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RINGKASAN

Analisis Molekuler Ekspresi Anomali Protein Mukosa Mulut dan Profil Subklas Antibodi Pada *Recurrent Aphthous Stomatitis(RAS)*

Diah Savitri Ernawati

Recurrent Aphthous Stomatitis (RAS) merupakan salah satu kelainan pada mukosa mulut yang ditandai adanya ulser yang rekuren. Lesi ini seringkali ditemukan di rongga mulut serta telah diteliti oleh banyak ahli, tetapi etiologi *RAS* secara molekuler sampai saat ini belum diketahui dengan pasti. Adanya ekspresi anomali protein mukosa mulut merupakan salah satu faktor yang dapat memodulasi reaksi lokal, sehingga dapat berperan sebagai salah satu faktor pemicu timbulnya *RAS*.

Penelitian ini secara umum bertujuan untuk mengungkap secara molekuler salah satu etiopatogenesis *RAS* melalui analisis ekspresi anomali protein pada mukosa mulut serta profil subklas antibodi dan gambaran klinis RAS. Tujuan khusus dari penelitian ini adalah untuk : 1) Mengkarakterisasi anomali protein spesifik *RAS* (predominan) yang terekspresi pada penderita *RAS.* 2) Menganalisis ekspresi anomali protein mukosa mulut dengan gambaran klinis *RAS.* 3) Menentukan profil subklas antibodi dengan gambaran klinis *RAS.* 4) Membuktikan antibodi monoklonal spesifik anomali protein mempunyai sensitivitas dan spesifisitas terhadap *RAS.*

Manfaat penelitian ini secara teoritis adalah memberi informasi tentang etiopatogenesis timbulnya *RAS* secara molekuler, melalui analisis ekspresi anomali protein anomali pada sel epitel mukosa mulut yang mempunyai sifat antigenik, sehingga dapat menginduksi antibodi humoral dan seluler. Oleh karena itu hasil penelitian ini dapat digunakan untuk mengembangkan IPTEK di bidang *Oral immunology.* Berdasar pada hasil penelitian diatas secara praktis dapat digunakan sebagai petanda untuk menentukan tipe *RAS* mayor, minor dan remisi sehingga tatalaksana penanganan *RAS* menjadi lebih sempurna. Selain

itu juga dengan ditemukannya anomali protein spesifik *RAS* yang mempunyai sifat reaktif terhadap antibodi, maka dapat digunakan sebagai bahan alternatif/ indikator dini untuk pencegahan atau pengobatan. Juga dapat digunakan sebagai salah satu marker dan kit diagnostik.

Penelitian ini merupakan penelitian observasional eksploratif dan analitik dengan menggunakan rancangan *cross sectional*, yang pelaksanaannya di bagi menjadi tahap pertama yaitu karakterisasi secara klinis penderita *RAS*, sehingga dapat ditentukan tipe *RAS*, kemudian di lakukan koleksi sampel. Tahap ke dua adalah mengkarakterisasi anomali protein yang diekspresikan oleh penderita *RAS*. Tahap ke tiga adalah analisis profil antibodi subklas pada serum penderita *RAS*. Tahap ke empat adalah analisis spesifisitas dan sensitivitas antibodi monoklonal spesifik *RAS*.

Karakterisasi klinis di lakukan dengan cara wawancara dan pemeriksaan klinis serta pengisian formulir kuesioner. Kasus *RAS* banyak ditemukan pada usia 10-59 tahun, dan paling banyak ditemukan pada wanita sebesar 65,7 % dari kasus *RAS* dan pria 12 penderita atau 34,2 %. Berdasarkan usia yang paling banyak menderita *RAS* adalah antara usia 20-29 tahun, dengan frekuensi kekambuhan setiap tahun 3-12 kali.

Karakterisasi anomali protein yang di ekspresikan pada permukaan epitel mukosa mulut di lakukan dengan analisis SDS-PAGE 12% dan *Westemblot* dari sampel hasil swab protein mukosa mulut dan serum, diambil dari 15 penderita *RAS* mayor, 20 penderita *RAS* minor, 15 penderita RAS fase remisi dan 15 penderita bukan *RAS* (kontrol), ditemukan beberapa protein yang kurang spesifik oleh karena itu diperlukan pemurnian dengan sephadex G 125 kromatografi. Hasil kromatografi protein dari pasien *RAS* ditemukan beberapa protein yang berbeda, tetapi menunjukkan kemurnian yang cukup baik. Hal ini dapat dilihat pada pita yang muncul setelah dilakukan pewarnaan *silver nitrat* ($AgNO_3$) terlihat hanya satu pita yang muncul pada setiap lajur.

Hasil karakterisasi protein setelah dimurnikan untuk menghilangkan kontaminan pada fraksi protein yang berasal dari kasus *RAS* mayor ditemukan 5 pita dengan berat molekul 87 kDa, 65 kDa, 30 kDa, 25 kDa dan 20 kDa. Begitu

juga fraksi protein dari kasus RAS minor ditemukan beberapa macam protein yang mempunyai berat molekul sama dengan kasus *RAS* mayor, hanya protein yang mempunyai berat molekul 30 kDa tidak ditemukan pada kasus *RAS* minor, demikian juga pada kasus *RAS* remisi.

Hasil analisis protein dengan *westernblot* menunjukkan, bahwa tidak semua protein dapat bereaksi dengan antibodi poliklonal anti *whole protein* pada serum kelinci. Gambaran pita protein yang dapat bereaksi dengan antibodi poliklonal adalah fraksi protein yang berasal dari kasus *RAS* mayor dan ditemukan empat macam pita protein dengan berat molekul 20 kDa, 25 kDa, 65 kDa dan 87 kDa. Sedang protein yang berasal dari fraksi protein kasus *RAS* minor hanya ditemukan dua macam pita protein yaitu protein dengan berat molekul 65 kDa dan 25 kDa. Walaupun sangat tipis, tetapi fraksi protein yang berasal dari kasus RAS remisi ditemukan dua macam protein yang mempunyai berat molekul beda dengan kasus *RAS* minor yaitu ditemukan protein dengan berat molekul 65 kDa dan 87 kDa. Hasil ini menunjukkan bahwa protein yang tampak setelah dilakukan analisis *Westernblot* mempunyai arti penting dalam respon imun, terutama daya reaktifitas protein terhadap antibodi.

Pemurnian protein predominan dengan elusi menunjukkan tingkat kemurnian yang tinggi, dan ditemukan satu pita protein dengan berat molekul 65 kDa yang mempunyai sifat reaktivitas tinggi dan kemungkinan protein ini yang mempunyai peranan penting dalam menginduksi antibodi pertamakali. Untuk mengetahui homogenitas molekul protein yang terkandung dalam protein mukosa mulut *RAS* dilakukan analisis menggunakan *scan densitometri*, didapatkan grafik satu puncak dengan ketinggian 522.5 mm dan luas area 39678.9 mm, ini menunjukkan suatu molekul protein dominan yang spesifik pada penderita *RAS* dan mempunyai sifat stabil. Juga dapat dibuktikan bahwa protein tersebut mampu menginduksi respon imun seluler dan terbukti dengan uji natif imunofloresen tampak sel T aktif.

Hasil uji analisis subklas imunoglobulin dari serum pasien menujukkan kesamaan dan sangat signifikan dengan imunoglobulin yang disekresikan oleh sel hibrid, jika dibandingkan dengan kontrol. IgG2a mempunyai titer yang paling tinggi dengan pengenceran 10^{-5} jika dibandingkan imunoglobulin lainnya.

Hasil analisis varians (Anova) satu jalur menunjukkan profil subklas antibodi IgG2a, IgG3 dan IgA pada serum penderita *RAS* menunjukkan perbedaan yang signifikan dibanding kontrol ($p<0.05$). Analisis varians satu jalur profil subklas antibodi IgG2a,IgG3 dan IgA pada *whole* protein maupun protein elusi penderita *RAS* menunjukkan perbedaan yang sangat signifikan ($p<0.05$). Hasil uji LSD kadar IgG2a,IgG3 dan IgA pada serum penderita antara *RAS* mayor dan minor tidak menunjukkan perbedaan yang signifkan, demikian juga untuk kadar kadar IgG2a,IgG3 dan IgA pada *whole* protein dan protein elusi penderita *RAS* mayor, minor maupun remisi

Penelitian tahap ke empat adalah *typing* imunoglobulin serta uji spesifisitas dan sensitivitas antibodi monoklonal spesifik anomali protein. Antibodi monoklonal anti anomali protein dengan berat molekul 65 kDa dilakukan dengan cara hibridisasi sel limfosit dan sel mieloma. Hasil hibridisasi sel limfosit dan sel mieloma ditemukan beberapa macam *subtyping* antibodi monoklonal IgG1, IgG2a, IgG2b, IgG3, IgA, IgM, *kappa chain* dan *lambda chain.* Hasil identifikasi tipe (isotyping) antibodi monoklonal terhadap klon sel hibrid dengan menggunakan uji ELISA ditemukan urutan titer yang tinggi yaitu mulai IgG2a, *kappa chain* (κ- chain), IgG3, IgG2b, IgG1, IgA,IgM dan *lambda chain* (λ-chain).

Uji sensitivitas dan spesifisitas subklas antibodi monoklonal IgG2a menunjukkan bahwa IgG2a mempunyai tingkat sensitivitas yang tinggi (94,3%) terhadap anomali protein mukosa mulut penderita *RAS* (mayor, minor dan remisi), sedang tingkat spesifisitasnya adalah 86,7 %. Untuk antibodi monoklonal subklas IgA mempunyai tingkat sensitivitas 88,6% dan spesifisitasnya 80%. Untuk subklas antibodi monoklonal igG3 mempunyai tingkat sensitivitasnya 91,4% dan spesifisitas 93,3%.

Semua hasil dari rangkaian tahapan penelitian telah di temukan protein anomali predominan yang di ekspresikan pada penderita *RAS* dengan berat

molekul (BM) 65 kDa. Anomali protein predominan spesifik *RAS* bersifat antigenik. Ekspresi anomali protein mukosa mulut pada penderita *RAS* tipe mayor secara kualitatif ditemukan beberapa pita protein dengan BM 87 kDa, 65 kDa, 25 kda dan 20 kDa. Untuk *RAS* minor ditemukan dua macam pita protein dengan BM 65 kDa dan 25 kDa sedang pada RAS remisi ditemukan dua pita protein masing-masing dengan BM 87 kDa dan 65 kDa. Profil subklas antibodi IgG2a dan IgG3 serta Ig A pada *RAS* mayor, *RAS* minor serta remisi mempunyai titer yang lebih tinggi dibanding penderita kontrol (normal). Antibodi monoklonal subklas IgG2a, IgG3 dan IgA spesifik protein 65 kDa mempunyai spesifisitas dan sensitivitas tinggi.

Sebagai saran masih perlu dilakukan penelitian lanjutan peranan protein predominan lainnya seperti protein 87 kDa, 30 kDa, 25 kDa, sehingga ditemukan etipatogenesis *RAS* secara molekuler yang komprehensif. Selain itu juga perlu dilakukan uji lapangan (*field trial*) dari antibodi monoklonal hasil hibridisasi terhadap pasien *RAS* sebagai kit diagnostik.

SUMMARY

THE MOLECULAR ANALYSIS ON THE EXPRESSION OF PROTEIN ANOMALY IN ORAL MUCOSA AND THE PROFILE OF ANTIBODY SUBCLASSES IN RECURRENT APHTHOUS STOMATITIS

Diah Savitri Ernawati

Recurrent Aphthous Stomatitis (RAS) is one of the abnormalities in oral mucosa, characterized by the presence of recurrent ulcer. The lesion, which is frequently found in oral cavity, has long been investigated by many researchers. However, the molecular etiology of RAS remains unclear. The expression of protein anomaly in oral mucosa is one of the factors that may modulate local reaction and plays role as a one of triggering factors in the emergence of RAS.

The general objective of this study was to disclose one of the etiopathogenesis of RAS at molecular level by analyzing the expression of protein anomaly in oral mucosa and the profile of antibody subclasses with the clinical presentation of RAS. The particular objectives of this study were as follows: 1). To characterize RAS-specific (predominant) protein resulting from the expression in RAS patients. 2) To analyze the expression of oral mucosa protein anomaly with the clinical presentation of RAS. 3) To determine the profile of antibody subclasses with the clinical presentation of RAS. 4) To prove that protein anomaly-specific monoclonal antibody has sensitivity and specificity against RAS. The theoretical benefit of this study were that it can provide information on the etiopathogenesis of RAS at molecular level through the analysis of protein anomaly expression in oral mucosal epithelial cells, which has antigenic characteristics, so that it can induce humoral and cellular immune response. Therefore, the results of this study can be used to develop science and technology in oral immunology. The practical benefit of this study was that the results can be used as marker to determine RAS types as major, minor, or remission, to improve RAS management. In addition, the antibody-reactive RAS-

specific protein anomaly can also be used as alternative material or early indicator for prevention and treatment, as a marker and diagnostic kit.

This was an explorative and analytic observational study using cross-sectional design. The implementation of this study was divided into four steps. The preparatory step consisted of the clinical characterization of patients with RAS to determine RAS type and sample collecting. The second was the characterization of protein anomaly expressed in RAS patients' oral mucosa. The third step was the analysis of subclass antibody profile in RAS patients' serum. The final step was the analysis of RAS-specific monoclonal antibody specificity and sensitivity.

Clinical characteristics was carried out by interview, clinical examination, and filling the questionnaire. RAS cases were mostly found in patients aged 10 - 59 years, most were female (65,7 %) from RAS cases, while male patients were only 34,2 %. Based on age, most patients were 20 - 29 years old, with recurrent frequency 3 - 12 times annually.

The characterization of protein anomaly expressed on the surface of oral mucosa epithelial cells was undertaken using 12% SDS-PAGE and Westernblot to samples taking from serum and oral mucosa protein swab from 15 patients with major RAS, 20 patients with minor RAS, and 15 non-RAS patients (control). The results revealed some less-specific protein, requiring purification by means of sephadex G 125. The results of protein chromatography from RAS patients showed some different proteins, but with sufficient purity, as proved from appearing bands after staining with silver nitrate ($AgNO_3$), in which only one band appeared in each line.

The results of characterization of protein, which had been purified to eradicate contaminants, showed that protein fractions from major RAS cases had 5 bands with molecular weights of 87, 65, 30, 25, and 20 kDa. Protein fractions from minor RAS cases revealed several types with the same molecular weights as those from major RAS cases, except protein with molecular weight of 30 kDa. Similar findings were also found in remission RAS cases.

The results of protein analysis using Westernblot showed that not all protein could react with anti-whole protein polyclonal antibody in rabbit serum. Protein bands that could react with polyclonal antibody were those from major RAS cases, in which there were four protein bands with molecular weights of 20, 25, 65, and 87 kDa. From protein of minor RAS cases, only two bands were found with molecular weights of 65 and 25 kDa. Although very thin, protein fractions from remission RAS cases revealed two proteins different from those in minor cases. They were those with molecular weights of 65 and 87 kDa. These results indicated that protein appeared after Westernblot analysis had an important role in immune response, particularly in protein reactivity against antibody.

Predominant protein purification using Elusion showed a high purity level, and one band with molecular weight of 65 kDa had a high reactivity and an important role in the first induction of antibody. To identify the homogeneity of protein molecules contained in RAS patients' oral mucosa protein, densitometry scan was carried out, revealing one-peak graph with a height of 522.5 mm and widht of 39678.9 mm. This indicated a stable predominant specific protein molecule in RAS patients, which was able to induce cellular immune response as proved from immunofluorescence native test that revealed active T cells.

The results of immunoglobulin subclass analysis from patients' serum showed significant similarities to those with immunoglobulins secreted by hybrid cells, as compared to control. The results of one-way variance analysis (Anova), which showed the profile of antibody subclasses IgG2a, IgG3 and IgA in the serum of RAS patients, indicated significant difference from that of control ($p < 0.05$). The results of LSD test showed that IgG2a,IgG3 and IgA levels in the serum of major and minor RAS patients were not significantly different, and neither were the levels of IgG2a,IgG3 dan IgA in whole-protein and eluted protein in major, minor, as well as remission RAS patients.

The fourth stage of this study was immunoglobulin typing and protein anomaly specific monoclonal antibody specificity and senstivity test. Anti protein anomaly monoclonal antibody with molecular weight of 65 kDa was identified

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ABSTRACT

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Diah Savitri Ernawati

The general purpose of this study was to disclose one of the etiopathogenesis of recurrent aphthous stomatitis (RAS) at molecular level by analyzing the expression of protein anomaly in oral mucosa, and the profile of antibody subclasses in clinical presentation of RAS.

This was a cross-sectional explorative and analytic observational study. Samples, who met inclusion and exclusion criteria, were taken from total population. Samples of protein swab obtained from oral mucosa, and serum were taken from 15 patients with major RAS, 20 patients with minor RAS, and 15 were control. The characterization of protein anomaly expressed on the surface of oral mucosa epithelium was carried out using SDS-PAGE 12% and Westernblot methods.

The result of oral mucosal protein anomaly expression analysis in patients with major RAS using SDS-PAGE 12% revealed five protein bands with molecular weights of 87, 65, 30, 25, and 20 kDa. In minor RAS cases with protein anomaly expression, there were four proteins with molecular weights of 87, 65, 25, and 20 kDa, and the protein in remission RAS had four protein bands with molecular weights of 87, 65, 25, and 20 kDa. The band disappearances, by using western blot test, of 30 kDa of major cases, 87 and 20 kDa of minor cases and 20 and 25 kDa of remission cases, indicated that those patients were not reacted with polyclonal antibodies of rabbit serum, there fore they had no role in the induction of RAS.

One-way variance analysis (Anova) revealed that the profile of antibody subclasses of IgG2a, IgG3 and IgA in RAS patients' serum had significant difference compared to control ($p<0.05$). Likewise, one-way variance analysis of

the antibody subclasses of IgG2a, IgG3 and IgA in whole-protein or eluted protein in RAS patients revealed highly significant differences ($p < 0.05$).

The results of LSD test no significant differences were observed in IgG2a, IgG3 and IgA levels between serum patients of major and minor RAS types, as well as in IgG2a, IgG3 and IgA levels in whole protein compared to eluted protein. Sensitivity and specificity test on IgG2a showed that this antibody had a high sensitivity level (94.3%) towards oral mucosal protein anomaly in RAS patients (major, minor, and remission), while the specificity level was 86.7%. For IgA monoclonal antibody, the sensitivity level was 88.6%, and the specificity was 80%. Finally IgG3 the sensitivity was 91.4% and specificity 93.3%.

In conclusion, the antigenic protein expressed in oral mucosa of major, minor, and remission RAS was predominantly 65 kDa molecular weight. Based on antibody level, IgG2a, IgG3 and IgA in mayor, minor and remission have significant higher compared to control. IgG2a, IgG3 and IgA MoAb have a high specificity and sensitivity to a protein anomaly of 65 kDa.

Keywords: RAS; predominant protein; protein anomaly 65 kDa; profile of antibody subclasses; sensitivity and specificity