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Prevalence of IgE against neuromuscular blocking agents in hairdressers and bakers

Running head: quaternary ammonium IgE in hairdressers

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Abstract

Background: Allergic IgE-mediated reactions to neuromuscular blocking agents (NMBAs) are the main cause of immediate hypersensitivity reactions in anaesthesia; their predominant occurrence in absence of previous exposure to NMBAs suggests a risk related to environmental exposure. **Objective:** to investigate the prevalence of specific IgE to quaternary ammonium ions in two populations professionally exposed to quaternary ammonium compounds, in the North-Eastern France. **Methods:** the study had a retrospective follow-up design whereby apprentices were assessed after their 2-year training period as apprentices. The populations professionally exposed, hairdressers (n=128) was compared with baker/pastry makers (n=108) and "non-exposed" matched control subjects (n=379). **Results:** we observed a 4.6-fold higher frequency of positive IgE against quaternary ammonium ions in hairdressers (HD), compared to baker/pastry makers (BP) and control (C) groups. The competitive inhibition of quaternary ammonium Sepharose-radioimmunoassay (QAS-IgE RIA) with succinyl-choline was significantly higher in HD, compared with BP and C groups, with inhibition percentage of 66.2 ± 7.4 , 39.7 ± 6.0 and 43.8 ± 9.9 , respectively ($P < 0.001$). The specific IgE against quaternary ammonium ions recognized also two compounds widely used by hairdressers, Benzalkonium chloride and Polyquaternium-10, in competitive inhibition of IgE-RIA. When considering the whole study population, hairdresser professional exposure hairdresser and total IgE > 100 kU/L were the two significant predictors of IgE-sensitization against quaternary ammonium ions in the multivariate analysis of a model that included age, sex, professional exposure, increased concentration of total IgE (IgE > 100 kU/L) and positive IgE against prevalent allergens (Phadiatop[®]) ($P=0.019$ and $P=0.001$, respectively). **Conclusion and clinical relevance:** the exposure to hair dressing professional occupational factors increases IgE-sensitization to NMBAs and quaternary ammonium ions compounds used in hairdressing. Beside the pholcodine hypothesis, our

study suggests that repetitive exposure to quaternary ammonium compounds used in hairdressing is a risk factor for NMBAs sensitization.

Key Words: allergy, anesthesia, neuromuscular blocking agents, IgE, cosmetics, Rh quaternary ammonium ion complex, hairdresser, baker/pastry make

Introduction

Allergic IgE-mediated reactions to neuromuscular blocking agents (NMBAs) are the main cause of immediate hypersensitivity reactions in anaesthesia; in most cases they occur in absence of previous exposure to the incriminated drug [1, 2]. Their incidence seems to be higher than previously estimated [1]. Up to 75% of reactions have been reported at the first known contact with a NMBA [3]. This suggests a previous environmental exposure to apparently unrelated chemical compounds, such as those present in cosmetics [3].

Almost all allergic reactions to NMBAs are IgE-mediated [4]. Structure-activity studies have established that quaternary and tertiary ammonium ions were the complementary allergenic sites on the reactive drugs [5]. Therefore, any attempt to prevent these reactions must address the question of the antigenic source that stimulates the production of IgE antibodies against NMBAs [6]. Neighbouring groups of different structures on the different NMBAs might, in addition to the substituted ammonium groups, form part of the allergenic determinant structure recognised by IgE antibodies [2].

The prevention of anaphylaxis to anaesthetic drugs requires determining the groups of patients who are at high risk of adverse reaction, in function of their exposure to sensitizing agents in their pre-operative history [6]. Exposure to pholcodine, a compound used for cough treatment, could lead to IgE-sensitization to quaternary ammonium ions and thereby increase the risk of allergic reaction to NMBAs, at least in Norway [7]. However, epidemiologic surveys also indicated that other type of exposure could be involved. Frequent exposure to

chemicals substances and cosmetics containing tertiary or quaternary ammonium groups [5, 8], exposure to phosphorylcholine or Rh-ammonium ion complex present in house dust mites or fungi [3] have been proposed as possible sensitizing antigens. This led us to investigate the prevalence of specific IgE to quaternary ammonium ions in two professionally exposed populations, hairdressers and baker/pastry makers, compared with control subjects [9].

The aim of this study was evaluate the prevalence of specific IgE antibodies to quaternary ammonium ions in hairdresser apprentices highly exposed to potential sensitizing compounds, compared to baker/pastry maker apprentices and to non-exposed matched control apprentices, during their 2-year training period.

Methods

Subjects and study design

This study has a retrospective follow-up design whereby 128 baker/pastry maker apprentices, 108 hairdresser apprentices and 379 "non-exposed" apprentices were assessed after their 2-year training period, in the Lorraine region, North-Eastern France. Its study design has been described elsewhere [10]. The exposed population was composed of hairdressers (HD group) who graduated very recently from nine vocational schools. It was compared to young bakers and pastry makers (BP group) and a "non-exposed" control group (C group) of all subjects who graduated in the sale or the food sectors (butcher, pork butcher, caterer, cook job,...) from the same vocational schools. Subjects were included provided they had no previous anaesthesia, no allergic manifestation, nor physician-diagnosed asthma. All those complying with these criteria are contacted by phone, with a short screening questionnaire to ascertain inclusion. None of them had pholcodine intake during the 2-years training period. Subsequently, they were subjected to a medical examination and an evaluation of occupational exposure, as described previously (10, 11,12). The training program typically

encompassed a period of 3 weeks of practical work, followed by one week at school for practical and academic classes. Every apprentice worked in the same salon until he/she graduates. Twice a year, all apprentices were asked to complete, in the classroom, a questionnaire describing their work environment and activities, including use of professional products. The research program was authorized by the Nancy University Hospital ethics committee (CPP n°08.07.01) and written consents are obtained from the young workers themselves.

Serum samples stored at -20°C were used for this study. The serum level of total IgE was measured by fluoroenzyme immunosorbent assay (UniCAP™; Phadia Diagnostics, Uppsala, Sweden). Specific IgE against prevalent allergens were assayed by Phadiatop® (Phadia Diagnostics, Uppsala, Sweden). Concentrations of specific IgE against quaternary ammonium compounds and muscle relaxant drugs were measured in the subjects and controls, with the quaternary ammonium Sepharose-radioimmunoassay (QAS) test. This assay uses a choline analog and radio-labeled anti-IgE. Results were expressed as percentage binding of the anti-IgE tracer to the solid phase, with a 2.3% positivity cutoff. The specificity of IgE binding was assessed by inhibition test, in which serum was mixed in equal volumes with succinyl-choline solution in buffer, with a final concentration of succinyl-choline at 120 nmol/L, as described previously [11, 12]. After gentle shaking for 2h at room temperature, QAS-IgE was assayed as described in the initial step. The inhibition percentage was calculated as $(\text{response of serum with buffer} - \text{response of serum with inhibitor}) / \text{response of serum with buffer} \times 100\%$. Percentage inhibition exceeding 20% was considered positive, as previously reported [11, 12].

Statistical analysis

The statistical analysis was designed to test the hypothesis of an association between the professional exposure and the risk of sensitization to quaternary ammoniums. The minimal size of our sample was estimated at 100 subjects per group, with a study power $1 - \beta = 0.8$ and $\alpha = 0.05$, assuming a 2.0-fold difference in the frequency of positive IgE against quaternary ammoniums among groups. Continuous variables were reported as means $\pm$ SD, median, and compared by the Mann-Whitney U-test and Kruskal Wallis test. Univariate correlation was tested by Rho-Spearman analysis and multivariate correlation by multiple regression analysis. The frequencies of categorical parameters were expressed as percentages and 95% exact confidence intervals. A chi-square test was used to assess percentage differences. Evaluation of the predictors of sensitization to quaternary ammoniums was performed by multivariate logistic regression analysis, using a model that included age, gender and the categorical variables significantly associated with the risk of sensitization to quaternary ammonium in univariate analysis, with *P*-value lower than 0.10. We considered a final *P*-value lower than 0.05 as indicating residual statistical significance. Data were collected and analyzed using the Stata 12.0 software (College Station, Texas 77845 USA).

Results

The apprentices were recruited during the same period and in the same area, in the North-East of France. We reported no significant difference of age between the three study groups. Control apprentices were apparently healthy subjects with a same sex distribution than the general population of this class of age (20-30 years old). The main characteristics of the 3 groups are presented in Table 1. The HD group was composed with females, excepted two males, and conversely, the BP group was largely composed with males, in agreement with the

sex distribution in these two professions, at least in France. When considering the HD and BP groups together, the exposed apprentices had a sex distribution, which was not different from that of controls ($P=0.633$). We reported cutaneous lesions (skin irritation and eczema) in 51 HD (47.2%, 95% confidence interval: 37.7-56.7) and in 38 BP (29.7%, 95% confidence interval: 21.7-37.7), respectively ($P=0.006$). The serum concentration of total IgE was similar in the three groups (Table 1). As expected, the detection rate of IgE against prevalent allergens (assessed by Phadiatop[®]) was significantly higher in the highly exposed BP group, compared to HD and C groups (Table 1). Conversely, we observed a 4.6-fold higher frequency of positive IgE against anti-quaternary ammonium in HD, compared to BP and C groups (Table 1). The competitive inhibition in presence of succinyl-choline was significantly higher in HD group, compared with BP and control group ($P<0.001$), with inhibition percentage of 66.2 ± 7.4 , 39.7 ± 6.0 and 43.8 ± 9.9 , respectively (Table 2). The level of specific IgE was also significantly higher in HD cases, compared to BP and control cases, when considering the cases from the three groups with positive RIA of IgE against quaternary ammonium. This result was in agreement with the hypothesis of a higher environmental exposure to quaternary ammonium compounds. We have listed in Table 3 the quaternary compounds, which were used in daily practice by HD apprentices, according to the results of the questionnaires. These quaternary ammonium compounds are commonly used by hairdressers in France. We performed competitive inhibition tests of QAS-IgE in presence of two of these compounds, Benzalkonium and Polyquaternium-10. Both compounds produced a significant inhibition of IgE binding to the QAS solid phase. In addition, the inhibition percentage of Benzalkonium was in the same order of magnitude than that reported with Succinylcholine, in HD, BP and control groups (Table 2).

The dramatic increase of the frequency of positive IgE against quaternary ammoniums in HD group was not related to the large predominance of females in this group. Indeed, the

frequency remained significantly higher when comparing the females of the HD group to those of the control group 7.6% (n=8) vs. 2.0% (n=4), $P=0.018$. In addition, there was no significant difference in the frequency of positive IgE against quaternary ammonium according to sex in the control group ($P=0.468$).

A significant correlation was found between serum levels of IgE against quaternary ammonium compounds and serum total IgE, in the whole study population ($Rho=0.21$, $P<0.001$) and in the control ($Rho=0.17$, $P<0.001$), BP (0.23 , $P=0.008$) and HD groups ($Rho=0.32$, $P<0.001$). No significant association was reported between the levels of IgE against quaternary ammonium and serum IgE against prevalent allergens, in control and HD groups, while a weak association was observed in BP group ($P=0.025$).

We considered further the risk to have positive IgE against quaternary ammonium compounds in the whole study population, in a multivariate model that included age, sex, professional exposure, increased concentration of total IgE ($IgE > 100$ kU/L) and positive IgE against prevalent allergens (assessed by Phadiatop®). In this model, professional exposure of hairdresser and total IgE > 100 kU/L were the two significant predictors in univariate analysis ($P=0.004$ and $P=0.001$, respectively) and in multivariate analysis ($P=0.019$ and $P=0.001$, respectively) (Table 4).

Discussion

The main finding of this study was the higher frequency of positive IgE against quaternary ammonium ions in hairdresser apprentices, compared to other apprentices, in the context of a 2-years high rate of professional exposure to cosmetics and absence of reported exposure to Pholcodine and anaesthetics. In contrast, professional exposure to inhalant allergens in bakers and pastries apprentices was not a risk factor for the presence of IgE against quaternary

ammonium compounds. Our data provide the first evidence in favour of a cross-sensitisation to NMBAs by repeated exposure to hairdresser professional chemical products. The increased risk of IgE-mediated sensitization against quaternary ammonium ions in hairdresser apprentices is consistent with the exposure of this population to quaternary and tertiary ammonium ion compounds widely used in cosmetics [7]. Hairdressers are also considered at high risk of contact dermatitis, rhinitis or asthma, which may increase the sensitization rate by repeated skin and air exposures to ammonium containing chemicals [13-17]. Consistently, we observed a higher rate of cutaneous lesions in HD group, compared to the other apprentices.

Although the levels of IgE against quaternary ammonium ions were significantly correlated with the levels of total IgE, in the 3 groups, no significant association was observed between IgE against quaternary ammonium ions and IgE against prevalent allergens, at least in the HD and control groups. These results are in agreement with previous reports on the epidemiology of allergic reactions to NMBAs [1]. The association between specific IgE-antibodies and total IgE has been previously reported in case control studies of patients with immediate-type allergic reactions against quaternary/tertiary ammonium ions [13, 19, 20] and against other drugs, such as betalactams and non-steroidal anti-inflammatory drugs [21, 22]. Consistently, gene determinants related to IgE production, IgE-class switching and atopy account for major genetic predictors of immediate-type allergic reactions to betalactams [21, 23, 24]. This suggests evaluating whether these determinants are also predictors of immediate-type allergy to NMBAs. We observed a weak association between levels of IgE against quaternary ammonium ions and IgE against prevalent allergens, in BP apprentices. Several possible sources of antigens reacting with quaternary/tertiary ammoniums have been suggested. Allergenic substances containing the tertiary ammonium compound

phosphorylcholine isolated from house dust mites, helminths, nematodes and fungi [18] are possible sources of stimulating antigens in subjects allergic to NMBAs [3]. However, the exposure with inhalant occupational allergens seemed to have little influence on the sensitization against quaternary ammoniums, at least in BP group, where the frequency of detectable IgE against quaternary ammoniums was similar to that reported in the C group.

It has been hypothesized that the striking difference in the rate of allergic reactions to neuromuscular blocking agents, which is more than six times as common in Norway as in Sweden, could be due to differences in preoperative exposure to the cough suppressant pholcodine [7]. Consistently, a higher prevalence of IgE antibodies to quaternary and/or tertiary ammonium ion among blood donors was reported in Norway, compared to Sweden. This study pointed out that amongst the common quaternary ammonium ion-containing household chemicals and drugs present in the environment of both populations, the only difference was the presence of pholcodine in cough mixtures in Norway but not in Sweden. This led to the hypothesis that pholcodine exposure increases the prevalence of sensitization to other quaternary ammonium ions, including NMBAs. This hypothesis was further supported by the results of an international prevalence study showing a significant association between pholcodine consumption and prevalence of IgE-sensitization to pholcodine and succinylcholine in several countries from Europe and in the USA. Withdrawal of pholcodine from the Norwegian market resulted in the decrease of IgE against quaternary ammonium ions in the population and in the number of reports of allergic reactions to NMBAs. However, epidemiologic surveys also indicated that other substances may be involved in IgE-sensitization towards NMBAs and remained to be identified [4, 7, 25-27]. Our results are in agreement with these observations and confirm that cosmetics should be other environmental factors involved in the cross-sensitization to quaternary ammonium ions and NMBAs.

Quaternary ammonium compounds are present in widely used cosmetics products. In this context, hairdressers are particularly exposed to these compounds in their professional practice. These compounds cross-react with NMBAs used in anaesthesiology, as illustrated by the results of inhibition tests of QAS-IgE RIA performed with Succinylcholine, Benzalkonium and Polyquaternium-10 in sera of cases with positive QAS-IgE RIA from hairdressers. These results highlight the need to evaluate the professional exposure against quaternary ammonium ions as a risk factor of allergic reactions against NMBAs.

Our report suffered a limitation inherent to studies of the relation between environmental factors and sensitisation against NMBAs. We could not evaluate the rate of sensitization specifically produced exclusively during the professional activity since we could not determine serum IgE against quaternary ammoniums at the beginning of the training period. In addition, the presence of serum IgE against quaternary/tertiary ammonium ions does not imply that a clinical hypersensitivity reaction will occur when administering an NMBA [25]. We were not allowed to perform skin tests and/or challenge tests to evaluate the risk of reactivity against NMBAs, in the subjects with positive QAS-IgE. However, it was probable that this risk was increased, according to the predictive value of the QAS-IgE assay previously reported in patients who underwent an anaphylactic reaction against NMBAs during anesthesia (evidenced by increased tryptase and positive skin testing) and in control subjects with no reaction to NMBAs during an uneventful anesthesia [20]. In these cases and controls, the positive predictive value of the QAS assay (i.e., the ability to predict positive skin tests) was estimated to 82.1% and the negative predictive value to 81.0 %. In addition, the frequency of positive QAS-IgE was 2.5-fold higher in skin test-negative reactors with increased tryptase, compared to controls [20].

In conclusion our study represents the first demonstration for a link between exposure to cosmetics used in hairdressing and cross-sensitization to quaternary ammonium ions. Beside the pholcodine hypothesis, it illustrates the importance of professional occupational factors in the genesis of sensitization to quaternary ammonium ions.

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Authors'contributions

SWD: performed biological analyses, statistical analyses and wrote paper; SAD: analyzed data, revised paper; RMGR and DZN: designed research, analyzed data, revised paper; PT: performed subject testing; TR: provided essential materials; *PMM: designed research, conducted research, analyzed data, wrote paper and supervised the whole project; *JLG: designed research, conducted research, supervised analyses, analyzed data, wrote paper, supervised the whole project and had primary responsibility for final content

*These authors have equal contribution to this manuscript

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Conflict of interest

No financial or other potential conflict of interest exists for any of the authors

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Table 1. Characteristics of the study population. Data are given as mean $\pm$ SD, number with frequency and 95% confidence interval (C.I.) in parenthesis or median with range in parenthesis.

	HD Group (n=108)	BP Group (n=128)	C Group (n=379)	<i>P</i>
Age (yr)	25.49 $\pm$ 2.63	24.71 $\pm$ 2.07	24.98 $\pm$ 3.52	0.476 *
				0.338 †
Female sex	105 (97.2%, 94.1-100.3)	13 (10.2%, 4.9-15.4)	197 (52.0%, 46.9-57.0)	0.000 *
				0.000

				†
Presence of specific IgE by QAS-RIA	8 (7.4%, 2.4-12.4)	2 (1.6%, 0.6-3.7)	6 (1.6%, 0.3-2.8)	0.001 *
				0.987 †
Total IgE (kU/L)	43 (2-1436)	52 (2-5000)	42 (2-5000)	0.709 *
				0.117 †
Phadiatop (kU/L)	0.19 (0.10-100.00)	0.25 (0.10-100.00)	0.15 (0.10-100.00)	0.450 *
				0.048 †

*Analysis between HD and C Group, †Analysis between BP and C Group, anti-IgE binding to solid phase of QAS-RIA (quaternary ammonium Sepharose radioimmunoassay) $\geq 2.3\%$ was considered positive.

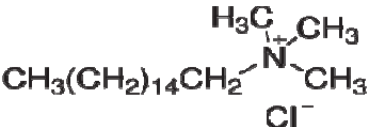
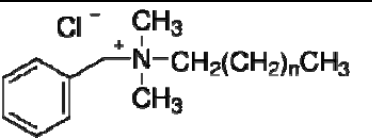
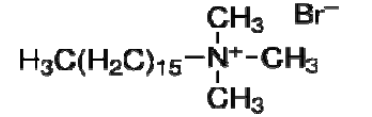
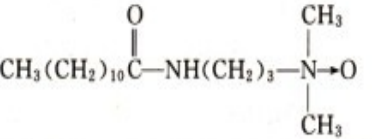
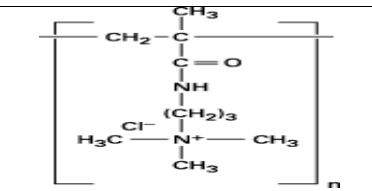
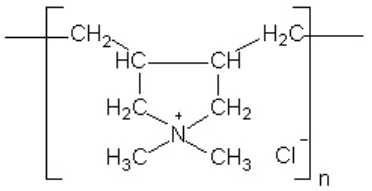
Table 2. Inhibition tests in sera of cases with positive QAS-IgE RIA from young bakers and pastry makers (BP group), hairdressers (HD group) and controls (C group) in presence of succinylcholine and two quaternary ammonium compounds commonly used in hairdressing, Benzalkonium and Polyquaternium-10.

Inhibition Tests (%)*	BP group (n=2)	HD group (n=7)	C group (n=6)	P†
Succinylcholine	39.6±0.6	68.7±10.1	43.8±10.9	0.005
Benzalkonium	62.5±3.5	53.2±9.9	47.3±7.2	0.075
Polyquaternium-10	37.5±0.7	32.7±9.1	45.8±11.3	0.213

*Each serum was tested in duplicate in presence of 120 nmol/L of the soluble compound as described previously [11]. Data are mean±SD

†P-value of Kruskal–Wallis test

Table 3: Names and formula of the quaternary ammonium compounds, which were commonly used by hairdresser apprentices

Common name	Chemical formula	Hairdressing use	Structure formula
Aliquat 6 Adogen 444	Cetyl trimethyl ammonium chloride	Shampoo Hair conditioner	
Benzalkonium chloride	N-Alkyl-N-benzyl-N,N-dimethylammonium chloride	Shampoo	
Cetrimonium bromide	Cetyl trimethyl ammonium bromide	Shampoo Hair dye and conditioner	
Lauramidopropyl dimethylamine	N- [3- (dimethylamino) propyl] dodecanamide;	Shampoo	
Polycare 133	Polymethacrylamidopropyl trimonium chloride	Hair Conditioner	
Polyquaternium-6	N,N-dimethyl-N-2-propenyl-2-propen-1-aminium chloride	Hair Conditioner	

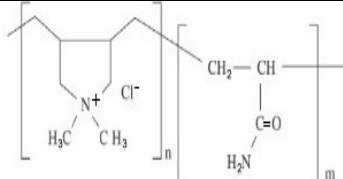
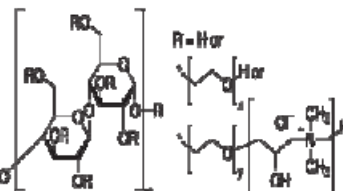
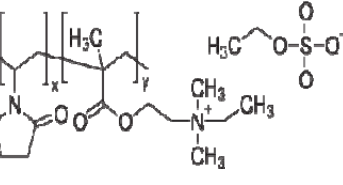
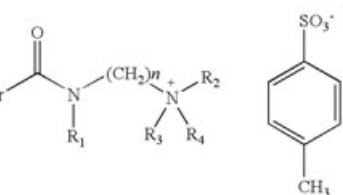
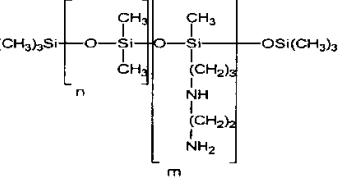
Polyquaternium-7	2-Propen-1-aminium,N,N-dimethyl-N-2-propenyl-,chloride	Shampoo Hair conditioner	
Polyquaternium-10	Hydroxyethylcellulose ethoxylate	Shampoo Hair conditioner	
Polyquaternium-11	Poly[(2-ethyl dimethyl ammonioethyl methacrylate ethyl sulfate)-co-(1-vinylpyrrolidone)]	Shampoo Hair conditioner	
Tosyquat	p-methoxy cinnamidopropyl dimethyl lauryl ammonium tosylate	Hair dye	
Trimethylsilyla modimethicone	methyl 3-(2-aminoethylamino)-2-methylpropyl	Shampoo Hair conditioner	

Table 4. Univariate and multivariate logistic regression of the determinants of the presence of anti-quaternary ammonium specific IgE detected by QAS-RIA (quaternary ammonium Sepharose radioimmunoassay).*

	Univariate analyses		Multivariate analysis†	
	Odds ratio (95% C.I.)	P-value	Odds ratio (95% C.I.)	P-value
Age	1.13 (0.95-1.33)	0.157	N/A	N/A
Sex	2.93 (0.93-9.19)	0.065	2.37 (0.44-12.70)	0.314
Increased Total IgE	7.10 (2.26-22.30)	0.001	7.47 (2.24-24.85)	0.001
Phadiatop positive	1.67 (0.61-4.53)	0.318	N/A	N/A
Hair dresser	4.97 (1.69-14.66)	0.004	4.32 (1.27-14.64)	0.019
Bakery and Pastry	0.99 (0.20-4.95)	0.987	N/A	N/A

* anti-IgE binding to solid phase of QAS-RIA (quaternary ammonium Sepharose radioimmunoassay) $\geq 2.3\%$ was considered positive

† multivariate logistic regression analysis was performed using a model that included the variables with P-value lower than 0.10 in univariate analysis.