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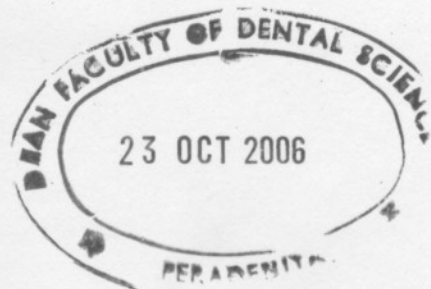
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**Cow's Milk Allergy – A study on the role  
of  $\beta$ -lactoglobulin in IgE mediated  
hypersensitivity reactions to cow's milk  
in infants**

**A THESIS PRESENTED  
BY**

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## ABSTRACT

Cow's milk allergy is usually the first allergic disease in the "atopic march", because cow's milk proteins are the first foreign proteins encountered by many infants. Mostly it is an immediate type hypersensitivity reaction to the major whey protein  $\beta$ -lactoglobulin.

In a previous study carried out in 1988, we showed that all the cow's milk based infant formulas available in the market contain  $\beta$ -lactoglobulin. Further, many pediatricians in Sri Lanka have observed an association between gastrointestinal, respiratory and cutaneous symptoms in infants with ingestion of cow's milk (personnel correspondence). Even though there is ample research conducted addressing this problem in Western countries, little information is available about cow's milk allergy in infants in Sri Lanka.

Therefore, research was conducted to study the role of  $\beta$ -lactoglobulin in infants with immediate type hypersensitivity reactions to cow's milk.

Experiments were conducted to determine the degree of *in vitro* hydrolysis of  $\beta$ -lactoglobulin at high pH values that prevail in infants' stomach and compare it with that at low pH values prevailing in adult stomach. Beta lactoglobulin was digested in simulated gastric fluid in a pH range of 2-5 followed by simulated intestinal fluid. Increase in pH of simulated gastric fluid resulted in a reduction of the degree hydrolysis and increment in the residual human IgE antigenicity of  $\beta$ -lactoglobulin.

Further, a sensitive double sandwich ELISA was developed to detect  $\beta$ -lactoglobulin in the infants' serum. Purified anti  $\beta$ -lactoglobulin rabbit IgG was used as the capture antibody and anti  $\beta$ -lactoglobulin goat serum was used as the primary antibody. Peroxidase conjugated anti goat rabbit IgG was the conjugate. The detection limit of the assay was 0.05  $\mu\text{g/l}$ . The recovery of  $\beta$ -lactoglobulin in infants' serum was 96%-115%. The intra assay CV was 5.4% and inter assay CV was 6.9%-8.8%. When the serum  $\beta$ -lactoglobulin concentration of infants with CMA and controls were measured using this assay, a significant difference ( $p < 0.05$ ) was observed.

To identify the milk proteins capable of eliciting type I hypersensitivity reactions, milk proteins were immunoblotted for IgE with the serum of infants with CMA and controls. The allergenic proteins found in the serum of CMA infants are  $\beta$ -lactoglobulin, casein,  $\alpha$ -lactalbumin and BSA. Most of them are found to be sensitized to more than one milk protein (19/30). They are more frequently positive to  $\beta$ -lactoglobulin (25/30) than caseins (15/30) and other whey proteins - ALA (4/30) and BSA (6/30)). Further, there was no correlation ( $p < 0.05$ ) between their serum  $\beta$ -lactoglobulin level and the  $\beta$ -lactoglobulin specific IgE level.