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Sensitization to bovine serum albumin as a possible cause of allergic reactions to vaccines

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ABSTRACT

Background: Immediate type hypersensitivity to vaccines containing bovine/porcine excipients, such as the measles, mumps and rubella (MMR) vaccine is probably due to sensitization to bovine/porcine gelatin. Most patients with such reactions in Sri Lanka have cow's milk (CM) or beef allergy.
Objectives: We investigated whether those who had beef and CM allergy had a higher incidence of hypersensitivity reactions to vaccines and the possible trigger of such reactions.
Material and methods: Twenty patients with immediate type hypersensitivity reactions to vaccines containing bovine/porcine excipients, controls with allergy to beef/pork (n = 11) or CM (n = 11), and 8 non atopic controls were recruited. Total serum IgE, specific IgE to beef, CM, casein, beta lactoglobulin, gelatin and bovine serum albumin (BSA) by Phadia ImmunoCap and IgE to porcine gelatin by Western blot were evaluated.
Results: 11/20, 5/20, 2/20, 2/20, 1/20 and 1/20 patients reported allergic reactions to measles containing, JE, rabies primary chick embryo, pentavalent, diphtheria and tetanus, and adult diphtheria and tetanus vaccines, respectively. Only one patient with allergy to vaccines had gelatin specific IgE, whereas IgE to BSA was seen in 73.3%, 90%, 66.6% and 0 of vaccine, beef or CM allergic and non-atopic controls, respectively. The mean IgE to BSA was higher in patients with allergy to vaccines, although not significant. Specific IgE to BSA was present in 54.7% of children with allergy to CM, of whom 11.8% had high levels (>17.5 kUA/L). In contrast, 66.6% of these children did not have specific IgE to β-lactoglobulin, which is one of the major components of whey protein.
Conclusion and clinical relevance: Gelatin does not appear to play a major role in Sri Lankan children with allergy to vaccines. In contrast, due to the higher levels of BSA specific IgE, sensitization to BSA is possibly playing a role.

1. Introduction

Anaphylaxis following immunization with the measles, mumps or rubella containing vaccines [1–5] (the measles, mumps and rubella (MMR), measles and rubella (MR) and measles vaccines), varicella [6] and live Japanese encephalitis vaccines [7] has been reported. Anaphylaxis in these instances was thought to occur due to an IgE mediated reaction to bovine or porcine gelatin or polygeline (a polymer of bovine gelatin) used as a stabilizer in these vaccines [1–3]. Some of those who developed anaphylaxis following vaccination were shown to have prior or subsequent allergy to gelatin containing foods [1–3]. High rates of allergic reactions to the MMR vaccine have been reported in Sri Lanka. For instance, in 2015, 119 cases were reported in the second quarter of the year, which gives a rate of 0.685% [8]. In 2008, two girls aged 13 years, died of anaphylaxis following immunization with the rubella vaccine in Sri Lanka (unpublished data). One of them was known to be allergic to cow's milk (CM) and the other allergic to beef and pork (confirmed by the presence of specific IgE to CM or beef and pork by Phadia ImmunoCap carried out with the assistance of the World Health Organization (WHO)). The gelatin used in the MMR is of porcine or bovine origin [9].

Patients with known allergy to beef or pork are at risk of developing immediate hypersensitivity reactions to gelatin containing

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vaccines [10]. Allergy to casein has also been implicated in patients with severe cow's milk allergy (CMA) who developed anaphylaxis following immunization with the diphtheria, tetanus and pertussis vaccine [11]. Although beef allergy is rare in the West, two to three percent of children are believed to have CMA [12,13]. In contrast, many adults and children with beef allergy have been referred to our clinical at the Medical Research Institute (MRI), Colombo and have been confirmed by detection of specific IgE. Therefore, the prevalence of beef allergy is likely to be higher in Sri Lanka when compared to many other countries. Although, beef consumption is less in Sri Lanka as the majority of individuals are either Buddhists or Hindus, it is possible that those with CMA become sensitized to the bovine serum albumin (BSA) component more frequently, thus reacting to BSA in vaccines.

Studies have shown that 73% [14] and 93% [15] of children allergic to beef also had CMA and 10% of patients with CMA were also allergic to beef [16]. The sensitivity to bovine serum albumin (BSA), a major allergen in beef was the main predictive marker of CMA in children with beef allergy [15]. The vaccines used in Sri Lanka have BSA and gelatin. The MMR contains trace amounts of BSA and porcine gelatin 2.5 mg/dose, live JE vaccine has BSA <50 ng/dose and gelatin and the rabies chick embryo vaccine (PCEC) contains polygeline. The pentavalent vaccine (against diphtheria, tetanus, whole cell pertussis, H influenza type b, and hepatitis B), manufactured by the Serum Institute, India has components produced in bovine protein containing media, including casein.

Most patients attending the Allergy Clinic at MRI, following anaphylactic reactions to vaccines gave a history of allergy to CM or beef. As bovine and porcine products are present in the MMR, MR, measles, live JE, pentavalent, adult diphtheria and tetanus (AdT) and PCEC vaccines, we sought to investigate if patients with CMA and concurrent beef allergy are at a higher risk of allergic reactions when immunized with such vaccines and the possible trigger of such reactions.

2. Material and methods

Patients who developed immediate hypersensitivity reactions, including anaphylaxis following immunization from 2014 to May 2016 were recruited following informed written consent. Ethical approval for the study was obtained from the Ethics Review Committee of the MRI (Research Project No 41/2014). Informed written consent was obtained from the parent if the patient was below 18 years, of age.

Anaphylaxis was diagnosed according to ICON guidelines [17]. A detailed clinical history was obtained, and clinical records were scrutinized. In addition, patients with CMA or allergy to beef/pork and who did not have reactions to vaccinations, as well as age matched controls who had no atopy related diseases who were attending the Thalassemia Clinic, at the Department of Haematology, MRI were recruited as controls.

The total serum IgE was assessed by ELISA (DRG instruments, GmbH, Germany) and specific IgE to CM, beef, β lactoglobulin, BSA, casein and gelatin was performed using the Phadia ImmunoCap system in all patients. A positive IgE titre was defined as specific IgE levels of >0.35 kUA/L. In order to determine the relationship between the presence of high titres of IgE to cow's milk and cow's milk components, the patients were grouped as previously described [18]. Accordingly, those with IgE levels of <3.5 kUA/L considered to have low levels, specific IgE levels of 3.5 to 17.5 kUA/L as moderate levels and levels >17.5 kUA/L were considered to be high.

The presence of IgE to gelatin was also evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and by Western blot [19] in patients who developed allergic reactions to the vaccine. BSA was used as a positive control, using serum from a patient who had specific IgE to BSA. SDS-PAGE was performed using a Mini Protein-R apparatus (Bio-Rad). Ten μl containing 10 μg of porcine gelatin (Bloom number 180-Sigma Aldrich) was added to 5 μl of 3X Lammeni sample buffer (80 mM Tris-HCl buffer (pH 6.8), containing 2% SDS and 10% glycerol, 0.1% Bromophenol Blue), incubated at 95 °C for 10 min and subjected to electrophoresis through a 15% polyacrylamide gel at 70 V at 4 °C for 3 h. Gels were stained with Coomassie stain for 1 h and de-stained using de-staining solution containing glacial acetic acid, methanol and deionized water. The process was repeated with the BSA standard (Sigma Aldrich).

For western blotting, the proteins separated by SDS-PAGE were first electro transferred from the gel to a nitrocellulose blotting membrane using a mini protein tetra system (Bio-Rad) for 2 h at 70 V constant voltage. The membrane was then washed with phosphate buffered saline (PBS) containing 0.05% Tween 20 (PBST) and blocked with blocking solution (5% non-fat milk in PBST) at 4 °C for 1 h. After washing with PBST for 5 min (x3), the membrane was reacted with patient's serum (diluted 1:40) in antibody diluting buffer (5% non-fat milk in PBST) at 4 °C for overnight. The membrane was washed with PBST for 5 min (x3) and reacted at 4 °C for 2 h with the peroxidase-labelled goat anti-human IgE antibody diluted in 1:1000 (Sigma Aldrich). After re-washing with PBST, antigen antibody binding was visualized using 4CN substrate.

2.1. Statistical analysis

PRISM version 6 was used in the statistical analysis. As the data were not normally distributed, differences in means were compared using the Mann-Whitney *U* test (two tailed). The degree of association between the development of anaphylaxis and the presence of high (>17.5 kUA/L) casein specific IgE was expressed as the odds ratio (OR), which was obtained from standard contingency table analysis by Haldane's modification of Woolf's method. Chi Square tests, Chi square for trend or the Fisher's exact test was used to determine the p value.

3. Results

Of the 20 patients 08 had immediate hypersensitivity reactions to the MMR, 02 for the MR, 01 for measles, 05 for live JE vaccine, 02 for rabies PCEC, 02 for the pentavalent vaccine, 01 for the diphtheria and tetanus (DT) and 01 for the AdT vaccine (Table 1 and 2). Two patients reacted to 2 vaccines. Fourteen patients developed anaphylaxis, of whom 8 developed anaphylaxis to measles containing vaccines (measles, MR, MMR), and 4 developed anaphylaxis following administration of the live JE vaccine, with one patient developing anaphylaxis to both MMR and live JE vaccines. Two patients developed anaphylaxis to the pentavalent vaccine, while 1 patient developed anaphylaxis to the PCEC. One patient who had anaphylaxis following the MMR vaccine previously had an anaphylactic reaction to the DT vaccine. Six patients developed immediate hypersensitivity reactions not considered to be anaphylaxis. Of the 6, two patients developed a dry cough immediately after the MMR vaccine, while 1 patient each developed cough and shortness of breath/wheezing after the MR and live JE vaccines. One patient each developed urticaria following the AdT and PCEC vaccines.

16/20 (80%) patients were known to have either CM and/or beef allergy. These included 7, 6 and 3 patients with CM, beef, or beef and CM allergy. One patient, who was 2 months at the time of the allergic reaction to the vaccine (pentavalent), had not been exposed to CM or beef. A total of 8 patients (40%) had never ingested beef.

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