Evaluation of the prevalence of antiwheat-, anti-flour dust, and anti–α-amylase specific IgE antibodies in US blood donors

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Background: Asthma in bakery workers is one of the most frequently occurring forms of occupational asthma in the world. Experience from other countries has shown the prevalence of sensitization (IgE) to bakery-associated allergens (BAAs) (wheat [W], flour dust [FD], α-amylase [AA]) in bakery workers to be 5% to 53%, whereas the prevalence in nonoccupationally exposed individuals was estimated to be 1.2% to 6.4%.

Objective: To estimate the prevalence of BAA sensitization by measuring BAA specific IgE in the residual serum tubes of volunteer blood donors.

Methods: Serum samples from 534 volunteer blood donors were measured for anti-W, anti-FD, and anti-AA specific IgE antibodies (in duplicate) using the AlaSTAT microplate assay. Samples with BAA IgE concentrations of 0.35 kU/L or greater were considered positive.

Results: Nineteen of 530 serum samples (3.6%; 95% confidence interval [CI], 3.3%–3.9%) were positive for W (range, 0.38–3.61 kU/L), whereas 31 of 534 (5.8%; 95% CI, 5.3%–6.3%) were positive for FD (range, 0.35–2.34 kU/L) and 5 of 529 (1.0%; 95% CI, 0.9%–1.1%) were positive for AA (range, 0.38–1.59 kU/L). Thirteen serum samples were positive for both W and FD; 1 sample each was positive for W and AA and FD and AA.

Conclusions: The prevalence of IgE sensitization in serum samples from a relatively large unselected population of volunteer blood donors is 1.0% for AA, 3.6% for W, and 5.8% for FD, which agrees well with data from other countries for sensitization prevalence rates for nonoccupationally exposed individuals.

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INTRODUCTION

The respiratory effects of exposure to cereal grains dates back to ancient Rome, where slaves employed in milling and in bakeries used face masks for respiratory protection.[1] Baker's asthma, first described in the literature approximately 300 years ago by Bernardini Ramazzini in his famous *De Morbis Artificum Diatriba*,[2] is now one of the most frequently occurring forms of occupational asthma in the world.[3–5] Wheat, α-amylase, flour dust, soy flour, rye flour, and xylanase are the allergens to which exposure has been most associated with developing baker's asthma.[5–9] Bakers seem to be at increased risk of sensitization (IgE) to bakery-associated allergens (BAAs) and also experience an increase in work-related respiratory symptoms.[4] Atopic status and elevated total IgE levels seem to be risk factors for the development of sensitization to BAAs and respiratory symptoms.[4,5] There is a clear concentration-effect relationship for both respiratory symptoms[10,11] and immunologic sensitization[7] with increasing levels of exposure to BAAs. In addition, the risk of developing baker's asthma seems to be increasing over time.[12] The prevalence of sensitization (IgE) to BAAs in bakers has been reported to range from 5% to 53%.[1,13,14] Bakery-associated allergen sensitization rates from nonoccupational exposures have been reported to range from 1.2% to 6.4%.[7,15] Most studies that describe the prevalence of sensitization to BAAs in bakers and nonoccupationally exposed individuals have been performed in Europe and Canada. To our knowledge, a systematic evaluation of the prevalence of sensitization to BAAs has not been reported for the general US population, and data on the prevalence of baker's asthma in US bakers is unknown.

Other investigators have used serum samples from volunteer blood donors to represent the general population of healthy adults in a region for specific IgE seroprevalence studies.[16] Similarly, in the present investigation, we evaluated volunteer blood donor serum samples for antiwheat, anti-flour dust, and anti–α-amylase specific IgE antibodies using commercially available diagnostic tests to estimate the background prevalence of sensitization to BAAs. Of all the serum samples, 80% were estimated to be obtained from individuals from Indiana and Ohio.

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METHODS

Serum Samples

Five hundred thirty-four samples were randomly collected from the residual serum tubes of volunteer blood donors (Indiana Blood Center, Indianapolis) during 1 week in 2001. The blood center processes samples from Ohio, Indiana, Kentucky, Maryland, the District of Columbia, and Illinois, but anonymity concerns precluded us from determining the demographics of the samples. Historical estimates of the source of the samples suggest that approximately 50% came from Indiana, approximately 30% came from Ohio, and approximately 20% came from Kentucky, Maryland, the District of Columbia, and Illinois combined. The samples were coded and stored frozen (−80° C) until used. Because of the anonymity of the samples, the study design of this project was determined to be exempt by the Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health Human Subjects Review Board.

Allergens and Serologic Analyses

Allergens (K87M, α-amylase [fungal]; K301M, flour dust; and F4M, wheat) were obtained from Diagnostic Products Corp (DPC) (Los Angeles, CA). The AlaSTAT assay was used for measuring anti-IgE antibodies according to the manufacturer's instructions using reagents purchased and run on a robotic microplate processing system (DPC). The AlaSTAT microplate assay for antiwheat specific IgE is US Food and Drug Administration cleared (section [510] of the Federal Food, Drug, and Cosmetic Act) as an in vitro diagnostic laboratory test. The AlaSTAT assays for flour dust and α-amylase specific IgE are supplied by DPC as Food and Drug Administration in vitro diagnostic analyte–specific reagents.[17] Briefly, the AlaSTAT assay is a liquid-phase immunoenzymometric assay in a microplate format. Allergens coupled to a soluble biotin-polymer/copolymer matrix were added to 50 μL of test serum in biotin-coated wells of the microtiter plate. After shaking for 1 hour at room temperature, avidin was added and incubated with shaking for another hour at room temperature. Plate-bound biotin-avidin-biotin-copolymer/antigen-antiallergen IgE was separated from the soluble components by washing, and horseradish peroxidase–labeled murine monoclonal anti-human IgE was added. Following a final incubation with shaking for 1 hour and buffer washes, chromogen (3,3′,5,5′-tetramethylbenzidine) in buffered H_2O_2 was added. The resulting color development was measured kinetically using the automatic microplate reader. Mean values from duplicate results, standard curve analyses, and interpolation of final values were calculated using WinMax software (DPC). Samples with BAA IgE concentrations of 0.35 kU/L or greater were defined as positive. A negative serum control (human serum with no detectable allergen specific IgE [catalog no. NGCM, DPC]) and an internal positive quality control (QC) serum sample, recommended by the manufacturer, were run in all assays. The positive QC serum sample is positive to dust mites (*Dermatophagoides farinae*) (catalog no. DC2M, DPC) and serves as an internal check of the performance of the AlaSTAT assays. Both negative and positive QC serum samples were run each day in triplicate duplicates. IgE calibrators (0, 0.35, 0.70. 3.5, 17.5, 52.5, and 100 kU/L IgE) (DPC) were run each day in duplicate.

Statistics

Simple linear regressions (SigmaPlot, SPSS, Chicago, IL) and Spearman correlations were used to evaluate the association between serum samples positive for more than 1 allergen. The Kolmogorov-Smirnov test (SigmaStat, SPSS) was used to evaluate the goodness of fit of positive antiallergen IgE values to a normal distribution. A Kruskal-Wallis analysis of variance (SigmaStat) was used to investigate any difference in positive control (*D farinae*) anti-IgE concentrations when investigated by each specific allergen. A type 1 error value of $P < .05$ was considered statistically significant.

RESULTS

Reproducibility and QC

Five hundred thirty, 534, and 529 serum samples were measured for antiwheat, anti-flour dust, and anti–α-amylase specific IgE levels, respectively. A total of 38 positive and negative control samples were run in this experiment. All negative control standards yielded anti-IgE concentrations of less than 0.35 kU/L. The interassay coefficient of variation (CV) was 11.0%, with a mean value of 2.43 kU/L (N = 38) for the *D farinae* QC serum samples. The intra-assay CV was 9.4% for all 1,593 duplicates. Individual duplicate results were positive/negative concordant for all antiwheat and anti–α-amylase specific IgE samples. One anti-flour dust sample yielded a discordant duplicate of less than 0.35

Table 1. Prevalence, Concentration, and Ranges of Positive Antiwheat, Anti-Flour Dust, and Anti–α-Amylase Specific IgE in Serum Samples from Volunteer Blood Donors

Allergen	Serum samples, No.		Prevalence, % (95% CI)	Antiallergen specific IgG, kU/L	
	Tested	Positive*		Mean ± SD	Range
Wheat	530	19	3.6 (3.3–3.9)	1.29 ± 0.78	0.38–3.61
Flour dust	534	31	5.8 (5.3–6.3)	0.84 ± 0.59	0.35–2.34
α-Amylase	529	5	1.0 (0.9–1.1)	0.75 ± 0.49	0.38 to 1.59

Abbreviation: CI, confidence interval.

* Concentration of 0.35 kU/L or greater.

and 0.36 kU/L, which the software categorized as positive (0.35 kU/L). There was no significant difference in mean ± SD positive control anti–*D farinae* specific IgE concentrations based on allergen (wheat, 2.38 ± 0.33 kU/L; flour dust, 2.44 ± 0.22 kU/L; and α-amylase, 2.49 ± 0.24 kU/L; $P = .34$).

Specific Bakery Allergen Anti-IgE Antibody Levels

Nineteen of 530 serum samples (3.6%; 95% confidence interval [CI], 3.3%–3.9%) were positive (≥0.35 kU/L of anti-allergen specific IgE) for wheat, 31 of 534 serum samples (5.8%; 95% CI, 5.3%–6.3%) were positive for flour dust, and 5 of 529 serum samples (1.0%; 95% CI, 0.9%–1.1%) were positive for α-amylase. The mean ± SD positive antiwheat specific IgE concentration was 1.29 ± 0.78 kU/L; range, 0.38–3.61 kU/L; n = 19). The 31 flour dust–positive serum samples had a mean ± SD anti-flour dust specific IgE value of 0.84 ± 0.59 kU/L (range, 0.35–2.34 kU/L). The 5 serum samples positive for anti–α-amylase had a mean ± SD anti–α-amylase specific IgE value of 0.75 ± 0.49 kU/L (range, 0.38–1.59 kU/L; Table 1). The frequency distributions for IgE-positive serum samples for the 3 allergens are shown in Figure 1. Results of normality tests indicated that positive IgE-specific antiwheat and anti–α-amylase concentrations were normally distributed ($P > .2$ and $P = .11$, respectively), whereas results of positive anti-flour dust specific IgE were not normally distributed ($P < .001$). Thirteen samples were doubly positive for both wheat and flour dust, and 1 serum sample each was doubly positive for flour dust and α-amylase and wheat and α-amylase. The 13 serum samples that were positive for both wheat and flour dust had anti-IgE concentrations that were highly correlated ($r = 0.704$; $P = .007$; Fig 2).

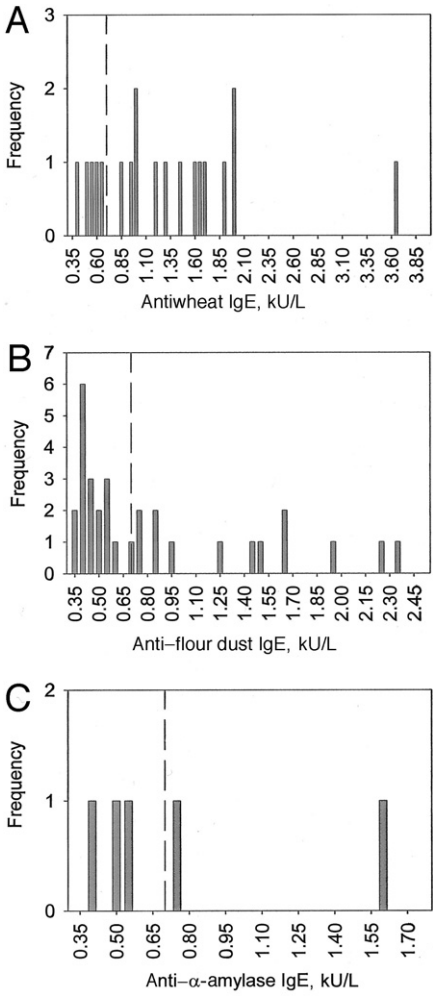

Figure 1. Frequency distribution of AlaSTAT-positive (≥0.35 kU/L) antiwheat (A), anti-flour dust (B), and anti-α amylase (C) specific IgE in serum samples from volunteer blood donors. The dashed vertical lines are at 0.70 kU/L, an anti-IgE concentration that the kit's manufacturer classifies as strongly positive for an individual allergen (see the "Discussion" section for details). Data points represent the mean of duplicate determinations.

DISCUSSION

Allergic sensitization to flour dust, wheat, or α-amylase in bakery workers has been studied in numerous cross-sectional evaluations outside the United States using either skin testing or in vitro testing for specific IgE antibody levels.[3,4,7,13,15,18–25] The prevalence of sensitization (IgE) to wheat flour in bakers has been reported to range from 5% to 53%.[1,13,14] This wide range in reported wheat flour specific IgE prevalence is thought to be due to the diagnostic sensitivity of methods used to measure specific IgE, with the larger prevalences being observed with contemporary commercial specific IgE test systems,[14] such as the AlaSTAT system used in the present study. Wheat flour dust is a complex mixture, containing not only numerous wheat flour antigens but also amylases, agglutinins, and gliadins,[6] as well as additives and other allergens from other sources, such as storage mites and cockroaches.[26] The prevalence of sensitization to α-amylase has been reported to occur in 2% to 15% of bakery workers.[10,27] The prevalence of sensitization to BAAs in US nonoccupationally exposed populations (except for the present work) has not been studied. The high correlation we observed in the present investigation between individuals with positive specific wheat and flour dust IgE antibody levels ($r = 0.704$; $P = .007$; Fig 2) suggests that nonoccupationally exposed individuals may have exposure to numerous BAAs or allergens that cross-react with BAAs. Estimates from the results of skin tests with wheat extracts in laboratory animal workers[7,10] suggest that 6.4% are sensitized. Estimates from apprentices in dental hygiene and animal health training programs suggest a prevalence of sensitization to wheat of 1.2% to 4.1%.[15] Background sensitization to BAAs in these nonoccupationally exposed cohorts is thought to be due to exposure to allergens either at home or through food[7] or cross-reactivity to other allergens, such as pollens.[15] In the present work, we showed a prevalence of specific IgE to wheat of 3.6% and to flour dust of 5.8%. The prevalence of specific IgE to α-amylase was 1.0%. Fungal α-amylase is known to

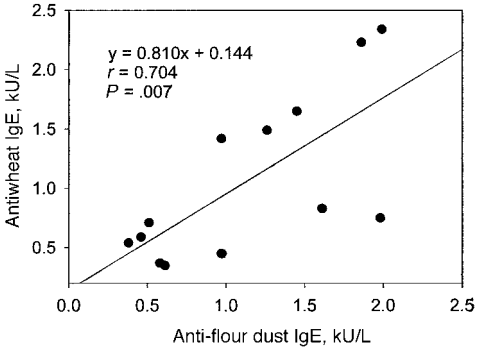

Figure 2. Linear regression of AlaSTAT-positive (≥0.35 kU/L) antiwheat and anti-flour dust specific IgE concentrations in serum samples from volunteer blood donors.

be present in flour dust.[28] The in vitro prevalences we report herein agreed well with the estimated prevalence in nonoccupationally exposed workers from other countries. Positive skin test results to flour dust and α-amylase simultaneously have been described in 9 of 21 bakery workers.[22] In the present work, we only observed 1 positive serum sample each for flour dust and α-amylase and wheat and α-amylase. These data strongly suggest that sensitizing exposures to α-amylase occur mainly in the bakery environment. The age, race, sex, and occupations of the donors in the present study were unavailable. It is unlikely, but not impossible, that the results we observed were due to occupational exposures in the bakery industry. Additionally, because the imputed geographic distribution of serum sample donors suggests that approximately 80% may have been from Ohio and Indiana, the observed results may represent regional bias and may not be representative of US prevalence.

These data must be evaluated with the knowledge that clinical tests may not perform optimally when used in low-prevalence populations for a condition such as IgE sensitization to BAAs in nonoccupationally exposed individuals. Clinical tests are optimized for use in evaluating patient populations where there is usually a high pretest probability for the condition of interest. Assuming constant sensitivity and specificity, the higher the true prevalence of a condition, the more accurately a test will identify the prevalence of that condition in a population. If a condition is present at low prevalence, a larger proportion of positive test results will be false positives, resulting in poor positive predictive value of the test and overestimation of prevalence.[29–31] The reproducibility of the AlaSTAT system has been investigated by repeatedly analyzing serum samples with low anti–latex IgE antibody concentrations (<0.40 kU/L) and evaluating the concordance of repeated results regarding positive and negative dichotomization. These investigators found that approximately 35% of repeatedly tested serum samples were discordant. At anti-IgE concentrations greater than 0.40 kU/L, concordances became much better.[32] In the present work, positive/negative concordance between sample duplicates was excellent (only 1 of 1,593 duplicates was discordant). The intra-assay and interassay CVs of 9.4% and 11.3%, respectively, found in the present work are within the less than 10% and less than 20% intra-assay and interassay guidelines for this type of assay and suggest extremely good precision.[33] In addition, most anti-IgE concentrations measured were greater than 0.40 kU/L (Fig 1), strongly suggesting that the results are true positives. If only positive data of 0.70 kU/L IgE or greater (a concentration interpreted as "strongly positive" by the manufacturer) are evaluated (Fig 1), the positive prevalences become 2.6% (95% CI, 2.4%–2.8%) for both antiwheat and anti-flour dust and 0.4% (95% CI, 0.37%–0.43%) for α-amylase, still generally in the range reported for nonoccupationally exposed individuals in other countries.[7,10,15]

It is unclear why there has been no reported study, to our knowledge, of BAA hypersensitivity in US bakers. Possibly, US bakers are not at an increased risk of BAA hypersensitivity owing to differences between the US and non-US (European and Canadian) bakery industries (large, centralized industrial bakeries vs small craft bakeries), processes, flour components, or types of exposures. Alternatively, baker's asthma may be underreported in the United States.

In conclusion, these data provide a baseline estimate of BAA sensitization in an unselected, nonoccupationally exposed regional US population. It is useful to have a baseline estimate of the prevalence of BAA sensitization in nonoccupationally exposed individuals to compare against prevalence estimates in US bakers. With this information, an estimate of the increased risk of BAA sensitization associated with being a US baker can be studied.

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