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Basic Fibroblast Growth Factor in Human Saliva Decreases With Aging

Anders Westermark, DDS, PhD; Ilmari Pyykkö, MD, PhD; Mikael Magnusson, DDS; Hisayoshi Ishizaki, MD, PhD; Pirkko Jäntti, MD, PhD; Gysbert van Setten, MD, PhD

Objective: **Basic fibroblast growth factor (bFGF) has significant properties in wound healing and tissue repair and is suggested to be of importance for the maintenance of mucosal integrity in the upper digestive tract. The purpose of the present study was to identify any age-dependent variations in the concentration of bFGF in human saliva.** ***Study Design:*** **Nonprospective, cross-sectional pilot study.** ***Methods:*** **The study was based on findings from 182 healthy volunteers with ages ranging from 4 to 97 years. Mixed saliva samples were obtained by drooling. The saliva concentration of bFGF was determined with a commercially available enzyme-linked immunosorbent assay kit.** ***Results:*** **The mean saliva concentration of bFGF was 0.41 pg/mL with no gender differences. In persons aged 4 to19 years, the mean concentration was 0.72 pg/mL; in those aged 20 to 65 years, 0.33 pg/mL; and in those aged 66 to 97 years, 0.005 pg/mL. These age-dependent differences were highly significant. In the youngest group the saliva concentration of bFGF varied more than in the other groups.** ***Conclusions:*** **The saliva concentration of bFGF varies with individual age, with the highest levels among young individuals, even levels during a mature phase of life, and low levels toward the end of the life cycle. This strongly suggests a physiological implication of bFGF in saliva.** ***Key Words:*** **Growth factor, saliva, age.**
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INTRODUCTION

Fibroblast growth factor basic or basic fibroblast growth factor (bFGF), also called fibroblast growth factor-2, belongs to a growth factor family that currently consists of nine members, and is on of the most extensively studied in its family.[1] It has been isolated from a variety of sources, such as neural tissue, pituitary, adrenal cortex, corpus luteum, and placenta. In previous studies we have demonstrated the presence of bFGF in tear fluids and in saliva.[2–4]

The bFGF has been demonstrated to stimulate proliferation of cells of mesodermal and neuroectodermal origin. Presence of bFGF accelerates proliferation of endothelial cells and fibroblasts; consequently, bFGF is considered to be highly important in wound healing and tissue repair.[5] The bFGF stimulates the proliferation of gastrointestinal tract epithelium, which has been shown to accelerate the healing of experimental intestinal ulcers in rats,[9] and it has been suggested that bFGF in the saliva may be partly responsible for maintenance of mucosal health in the entire upper digestive tract.

In a previous study, we demonstrated that saliva concentration of bFGF was higher among smokers than among nonsmokers, but also that there was no age-related differences in saliva concentration of bFGF among individuals aged 22 to 49 years.[4] In the current study, we evaluated saliva concentration of bFGF in a larger population of healthy nonsmoking individuals covering an age span from young children to a geriatric population.

MATERIAL AND METHODS

Saliva samples were obtained from 185 healthy volunteers after informed consent according to the Declaration of Helsinki. Samples from the youngest children were obtained in a kindergarten after informed consent from their parents. Samples from the geriatric part of the population were collected in a long-term care facility for elderly individuals.

The saliva was nonstimulated and was collected by drooling or spitting into a mug, from which specimen approximately 1.5 mL of saliva was collected in a test tube; the test tube was sealed immediately and thereafter stored at −80°C until the day of analysis. Information was registered about gender and age of the tested individuals.

At the day of analysis the samples were thawed at room temperature. Then the samples were centrifuged at 14,000 rpm for 8 minutes. Thereafter, the water phase of the saliva was separated for the assay procedure. The samples were analyzed with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (R&D System, Minneapolis, MN) and the instruction manual provided by the company was followed, as in our previous studies.[3,4] The system uses antibodies specific for fibroblast growth factor bound to wells in microtiter plates. A 2-hour incubation with the saliva samples was followed by repeated

From the Departments of Maxillofacial Surgery (A.W., M.M.) and Otolaryngology (I.P.), Karolinska Hospital, Stockholm, Sweden; the Department of Otolaryngology, Kikugawa Hospital (H.I.), Shijuoka, Japan; the Department of Otolaryngology, Health Center Hospital of Oulu (P.J.), Oulu, Finland; and the Department of Ophthalmology (G.V.S.), St. Erik Eye Hospital, Stockholm, Sweden.

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Send Correspondence to Anders Westermark, DDS, PhD, Department of Maxillofacial Surgery, Karolinska Hospital, SE 171 76 Stockholm, Sweden. E-mail: anders.westermark@ks.se

washings. Thereafter, an enzyme-linked polyclonal bFGF-specific antibody was added to the wells. A substrate solution was added, followed by incubation for 1 hour. An amplifier solution was added before the reaction was stopped with 2N sulfuric acid. Optical densities of the wells were determined with a spectrophotometer at 490 nm with a correction wavelength of 690 nm. A standard curve was obtained according to the values of a standard dilution of recombinant human bFGF dilutions ranging from 0.0 to 36 pg/mL, in duplicate determination. The statistical evaluation was performed with unpaired Student *t* test, and the level of significance was set at *P* levels below .05.

RESULTS

Of the 185 samples, 182 were included in the study, representing 69 male and 113 female individuals with ages ranging from 4 to 97 years. The distribution of bFGF in saliva is illustrated in Table I and in Figures 1 and 2. The mean saliva concentration of bFGF was 0.41 pg/mL. There was no significant difference between male and female individuals. Among male individuals the mean concentration was 0.46 pg/mL, and among female individuals, 0.38 pg/mL. Young individuals (aged 4–19 y) had a mean concentration of 0.72 pg/mL; individuals aged 20 to 65 years, 0.33 pg/mL; and those aged 66 to 97 years, 0.005 pg/mL (Table I). These age-related differences were highly significant, with *P* levels below .01 both between the young group and the middle group and between the middle group and the old group. There was a large variation in saliva concentration of bFGF among the young individuals (Table I; Figs. 1 and 2). Three elderly individuals possessed high levels (4.23, 3.53, and 2.59 pg/mL), but they were excluded from the study because of the presence of intraoral pathological processes.

DISCUSSION

The present study repeated our previous finding that there seems to be an even distribution of saliva concentration of bFGF among individuals in the third, fourth, and fifth decades of life. A new finding was that there were significantly higher bFGF concentrations in younger individuals, but also with large variations in bFGF concentrations. Moreover, there were low concentrations of bFGF in the saliva of the older individuals and the geriatric population. Because bFGF is considered to be one of the most potent growth factors for fibroblast and endothelial cell proliferation, it seems natural that growing individuals with intense proliferative activity of intraoral tissues possess the highest amounts of bFGF in their saliva. During the more mature part of the life cycle with well-balanced regulation of cell proliferation, bFGF levels seem to be relatively stable, and toward the end of the life cycle very little bFGF seems to be normally produced, unless significant disease is present.

TABLE I.
Distribution of bFGF in Saliva in Individuals Aged 4 to 97 Years.

Age Group		Mean	Median	Range
Years	Number	(pg/mL)	(pg/mL)	(pg/mL)
4–19	55	0.72	0.55	0.01–2.58
20–65	101	0.33	0.27	0.00–0.90
66–97	26	0.005	0.00	0.00–0.94

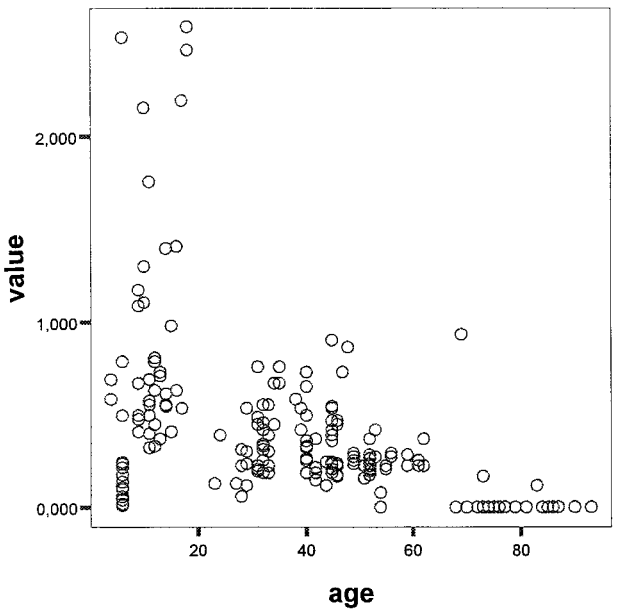

Fig. 1. Diagram of the distribution of basic fibroblast growth factor in saliva (picograms per milliliter saliva). Age = years.

Interesting was the wide variation observed in the saliva concentration of bFGF in the young individuals. However, there may be an obvious explanation for that, emphasizing the physiological importance of bFGF in saliva. Growth factors stimulate target cells to chemotaxis and mitosis.[5] One of these factors is bFGF, which is released partly in wounded tissues.[6] Intraoral wounds such as teething are present during a relatively long age-period, from approximately 2.5 to 13 or 14 years. During this time, there are frequent periods with tooth eruption or tooth exchange. Thereafter, there is a relatively wide period of the upper teens when the wisdom teeth start to erupt. Such conditions represent rapidly healing wounds and therefore may be involved in the high and varying saliva concentrations of bFGF among the young individuals. However, no clinical records were taken for the young individuals examined.

Another cause of high salivary concentration of bFGF may be smoking habits.[4] Many of the children aged 12 to 15 were examined in connection with a visit at an orthodontist's evaluation for possible orthodontic treatment. At that occasion, most of the patients were accompanied by one parent or both, and it seems unlikely that any smoking would have been confessed under those circumstances. In our previous study on bFGF in human saliva, we observed significantly higher saliva concentrations of bFGF among smokers than among nonsmokers.[4] However, those differences were not, by far, of the magnitude that we observed in the group of young individuals in the present study. Therefore, we think that the high concentrations of salivary bFGF in the two youngest decades in the current study represent a true age-dependent phenomenon, just as the low concentrations in the older and

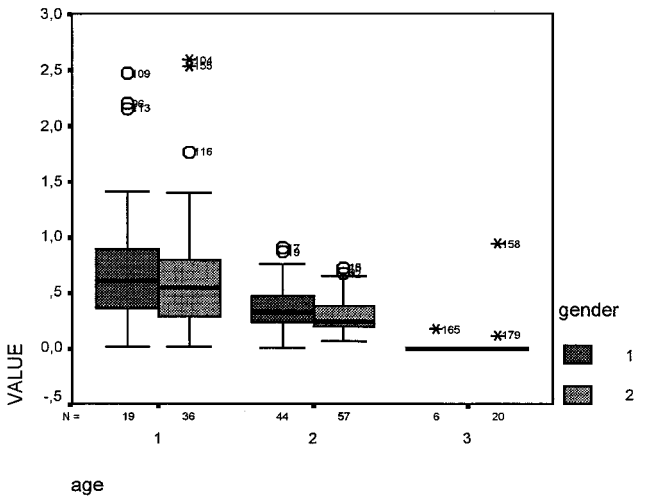

Fig. 2. Basic fibroblast growth factor in saliva (picograms per milliliter saliva) in the following age groups: Group 1 = 4 to 19 y; group 2 = 20 to 65 years; and group 3 = 66 years and older. Gender: 1 = male individual; 2 = female individual.

geriatric population do. It is interesting, but not surprising, that serum levels of insulin growth factor-I vary with age in a somewhat similar pattern with a peak around the period of maximal growth during youth and, thereafter, a slow reduction over the mature and aging periods of the life cycle.[7] It was also demonstrated previously that with increasing age there is a decline in gastrointestinal absorption of growth factors in the rat.[8] Just as growth factors are most active during growth, a physiological downregulation of their presence toward the end of the life cycle may be assumed. Obviously, however, because three individuals with oral pathological conditions demonstrated high levels, the potential to produce bFGF remains, despite aging. Such association between bFGF and pathological processes has been previously described.[1]

Experimentally, bFGF in saliva has been described to heal duodenal ulcers in rodents,[9] and similar findings in humans have been reported with epidermal growth factor.[10] Therefore, it has been suggested that growth factors in saliva may be responsible for the maintenance of mucosal integrity in the entire upper digestive tract. In their report, Sabbadini and Berczi[11] speculated that the submandibular gland may be a key organ in the neuroimmunoregulatory network. They reported that in rodents the submandibular gland is fully integrated into the neuroendocrine system.[11]

The bFGF also may be present under pathological conditions. Kaban et al.[12] reported on a case of giant cell tumor of the mandible in which the patient possessed abnormally elevated levels of bFGF in the urine. As the tumor was treated with interferon alfa-2a, the tumor regressed and disappeared and, simultaneously, the urinary bFGF fell to normal levels. However, no evaluation of salivary bFGF was mentioned by Kaban et al.[12]

Today, we still lack much information on growth factors in human saliva. Further studies should include not only mixed saliva but also gland-specific saliva and should be directed to study a vast array of growth factors in the saliva. Growth factor responses to aging, radiation treatment, and the abuse of tobacco and alcohol might give indications about the roles of growth factors in health and disease. However, the interactions between various growth factors are poorly understood, and the interactions between growth factors and upregulation or downregulation of their receptors appear to make the impression of the system even more complex.

CONCLUSION

The saliva concentration of bFGF seems to be age dependent with high levels during childhood and youth, even levels during the mature period of life, and low levels during the last decades of life. These differences may be linked to age-dependent mucosal degeneration in the upper digestive tract.

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