Immunoglobulin-Y Effect On Protein Of Streptococcus Mutans Isolated From Caries And Caries-Free Subjects

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Abstract

The main microbial culprit in dental caries is *Streptococcus mutans* (*S.mutans*), virulence of which can be observed by its differential protein expression between caries and caries-free subjects.The success of Immunoglobulin-Y (IgY) anti *S.mutans* as a passive immunization agent in eliminating *S.mutans* has been reported. The aim of this study is to analyze the effect of IgY anti *S.mutans* on the protein expression of *S.mutans* isolated from caries and caries-free subjects. Each dental plaque was collected by swabbing the buccal surface of the first lower permanent molar of caries and caries-free subjects. The plaques were then cultured on agar medium TYS20B. After 72 hours, the colonies from each of them were cultured in liquid medium TYS *Broth* for 72 hour. Each collected bacteria (whether from caries or caries-free subjects) were grouped into control and exposure group. In exposure group, *S.mutans* was exposed by pre-incubated (for one hour at 37°C) IgY anti *S.mutans* for one hour at 37°C. Protein expression of *S.mutans* was analyzed with SDS PAGE after the preparation of its antigen and Bradford protein assay. Our result shows that *S.mutans* 41.3 kilodalton protein expression of caries subjects, are up-regulated in comparison to the control group. Meanwhile, the *S.mutans* 41.3 kilodalton protein expression of caries-free subjects, are down-regulated in comparison to the control group. This study suggests that IgY anti *S.mutans* up-regulates 41.3 kilodalton protein expression of *S.mutans* in the caries subjects. However IgY anti *S.mutans* down-regulates 4.13 kilodalton protein expression of *S.mutans* in the caries-free subjects.

Key words: Streptococcus mutans, protein expression of Streptococcus mutans, imunoglobulin-Y anti Streptococcus mutans, caries and free caries subjects.

Pengaruh Imunoglobulin-Y Terhadap Protein *Streptococcus Mutans* Yang Diisolasi Dari Subjek Karies Dan Bebas Karies

Abstrak

Mikroba penyebab utama karies pada gigi adalah *Streptococcus mutans* (*S.mutans*). Virulensi *S.mutans* dapat diketahui melalui ekspresi proteinnya yang

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berbeda antara subjek karies dan bebas karies. Keberhasilan Imunoglobulin-Y (IgY) anti *S.mutans* sebagai media imunisasi pasif dalam mengeliminasi *S.mutans* telah banyak dilaporkan. Tujuan penelitian ini yaitu untuk menganalisis efek IgY anti *S.mutans* terhadap ekspresi protein *S.mutans* yang diisolasi dari subjek karies dan bebas karies. Masing-masing plak pada gigi dikumpulkan dengan cara dilakukan pengulasar pada permukaan bukal gigi molar pertama permanen dari subjek karies dan bebas karies. Plak lalu dikultur di atas medium agar TYS20B . Setelah 72 jam, koloni yang dihasil dari keduanya dikultur pada medium cair TYS *Broth* selama 72 jam. Masing-masing bakteri yang telah terkumpul (yang berasal dari subjek karies maupun subjek bebas-karies) dikelompokkan menjadi kelompok kontrol dan kelompok pemaparan. Pada kelompok pemaparan, *S.mutans* dipapar oleh IgY anti *S.mutans* (yang telah sebelumnya dipreinkubasi selama 1 jam pada suhu 37°C) selama 1 jam pada suhu 37°C. Ekspresi protein dianalisis dengan SDS PAGE setelah sebelumnya dilakukan preparasi antigen dan uji kadar protein Bradford. Hasil penelitian ini adalah ekspresi protein *S.mutans* 41.3 kilodalton pada subjek karies meningkat dibandingkan dengan kelompok kontrol. Sementara itu, ekspresi protein *S.mutans* 41.3 kilodalton pada subjek bebas karies menurun dibandingkan dengan kelompok kontrol. Dalam penelitian ini disimpulkan bahwa IgY anti *S.mutans* 41.3 kilodalton meningkat . Akan tetapi , IgY anti *S.mutans* menurunkan ekspresi protein 41..3 kilodalton dari *S.mutans* pada subjek bebas-karies.

Kata Kunci: Streptococcus mutans, ekspresi protein Streptococcus mutans, imunoglobulin-Y anti Streptococcus mutans, caries and free caries subjects

Introduction

Dental caries remains as one of the most widespread diseases of a mankind. [1] Dental caries is a continuing chronic loss of mineral ion from the enamel or root surface of the tooth, stimulated mostly by certain bacterial flora and their byproducts. [2] *Streptococcus mutans* is the main microbial agent in pathogenesis of dental caries. [3] However, *S.mutans* is widely distributed not only in populations with moderate or high caries prevalences, but also in populations having no or low caries experiences. Possible explanation for their presence in subjects with low caries experience are the virulence factors. [4] The virulence factors associated with *S.mutans* cariogenicity include adhesion, acidogenicity, and acid tolerance. [5,6] One of virulence representing factors that can induce dental caries is the expression of antigen protein because several proteins involved in *S.mutans* pathogenicity are located on the cell surface. [7] Emteta reported that protein expression of *S.mutans* isolated from dental caries and caries-free subjects was different. [8]

A key to understand how *S.mutans* colonizes in the oral cavity is discerning how the various molecular components comprising the bacterial cell-surface interact with acquired dental pellicle. *S.mutans* possesses cell surface

substances, including antigen I/II (AgI/II), glucosyltransferase (gtf), and glucan-binding protein (gbp). These cell-surface molecules are thought to play important roles in interaction between the organism and its host, and have been given much attention as vaccine candidates against dental caries. [9] Sucrose-independent adherence is thought to be most profoundly influenced by Antigen I/II, a 185 kDa surface protein. The action of gtf in the synthesis of glucans is the major mechanism behind sucrose-dependent adhesion. The glucan-binding proteins are served as the receptor for glucans synthesized by Gtf. [5]

Immunoglobulin-Y (IgY) is an antibody largely found in chicken eggs. The use of chicken egg yolk as a source for antibody production represents a reduction in animal use, as chicken produces larger amounts of antibodies than laboratory rodents. It also makes it possible to eliminate the collection of blood which is painful for the animal. [10] The success of IgY anti *S.mutans* as an agent of passive immunization in eliminating *S.mutans* has been previously reported. Otake *et al*. reported that specific pathogen-free rats infected with *S.mutans* MT8148 (c) and fed with a cariogenic diet containing more than 2% immune yolk powder developed significantly lower caries scores than did the ones infected with the same strain and fed with a diet containing only control yolk powder obtained from non-immunized hens. [11] Then almost two decade after previous study, Anggraeni also reported that swabbed gel containing IgY anti *S.mutans* to rats teeth reduced biofilm formation of *S.mutans*. The studies are not only conducted in animal, but also in human using mouthwash and toothpaste containing IgY anti *S.mutans*. [12] Hatta *et al*. reported that in the short-term (4-hour) test using a mouthwash containing 10% sucrose, IgY decreased the ratio of the percentage of *S.mutans* per total streptococci in saliva. [13] Meanwhile, Paau *et al*. reported that experimental group in which using toothpaste containing IgY anti *S.mutans* twice a day showed significant percentage reduction of *S.mutans* level in saliva and plaque in comparison to the control group in which using conventional toothpaste. [14]

The efficacy of IgY anti *S.mutans* in reducing dental caries development by eliminating *S.mutans* factor was clinically proven. *S.mutans* protein expression that represents the virulence factors of *S.mutans* was apparently different between *S.mutans* isolated from caries and caries-free subjects after the exposure of IgY anti *S.mutans*. Moreover, the difference protein expression of *S.mutans* isolated from caries and caries-free subjects after the exposure of IgY anti *S.mutans* emerges the possibility of different response between them, it was of interest to analyze the effect of IgY anti *Streptococcus mutans* on protein of *S.mutans* isolated from dental caries and caries-free subjects.

Materials and Methods

This study has been approved by Research Ethical Committee of Faculty of Dentistry, Universitas

Indonesia. Participation of the program is voluntary. The study was carried out at Faculty of Dentistry, Universitas Indonesia. The subjects were 30 students of this faculty with age between 17-23 years, capable of informed consent and free from terminal and serious illness. These subjects were divided into 2 groups, 15 of which were dental caries subjects and the other 15 were dental caries-free subjects.

Streptococcus mutans were isolated from dental plaque at the buccal of the first lower molar of both dental caries and caries subjects using sterile cotton bud, dispersed into micro centrifuge tubes containing 1 ml PBS and stored at 4^oC until used. The plaques were then cultured on agar medium TYS20B. After 72 hours, the colonies from each of them were cultured in liquid medium TYS *Broth* for 72 hour. Each of collected bacteria (whether from caries or caries-free subjects) were grouped into control and exposure group after its concentration had been adjusted to 10^9 CFU/ml. In exposure group, 5 ml of *S.mutans* 10^9 CFU/ml was exposed by 1 ml pre-incubated (for one hour at 37°C) IgY anti *S.mutans* 120 µg/ml for one hour at 37°C. In the other hand, 5 ml of *S.mutans* 10^9 CFU/ml without any exposure was prepared as control group. Both *S.mutans* in control and exposure group were collected by centrifugation (13000 rpm, 1 min). Each of the collected pellets was washed once with 200 µl PBS and homogenized by ultrasonic homogenizer (Omni-Ruptor 250, OMNI International Inc.). Protein expression of *S.mutans* was analyzed with sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS PAGE) after protein concentration of the homogenized samples had been minimally adjusted to 2000 µg/ml with Bradford protein assay.

Results

Protein expression that was attempted to be analyzed referred to protein regarding to the virulence factors of *S.mutans*. Those proteins includes 185-190 kDa protein, 162 kDa protein, 145 kDa protein, 155 kDa protein, 59 kDa protein, 41.3 kDa protein, 63.5 kDa protein, and 76 kDa protein. Our result showed that multiple band protein of 41.3 ± 2 kDa, 59 ± 2 kDa, 63.5 ± 2 kDa, and 76 ± 2 kDa were predominantly emerged (Figure 1).

Our result shows that there are *S.mutans* ± 41.3 kDa protein expressions from 8 of 15 samples in control groups of caries subjects and 10 of 15 samples in control groups of caries-free subjects. In exposure groups of caries subjects, there are *S.mutans* ± 41.3 kDa protein expressions from 10 of 15 samples whereas in exposure groups of caries-free subjects, there are *S.mutans* ± 41.3 kDa protein expressions from 8 of 15 samples (Figure 2). On the other hand, there are *S.mutans* ± 59 kDa protein expressions from 8 of 15 samples in both control groups of caries and caries-free subjects. In exposure groups of caries subjects, there are *S.mutans* ± 59 kDa protein expressions from 6 of 15 samples whereas in exposure groups of caries-free subjects there are

S.mutans ± 59 kDa protein expressions from 3 of 15 samples (Figure 3).

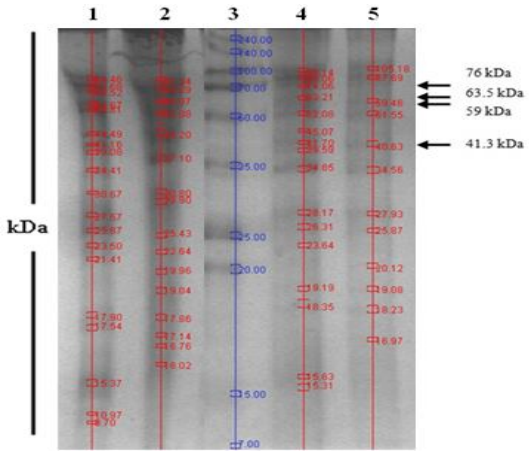

Fig.1. Protein profile of *S.mutans* identified as molecular weight from Gel-doc Bio-Rad. Lane 1: Caries-free subject (control), Lane 2: Caries subject with IgY anti *S.mutans*, Lane 3: Marker, Lane 4: Caries-free subject with IgY anti *S.mutans*, Lane 5: Caries subject (control)

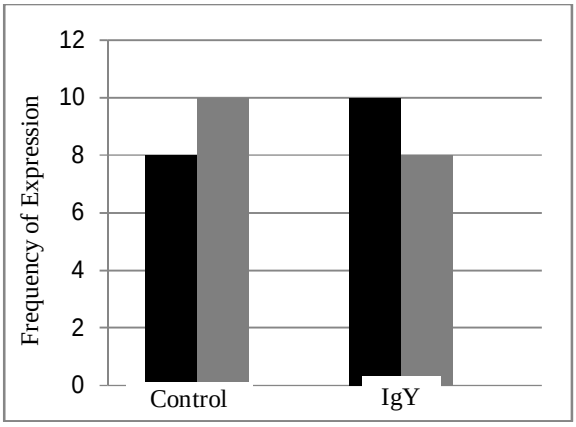

Fig.2. IgY anti *S.mutans* effect on *S.mutans* ± 41.3 kDa protein expression. Black bars, caries subjects; Grey bars, caries-free subjects.

Moreover, there are *S.mutans* ± 63.5 kDa protein expressions from 7 of 15 samples in both control groups of caries and caries-free subjects. In exposure groups of caries subjects, there are *S.mutans* ± 63.5 kDa protein expressions from 2 of 15 samples whereas in exposure groups of caries-free subjects there are *S.mutans* ± 63.5 kDa protein expressions from 4 of 15 samples (Figure 4). Meanwhile, there are *S.mutans* ± 76 kDa protein expressions from 2 of 15 samples in

both control groups of caries and caries-free subjects. In exposure groups of caries subjects, there are *S.mutans* ± 76 kDa protein expressions from 6 of 15 samples whereas in exposure groups of caries-free subjects there are *S.mutans* ± 76 kDa protein expressions from 4 of 15 samples (Figure 5).

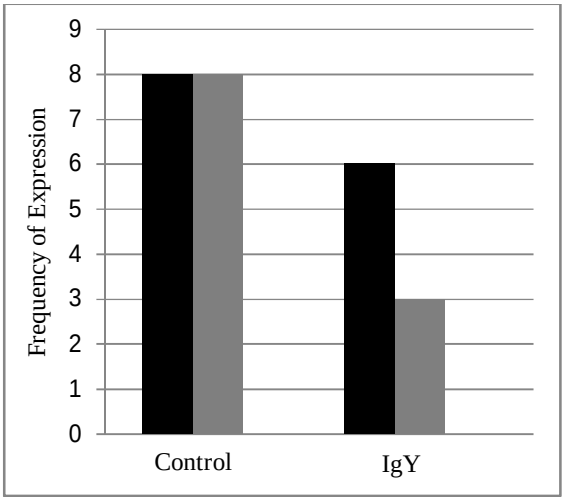

Fig.3. IgY anti *S.mutans* effect on *S.mutans* ± 59 kDa protein expression. Black bars, caries subjects; Grey bars, caries-free subjects.

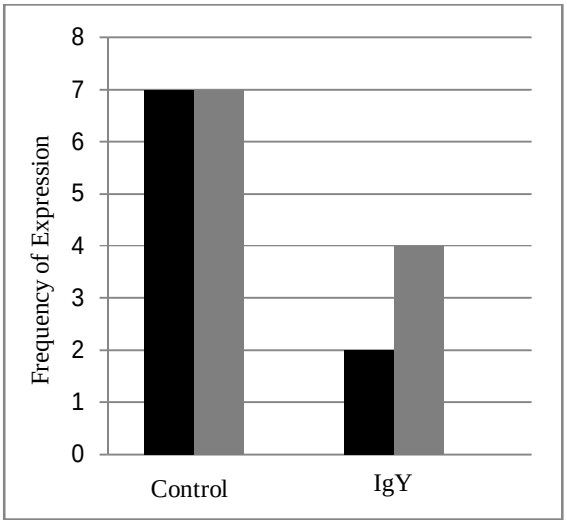

Fig.4. IgY anti *S.mutans* effect on *S.mutans* ± 63.5 kDa protein expression. Black bars, caries subjects; Grey bars, caries-free subjects.

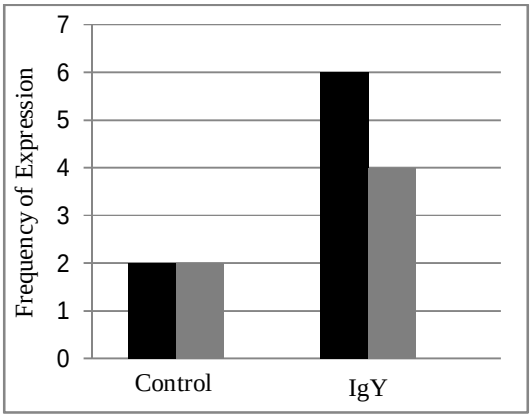

Fig.5. IgY anti *S.mutans* effect on *S.mutans* ± 76 kDa protein expression. Black bars, caries subjects; Grey bars, caries-free subjects.

Discussion

The result of this study suggests that changes in protein expression of *S.mutans* are caused by the effect of IgY anti *S.mutans.* In both control and exposure groups whether caries and caries free subjects, multiple band proteins that were predominantly emerged are Gbp. *S,mutans* secretes distinct proteins with glucan-binding activity: GbpA, GbpB, GbpC, and GbpD. [5] Mattos-Granner *et al.* reported that a calculated molecular weight of 41.3 kDa is GbpB protein and Banas *et al*. reported that GbpA protein has molecular weight of 59 kDa. [1] Moreover, molecular weight of 63.5 kDa is reported as GbpC by Sato *et al.* and Shah *et al.* reported that GbpD protein has molecular weight of 76 kDa. [15, 16]

The absence of AgI/II is probably caused by the role of AgI/II that is required in the initial colonization of dental biofilm in which it interacts with receptor of adhesin on the tooth pellicle. [17] Besides, AgI/II interacts with salivary glycoprotein prevented its attachment to hydroxyapaptite, so that AgI/II expression can be found in planktonic *S.mutans*. [18] Meanwhile, the explanation that supports the absence of Gtf expression in this study is that Gtf expression is only found in *S.mutans* in saliva because glucan synthesized from sucrose by Gtf is present in human saliva. [9]

GbpA and GbpC protein expression in both exposure groups of caries and caries-free subjects are down-regulated in comparison to the control groups. Meanwhile, in both exposure groups of caries and caries-free subjects are up-regulated in comparison to the control groups. On the other hand, GbpB protein expression of exposure groups in caries subjects up-regulated in comparison to the control groups. However GbpB protein expression of exposure groups in caries-free subjects is down-regulated.

This present study provides an information that there is different response in *S.mutans* isolated from dental caries and caries-free subjects after the exposure of IgY anti

S.mutans, especially in expression of GbpB. Smith *et al*. demonstrated that IgY anti *S.mutans* GBP-B developed reduction of *S.mutans* colonies and caries index relative to the control. [19] The present study, GbpB expression in exposure groups of caries-free subjects is down-regulated in comparison to the control groups. Regarding to Smith *et al*. demonstration, down-regulation of GbpB expression can cause the reduction of *S.mutans* colonies. This is consistent with the role of GbpB in growth and construction of cell wall of *S.mutans*. According to the literature, GbpA and GbpC are present on the cell wall surface of *S.mutans*. [5] In this present study, GbpA and GbpC expressions are dow-regulated probably because of *S.mutans* colonies reduction.

However, in exposure groups of caries subjects, GbpB protein expressions are up-regulated in comparison to the control groups. This is probably because of IgY anti *S.mutans* that is derived from whole cell mutans or not GbpB specific, so that probably cause IgY anti *S.mutans* binds with another protein epitop, because of which there is changes in molecular weight of those proteins resembling the molecular weight of GbpB.

Conclusions

IgY anti *S.mutans* up-regulates ± 41.3 kDa protein expression of *S.mutans* in the caries subject and down-regulates ± 41.3 kDa protein expression of *S.mutans* in the caries-free subjects. Western blot analysis needs to be performed in order to confirm the specific protein that bind to IgY anti *S.mutans*.

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