The Protein Level and Molecular Weight Analysis in Different Children's Toothpaste, which Probably Induced Hypersensitivity

by Hendrik Setia Budi

Submission date: 13-Jun-2022 07:09AM (UTC+0800)
Submission ID: 1855527712
File name: ContempClinDent113245-5262742_143707.pdf (716.62K)
Word count: 3122
Character count: 16859

Original Article

The Protein Level and Molecular Weight Analysis in Different Children's Toothpaste, which Probably Induced Hypersensitivity

Hendrik Setia Budi[1,2], Indah Pebriani[2]

[1]Department of Oral Biology, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, [2]Research Center, Faculty of Dental Medicine, Universitas Airlangga, Surabaya, Indonesia

Abstract

Context: A new case of an allergic reaction due to the use of toothpaste was found in California, United States, causing the death of an 11-year-old girl. Ingredients contained in toothpaste suspected as a cause of allergies are milk protein compounds. **Aims:** The aim is to compare the protein level and molecular weight in children's toothpaste. **Settings and Design:** Stratified random sampling. **Subjects and Methods:** Samples used were children's toothpaste products in society, namely, Pepsodent®, Cussons®, Enzyme®, Kodomo®, Formula®, Colgate®, the toothpaste contain recaldent of GC Tooth Moose® and pure cow's milk. Those samples were divided into eight groups, randomly selected according to the purpose (stratified random sampling), and then coded to maintain product confidentiality. Meanwhile, samples used as comparison groups were recaldent paste and pure cow's milk. **Results:** Each sample was analyzed for protein content using a biuret test and protein molecular weight using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis test. The protein content in toothpaste is compared with the similarity of the molecular protein weight in toothpaste that contains recaldent. Protein was found in samples 2, 3, 5, 6, 7, and 8 through a biuret test, with a concentration of 1.82; 3.53; 2.76; 1.92; 1.85; and 3.2 µg/mL. However, the protein bands were only found in sample 5 with a molecular weight of 20.7 kDa, sample 6 with a molecular weight of 19.1 kDa, sample 7 with a molecular weight of 17.7 kDa, and sample 8 with 2 bands, namely, 28.7 and 39.7 kDa. **Conclusions:** We found the presence of protein in children's toothpaste. The protein molecular weight of recaldent paste is 17.7 kDa. Toothpaste containing protein with its molecular weight similar to recaldent's are found in samples 5 and 6.

Keywords: *Children's toothpaste, hypersensitivity, protein molecular weight, recaldent, sodium dodecyl sulfate-polyacrylamide gel electrophoresis*

Introduction

Hypersensitivity or allergy is an excessive antigenic response, which occurs in individuals who have previously experienced sensitization with certain antigens or allergens. In the last 20 years, the incidence of hypersensitivity has increased, which can cause health problems.[1,2] Allergies are caused by changes in the body's reaction (becoming vulnerable) to material in our daily environment. Allergic contact dermatitis due to cosmetics occurs as much as 2%–4% of all dermatitis cases in the clinic, or even more.[3]

Allergens are nonparasitic foreign substances that can cause certain immune reactions in the body when they pass into the body. Conditions caused by allergens are called allergies. Allergies can cause several disorders of the mucosa, skin, digestive tract, respiratory tract, and blood vessels, leading to certain symptoms, such as urticaria, dermatitis, edema, asthma, and even death.[4,5] Cosmetic contact with the skin for a long time initiates the sensitization process of some chemicals contained. Some cosmetic products that are commonly used by the public are soap, shampoo, deodorant, toothpaste, facial cream, sunscreen, and perfume.

Many cases of contact dermatitis due to allergies are caused by various cosmetic products. Irritant reactions to cosmetics usually occur in patients who have sensitive skin or a history of atopy.[6] A new and rare occurrence of an allergic reaction due to the use of toothpaste was found in California, United States, causing the death of an 11-year-old girl. Ingredients contained in toothpaste suspected as a cause of allergies are milk protein compounds used, namely, recaldent

How to cite this article: Budi HS, Pebriani I. The protein level and molecular weight analysis in different children's toothpaste, which probably induced hypersensitivity. Contemp Clin Dent 2020;11:245-8.

Submitted : 11-Nov-2019
Revised : 16-Apr-2020
Accepted : 29-May-2020
Published : 26-Nov-2020

Address for correspondence:
Hendrik Setia Budi, Faculty of Dental Medicine, Universitas Airlangga, Jl. Prof. Dr Moestopo 47, Surabaya, Indonesia.
E-mail: hendrik-s-b@fkg.unair.ac.id

Access this article online

Website: www.contempclindent.org

DOI: 10.4103/ccd.ccd_394_19

Quick Response Code:

or casein phosphopeptide-amorphous calcium phosphate (CPP-ACP).[7] These materials are used for toothpaste in patients who have teeth hypersensitivity with remineralization efficacy. Recaldent is the commercial name of CPP-ACP, a natural protein found in cow's milk.[8-10] The molecular weight of protein in cow's milk is 37 kDa is α-casein, 33 kDa is β-casein, 46 kDa is κ-casein.[11]

Improving dental health at an early age is actually very important since it is one of the important elements to support holistic health, namely by maintaining dental hygiene and mouth brushing regularly at least twice a day using toothpaste. Toothpaste is generally made from a mixture of several ingredients, each of which has different functions, namely as therapy, moisturizer, water, flavor, preservative, detergent, and whitener.[12] Variations in toothpaste products sold in the market may have side effects, so parents should be able to choose the right toothpaste for children, regarding the composition of toothpaste and the age of the child.[13]

Therefore, this study aims to reveal the possibility of milk protein found in some children's toothpaste. This can be useful for the public to get as much information related to food and cosmetics that would be used, especially for children who are vulnerable to allergens, related to the development of the immune system. Toothpaste samples used were (Pepsodent®, Cussons®, Enzyme®, Kodomo®, Formula®, Colgate®).

Subjects and Methods

Toothpaste sample preparation

Samples used were toothpaste products easily found in society and used by children, namely Pepsodent®, Cussons®, Enzyme®, Kodomo®, Formula®, Colgate®. Those samples were then divided into six groups randomly selected according to the objectives (stratified random sampling) and coded to maintain product confidentiality. Meanwhile, samples used as comparison groups were GC tooth moouse® (toothpaste containing recaldent) and pure cow's milk, so that totally were eight groups.

Analysis of protein levels with biuret test

Measurement of protein level was carried out according to the method of Lowry *et al*.[14] First, 1 gof each sample was added with 10 ml of distilled water. Second, 4 ml of each sample was added with 6 ml of biuret solution. The solution was rested for 10 min at 37°C, and then, the protein content was analyzed by reading its absorption using a visible spectrophotometer (Thermoscientific, Germany) with a wavelength of 595 nm.

Protein profile

The protein was isolated from each sample before its protein profile was analyzed. Next, each sample was taken as much as 5 ml, and then dissolved with distilled water as much as five times the volume. Afterward, the solution was sonicated with an amplitude of 20% for 10 min, and then centrifuged at 6000 rpm at 4°C for 15 min. Subsequently, the supernatant was taken and cold ethanol solution (1:1) was added. It was then stored at 4°C for 12 h. After that, the samples were centrifuged at a speed of 6000 rpm at 4°C for 15 min. The pellets were then dried until ethanol was disappeared. Finally, tris HCl pH 6.8 (1:1) was added, and then stored at −20°C.[15]

Sodium dodecyl sulfate-page examination

The protein profile was analyzed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with a 10% separating gel. Protein samples that had been measured for protein levels with a nanospectrophotometer (Thermoscientific, Germany) were added to the buffer sample. The samples were then heated at 70°C for 5 min with a water bath. Next, electrophoresis was run at a constant current of 150 mA for 80 min. The distribution of the ribbon then was detected by coloring the coomassie brilliant blue gel-250.[15]

Results

The examination results of protein content in the toothpaste groups and the comparison groups showed that samples 2, 3, 5, 6, 7, and 8 contained protein, but there was no protein in samples 1 and 4 with the biuret test [Table 1].

Electrophoresis gel shown protein bands in sample 5 with a molecular weight of 20.7 kDa, sample 6 with a molecular weight of 19.1 kDa, sample 7 with a molecular weight of 17.7 kDa, and sample 8 with 2 bands, namely. 28.7 and 39.7 kDa, but no protein band in samples 2 and 3 [Figure 1].

Discussion

Mouth and gum are parts of the digestive system contacting food or drink first. This system has the best absorbent point of the body; thus, if there are chemicals in toothpaste, it will quickly circulate throughout the body through the

Table 1: Protein levels of children's toothpaste compared to the comparison groups

Code	Absorption	Level (μg/mL)	Molecular weight (kDa)
Sample 1	-	-	-
Sample 2	2.07	1.82	-
Sample 3	1.73	1.53	-
Sample 4	-	-	-
Sample 5	3.18	2.76	20.7
Sample 6	2.19	1.92	19.1
P1	2.11	1.85	17.7
P2	3.71	3.20	28.7 and 39.7

Sign - indicates the absence of protein through biuret test and sodium dodecyl sulfate-polyacrylamide gel electrophoresis. P1 is a recaldent paste, and P2 is pure cow's milk. SDS: Sodium Dodecyl Sulfate

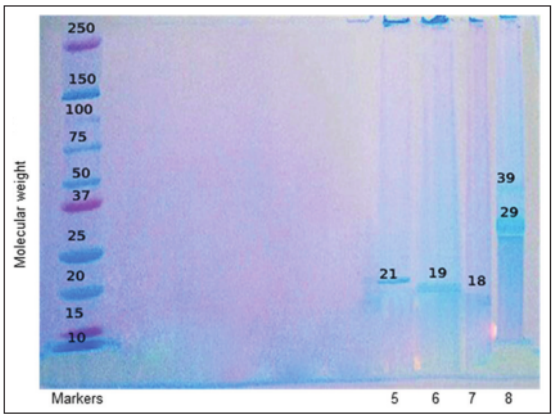

Figure 1: Sodium dodecyl sulfate profile of prostein from the children's toothpaste separated in polyacrylamide gels

bloodstream.[16,17] In some toothpaste samples, there were proteins contained with different concentrations [Table 1]. Allergy caused by a chemical material does not depend on its amount or dose. In people who have a history of allergy to a certain chemical material, making contact with a small amount of the chemical material still can cause allergic reactions.[18] Most allergens are proteins that can stimulate the body's immune response through enzymatic reactions or receptor activations on mucosal epithelial cells directly.[19]

Most food allergens contain glycoprotein. Carbohydrates covalently attach to proteins to form glycoproteins. The glycemic portion of mammalian glycoprotein is generally not immunogenic because it can be tolerated by most mammals. The mammalian immune system can develop tolerance to this group of carbohydrates. In contrast, nonmammalian glycoproteins, which are n@different from mammalian glycoproteins, can induce immunoglobulin G (IgG) formation and sometimes IgE antibodies.[16]

These glycoproteins are recognized as certain antigens in the body by the immune system. The severity of allergic reactions can vary from person to person (genetic susceptibility).[20] These reactions occur after skin or mucosal contact with allergens. The allergens usually have small molecules (picric acid, dinitrochlorobenzene, different herbal ingredients, cosmetics, some drugs, metals, and other substances). After being absorbed into the epidermis/epithelium, the substance binds to protein as a carrier and becomes immunogenic, then leading to a hypersensitivity response.[21]

Based on the mechanism of immunologic reactions that occur, hypersensitivity reactions are divided into four Groups. First, Type I hypersensitivity reactions (anaphylactic reaction) participate in IgG, IgE, and Histamine. The general mechanism of this reaction is that the allergens crosslink with IgE. Mast cells and basophils then secrete vasoactive amines and other chemical mediators. Second, Type II hypersensitivity reactions (cytotoxic reactions) are generally derived from the activation of the complement system after stimulation from the presence of antigen-antibody complexes. IgG, IgM, and complement play a role in Type 2 hypersensitivity reactions. Third, Type 3 hypersensitivity reactions (immune complex reactions) occur because of the deposition of immune complexes (antigen-antibodies) that are difficult to phagocyte so that they activate complement and accumulate polymorphonuclear leukocytes in the tissues. Moreover, Type IV hypersensitivity reactions can also be called as slow cellular immune reactions since they are mediated by T CD4+ and CD8+ cells. The mechanism for this reaction, in general, is triggered by sensitized T-lymphocytes. Cytokines and other mediators or cytotoxic then are released, mediated by direct T-cells.[22-25]

The results of measurements of protein levels in Groups 2, 3, 5, 6, 7, and 8 detected the presence of protein through the biuret test, but only in samples 5, 6, 7, and 8 the protein molecular weight can be determined. This can be due to low protein levels in samples 2 and 3, so the molecular weight could not be measured using the SDS-PAGE. The molecular weight of children's toothpaste proteins in samples 5 and 6 showed molecular weights of 20.7 and 19.1 kDa, quite close to the molecular weight of recaldent paste, 17.7 kDa. This means that the protein contained in the toothpaste samples is likely similar to recaldent paste, so a child has a history of allergies should be warned. Meanwhile, in the cow's milk group, two bands were found, 28.7 and 39.7 kDa, which showed very close to α-casein and β-casein [Figure 1]. The addition of protein to toothpaste is intended to make toothpaste softer and can be used as antibacterial, such as lysozyme and lactoferrin.[26]

Allergies that occur in children are considered to be dangerous and need serious attention. Based on the results of this study, there are some toothpastes containing protein with its molecular weight similar to recaldent, but the specific further investigation is required to analyze deeper whether protein used is recaldent or not.

Acknowledgment

We would like to thank our department colleagues for supporting this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflict of interest.

References

1. Rubianti MA, Rosita C. Profile of allergic contact dermatitis patients due to cosmetic. Periodic Dermatol Venereol 2019;31:35-40.

2. Tanno LK, Calderon MA, Smith HE, Sanchez-Borges M, Sheikh A, Demoly P, *et al*. Dissemination of definitions and concepts of allergic and hypersensitivity conditions. World Allergy Organ J 2016;9:24.
3. González-Muñoz P, Conde-Salazar L, Vañó-Galván S. Allergic contact dermatitis caused by cosmetic products. Actas Dermosifiliogr 2014;105:822-32.
4. Bernstein JA, Cremonesi P, Hoffmann TK, Hollingsworth J. Angioedema in the emergency department: A practical guide to differential diagnosis and management. Int J Emerg Med 2017;10:15.
5. Venter C, Pereira B, Voigt K, Grundy J, Clayton CB, Higgins B, *et al*. Prevalence and cumulative incidence of food hypersensitivity in the first 3 years of life. Allergy 2008;63:354-9.
6. Broeckx W, Blondeel A, Dooms-Goossens A AG. Cosmetic intolerance. Contact Dermat 2012;16:189–94.
7. Sanchez EB. 2019. Girl's Deadly Reaction to Toothpaste is Extremely Rare, Allergist Reminds Parents. CNN Online. Available from: https://edition.cnn.com/2019/04/22/health/toothpaste-deadly-allergic-reaction-perspective/index.html. [Last accessed on 2019 June 22].
8. Kowalczyk A, Botuliński B, Jaworska M, Kierklo A, Pawińska M, Dabrowska E. Evaluation of the product based on Recaldent technology in the treatment of dentin hypersensitivity. Adv Med Sci 2006;51 Suppl 1:40-2.
9. Gurunathan D, Somasundaram S, Kumar S. Casein phosphopeptide-amorphous calcium phosphate: A remineralizing agent of enamel. Aust Dent J 2012;57:404-8.
10. Huq NL, Myroforidis H, Cross KJ, Stanton DP, Veith PD, Ward BR, *et al*. The Interactions of CPP-ACP with Saliva. Int J Mol Sci 2016;17:915.
11. Nitsche R. Milk Protein Analysis with the Agilent 2100 Bioanalyzer and the Agilent Protein 80 kit. Germany: Agilent Technologies, Inc.; 2011.
12. Sitthisettapong T, Phantumvanit P, Huebner C, DeRouen T. Effect of CPP-ACP paste on dental caries in primary teeth. J Dent Res 2012;91:847-52.
13. Emanuela BM. Role and Influence of the Toothpaste Components in the Oral Biochemistry. Thesis. Master Dentistry of Porto University; 2011.
14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
15. Barsotti O, Comby C, Morrier JJ, Benay G, Decoret D, Guinet R, *et al*. Improved SDS-PAGE method for protein profiles analysis of actinomyces species. J Biol Buccale 1988;16:219-23.
16. Soh JY, Huang CH, Lee BW. Carbohydrates as food allergens. Asia Pac Allergy 2015;5:17-24.
17. Kaplan DH, Igyártó BZ, Gaspari AA. Early immune events in the induction of allergic contact dermatitis. Nat Rev Immunol 2012;12:114-24.
18. Khan DA, Solensky R. Drug allergy. J Allergy Clin Immunol 2010;125:S126-37.
19. Dubois AEJ, Turner PJ, Hourihane J, Ballmer-Weber B, Beyer K, Chan CH, *et al*. How does dose impact on the severity of food-induced allergic reactions, and can this improve risk assessment for allergenic foods? Report from an ILSI Europe Food Allergy Task Force Expert Group and Workshop. Allergy 2018;73:1383-92.
20. Simons FE, Ebisawa M, Sanchez-Borges M, Thong BY, Worm M, Tanno LK, *et al*. 2015 update of the evidence base: World Allergy Organization anaphylaxis guidelines. World Allergy Organ J 2015;8:32.
21. Bakula A, Lugović-Mihić L, Situm M, Turcin J, Sinković A. Contact allergy in the mouth: Diversity of clinical presentations and diagnosis of common allergens relevant to dental practice. Acta Clin Croat 2011;50:553-61.
22. Paul WE. Fundamental Immunology. 7th ed.. Philadelhpia: Lippincott Williams & Wilkins. 2013. p. 1379-81.
23. Tomasiak-Łozowska MM, Klimek M, Lis A, Moniuszko M, Bodzenta-Łukaszyk A. Markers of anaphylaxis-a systematic review. Adv Med Sci 2018;63:265-77.
24. Son JH, Park SY, Cho YS, Chung BY, Kim HO, Park CW. Immediate hypersensitivity reactions induced by triamcinolone in a patient with atopic dermatitis. J Korean Med Sci 2018;33:e87.
25. Koike Y, Sato S, Yanagida N, Asaumi T, Ogura K, Ohtani K, *et al*. Predictors of persistent milk allergy in children: A retrospective cohort study. Int Arch Allergy Immunol 2018;175:177-80.
26. Adams SE, Arnold D, Murphy B, Carroll P, Green AK, Smith AM, *et al*. A randomised clinical study to determine the effect of a toothpaste containing enzymes and proteins on plaque oral microbiome ecology. Sci Rep 2017;7:43344. https://doi.org/10.1038/srep43344.

The Protein Level and Molecular Weight Analysis in Different Children's Toothpaste, which Probably Induced Hypersensitivity

ORIGINALITY REPORT

13%	9%	13%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	A Aulanni'am, T Z Anita, D S Nahari, I A Aluka, E I Agustine, T Novita, A A Pentaloka, D K Wuragil, W Riawan, M A G Beltran. "The potency of Polylathia longifolia from Indonesia and the Philippines as therapeutic agents on inflammatory bowel disease (IBD) in Rats (Rattus norvegicus) induced by Indomethacin", IOP Conference Series: Materials Science and Engineering, 2020 Publication	3%
2	iapindia.org Internet Source	3%
3	Abdul S. Ethayathulla, Devendra B. Srivastava, Janesh Kumar, Kolandaivelu Saravanan et al. "Structure of the buffalo secretory signalling glycoprotein at 2.8 Å resolution", Acta Crystallographica Section F Structural Biology and Crystallization Communications, 2007 Publication	2%

4	repository.unair.ac.id Internet Source	1 %
5	"Practical Immunodermatology", Springer Science and Business Media LLC, 2017 Publication	1 %
6	jnsmonline.org Internet Source	1 %
7	bio-protocol.org Internet Source	1 %
8	Pratiwi Soesilawati, Harianto Notopuro, Yuliati Yuliati, Maretaningtias Dwi Ariani, Muhammad Alwino Bayu Firdauzy. " The role of salivary sIgA as protection for dental caries activity in Indonesian children ", Clinical, Cosmetic and Investigational Dentistry, 2019 Publication	1 %
9	Mahdi Jalaeian, Mozhgan Mohammadzadeh, Mohammad Mohammadzadeh, Ehsan Borzoui. "Rice cultivars affect fitness-related characteristics and digestive physiology of the rice weevil, Sitophilus oryzae (L.) (coleoptera: Curculionidae)", Journal of Stored Products Research, 2021 Publication	1 %

10	Zacharias A. Pretorius van der Walt, Renée Prins, Elsabet Wessels, Cornel M. Bender, Botma Visser, Willem H.P. Boshoff. "Accomplishments in wheat rust research in South Africa", South African Journal of Science, 2020 Publication	<1 %
11	"Biomineralization", Springer Science and Business Media LLC, 2018 Publication	<1 %
12	Irene Garcia-Maquilon, Alberto Coego, Jorge Lozano-Juste, Maxim Messerer et al. " PYL8 ABA receptors of play a crucial role in response to abiotic stress and are stabilized by ABA ", Journal of Experimental Botany, 2021 Publication	<1 %

Exclude quotes	Off	Exclude matches	< 10 words
Exclude bibliography	On		

The Protein Level and Molecular Weight Analysis in Different Children's Toothpaste, which Probably Induced Hypersensitivity

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4