

Age-Related changes of flow rate, immunoglobulins, lactoferrin and electrolytes in human whole saliva

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CONTENTS

- I. INTRODUCTION
- II. MATERIALS AND METHODS
- III. RESULTS
- IV. DISCUSSION
- V. CONCLUSION
- REFERENCES
- KOREAN ABSTRACT

I. INTRODUCTION

Saliva is regarded as one of the important factors in regulating oral health, with respect to both the volume produced and the constituents it contains.¹⁾ The mucous membrane are lubricated and protected by the salivary glycoproteins and mucoids. Furthermore, saliva keeps the mouth moist, and softens the food, thereby facilitating chewing and swallowing.²⁾ Also, human saliva contains a number of antimicrobial agents which are either synthesized in the salivary glands or leak into the mouth from blood, usually via gingival crevices. Examples of purely glandular antimicrobial products are secretory IgA, salivary peroxidase, and histidine-rich polypeptides, whereas, *e.g.*, lysozyme, lactoferrin, and IgM may originate from both saliva and gingival fluids.³⁾

It is commonly believed that decreased

salivation, "dry mouth" is characteristic of the later years of aging. This is mainly based on clinical experience. But, the effect of aging on salivary flow rate remains unclear, since conflicting observations exist in the literature. Bertram (1967) reported significant reduction in the flow rate of unstimulated whole saliva when healthy males aged 65 years or older were compared with those of younger than 65 years.⁴⁾ And, an age-related reduction in secretion rates of both resting and stimulated whole saliva(Heintze *et al.*, 1983), a decrease in whole salivary flow rates with age(Gutman and Ben-Aryeh, 1974), and a reduction rate with aging(Yaegaki *et al.*, 1985) have all been reported.⁵⁻⁷⁾ On the other hand, many recent studies have failed to find significant effects of age on unstimulated or stimulated parotid as well as whole salivary flow rate. Age-related decline was not observed in other studies with stimulated whole salivary flow rate(Parvinen and Larmas, 1982), stimulated parotid saliva flow (Baum, 1981), or unstimulated whole and stimulated whole and parotid saliva (Gandara *et al.*, 1985).⁸⁻¹⁰⁾ The variation in results might be the difficulties associated with measuring resting saliva, and some studies may have included subject on systemic medications.

In saliva the predominant immunoglobulin is dimeric secretory immunoglobulin A (S-IgA), but

monomeric IgA, which diffuses from serum, may also be detected in whole saliva.¹¹⁾ Other immunoglobulin classes (IgG and IgM) are also present in saliva but in markedly lower amounts. Secretory IgA antibody is found in human external secretions which includes saliva, tears, milk and the mucus secretions of the gut and upper respiratory tracts.¹²⁾ It is generally accepted that S-IgA antibodies act in a "first line" of mucosal defense principally by simple binding to soluble or particulate antigens. This function of "immune exclusion" is probably enhanced by cooperation of S-IgA with nonspecific defense factors.¹³⁻¹⁶⁾

Most salivary IgG reaches the oral cavity through the gingival crevice and is mainly derived from serum, although a minor fraction of the crevicular IgG may originate in local plasma cells when the gingiva is inflamed. Crevicular IgG antibodies have definitely been shown to inhibit colonization of *S. mutans* and can protect significantly against tooth decay in monkeys.¹⁴⁾

Immunoglobulin levels in saliva are not constant, and changes in salivary flow can significantly influence IgA level.¹⁷⁾ And aging is also believed to affect the levels of salivary immunoglobulins. However, there is no clear relationship regarding the influence of the aging process on the secretory immune responses. Alford reported an age-associated decrease in IgA levels in the nasopharyngeal washing of elderly people,¹⁸⁾ but Challacombe reported that the IgA and IgG levels in whole saliva increased significantly in the oldest age group, but no changes were detected in IgM levels.¹⁹⁾ And other workers found that salivary immunoglobulin concentrations did not change with age when young and old population were compared.^{20,21)}

Lactoferrin, an iron binding glycoprotein with antibacterial properties, is common to all exocrine gland secretions including tears, milk and saliva. The biological function for lactoferrin has been attributed to its high affinity for iron and its con-

sequent deprivation of this essential metal from pathogenic microorganisms. Although it was initially thought that the antibacterial effect was due to the iron binding capacity of lactoferrin, recent studies indicate that lactoferrin also has a direct effect on certain bacterial strains.²²⁾ Besides its role in bacterial immunity, lactoferrin has been shown to play a role in the regulation of the production of granulocyte and macrophage-derived colony-stimulating factors.²³⁾

In the lactoferrin levels in whole saliva, Tenovuo *et al.* reported that the lactoferrin concentrations are significantly lower than in adults.²⁴⁾ But Cole *et al.* reported that among 7 to 12-years-old children the whole saliva values are already close to those of adults although the salivary concentrations slightly increase with age.²⁵⁾

Salivary electrolytes have been extensively studied, and the normal concentrations of the principal electrolytes are well documented. The concentrations of electrolytes alter as salivary flow rate alters and may vary with time of day in any one individual, while age and possibly sex may be sources of variation between individuals. The ionic composition of unstimulated whole saliva in infants was studied by Ben-Aryeh *et al.* They observed that calcium, magnesium and chloride concentrations were higher in infants than in adults, calcium and magnesium concentrations remaining high throughout the first year of life while chloride concentrations fell toward adult levels.²⁶⁾ And Chaucey reported that sodium, calcium and chloride concentrations were lower in acid-stimulated parotid saliva from older subjects.²⁷⁾

Sodium and chloride ions reach saliva in the acinus, and their concentrations in acinar fluid are similar to those in interstitial fluid and plasma. The final concentrations of sodium and chloride in saliva depend upon the extent of reabsorption of these two ions which occur in the striated ducts. The mechanism of reabsorption is a Na^+/K^+ -ATP-ase system situated in the walls of the

ductal cells distant from the lumen. The extent of reabsorption is directly related to the time that the secretion takes to traverse the ducts and is therefore inversely related to flow rate.¹⁴⁾ Thus damage to the acinar cells reduces flow rate but does not affect sodium and chloride concentrations in the primary secretion; damage to the ductal cells may affect reabsorption of these ions and therefore result in inappropriately high concentrations of sodium and chloride relative to flow rate. Potassium concentrations in saliva are five to ten times high as in plasma. This ion reaches saliva by active processes in both the acini and the ducts, and sodium-potassium exchange in the ducts accounts for only a part of the secretory processes in that situation.²⁸⁾ Thus potassium concentration are relatively flow rate independent. But, little is known of how magnesium reaches saliva or of how far concentrations are related to blood vessels.

In this study, we examined a healthy, non-medicated aged population, and the aims were to investigate and compare age-related changes in mucosal immunoglobulins, lactoferrin and electrolytes.

II. MATERIALS AND METHODS

1. Study population

Fifty nine healthy unmedicated subjects participated in this study and were divided among the following four groups according to their age : group A, 10~15 years (7 males and 7 females) ; group B, 20~30 years (8 males and 7 females) ; group C, 40~50 years (7 males and 7 females) ; group D, ≥ 60 years (7 males and 9 females). (Table 1)

2. Saliva collection

Unstimulated (resting) whole saliva was colle-

Table 1. Age distribution of the unmedicated subjects

Group	Age	Sex	Number	Mean Age
A	10~15	M	7	14.25 \pm 0.96
		F	7	13.50 \pm 1.52
B	20~30	M	8	26.43 \pm 2.70
		F	7	24.43 \pm 2.76
C	40~50	M	7	46.20 \pm 3.83
		F	7	45.29 \pm 3.90
D	60~	M	7	69.86 \pm 8.28
		F	9	66.71 \pm 4.59

cted from all subjects by direct expectoration into a sterile container over a period of 5 minutes to enable the calculation of flow rate. The flow rates of unstimulated whole saliva was expressed as ml/min.

Saliva collection was performed between 9:00 a.m. and 11:30 a.m. to minimize the effects of diurnal variability in salivary composition. The subject refrained from eating, drinking and brushing prior to sample collection.

Unstimulated whole saliva was clarified by centrifugation at 2,500 \times g for 15 min; sediments were discarded and aliquots of clarified saliva were stored at -20°C.

3. Quantification of salivary immunoglobulins

All salivary immunoglobulins (IgA, IgG, IgM) were identified and quantitated by an ELISA. In this study unstimulated whole saliva was diluted with 50mM Tris buffer, 0.15M NaCl (10 \times , 30 \times , 100 \times , 300 \times , 1000 \times). Microtiter plates were coated with 100 μ l of diluted samples and standards (Human IgA, Human IgG, Human IgM, Sigma Chemical Co.) and incubated at 37°C for 2 hours. All samples were tested at least in duplicate. After washing the well 6 times with 0.1% Tween 20, 50mM Tris buffer, 0.15M NaCl solution (washing solution), the microtiter plates were coated 200ml with 5% BSA, 50mM Tris buffer,

0.15M NaCl (blocking solution). Incubation and washing buffers contained Tween 20 for minimization of nonspecific binding in the assay. After incubation for 1 hour and washing, each well were coated with 100 μ l of either a 1:1000 dilution of goat anti-human IgA α -chain specific (peroxidase conjugate) or 1:3000 dilution of goat anti-human IgG (peroxidase conjugate) or 1:1000 of anti IgM μ -chain specific (Sigma Chemical Co) diluted in phosphate-buffered saline (PBS) solution containing azide.

For immunoglobulin estimation, a standard curve was included in each plate composed of 6 doubling dilutions in duplicate of purified immunoglobulins starting at 0.391 μ g/ml (0.391 μ g/ml ~ 12.50 μ g/ml).

After further incubation and washing, the assay was developed with OPD tablet (O-phenylenediamine dihydrochloride) substrate (Sigma Chemical Co.) for 30 min for all isotypes. The optical density of each well was determined with EIA reader model 312A (Bio-Rad Lab. Hemel Hempstead. UK). Concentrations were expressed as μ g/ml. The value for each sample was taken as the mean of the doubling dilutions falling within the standard curve.

4. Quantification of lactoferrin

The lactoferrin concentration was determined in an indirect ELISA. Briefly, a microtiter plates was coated with 100 μ g sample. After incubation and rinsing, the well coated with 5% BSA, 50mM Tris buffer, 0.15M NaCl Solution (blocking solution). After further incubation and washing, 1:300 dilution of anti-human lactoferrin (developed in rabbit) was added to each well and the plate incubated at 37°C. Then 1:3000 dilution of anti-rabbit IgG to peroxidase conjugate was added and the plate incubated for 2 hours. After rinsing, residual peroxidase activity was measured as described in the ELISA for salivary

immunoglobulins.

5. Electrolytes analysis

Sodium, potassium and magnesium contents were determined with atomic absorption spectrophotometer (spectr AA-20, Varian Techtron Pty. Limited, Mulgrave, Victoria, Australia). And chloride content was measured by ionchromatography (Dionex DX 500 chromatography systems, Dionex Corp., Sunnyvale, CA, USA).

6. Statistical analysis

Data are expressed as mean \pm SEM. Differences among means were analyzed by T-test and ANOVA with SAS program.

III. RESULTS

Although the unstimulated salivary flow rate fluctuated with age, the analysis of variance did not reveal any significant influence of age on the salivary secretion rate. Females had a lower mean flow rate than did males in every age group. Especially in group B, females had a significant lower flow rate than male ($p < 0.05$). In the male group B (20~30 years) has a maximal flow rate and Group D (60-years) had in the female group (Table 2). This was not, however, statistically significant.

The concentrations of salivary immunoglobulins (IgA, IgG and IgM) in the 4 age groups are presented in Table 3.

The IgA levels were maximal in Group B and then declined and in the male groups. Group Band C had significantly higher value than group A. In the group A, females had a higher IgA levels than males ($p < 0.05$). There was no apparent trend in the IgG with age and sex, whereas IgM levels were significantly reduced in males of group D ($p < 0.05$).

Table 2. Flow rates of unstimulated whole saliva in relation to age. (ml/min)

Group	Male	Female	Total	Difference between males and females
A	0.368±0.132	0.294±0.124	0.331±0.129	N.S.
B	0.449±0.111	0.318±0.069	0.388±0.113	*
C	0.345±0.059	0.287±0.166	0.316±0.124	N.S.
D	0.416±0.169	0.333±0.270	0.370±0.228	N.S.
Difference in each groups	N.S.	N.S.	N.S.	

* : Statistically significant (p<0.05)

** : Statistically significant (p<0.01)

N.S. : Not significant

Table 3. Immunoglobulin concentrations in whole saliva in relation to age. (µg/ml)

	Group	Male	Female	Total	Difference between sexes
IgA	A	56.73±23.28	90.67±23.28	73.70±27.73	*
	B	91.95±34.50	98.38±21.70	94.95±28.42	N.S.
	C	91.16±21.14	86.45±26.76	88.81±23.30	N.S.
	D	75.60±32.09	75.45±27.73	75.52±28.67	N.S.
	Difference in each groups	(A,B) * (A,C) *	N.S.	N.S.	
IgG	A	1.277±0.332	1.231±0.556	1.254±0.440	N.S.
	B	1.034±0.605	1.103±0.756	1.066±0.665	N.S.
	C	1.731±0.596	1.677±0.679	1.704±0.615	N.S.
	D	1.253±0.947	1.447±0.879	1.362±0.884	N.S.
	Difference in each groups	N.S.	N.S.	N.S.	
IgM	A	1.631±0.898	2.633±1.597	2.132±1.349	N.S.
	B	1.045±0.846	1.115±1.212	1.078±0.994	N.S.
	C	2.137±1.423	2.224±2.797	2.181±2.133	N.S.
	D	0.669±0.493	1.663±1.210	1.228±1.067	*
	Difference in each groups	(C,D) *	N.S.	N.S.	

* : Statistically significant (p<0.05)

** : Statistically significant (p<0.01)

N.S. : Not significant

The lactoferrin levels in saliva was maximal in group B. In the males group, the lactoferrin level in group A was significantly lower than in group B (p<0.05) and males had lower lactoferrin levels

than females in group A (Table 4).

There was no significant differences in sodium, potassium, calcium and magnesium between males and females. The concentrations of ele-

Table 4. Lactoferrin levels in whole saliva in relation to age.($\mu\text{g/ml}$)

Group	Male	Female	Total	Difference between sexes
A	27.83 \pm 9.75	47.18 \pm 13.64	37.51 \pm 15.18	*
B	46.23 \pm 14.70	50.15 \pm 12.40	48.06 \pm 13.34	N.S.
C	37.73 \pm 8.04	42.05 \pm 12.31	39.89 \pm 10.24	N.S.
D	39.00 \pm 15.16	38.98 \pm 15.70	38.99 \pm 14.95	N.S.
Difference in each groups	(A,B) *	N.S.	N.S.	

* : Statistically significant ($p < 0.05$)** : Statistically significant ($p < 0.01$)

N.S. : Not significant

Table 5. The concentrations of electrolytes in whole saliva in relation to age.($\mu\text{mol/ml}$)

	Group	Male	Female	Total	Difference between sexes
Sodium	A	4.694 \pm 2.074	4.454 \pm 1.117	4.574 \pm 1.605	N.S.
	B	5.009 \pm 2.221	3.211 \pm 1.621	4.170 \pm 2.111	N.S.
	C	5.251 \pm 1.261	6.883 \pm 3.876	6.067 \pm 2.895	N.S.
	D	4.521 \pm 1.747	5.177 \pm 2.349	4.890 \pm 2.068	N.S.
	p	N.S.	(B,C) *	N.S.	
Chloride	A	14.91 \pm 2.30	12.10 \pm 1.95	13.51 \pm 2.52	*
	B	15.02 \pm 5.76	11.03 \pm 5.51	13.16 \pm 5.82	N.S.
	C	15.29 \pm 4.52	19.17 \pm 5.95	17.23 \pm 5.46	N.S.
	D	16.39 \pm 7.03	19.43 \pm 8.76	18.10 \pm 7.95	N.S.
	p	N.S.	(A,C) *, (A,D) *	(A,C) *, (A,D) *	
Potassium	A	24.10 \pm 2.96	22.95 \pm 2.96	23.52 \pm 2.91	N.S.
	B	22.28 \pm 5.57	18.63 \pm 5.92	20.57 \pm 5.84	N.S.
	C	29.65 \pm 6.84	25.01 \pm 7.20	27.33 \pm 7.15	N.S.
	D	24.73 \pm 6.76	27.32 \pm 9.09	26.18 \pm 8.01	N.S.
	p	(B,C) *	N.S.	(B,C) *	
Magnesium	A	0.136 \pm 0.077	0.229 \pm 0.139	0.182 \pm 0.118	N.S.
	B	0.244 \pm 0.116	0.187 \pm 0.058	0.217 \pm 0.095	N.S.
	C	0.309 \pm 0.211	0.293 \pm 0.203	0.301 \pm 0.199	N.S.
	D	0.336 \pm 0.168	0.333 \pm 0.471	0.334 \pm 0.360	N.S.
	p	(A,C) * (A,D) *	N.S.	N.S.	

* : Statistically significant ($p < 0.05$)** : Statistically significant ($p < 0.01$)

N.S. : Not significant

ctrolytes showed the increasing pattern with aging.

Although the result was not statistically significant, the concentration of sodium and potassium in unstimulated whole saliva was maximal in

group C, and the concentration of chloride and magnesium was maximal in group D. But there was no apparent trend in the concentration of electrolytes with aging. And there was no significant difference between both sexes. The

only exception was that male had a higher chloride levels than females in group A (Table 5).

IV. DISCUSSION

The results of the present study have demonstrated that there is no age-associated diminution in fluid output for unstimulated states in unmedicated healthy elderly subjects of aged population when compared with the younger age groups. This result is in agreement with those of previous studies where no significant changes in flow rate of unstimulated whole saliva with aging has been reported,^{8,10)} but not with others.^{29,30)} The unexpectedly high flow rate recorded in the oldest age group of both sexes is thought to be related to the fact that unmedicated subjects of this age group are probably in very good health, since a weakened condition has been reported to reduce salivary output in the elderly, as do many drugs.^{4,31)}

The high values in the elderly also show that the suppressing effect on flow rate caused by the structural changes in salivary glands is not as pronounced as commonly believed. The belief that salivary flow rate is low in the elderly is probably due to side-effects from medication.

Multiple investigators have determined that parotid gland function is not decreased with aging.^{9,32)} And we know from several studies that the actual amount of salivary acinar tissue in human submandibular glands is reduced by about 30% in older persons.^{33,34)} Further, it appears that all salivary flow is secreted by acinar elements.³⁵⁾ And the majority (66%) of unstimulated whole saliva is contributed by secretions from submandibular and sublingual glands.³⁶⁾

Based on the previous study and our results, we conclude that despite marked reductions in acinar volume, the human submandibular gland retains what may be termed a "reserve secretory capacity", enabling it to perform normally. This

is also exist in the human parotid gland.³⁷⁾ The results of this study suggest that all salivary parenchymal tissue is not functionally active at all times.

Our finding that females had lower secretion rates of unstimulated whole saliva compared with males is in agreement with other reported results for whole saliva.^{30,38)} This difference between the sexes have been explained on the basis of female salivary glands being smaller than those of males.³⁹⁾ The hormonal status is considered to be another factor.⁸⁾ But this loss of estrogens would not be sufficient to account for reduced flow in females of all age group, since women in older age group is similar flow rate to younger group.

In the present study, there is no apparent trend in the levels of salivary immunoglobulins and lactoferrin with aging. And the groups of relatively healthy geriatric individuals (Group D: 60- years) have a similar immunoglobulins and lactoferrin contents in their saliva to healthy younger individuals.

A correlation was found between the salivary immunoglobulin levels of this study and serum immunoglobulin levels with aging in the earlier study.^{40,41)} The mucosal surfaces are important because they are the initial contact areas between the host and potentially pathogenic microorganisms.⁴²⁾ Our findings that levels of secretory IgA are not diminished in whole saliva of the oldest age group would appear to indicate that the mucosal surfaces in elderly people are well protected. And there was no consistent trends in relation to age in the levels of IgG and IgM antibodies in whole saliva. Assuming maintained level of antibodies, both this and earlier observations would therefore suggest that the elderly should be more resistant to infections, not more susceptible. However, increased immunoglobulin levels do not necessarily mean that this age group has normal immune function, since it has been demonstrated that aberrations in immunog-

lobulin synthesis and appearance of ineffective broken IgA fragments increasingly occur with age.⁴³⁾ The total secretory antibody response, including affinity, avidity and memory,¹⁶⁾ and not just antibody levels *per se* may be more important parameters to consider when relating secretory antibody to protection of mucosal surfaces. An earlier study has shown that salivary antibody functions were altered in elderly subjects where an impairment in the opsonic activity, as demonstrated by reduced phagocytosis of yeast, was observed despite subjects having normal salivary IgA levels.²⁰⁾ Both salivary IgA and IgM are known to be important for the protection of mucosal surfaces, and in the absence of IgA synthesis, compensatory mechanism for IgM production can occur.⁴⁴⁾

In this study, the secretion rates of salivary immunoglobulin are not measured. Challacombe *et al.* reported that secretion rates of salivary IgG and IgM in elderly people were significantly reduced, whereas the immunoglobulin concentration were not affected.¹⁹⁾ And it probably reflects age-related changes in secretory and removal rates, which may lead to inadequate protection of mucosal surfaces in elderly people.

Aging appears to modify several aspects of mucosal immunity. Systemic or mucosal immunization of rodents or primates with purified antigen resulted in significantly lower levels of IgA antibody in secretion of old *vs.* young animals, although the output of total IgA was not diminished in any of these animal studies.⁴⁵⁾ Kawanishi *et al.* have demonstrated intrinsic defects in dimeric IgA production and in suppressor T-cell populations in lymphatic tissue of senescent mice.⁴⁶⁾ Taken together, these observations suggest that the quality of mucosal immunity may become compromised with aging despite apparently "normal" quantities of salivary immunoglobulin.²¹⁾

The lactoferrin level in unstimulated whole saliva have no significant changes with those study of Tenovuo *et al.* and Cole *et al.*^{24,25)} They reported that the lactoferrin concentrations were significantly lower than in adults. And among 7-to-12 years old children the whole saliva values were already close to those of adults although the salivary concentrations slightly increase with age. Lactoferrin is considered to play an important role in nonspecific defence against a variety of bacteria.⁴⁷⁾ Although it was initially thought that the antibacterial effect was due to the iron binding capacity of lactoferrin, recent studies indicate that lactoferrin also has a direct effect on certain bacterial strains²²⁾ and can inhibit the formation of the classical C3 convertase of the complement system and thus can prevent the formation of the biologically active complement fragments C3a and C5a.⁴⁸⁾ Although our results have demonstrated that salivary lactoferrin levels in whole saliva was no significant change between young and old age populations, this does not eliminate the possibility of impairment in lactoferrin functions, which will require further investigation.

In this study, the electrolytes (sodium, potassium, chloride, magnesium) concentration of unstimulated whole saliva in different age groups is increased with aging, but there was no significant difference. This result is in agreement with the study of Gutman and Ben-Aryeh that sodium concentrations were slightly lower in the young adults than in the older age group.⁶⁾ The sodium and chloride ions reach saliva in the acinus, and their concentrations in acinar fluid are similar to those in interstitial fluid and plasma.¹⁴⁾ The final concentrations of sodium and chloride reabsorption of these two ions which occurs in the striated ducts.

Damage to the acinar cells, therefore, reduces flow rate but does not affect sodium and chloride concentrations in the primary secretion results in

inappropriately high concentrations of these ions relative to flow rate. Although the level of sodium and chloride is directly related to the secretion rate the high concentration of these two ions in older age groups suggest that there are impairment in ductal cell in the elderly.¹⁴⁾ Consequently damage to the ductal cells may affect reabsorption of sodium and chloride therefore result in high concentrations in the older age group.

Potassium reaches saliva by active processes in both the acini and the ducts and sodium-potassium exchange in the ducts accounts for only a part of the secretory processes in that situation.²⁸⁾ Thus, potassium concentrations are relatively flow rate independent. The concentrations of magnesium fall as flow rate increases,⁴⁹⁾ but little is known of how magnesium reaches saliva or of how far concentrations are related to blood vessels. The levels of potassium and magnesium are increased with age, although this is not statistically significant, but the meaning of this increase has not been well introduced. Future researches on oral and salivary changes with aging will give us more information on the changes of salivary components with aging. Thus these study will give us basic information which is essential for developing artificial saliva close to actual human saliva.

V. CONCLUSIONS

Saliva is regarded as one of the important factors in regulating oral health, with respect to both the volume produced and the constituents it contains. Although numerous studies on the secretion and components of saliva have been published, the effects of aging on salivary flow and composition remains unclear, since conflicting observations exist in the literature. Also, there are few published data about age related changes in concentration of lactoferrin and

electrolytes.

To provide the effects of aging on salivary secretion and salivary constituents, the author investigated fifty nine healthy unmedicated subjects. The subjects in this study were divided among the following four groups according to their age : group A, 10~15 years (7 males and 7 females); group B, 20~30 years (8 males and 7 females); group C, 40~50 years (7 males and 7 females); group D, ≥ 60 years (7 males and 9 females). And the author collected unstimulated saliva from all subjects and investigated salivary flow rate and the concentration of immunoglobulins, lactoferrin and electrolytes(sodium, chloride, potassium, magnesium) in each subjects.

The author came to the following conclusions after having examined the results of this study.

1. There were no significant differences of the whole salivary flow rates in each age group. Within each age group, female had lower mean flow rates than males, but these differences were significantly only in the 20-30 age group(Group B). ($p < 0.05$)
2. Although there was no apparent trend with age and sex, the levels of IgA and lactoferrin in whole saliva were significantly lower in 10-15 (Group A) male group.
3. The concentration of IgG in human whole saliva was not significantly affected by aging and sex.
4. The concentration of IgM was significantly lower in 60-(Group D) male group.
5. The concentration of electrolytes (sodium, chloride, potassium, magnesium) showed the increasing pattern with aging. The concentration of magnesium and chloride displayed the highest value in 60- age group (Group D), and the conc. of sodium and potassium were maximal in 40-50 age group (Group C). But there was no significant difference between both sexes.

REFERENCES

1. Mandel ID, Wotman S. The salivary secretions in health and disease. *Oral Sci Rev* 1976; 4: 25-47.
2. Tenovuo JO. Human Saliva: Clinical Chemistry and Microbiology Vol. I, CRC Press, Inc, Boca Ration, Florida, U.S.A. 1989.
3. Tenovuo JO, Grahn E, Lehtonen OP. Antimicrobial factors in saliva : ontogeny and relation to oral health. *J Dent Res* 1987; 66(2): 475-479.
4. Bertram V. Xerostomia : studies on salivary secretion(Thesis). *Acta Odontol Scand* 1967; 25(49 suppl): 1-126.
5. Heintze U, Birkhed D, Bjorn H. Secretion rate and buffer effect on resting and stimulated whole saliva as a function of age and sex. *Swed Dent J* 1983; 7: 227-238
6. Gutman D, Ben-Aryeh G. The influence of age on salivary content and rate of flow, *Int J Oral Surg* 1974; 3: 314-317.
7. Yaegaki K, Ogura R, Kameyama T, Sujaku C. Biochemical diagnosis of reduced salivary gland function. *Int J Oral Surg* 1985; 14: 47-49.
8. Parvinen T, Larmas M. Age dependency of stimulated salivary flow rate, pH and lactobacillus and yeast concentrations, *J Dent Res* 1982; 61: 1052-1055.
9. Baum BJ. Evaluation of stimulated parotid saliva floe rate in different age groups. *J Dent Res* 1981; 60: 1292-1296.
10. Gandara BK, Izutsu KT, Truelove EL, Ensign WY, Sommerce EE. Age related salivary flow rate changes control and patients with oral lichen planus. *J Dent Res* 1985; 64: 1149-1151.
11. Challacombe SJ, Russel MW, Hawkes JE, Bergmeier LA, Lehner T. Passage of immunoglobulins from plasma to the oral cavity in rhesus monkeys. *Immunology* 1978; 35: 923-931.
12. Tomasi TB. The immune system of secretions. Englewood Cliffs, NJ: Prentice-hall, 1976.
13. Brandtzaeg P. Immune functions of human nasal mucosa and tonsils in health and disease, in *Immunology of the Lung and Upper Respiratory Tract*, Bienenstock, J., Ed., McGraw-Hill, New York, 1984.
14. Tenovuo JO. Human Saliva: Clinical Chemistry and Microbiology Vol.II, CRC Press, Inc, Boca Ration, Florida, U.S.A. 1989.
15. Brandtzaeg P. The oral secretory immune system with special emphasis on its relation to dental caries. *Proc Finn Dent Soc* 1983; 79: 71-84.
16. Smith DJ, King WF, Taubman MA. Salivary IgA antibody to oral streptococcal antigens in predentate infants. *Oral Microbiol Immunol* 1990; 5: 57-62.
17. Brandtzaeg P. Human secretory immunoglobulin VII. Concentration of parotid IgA and other secretory proteins in relation to the rate of flow and duration of secretory stimulus. *Arch Oral Biol* 1971; 16: 1295-1310.
18. Alford RH. Effects of chronic bronchopulmonary disease and aging on human nasal secretion IgA concentrations. *J Immunol* 1968; 101: 984-988.
19. Challacombe SJ, Percival RS, Marsh PD. Age-related changes in immunoglobulin isotypes in whole and parotid saliva and serum in healthy individuals. *Oral Microbiol Immunol* 1995; 10: 202-207.
20. Ganguly R, Stablien J, Lockey RF, Shamblin P, Vargas L. Defective antimicrobial functions of oral secretions in the elderly. *J Infect Dis* 1986; 153: 163-164.
21. Smith DJ, Joshipura K, Kent R, Taubman MA. Effect of age on immunoglobulin content and volume of human labial gland saliva. *J Dent Res* 1992; 71: 1891-1894.
22. Arnold RR, Russell JE, Champion WJ, Brewer M, Gauthier JJ. Bactericidal activity of human lactoferrin : differentiation from the stasis of iron deprivation. *Infect Immun* 1982; 35: 792-799.
23. Broxmeyer HE, Smithyman A, Eger RR, Meyer PA, de Sousa M. Identification of lactoferrin as the granulocyte-derived inhibitor of colony-stimulating production. *J Exp Med* 1978; 148: 1052.
24. Tenovuo JO, Lehtonen OP, Aaltonen AS, Vilja P, Tuohimaa P. Antimicrobial factors in whole saliva of human infants. *Infect Immun* 1986; 51: 49.
25. Cole MF, Hsu SD, Baum BJ, Bowen WH, Sierra LI, Aquirre M, Gillespie G. Specific and nonspecific immune factors in dental plaque fluid and saliva from young and old populations. *Infect Immun* 1981; 31: 998.
26. Ben-Aryeh H, Lapid S, Szargel R, Benderly A,

- Gutman D. Composition of whole unstimulated saliva in infants. *Arch Oral Biol* 1984; 29: 357.
27. Baum BJ, Costa PT Jr, Izutsu KT. Alteration in sodium handling by human parotid glands during aging: failure to support a simple two stage secretion model. *Am J Physiol* 1984; 246: R35.
28. Schneyer LH, Young JA, Schneyer CA. Salivary secretion of electrolytes. *Physiol Rev* 1972; 53: 720
29. Navazesh M, Mulligan RA, Kipnis V, Denny PA, Denny PC. Comparison of whole saliva flow rates and mucin concentrations in healthy Caucasian and young aged adults. *J Dent Res* 1992; 71: 1275-1278.
30. Percival RS, Challacombe SJ, Marsh PD. Age-related microbiological changes in the salivary and plaque microflora of healthy adults. *J Med Microbiol* 1991; 35: 5-11.
31. Makila E. Oral health among the inmates of old people's homes. II. Salivary secretion. *Proc Finn Dent Soc* 1977; 75: 64-69.
32. Chaucey HM, Brokan GA, Wayler AH, Feller RP, Kapur KK. Parotid fluid composition in healthy aging males. *Adv Physiol Sci* 1981; 28: 323-328.
33. Scott J. Quantitative age changes in the histological structure of human submandibular salivary glands. *Arch Oral Biol* 1977; 22: 221-227.
34. Waterhouse JP, Chisholm DM, Winter BB, Patel M, Yale RS. Replacement of functional parenchymal cells by fat and connective tissue in human submandibular salivary glands: An age related change *J Oral Pathol* 1973; 2: 16-27.
35. Young JA, Van Lennep EW. Salivary and salt glands, In: Membrane Transport in Biology, Giebisch G, Tosteson DC and Ussing HH, Eds., Berlin : Springer-Verlag.
36. Kerr AC. The physiological regulation of salivary secretion in man. In: International series of monographs on oral biology. Oxford (UK): Pergamon Press, 9.
37. Scott J. Structure and function in aging human salivary glands, *Gerodontology* 1987; 5: 149-158.
38. Fure S, Zickert I. Prevalence of root surface caries in 55, 65 and 75-year-old Swedish individuals. *Community Dent Oral Epidemiol* 1990; 18: 100-105.
39. Ericson S. The normal variation of the parotid size. *Acta Otolaryngol* 1970; 70: 294-300.
40. Buckley CE, Buckley EG, Dorsey FC. Longitudinal changes in serum immunoglobulin levels in older humans. *Fed Proc* 1974; 33: 2036-2039.
41. Grundbacher FJ, Shreffler DC. Changes in human serum immunoglobulin levels with age and sex. *Z Immun Forsch Bd* 1970; 141: s20-26.
42. Tomasi TB, Plaut AG. Humoral aspects of mucosal immunity. *Adv Host Defence Mechanism* 1985; 4: 31-61.
43. Arranz E, O'Mahoney S, Ferguson A. Serum and salivary immunoglobulins and food antibodies in normal elderly subjects. In: macdonald T, Challacombe SJ, Bland P, Strokes C, Heatly R, NelMowat A, Ed. Advances in mucosal immunology. Dordrecht: Kluwer Academic Publisher, 1970.
44. Smith DJ. The oral cavity as an immunological entity. In: Slots J, Taubman MA, ed. Contemporary oral microbiology and immunology. St.Louis: Mosby-Year Book, 1992.
45. Smith DJ, Ebersole JL, Taubman MA. Local and systemic immun response in aged hamsters. *Immunol* 1983; 50: 407-413.
46. Kawanishi H, Ajitsu S, Mirabella S. Impaired humoral immune responses to mycobacterial antigen in aged murine gut-associated lymphoid tissues. *Mech Ageing & Devel* 1990; 54: 143-161.
47. Arnold RR, Cole MF, McGhee JR. A bacterial effect for human lactoferrin. *Science* 1977; 197: 263-265.
48. Kijlstra A, Jeurisen SHM, Koning KM. Lactoferrin levels in normal human tears. *Br J Ophthalmol* 1983; 67: 199-202.
49. Dawes C. The secretion of magnesium and calcium in human parotid saliva. *Caries Res* 1967; 1: 133.

증령에 따른 인체내 전타액의 타액분비율, 면역글로불린, 락토페린 및 전해질의 변화에 관한 연구

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타액은 분비율과 그 구성성분으로 인해 구내환경을 조절하는데 있어 가장 중요한 요인으로 여겨진다. 타액 분비율과 그 성분에 관한 많은 연구가 이루어 졌지만, 증령이 타액과 그 성분에 미치는 영향에 대한 연구는 상반된 결과를 보고하고 있으며 현재까지도 논란의 여지가 많다. 또한 증령에 따른 lactoferrin과 전해질의 변화는 거의 보고되지 않은 실정이다.

이에 저자는 증령이 타액분비량과 타액성분에 미치는 영향을 연구하기 위하여 59명의 투약력이 없고 건강한 사람을 대상으로 연구를 시행하였다. 연구대상을 그들의 나이에 따라 A군, 10~15세 (남자7명, 여자7명); B군, 20~30세 (남자8명, 여자7명); C군, 40~50세 (남자7명, 여자7명); D군, 60세이상 (남자7명, 여자9명) 등의 4군으로 구분하여 각각의 비자극성 전타액을 표준화된 방법으로 채취한후 타액분비량과 immunoglobulin, lactoferrin 및 전해질의 변화를 측정하였다. 이와 같은 실험을 통해 다음과 같은 결론을 얻었다.

1. 비자극성 타액분비량은 각 연령군간의 유의한 차이가 관찰되지 않았으며, 20-30세 군(B군)에서만 남성에게 비해 여성에서 유의하게 낮았다.
2. 인체 전타액내 IgA와 lactoferrin 농도는 연령이나 성별에 따른 뚜렷한 변화는 없었지만, 10-15세 군(A군) 남성에서 유의하게 낮았다.
3. 인체 전타액내 IgG의 농도는 연령이나 성별에 따른 차이가 관찰되지 않았다.
4. 인체 전타액내 IgM의 농도는 60세이상 군(D군) 남성에서 유의하게 낮은 농도를 보였다.
5. 인체 전타액내 전해질(sodium, chloride, potassium, magnesium)의 농도는 증령에 따라 증가하는 경향을 보였다. magnesium과 chloride는 60세이상 군(D군)에서, sodium과 potassium은 40-50세 군(C군)에서 최대치를 보였다. 성별간의 유의성 있는 차이는 발견되지 않았다.

주요어 : 증령, 타액, 분비율, 면역글로불린, 락토페린, 전해질