patients with Sjogren's syndrome. Possible involvement of a novel strain of Epstein-Barr virus in disease pathogenesis. Arthritis Rheum 1993;36:827–835.
45 Sullivan JL: Epstein-Barr virus and lymphoproliferative disorders. Semin Hematol 1988; 25:269–279.
46 Inoue N, Harada S, Miyasaka N, Oya A, Yanagi K: Analysis of antibody titers to Epstein-Barr virus nuclear antigens in sera of patients with Sjogren's syndrome and with rheumatoid arthritis. J Infect Dis 1991;164: 22–28.
47 Yamaoka K, Miyasaka N, Yamamoto K: Possible involvement of Epstein-Barr virus in polyclonal B cell activation in Sjogren's syndrome. Arthritis Rheum 1988;31:1014–1021.
48 Rickinson AB, Moss DJ, Wallace LE, Rowe M, Misko IS, Epstein MA, Pope JH: Long-term T-cell-mediated immunity to Epstein-Barr virus. Cancer Res 1981;41:4216–4221.
49 Slaughter L, Carson DA, Jensen FC, Holbrook TL, Vaughan JH: In vitro effects of Epstein-Barr virus on peripheral blood mononuclear cells from patients with rheumatoid arthritis and normal subjects. J Exp Med 1978;148:1429–1434.
▶50 Cerimele F, Battle T, Lynch R, Frank DA, Murad E, Cohen C, Macaron N, Sixbey J, Smith K, Watnick RS, Eliopoulos A, Shehata B, Arbiser JL: Reactive oxygen signaling and MAPK activation distinguish Epstein-Barr virus (EBV)-positive versus EBV-negative Burkitt's lymphoma. Proc Natl Acad Sci USA 2005;102:175–179.
51 Yoshimasu T, Hiroi A, Ohtani T, Uede K, Furukawa F: Comparison of anti 60 and 52 kDa SS-A/Ro antibodies in the pathogenesis of cutaneous lupus erythematosus. J Dermatol Sci 2002;29:35–41.
52 Saegusa J, Kawano S, Koshiba M, Hayashi N, Kosaka H, Funasaka Y, Kumagai S: Oxidative stress mediates cell surface expression of SS-A/Ro antigen on keratinocytes. Free Radic Biol Med 2002;32:1006–1016.
53 Krapfenbauer K, Birnbacher R, Vierhapper H, Herkner K, Kampel D, Lubec G: Glycoxidation, and protein and DNA oxidation in patients with diabetes mellitus. Clin Sci (Lond) 1998;95:331–337.
54 Erhola M, Toyokuni S, Okada K, Tanaka T, Hiai H, Ochi H, Uchida K, Osawa T, Nieminen MM, Alho H, Kellokumpu-Lehtinen P: Biomarker evidence of DNA oxidation in lung cancer patients: association of urinary 8-hydroxy-2′-deoxyguanosine excretion with radiotherapy, chemotherapy, and response to treatment. FEBS Lett 1997;409: 287–291.
▶55 Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Oshima T, Chayama K: Endothelial function and oxidative stress in renovascular hypertension. N Engl J Med 2002;346:1954–1962.
▶56 Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K, Poulsen HE: Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. Carcinogenesis 1992;13:2241–2247.