

A specific hygiene hypothesis

Cliff Shunsheng Han

Los Alamos National Laboratory, Bioscience Division and Quality Performance Assurance Division, MS, M888, Los Alamos, NM 87545, USA



ARTICLE INFO

Article history:

Received 25 November 2015

Accepted 24 May 2016

ABSTRACT

Allergic diseases have reached epidemic proportions in Western populations in the last several decades. The hygiene hypothesis proposed more than twenty years ago has helped us to understand the epidemic and has been verified with numerous studies. However, translational measures deduced from these studies to prevent allergic diseases have not proven effective. Recent studies on immigrants' allergies and any potential association between oral infection and allergic diseases prompt me to propose a specific hygiene hypothesis to explain how oral hygiene practices might have contributed to the uprising of hay fever, the most common allergic disease. The historic oral hygiene level in US is closely associated with the emerging allergic epidemic. Future studies to test the hypothesis are needed and verification of the hypothesis can potentially yield highly effective measures to prevent allergic diseases.

Published by Elsevier Ltd.

Introduction

Allergies are a significant health concern. Allergic diseases have reached epidemic proportions in Western populations in the last decades. Hay fever (allergic rhinitis or seasonal allergy) is the most common allergic disease, affecting 13.5% of the US population [1]. Its symptoms include any combination of the following: a runny or stuffy nose, sneezing, itchiness, red eyes, and congestion. The hygiene hypothesis was proposed by Strachan in 1989 to explain the cause [2], which is referred to here as general hygiene hypothesis (GHH). Since then, a variety of studies reported results consistent with GHH, including that rural farm living significantly reduces the risk of developing hay fever and atopic sensitization [3,4]. These environmental cues point to lack of access to microbial diversity as a possible cause of allergies and autoimmune diseases [5–8]. The observed link between allergy and commensal bacteria has provided the scientific rationale for microbiome reconstitution as measure against the allergy epidemic [9]. However, the results from probiotic treatments have been conflicting [10–13]. The continuous confirmation of the hygiene hypothesis [14] and failure in current intervention methods suggest a need to research in new directions [15,16].

Recent studies on immigrants indicated that environmental changes later in life alone could lead to allergic diseases for adults who are equipped with immune systems developed in a relatively traditional lifestyle [17–19]. In several studies of Italian populations, immigrants who visited doctors with complaints of allergies had little family history of atopy [18,19]. Only 16% had a clinical history of allergies before migration. The time that elapsed between migration and onset of symptoms was 5.3 ± 3.1 years,

with a minimum of 0.5 and a maximum of 7 years. These immigrants were from Asia, Africa, South America, and Eastern Europe. A study of US immigrant children showed that children born outside the United States have a lower prevalence in allergic diseases that increases after residing in the United States for a decade [20].

Hypothesis

The combination of these research results prompted me to propose a specific hygiene hypothesis (SHH) and a capacity competition theory (CCT) to explain the rising of hay fever. SHH posits that continuous improvement in oral hygiene practices in modern society is the major cause of the increase in the prevalence of hay fever and perhaps some other allergic diseases as well (Fig. 1A). CCT is proposed to explain how reduction of oral infection, one of the results of modern oral hygiene, leads to development of allergy (Fig. 1B). CCT assumes that bacterial antigens under chronic oral infections have a much higher concentration than pollens do in oral-rhino cavities. The dominant bacterial antigens overwhelm the acquired immune system residing in lymph nodes at the back oral-rhino opening or nearby places, which limits the chance of immune system to access allergens and to produce B cell clones with efficient antibodies against allergens. After removing oral infections with persistent modern oral hygiene practices, reduced bacterial antigen concentration leaves extra antibody-generating capacity to allergens, with relative concentration becoming higher. The probability to produce B cell clones with highly efficient antibodies against allergens increases. The elevated innate immune system under infectious conditions can also clean allergens faster than that under conditions without infections, which further

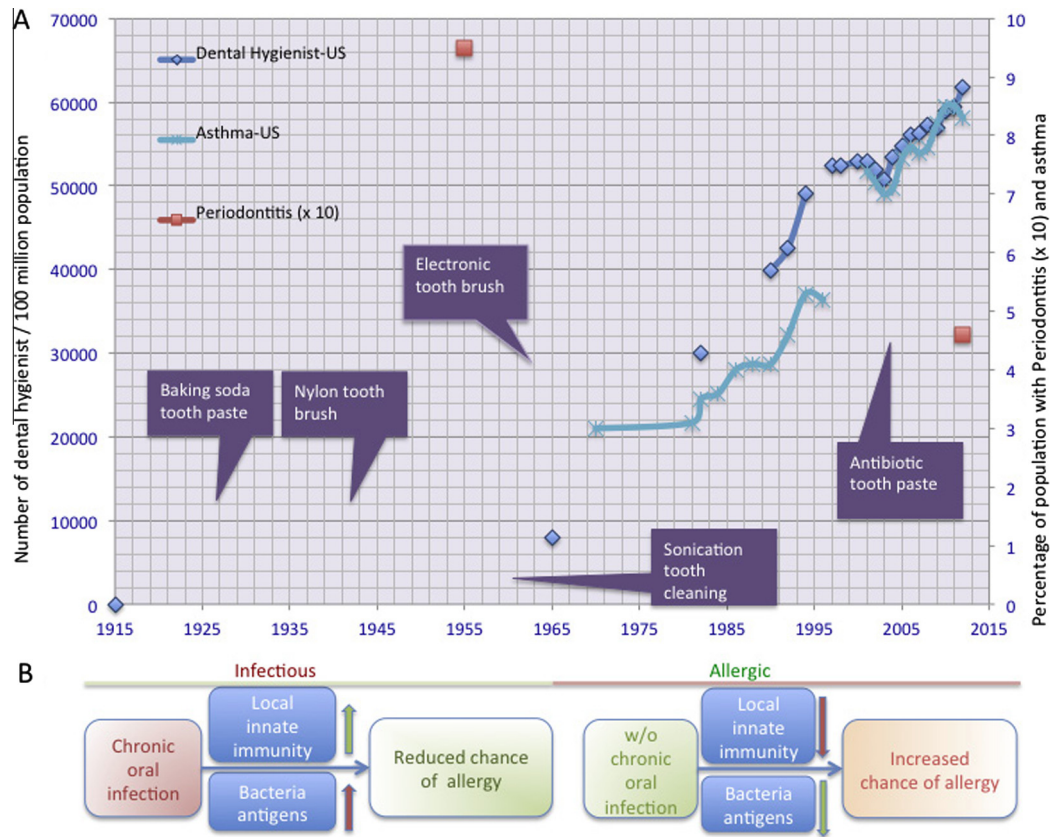


Fig. 1. The specific hygiene hypothesis and capacity competition theory. (A) Correlation between the number of dental hygienists, asthma prevalence in the US ($\text{cor} = 0.96$, $p < 0.01$), and reduction in prevalence of periodontitis suggest a relationship between oral hygiene and allergy. (B) The capacity competition theory (see text).

reduces the competitive edge of allergens (Fig. 1B). For the same reason, the stimulated innate immunity can likely protect against acute infections [21]. CCT indicates that the competing bacterial antigens do not have to be from a/multiple specific species.

SHH is different from GHH in the following aspects: 1) it specifies modern oral hygiene practices as the cause for the high rise of hay fever through the reduction of oral infection (Fig. 1B). 2) This hypothesis suggests actionable practices to prevent allergy if proved. 3) CCT indicates the importance of the local immune system in allergy development, though some bacteria might systematically relieve allergic reaction [22]. 4) CCT indicates lasting chronic infections have more protection against allergy than acute infections.

Besides the observed negative association between periodontitis, or periodontal microflora, and respiratory allergic diseases, studies on the dose-response effects of allergens also support the capacity competition theory [23–26]. A study found there is a sigmoid dose-response curve for subsequent reactivity [27]. Another study shows that local pollen counts are positively related to the prevalence of hay fever in children of different states in the United States [23].

The first evidence for the SHH is a high correlation between oral hygiene and the prevalence of allergic diseases (Fig. 1 and Supplementary Table 1). The oral hygiene level in a population can be measured in several ways, such as the consumption of oral hygiene products, frequency of dental visits, percentage of the population with dental insurance, and/or dental health condition. I choose to use the historical number of dental hygienists as the indicator of oral hygiene levels in US as data is readily available at the US Bureau of Labor Statistics. The profession of dental hygienist is a relatively new one, appearing first in 1915 with the main purpose

of cleaning and polishing teeth to prevent dental diseases. They also give visitors advice on their daily oral hygiene practices. The employment in this occupation has been steadily increasing since its creation. There were about 15 thousand dental hygienists in the US in 1965, before the increase in allergic diseases, and now there is a total of more than 193 thousand dental hygienists in US. The asthma prevalence data was collected from the Center for Disease Control. Linear regression shows that the two data sets are closely associated with each other ($r = 0.96$, $p < 0.01$, Fig. 1A and Supplementary Table 1).

The traditional oral hygiene status is infectious. The oral cavity is the entry to the digestive tract, which is where the battle between foreign bio-agents and our immune system begins. Without intentional help from the human host, the battle between foreign agents and the immune system is continuously visible. Oral infections are common in the population without modern hygiene practices. The study of an indigenous Native American population in Central America indicated a high prevalence of pocketing, and 90% of adults (≥ 35 years) had at least one site with clinical attachment levels (CAL) ≥ 6 mm [28]. A study based on the US population indicated that close to 80% of youth (13–15 years old) have gingivitis and more than 95 percent of the older population (≥ 35 years old) had periodontitis in the 1950s [29].

Oral hygiene practices began in ancient times, before recorded human history. Various excavations done throughout the world have recovered sticks, tree twigs, bird feathers, animal bones and porcupine quills that were chewed on to clean teeth [30,31]. However, only recently are we gradually winning the war against oral microbiota, with better and better toothbrushes, toothpaste, mouthwash, and floss. Early toothbrushes made of animal hair were neither effective, due to contamination of bacteria, nor

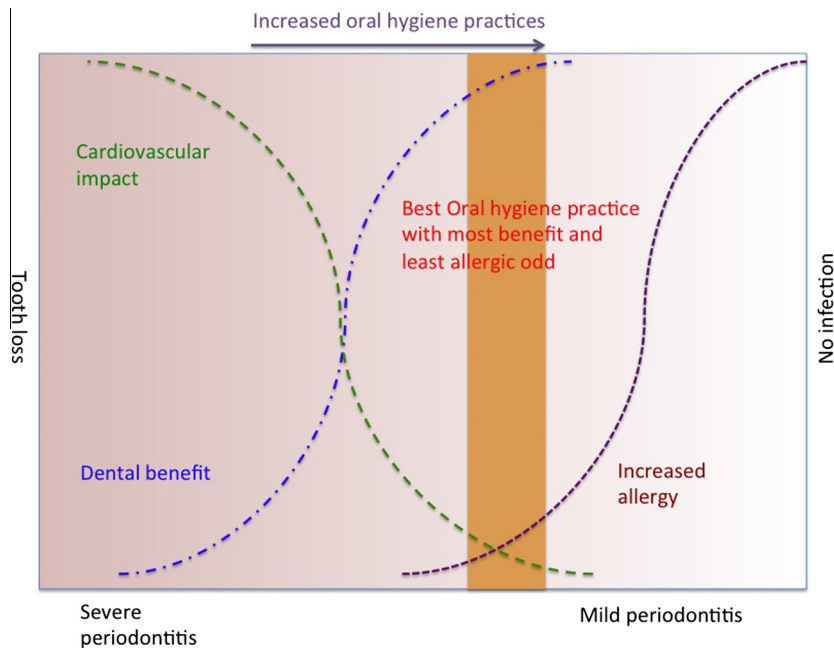


Fig. 2. Hypothetical relationships between outcomes of modern oral hygiene. Benefits of oral hygiene practice include reducing the severity of oral infection and its impact on cardiovascular health, increasing dental health. Potential harm according to SHH is the increasing odds of hay fever. Future studies have to be designed to not only verify SHH, but also identify the window of the best oral hygiene practices that yield the most benefits and are the least harmful.

popular, due to rare availability and ignorance of oral hygiene issues. DuPont introduced modern toothbrushes made of nylon around World War II with a dime price. Early toothpaste made of vinegar or urine did as much harm as good. The dust of brick or china, mixed with soap, was later more effective. Modern toothpastes were introduced after WWII with synthetic detergent agents and fluoride. Antibiotic triclosan was introduced into toothpaste in the 1980s [32–35]. Floss, introduced in the 19th century, was originally made of silk and later replaced by nylon floss, to which antibacterial agents were added relatively recently. Water used in rinsing one's mouth was replaced with mouthwashes that contain much stronger antibiotic effects [36]. Now trending in oral hygiene is Oil-pulling, which is an ancient Ayurvedic dental technique that involves swishing a tablespoon of oil in one's mouth, which proves effective in reducing oral infection [37–39].

The results of oral cleaning include significant reductions in oral infection and higher teeth retention. From 2009 to 2012, only 46% (compare to more than 95% in the 1950s) of US adults, had periodontitis [40]. Another study in Sweden showed that, in all age groups, the number of edentulous individuals decreased dramatically as the number of teeth increased [41]. Over 30 years, the proportion of periodontally healthy individuals increased from 8% in 1973 to 44% in 2003, and the proportion of individuals with gingivitis and moderate periodontitis decreased in Sweden. With the help of the hygiene industry and the promotion of health organizations, modern societies are winning the war against oral microbiota. As an unintentional result, oral microbiota might be among the most interrupted human microbiota. Are there any unwanted consequences besides those beneficial outcomes? SHH and CCT are proposed here to explain the possible link oral hygiene and allergy. SHH and CCT can not only explain the high rising of hay fever, but also explain other characters of allergy epidemics, such as the crucial time in early life and impact of antibiotic use.

Conclusion

This specific hygiene hypothesis, if proven, suggests a simple and economical way to prevent allergy – personalized oral hygiene

practices that maintain an oral microbiota with controlled mild oral infections. We acknowledge great benefits of modern oral hygiene, such as healthy teeth and likelihood in preventing cardiovascular diseases, and speculate that there is likely an optimal level of oral hygiene that will give intended benefits and the smallest odds to allergic diseases (Fig. 2). Testing this, SHH and CCT will be a significant step forward in understanding the current allergy epidemic. Proving or disproving the hypothesis will provide novel insights into the mechanism of the observed trends and help to define future research directions.

Conflicts of interest

The author does not have any financial interests associated with this publication.

Acknowledgements

This project was supported by Los Alamos National Laboratory, Laboratory Directed Research and Development, 20130779PRD1. I thank Joe Alcock, Melanie Martin, and Armand Dichosa for previous stimulating collaborations in the microbiome study of the Tsimane people and valuable comments during the preparations of the manuscript. I am grateful to Ruy Ribeiro for his encouraging and constructive discussions. I am grateful that the critics for a grant review from NIH have also helped to strengthen the discussions.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.mehy.2016.05.029>.

References

- [1] Li C, Cheung CL, Cheung TT, Samaranyake NR, Cheung BM. Hay fever and hypertension in the US adult population. *Clin Exp Hypertens* 2014;36:206–10. <http://dx.doi.org/10.3109/10641963.2013.804545>.

- [2] Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989;299:1259–60.
- [3] von Mutius E, Radon K. Living on a farm: impact on asthma induction and clinical course. *Immunol Allergy Clin North Am* 2008;28:631–47. <http://dx.doi.org/10.1016/j.jac.2008.03.010> ix–x.
- [4] Ege MJ et al. Not all farming environments protect against the development of asthma and wheeze in children. *J Allergy Clin Immunol* 2007;119:1140–7. <http://dx.doi.org/10.1016/j.jaci.2007.01.037>.
- [5] Lluís A, Schaub B. Lesson from the farm environment. *Curr Opin Allergy Clin Immunol* 2012;12:158–63. <http://dx.doi.org/10.1097/ACI.0b013e32835109a8>.
- [6] von Mutius E. Maternal farm exposure/ingestion of unpasteurized cow's milk and allergic disease. *Curr Opin Gastroenterol* 2012;28:570–6. <http://dx.doi.org/10.1097/MOG.0b013e32835955d3>.
- [7] Ege MJ et al. Exposure to environmental microorganisms and childhood asthma. *N Engl J Med* 2011;364:701–9. <http://dx.doi.org/10.1056/NEJMoa1007302>.
- [8] Ege MJ. Gene-environment interaction for childhood asthma and exposure to farming in Central Europe. *J Allergy Clin Immunol* 2011;127:138–44. <http://dx.doi.org/10.1016/j.jaci.2010.09.041> 144 e131–134.
- [9] Parker W, Ollerton J. Evolutionary biology and anthropology suggest biome reconstitution as a necessary approach toward dealing with immune disorders. *Evol Med Public Health* 2013;89–103:2013. <http://dx.doi.org/10.1093/emph/eot008>.
- [10] Toh ZQ, Anzela A, Tang ML, Licciardi PV. Probiotic therapy as a novel approach for allergic disease. *Front Pharmacol* 2012;3:171. <http://dx.doi.org/10.3389/fphar.2012.00171>.
- [11] Pelucchi C et al. Probiotics supplementation during pregnancy or infancy for the prevention of atopic dermatitis: a meta-analysis. *Epidemiology* 2012;23:402–14. <http://dx.doi.org/10.1097/EDE.0b013e32824d5da2>.
- [12] Jeurink PV, Rijnierse A, Martin R, Garssen J, Knippels LM. Difficulties in describing allergic disease modulation by pre-, pro- and synbiotics. *Curr Pharm Des* 2012;18:2369–74.
- [13] Gore C et al. Treatment and secondary prevention effects of the probiotics *Lactobacillus paracasei* or *Bifidobacterium lactis* on early infant eczema: randomized controlled trial with follow-up until age 3 years. *Clin Exp Allergy* 2012;42:112–22. <http://dx.doi.org/10.1111/j.1365-2222.2011.03885.x>.
- [14] Olszak T. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science* 2012;336:489–93. <http://dx.doi.org/10.1126/science.1219328>. New York, NY.
- [15] Okada H, Kuhn C, Feillet H, Bach JF. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. *Clin Exp Immunol* 2010;160:1–9. <http://dx.doi.org/10.1111/j.1365-2249.2010.04139.x>.
- [16] Reddy BV, Chava VK, Nagarakanti S, Gunupati S, Samudrala P. Hygiene hypothesis and periodontitis – a possible association. *Med Hypotheses* 2014;82:60–3. <http://dx.doi.org/10.1016/j.mehy.2013.11.006>.
- [17] Lombardi C, Penagos M, Senna G, Canonica GW, Passalacqua G. The clinical characteristics of respiratory allergy in immigrants in northern Italy. *Int Arch Allergy Immunol* 2008;147:231–4. <http://dx.doi.org/10.1159/000142046>.
- [18] Lombardi C, Canonica GW, Passalacqua G. The possible influence of the environment on respiratory allergy: a survey on immigrants to Italy. *Ann Allergy Asthma Immunol* 2011;106:407–11. <http://dx.doi.org/10.1016/j.anai.2011.01.023>.
- [19] Burastero SE, Masciulli A, Villa AM. Early onset of allergic rhinitis and asthma in recent extra-European immigrants to Milan, Italy: the perspective of a non-governmental organisation. *Allergol Immunopathol (Madr)* 2011;39:232–9. <http://dx.doi.org/10.1016/j.allerg.2010.07.004>.
- [20] Silverberg JL, Simpson EL, Durkin HG, Joks R. Prevalence of allergic disease in foreign-born American children. *JAMA Pediatr* 2013;167:554–60. <http://dx.doi.org/10.1001/jamapediatrics.2013.1319>.
- [21] Abt MC et al. Innate immune defenses mediated by two ILC subsets are critical for protection against acute clostridium difficile infection. *Cell Host Microbe* 2015;18:27–37. <http://dx.doi.org/10.1016/j.chom.2015.06.011>.
- [22] Card JW et al. Modulation of allergic airway inflammation by the oral pathogen *Porphyromonas gingivalis*. *Infect Immun* 2010;78:2488–96. <http://dx.doi.org/10.1128/IAI.01270-09>.
- [23] Silverberg JL, Braunstein M, Lee-Wong M. Association between climate factors, pollen counts, and childhood hay fever prevalence in the United States. *J Allergy Clin Immunol* 2015;135:463–9. <http://dx.doi.org/10.1016/j.jaci.2014.08.003>.
- [24] Camouse MM et al. Determination of in vivo dose response and allergen-specific T cells in subjects contact-sensitized to squaric acid dibutyl ester. *Dermatitis* 2008;19:95–9.
- [25] Arts JH, Mommers C, de Heer C. Dose-response relationships and threshold levels in skin and respiratory allergy. *Crit Rev Toxicol* 2006;36:219–51.
- [26] Riu E et al. Occupational risk factors for rhinitis in greenhouse flower and ornamental plant growers. *Am J Rhinol* 2008;22:361–4. <http://dx.doi.org/10.2500/ajr.2008.22.3186>.
- [27] Friedmann PS. The relationships between exposure dose and response in induction and elicitation of contact hypersensitivity in humans. *Br J Dermatol* 2007;157:1093–102. <http://dx.doi.org/10.1111/j.1365-2133.2007.08162.x>.
- [28] Dowsett SA, Archila L, Segreto VA, Eckert GJ, Kowolik MJ. Periodontal disease status of an indigenous population of Guatemala, Central America. *J Clin Periodontol* 2001;28:663–71.
- [29] Marshall-Day CD. The epidemiology of periodontal disease. *J Periodontol* 1951;22:13–22. Passim.
- [30] Levy J. In: Bennett Anna, editor. Really useful: the origins of everyday things. Firefly Books Inc.; 2002. p. 68–71.
- [31] Panati C. Extraordinary origins of everyday things. Harper & Row Publishers, Inc.; 1987. p. 208–13.
- [32] Marsh PD. Dentifrices containing new agents for the control of plaque and gingivitis: microbiological aspects. *J Clin Periodontol* 1991;18:462–7.
- [33] Wade WG, Addy M. Antibacterial activity of some triclosan-containing toothpastes and their ingredients. *J Periodontol* 1992;63:280–2. <http://dx.doi.org/10.1902/jop.1992.63.4.280>.
- [34] Rosling B et al. Effect of triclosan on the subgingival microbiota of periodontitis-susceptible subjects. *J Clin Periodontol* 1997;24:881–7.
- [35] Furuichi Y, Ramberg P, Krok L, Lindhe J. Short-term effects of triclosan on healing following subgingival scaling. *J Clin Periodontol* 1997;24:777–82.
- [36] Quintas V, Prada-Lopez I, Donos N, Suarez-Quintanilla D, Tomas I. In situ neutralisation of the antibacterial effect of 0.2% chlorhexidine on salivary microbiota: quantification of substantivity. *Arch Oral Biol* 2015;60:1109–16. <http://dx.doi.org/10.1016/j.archoralbio.2015.04.002>.
- [37] Asokan S, Rathana J, Muthu MS, Rathana PV, Emmadi P. Effect of oil pulling on *Streptococcus mutans* count in plaque and saliva using Dentocult SM strip mutans test: a randomized, controlled, triple-blind study. *J Indian Soc Pedod Prev Dent* 2008;26:12–7.
- [38] Asokan S, Emmadi P, Chamundeswari R. Effect of oil pulling on plaque induced gingivitis: a randomized, controlled, triple-blind study. *Indian J Dent Res* 2009;20:47–51.
- [39] Asokan S et al. Mechanism of oil-pulling therapy – in vitro study. *Indian J Dent Res* 2011;22:34–7. <http://dx.doi.org/10.4103/0970-9290.79971>.
- [40] Eke PI et al. Update on prevalence of periodontitis in adults in the United States: NHANES 2009–2012. *J Periodontol* 2015;86:611–22. <http://dx.doi.org/10.1902/jop.2015.140520>.
- [41] Hugoson A, Sjödin B, Norderyd O. Trends over 30 years, 1973–2003, in the prevalence and severity of periodontal disease. *J Clin Periodontol* 2008;35:405–14. <http://dx.doi.org/10.1111/j.1600-051X.2008.01225.x>.

Supplementary Table 1. Increase of dental hygienist and prevalence of asthma and reduction of periodontitis.

Year	Dental Hygienist	Asthma (%)	Periodontitis (%)
1915	1 ^{#, 1}		
1955			95 ²
1964			
1965	7969 ³		
1970		3 ⁴	
1981		3.1 ⁴	
1982	29947 ⁵	3.5 ⁶	
1984		3.6 ⁶	
1986		4 ⁶	
1988		4.1 ^{6, 7}	
1990	39946 ⁸	4.1 ⁶	
1992	42585 ⁹	4.6 ⁶	
1994	49058 ¹⁰	5.3 ⁶	
1996		5.2 ⁶	
1997	52461 ¹¹		
1998	52356 ¹²		
2000	52900 ¹³		
2001	52861 ¹⁴	7.4 ¹⁵	
2002	51890 ¹⁶	7.2 ¹⁵	
2003	50687 ¹⁷	7 ¹⁵	
2004	53459 ¹⁷	7.1 ¹⁵	
2005	54782 ¹⁷	7.6 ¹⁵	
2006	56026 ¹⁷	7.8 ¹⁵	
2007	56211 ¹⁷	7.7 ¹⁵	
2008	57178 ¹⁷	7.8 ¹⁵	
2009	56953 ¹⁷	8.2 ¹⁵	
2010	58986 ¹⁷	8.5 ¹⁵	
2011	59410 ¹⁷	8.5 ¹⁵	
2012	61754 ^{17, 18}	8.3 ¹⁵	46 ⁷

#Normalized to US population in 1915, about 100 million.

1. American Dental Hygienist Association, 100 Years of Dental Hygienist.
<http://www.adha.org/timeline>.

2. Marshall-Day, C.D. The epidemiology of periodontal disease. *The Journal of periodontology* 22, 13-22; passim (1951).
3. Danner, V. Looking back at 75 years of the Journal: former editors reflect on their time with the Journal. *J Dent Hyg* 76, 4 (2002).
4. Evans, R., 3rd, Mullally, D.I., Wilson, R.W., Gergen, P.J., Rosenberg, H.M., Grauman, J.S., Chevarley, F.M. & Feinleib, M. National trends in the morbidity and mortality of asthma in the US. Prevalence, hospitalization and death from asthma over two decades: 1965-1984. *Chest* 91, 65S-74S (1987).
5. United States, Bureau of Labor Statistics. (ed.) *Dental Hygienists in Occupational outlook handbook*, Issue (1984). p. 32-32.
<https://books.google.com/books?id=k3pVBYkWnwMC&pg=PA32>.
6. Center for Disease Control. *Asthma Prevalence in the United States* (2014),
http://www.cdc.gov/asthma/asthma_prevalence_in_us.pptx.
7. Eke, P.I., Dye, B.A., Wei, L., Slade, G.D., Thornton-Evans, G.O., Borgnakke, W.S., Taylor, G.W., Page, R.C., Beck, J.D. & Genco, R.J. Update on Prevalence of Periodontitis in Adults in the United States: NHANES 2009 to 2012. *J Periodontol* 86, 611-622 (2015).
8. United States, Bureau of Labor Statistics. (ed.) *Dental Hygienists in Occupational outlook handbook*, Issue 2400 (1992). p.187-188,
<https://books.google.com/books?id=13sgzVmjlCUC>.
9. United States, Bureau of Labor Statistics. (ed.) *Dental Hygienists in Occupational outlook handbook* (1994). p. 255-256 .
<https://books.google.com/books?isbn=156370160X>.
10. United States, Bureau of Labor Statistics (ed.) *Dental Hygienists in Occupational Outlook Handbook, 1996-1997* (1996). p. 202-203.
<https://books.google.com/books?isbn=0788129058>.
11. United States, Bureau of Labor Statistics. *OCCUPATIONAL EMPLOYMENT AND WAGES, 1997* (1998),
http://www.bls.gov/news.release/history/ocwage_010499.txt.
12. United States, Bureau of Labor Statistics. *OCCUPATIONAL EMPLOYMENT AND WAGES, 1998* (1999).
http://www.bls.gov/news.release/history/ocwage_12221999.txt.
13. United States, Bureau of Labor Statistics. *Occupational Employment Statistics* (2000). <http://www.bls.gov/oes/2000/oes292021.htm>.
14. United States, Bureau of Labor Statistics. *Occupational Employment Statistics* (2001). <http://www.bls.gov/oes/2001/oes292021.htm>.

15. Center for Disease Control. National Health Interview Survey (NHIS) Data. <http://www.cdc.gov/asthma/nhis/default.htm>.
16. United States, Bureau of Labor Statistics. Occupational Employment Statistics (2002). <http://www.bls.gov/oes/2002/oes292021.htm>.
17. United States, Bureau of Labor Statistics, Occupational Employment Statistics: Dental Hygienists. <http://www.bls.gov/oes/20YY/may/oes292021.htm> (Replace YY to corresponding years.)
18. Brandt, O., Zuberbier, T. & Bergmann, K.C. Risk of sensitization and allergy in Ragweed workers - a pilot study. *Allergy Asthma Clin Immunol* 10, 42 (2014).