

## Salivary Immunoglobulin A and Lysozyme in Patients with Psoriasis

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### Abstract

**Introduction:** We compared the salivary immunoglobulin A (IgA) and lysozyme concentration and secretion rates among mild and severe psoriasis patients and controls in Singapore. **Materials and Methods:** Fifty-one psoriasis patients and 24 controls participated in the study. None of the patients were on immunosuppressive therapy. The Psoriasis Area and Severity Index (PASI) was used to assess the severity of psoriasis. Patients were divided into mild and severe groups by the median PASI score. Each subject contributed a 5-minute unstimulated salivary sample. Enzyme-linked immunosorbent assay method was used to determine the salivary IgA and lysozyme levels. **Results:** Psoriasis patients had lower concentration and secretion rate of IgA (geometric mean [GM], 97.5 µg/mL and 32.3 µg/min) and lysozyme (GM, 127.6 µg/mL and 42.1 µg/min) than controls (IgA GM 256.3 µg/mL, 79.1 µg/min; lysozyme GM 180.9 µg/mL, 55.8 µg/min) [ $P = 0.000$  (IgA concentration),  $P = 0.000$  (IgA secretion rate),  $P = 0.015$  (lysozyme concentration) and  $P = 0.150$  (lysozyme secretion rate)]. However, no significant differences were observed between mild and severe patients for both IgA and lysozyme concentrations and secretion rates. PASI score showed negative, but non-significant, correlations with either log salivary IgA ( $r = -0.22$ ,  $P = 0.13$ ) or log lysozyme ( $r = -0.09$ ,  $P = 0.53$ ) secretion rates. **Conclusion:** Psoriasis patients had lower concentrations and secretion rates of salivary IgA and lysozyme compared to controls. However, among patients, the salivary IgA and lysozyme levels are variable and not related to severity of psoriasis.

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**Key words:** Psoriasis, Salivary IgA, Salivary lysozyme

### Introduction

The pathophysiological mechanisms in psoriasis are still unclear. Many factors, such as infection, trauma and stress, could cause psoriasis. Upper respiratory tract infections (URTIs) are a recognised triggering stimulus for psoriasis,<sup>1</sup> while continuing, sub-clinical infections, which may result from lowered immunity, might be responsible not only for relapse but also for a new episode of psoriasis.<sup>2</sup>

Immunoglobulin A (IgA) is the most important immunoglobulin in saliva and serves as a main immunological defence of mucosal surfaces.<sup>3</sup> Studies have shown that regular, moderate exercise result in increased salivary IgA (SIgA) level, and decreased total URTI symptoms and

number of days of sickness.<sup>4,5</sup>

Lysozyme is a low-molecular-weight cationic protein that is synthesised in and continuously released from monocytes or macrophages,<sup>6,7</sup> and widely distributed in human tissues and secretions.<sup>8</sup> It has enzymatic activity, cleaving beta-1, 4 glycosidic bonds between muramic acid and *N*-acetylglucosamine residues in the peptidoglycan of the bacterial cell wall.<sup>9</sup>

This study compares the salivary IgA and lysozyme secretion rates among patients with mild and severe psoriasis and controls. It also examines the relationship between salivary biomarkers and the severity of psoriasis among patients.

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## Materials and Methods

The study was approved by the ethics committee of the National Skin Centre of Singapore. Male psoriasis patients who were seen in the Centre between May and September 2001 were invited to participate in the study. After a complete description of the study to the subjects, written informed consent was obtained. None of the patients refused to participate in the study.

Twenty-four healthy adult male subjects, free from skin disease, were recruited as controls. They were asymptomatic, healthy male workers who were reviewed during periodic occupational medical examinations.

Patients on treatment with systemic steroid or immunosuppressive drugs within the last 4 weeks, as well as those with current or recent (within 1 week) URTI were excluded. Age, smoking status, family history of psoriasis, duration of psoriasis, medication history in the last 6 months and presence of arthropathy or other diseases were recorded. Disease severity was assessed using the Psoriasis Area and Severity Index (PASI),<sup>10</sup> which measures both the extent of psoriatic involvement and clinical manifestations of psoriasis (erythema, infiltration and desquamation). Four main body areas were assessed: head, trunk, upper extremities and lower extremities corresponding to 10%, 20%, 30% and 40%, respectively, of the body area. This yields a range of scores from zero (no psoriasis) to 72 (very severe psoriasis). The PASI was assessed by a dermatologist. The median PASI score of 10.5 was used to divide the patients into 2 groups: mild and more severely affected patients.

The patients were briefed and supervised on the collection method by a trained field worker on the day of collection. They were instructed to refrain from eating or drinking (except for water) 1 hour before saliva collection to minimise possible food debris and stimulation of salivation. The sample was collected in the morning during weekdays, to minimise possible circadian variation of salivary biomarkers. A single, 5 min, unstimulated total saliva sample was obtained. The flow rate of saliva of valid subjects should not be <0.1 mL/min. Under basal conditions, the rate of saliva production is 0.5 mL/min.<sup>11</sup> Subjects with a flow rate <0.1 mL/min would not have collected the saliva properly after the 5-min period. After collection, the samples were frozen at -70°C until assay.

A Salimetrics HS-IgA kit (Salimetrics LLC, State College, PA, USA) was used for the quantitative measurement of salivary IgA. The intra- and inter-assay variability were 4.5% and 8.9%, respectively. The sensitivity of the kit was 2.5 µg/mL. The salivary lysozyme concentration was measured using an ELISA method. The details of the laboratory procedure were described in an earlier publication.<sup>12</sup>

As the distributions of salivary IgA and lysozyme were positively skewed, logarithmic transformations were performed for the data prior to further statistical analyses. A *P* value of <0.05 was considered statistically significant. Two-sample independent *t*-tests were used in the univariate analysis. General linear models were used to control for the age of the subjects as it may confound in the analysis. The data were analysed using SPSS version 10 (SPSS Inc, Chicago, USA).

## Results

Fifty-one psoriasis patients and 24 controls were recruited. The former were slightly older (7.1 years) than the later (Table 1). Forty-five (88%) psoriasis patients and 18 (75%) controls were Chinese. No difference was found in the ethnic groups and smoking status of patients and controls.

The mean PASI score was 10.5 (range, 0.4 to 19) and the interquartile range was 8.3. The mean duration of psoriasis in all the patients was 11.8 years (range, 1 to 35 years). Eleven (21%) psoriasis patients had arthropathy.

Salivary immunoglobulin A (SIgA) concentration and secretion rate were lower in psoriasis patients (geometric mean [GM] of 97.5 µg/mL and 32.2 µg/min, respectively; *P* = 0.000) than in controls (GM of 256.3 µg/mL and 79.1 µg/min, respectively; *P* = 0.000). The differences were present even after adjustment for age (*P* = 0.001) (Table 2). In psoriasis patients, the SIgA concentration and secretion rate were lower among those with more severe psoriasis (GM of 93.6 µg/mL and 30.7 µg/min, respectively) than those with milder psoriasis (GM of 102.1 µg/mL and 34 µg/min, respectively) (Table 3). However, the difference was not statistically significant for both concentration (*P* = 0.427) and secretion rate (*P* = 0.577).

Salivary lysozyme concentration was lower in psoriasis patients (GM, 127.6 µg/mL) than controls (GM, 180.9 µg/mL) (*P* = 0.005). The difference was statistically significant even after adjustment for age (*P* = 0.015). The salivary lysozyme secretion rate was also lower in the patients (GM,

Table 1. Demographic Information of Psoriasis Patients and Controls

Variables	Patients (n = 51)	Controls (n = 24)	P-C (95% CI)
Age (y)*	41.2 ± 10.6	34.1 ± 8.3	7.1 (2.2 to 12.0)
Smoking status†			
Smoker (%)	35.3	37.5	-2.2 (-1.9 to -2.5)
Non-smoker (%)	64.7	62.5	
Mean PASI	10.5		
Interquartile range	8.3		
Duration of psoriasis (years)	11.8 ± 7.8		

CI: confidence interval; PASI: Psoriasis Area and Severity Index

\* Two-sample *t*-test (*P* = 0.005)

† Chi-square test ( $\chi^2$  = 0.85, *P* = 0.525)

42.1 µg/min) than controls (GM, 55.8 µg/min) ( $P = 0.07$ ). No significant difference was observed after adjustment for age ( $P = 0.15$ ) (Table 2).

Salivary lysozyme concentration and secretion rate were lower in severe psoriasis patients (GM, 123 µg/mL and 40.3 µg/min) than mild psoriasis patients (GM, 132.9 µg/mL and 44.3 µg/min) (Table 3). However, no significant differences were found between the 2 patient groups for both lysozyme concentration ( $P = 0.596$ ) and secretion rate ( $P = 0.585$ ).

Log SIgA and lysozyme were negatively, but non-significantly, correlated with PASI score (SIgA,  $r = -0.22$ ,  $P = 0.13$ ; lysozyme,  $r = -0.09$ ,  $P = 0.53$ , respectively). However, SIgA secretion rate was moderately correlated with that of lysozyme in all subjects ( $r = 0.57$ ,  $P < 0.001$ ).

## Discussion

Results of previous studies on salivary IgA and lysozyme among patients with psoriasis are inconclusive and contradictory, possibly because of several reasons. Firstly, the number of subjects in each study is generally small. Secondly, saliva collection times were not mentioned, so a possible circadian effect could not be ruled out. Furthermore, the assays for IgA did not always use the more sensitive enzyme-linked immunosorbent assay method, which is currently available.

One of the differences between psoriasis patients and controls in this study was age. The mean difference was 7 years. Previous reports had indicated no significant age effect on SIgA and immunity in adults aged between 15 and 70 years.<sup>4,13</sup> However, age was still used as a covariate to adjust for its possible effects.

In this study, psoriasis patients had a significantly lower SIgA concentration and secretion rate than controls. Patients with more severe psoriasis had lower levels of SIgA compared to patients with milder disease, but the difference was not statistically significant. The SIgA secretion rate was also negatively (but not significantly) correlated with PASI score.

Previous studies produced different results. For example, SIgA concentration and secretion rates were not significantly different in 2 previous studies of 15 and 10 psoriasis patients and a similar number of controls.<sup>14,15</sup> Among 12 psoriasis patients, Oon et al<sup>16</sup> found elevated IgA level in stimulated parotid saliva, while Guilhou et al<sup>17</sup> found increased SIgA in 28 psoriasis patients ( $19.2 \pm 5.2$  mg/dL) compared to 40 controls ( $5.2 \pm 1.19$  mg/dL). In another study, Syrjanen<sup>18</sup> showed significantly elevated level of SIgA in 28 psoriasis patients ( $23.8 \pm 7.4$  mg/dL) compared to 28 controls ( $19.4 \pm 4.5$  mg/dL).

In this study, psoriasis patients had a significantly lower level of salivary lysozyme than controls. Psoriasis patients

Table 2. Salivary IgA and Lysozyme of Psoriasis Patients and Controls

Biomarkers	GM		P-C 95% CI	<i>P</i> value*	Adjusted <i>P</i> value†
	Patients (n = 51)	Controls (n = 24)			
IgA					
Concentration (µg/mL)	97.5	256.3	-195.7 to -122.7	<0.001	<0.001
Secretion rate (µg/min)	32.2	79.1	-72.3 to -31.3	<0.001	<0.001
Lysozyme					
Concentration (µg/mL)	127.6	180.9	-141 to -43.7	0.005	0.015
Secretion rate (µg/min)	42.1	55.8	-43.4 to 2.3	0.073	0.15

CI: confidence interval; GM: geometric mean; IgA: immunoglobulin A

\* Calculated using two-sample *t*-tests.

† Calculated using general linear model to adjust for age.

Table 3. Salivary IgA and Lysozyme of Patients with Severe and Mild Psoriasis

Biomarkers	GM		S-M 95% CI	P value*	Adjusted P value†
	Severe (n = 27)	Mild (n = 24)			
IgA					
Concentration (µg/mL)	93.6	102.1	-27.1 to 39.1	0.427	0.442
Secretion rate (µg/min)	30.7	34	-13 to 31.5	0.577	0.583
Lysozyme					
Concentration (µg/mL)	123	132.9	-21.2 to 35.5	0.596	0.528
Secretion rate (µg/min)	40.3	44.3	-13.4 to 37.1	0.585	0.426

CI: confidence interval; GM: geometric mean; IgA: immunoglobulin A

\* Calculated using two-sample *t*-tests.

† Calculated using general linear model to adjust for age.

with milder disease showed lower (but not statistically significant) lysozyme level than patients with more severe psoriasis. There was no linear relationship between salivary lysozyme secretion rate and PASI score among psoriasis patients.

These results are similar to findings from other studies. Gasior-Chrzan and Falk<sup>14</sup> showed that psoriatic patients had a lower level of salivary lysozyme than controls. In another study of 28 psoriasis patients, Syrjanen<sup>18</sup> showed reduced levels of salivary lysozyme compared to controls.

It has been suggested that the lower level of lysozyme in saliva is most likely due to the disease process and might reflect either a higher consumption of the enzyme or its reduced production.<sup>14,18</sup>

In our study, we had tried to overcome the above-mentioned shortcomings. However, we still could not find significant correlation between salivary biomarkers and PASI scores. The result might reflect the mechanism of the disease. Lowered immunity could lead to streptococcal infection, and its superantigen was a triggering factor that leads to psoriasis. However, lowered immunity might lead to not only streptococcal infection but also other infections. This might explain the non-significant relationship between PASI and salivary IgA and lysozyme secretion rate. Further study, especially prospective study, is warranted to fully reflect the real situation of mucosal immunity in psoriasis patients.

One limitation of this study was that the cross-sectional research paradigm did not permit examination of the possible sequential relationship between the salivary biomarkers and psoriasis to establish causality. Second, the sample was restricted to male tertiary clinic attendees. As a convenience sample of patients treated at a single site, it might not fully represent the population of individuals with psoriasis. For instance, results may not be applicable to female patients. Third, only salivary samples were studied in our study. SIgA and lysozyme levels reflect only mucosal immunity. Lowered levels of these biomarkers may lead to increased susceptibility to streptococcal and other URTIs, which could act as a trigger factor for psoriasis. Other studies<sup>19</sup> demonstrate increased cortisol levels in psoriasis patients, which would also suppress host immunity. However, the weak or absent relationship between SIgA and lysozyme levels with disease severity might indicate that the disease severity is independent of the trigger.

## Conclusion

Psoriasis patients showed significantly lower concentrations and secretion rates of SIgA and lysozyme compared to controls. As these salivary biomarkers are indicative of mucosal immunity, psoriasis patients might be at higher risk of microbial infections. The infections could act as triggers for psoriasis.

However, no differences in these salivary biomarkers were found between patients with mild and severe psoriasis, and no significant correlations were found between PASI score and salivary biomarkers. The results confirm that salivary IgA and lysozyme levels are variable in patients and not associated with severity of psoriasis.

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