

Serological prevalence of anti-latex IgE antibodies in unselected blood donors in Argentina

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Abstract. The prevalence of specific IgE to natural rubber latex proteins in the general population remains uncertain. The purpose of this study was to determine the prevalence of sera containing specific IgE antibodies to latex proteins using immunoenzymatic methods.

A population of 500 unselected adult voluntary blood donors was the source of the sera used in this study. Two different immunoenzymatic methods (EAST and CARLA) were used to analyze the presence of specific IgE antibodies. Confirmation assay was carried out by inhibition ELISA and immunoblotting. Sera from healthy non-atopic individuals were also used as control.

Two hundred and twenty five sera showed higher than normal total IgE levels. Of those, three presented latex specific IgE antibodies, which could be inhibited in a dose-response manner with the natural rubber latex and glove extracts. Several latex allergens were recognized by the IgE antibodies from these positive sera.

This low seroprevalence (0.66 %) indicates that latex hypersensitivity is not an important problem in the general population. We believe that prevention of latex exposure is only necessary in high risk groups of patients.

Key words: Latex allergy, IgE, ELISA, serology

Introduction

The information available in the literature on the prevalence of latex allergy (LA) in the general population is scarce. These data may be relevant since there exists permanent contact with rubber products. Under certain circumstances (surgical procedures, medical practice, intake of cross-reactive fruits and vegetables, etc.) prevention may be mandatory in subjects sensitized to latex and with no clinical reactivity [1,2]. The presence of latex specific IgE may indicate a pre-clinical sensitization. *In vitro* assays are appropriate for seroepidemiological studies, although they may not be reliable clinical screening tools for individuals taken at random. The aim of this study was to assess the proportion of the general population that might be sensitized to latex. We believed that the study population

that would best represent a general cohort of healthy adults in an area could be a sample of unselected adult voluntary blood donors.

Materials and methods

Sera from 500 blood donations from unselected adult Caucasians were obtained at La Plata Blood Institute. Patients completed a preliminary questionnaire. No information on their personal allergic history nor on potential risk factors for latex allergy was required. Most of them were unemployed and civil servants. Samples were tested for NRL (natural rubber latex)-specific IgE antibodies using two different methods: the Enzyme Allergo Sorbent Test (EAST) [3], and the Capture Assay

Radim Liquid Allergen (CARLA) [4]. The EAST assay offered maximal specificity (98.7%) with good sensitivity (82.9 %), positive predictive value (93.1 %) and negative predictive value (93.9 %).

Total water-extractable proteins were obtained from ammoniated NRL [3] or from non-powdered latex examination gloves [5] as previously described. Protein extracts were analyzed by SDS-PAGE and immunoblotting. Membranes were revealed using human sera diluted 1:5, biotinylated goat anti-human IgE (e-chain specific) antibody 1/2000 (Vector, California, USA) and ExtrAvidin-alkaline phosphatase conjugate 1/1000 (Sigma).

Total IgE levels of all sera were measured by a capture ELISA [3].

Inhibition assays for latex-specific IgE antibodies were performed by pre-incubating the samples of undiluted latex positive serum, each with different concentrations (1 mg/ml, 0.1 mg/ml and 1 ng/ml) of specific- (ammoniated NRL), related-(non-powdered latex glove) or non related-allergens (skimmed cow milk), for 2 hours at 37 °C, prior to the assay for latex-specific IgE. The inhibition score was provided by the comparison with the uninhibited specific IgE assay,

(41/500) of the sera showed values above 1000 IU/ml. Antilatex-specific IgE antibodies were detected in 3 sera by both methods (Table 1). As discussed by Yeang [6] a 0.66 % of prevalence may be an overestimated data since sensitivity and specificity of the EAST are 82.9 % and 98.73 % respectively.

The specificity of the latex IgE antibodies detected was confirmed by the EAST inhibition assay (Figure 1). The cow's milk protein control extract rendered no relevant inhibitions (around 10%), while significant inhibitions either with NRL or the glove extract were found with the three positive sera. As the concentration of the inhibitor increased higher values of inhibition were obtained. IgE antibodies may have higher relative affinity for glove-derived proteins than for NRL proteins, since higher values of inhibition were obtained using the glove extract as inhibitor. A possible explanation could be that primary sensitization in these patients occurred due to NRL-derived antigens present in manufactured products. Total inhibition IgE antibodies binding to the solid-phase antigen of glove proteins could not be reached. This could be explained because apparently homologous coated-antigens bind free IgE antibodies with higher avidity than liquid-phase

Table 1. Serological results from latex positive sera.

Serum number	EAST (class)	CARLA (class)	CARLA (IU/ml)	Total IgE level (IU/ml)*	Components recognized by Ib (kDa)
1	3	3	20.8	580	14, 15, 27-33, 69
2	3	2	4.6	431	14, 27-30
3	1	1	0.92	237	14,30

* Normal IgE value for adults: lower than 100 IU/ml.

• Ib: IgE immunoblotting

containing equal volume of phosphate-buffered saline as control. Residual IgE antibodies to NRL proteins were detected by incubating a volume of 100 ml of each of the above mentioned pre-incubated samples in NRL-coated (1 mg/well of ammoniated natural latex protein) wells (NUNC, Maxisorp, Denmark). A monoclonal anti-human IgE FAL conjugate (SIGMA) diluted in 1/6000 in blocking buffer, was added to detect IgE antibodies.

Results and Discussion

Two hundred and thirty six out of the five hundred (47.2%) sera had total IgE levels above normal according to age. Many individuals, 26.8% (134/500), showed total IgE levels ranging from 101 to 300 IU/ml, while 8.2 %

antigens. Besides, the inhibition capacity of the glove extracts may be different compared to NRL due to neo-antigens created during processing of the ammoniated latex. Due to differences in the allergen composition between NRL and the glove extract (Figure 2), a restricted population of specific antibodies could not be bound by proteins present in the glove extract employed as inhibitor. These antibodies may result from a primary sensitization to food or pollen allergens [7].

The main allergens of NRL were also detected in the glove extract. Components localized in the 14 kDa-(Hev b1 as judge by its MW), and 30 kDa-regions (hevamine) were bound by the IgE antibodies from the three positive sera (Figure 2).

Limited data are available on the prevalence of latex allergy in the general population. Several laboratories in the United States and Europe used *in vitro* tests to

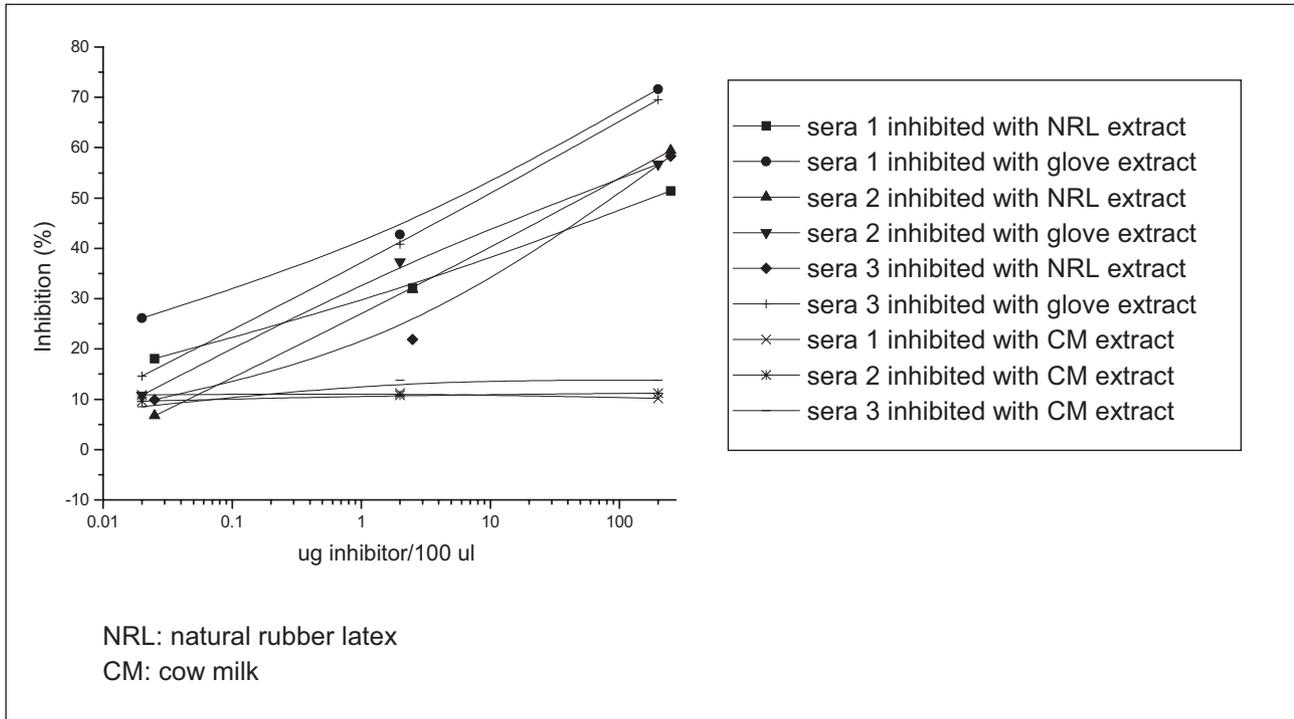


Figure 1. EAST-inhibition results of the three latex positive sera using latex, glove or cow milk extracts as inhibitors.

detect specific IgE antibodies. None of these papers provided information on the occupation of the patients. Prevalence data between 4 and 7.6 % were reported [1,7,8]. On the other hand, when the skin prick test and the questionnaire were included in the study, a lower prevalence was found [9,10,11].

Most of the subjects who tested positive for IgE to NRL are atopic individuals, however most of them are asymptomatic [9,12]. This likely reflected that these individuals are not primarily sensitized to NRL and this fact may explain the higher values of prevalence found by other authors. Therefore, *in vitro* and *in vivo* tests may detect IgE cross-reacting antibodies with no clinical relevance, and this may result in false positive test results.

Although it is known that the most important factor for LA is the frequency of exposure to latex products [13], only in well-defined population groups repeated latex exposure appears to be an important factor for latex sensitization. The question would be if it is necessary to implement LA prevention strategies in the groups not at risk since awareness for the development of LA may involve difficult and expensive lifestyle changes. The low prevalence ascertained in the general population in this study (0.66 %) might indicate that the sporadic exposure to manufactured products is not sufficient for latex sensitization.

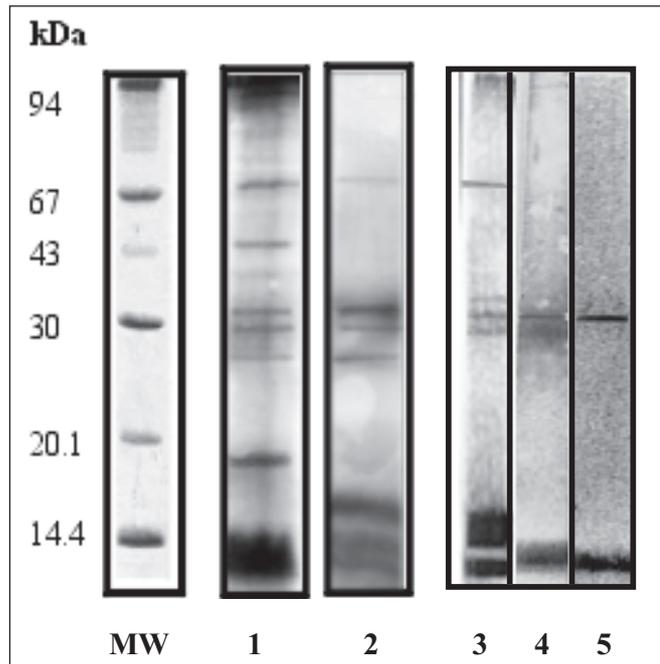


Figure 2. SDS-PAGE and immunoblotting analysis. MW: molecular weight markers, lane 1: SDS-PAGE of natural rubber latex extract: lane 2: SDS-PAGE of globe protein extract (silver staining). Lanes 3-5: IgE immunoblotting of positive sera against NRL proteins. Protein loaded: 10 µg/lane.

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