RINGKASAN

MUTASI GEN PENYEBAB DEFISIENSI GLUKOSA 6 FOSFAT DEHIDROGENASE (G6PD) DI SURABAYA DAN KEPULAUAN MALUKU TENGGARA

Suhartati

Defisiensi G6PD merupakan suatu kelainan metabolik bawaan sejak lahir (*inborn error of metabolism)* yaitu cacat genetik akibat mutasi gen yang bersifat resesif terkait X. Mutasi ini dapat menyebabkan cacat pada mekanisme katalitik, tempat pengikatan substrat (*substrat binding* atau *koenzim binding*) sehingga dapat menurunkan aktivitas G6PD.

Populasi Indonesia memiliki variasi genetik beraneka ragam. Daerah pesisir merupakan daerah terbuka misalnya Surabaya dengan penduduk yang mempunyai mobilitas tinggi, sehingga terdapat percampuran berbagai variasi genetik dan jarang ditemukan malaria. Di daerah tersebut besar kemungkinan terjadi pembauran (perkawinan) antar satu populasi dengan populasi lain yang berbeda, sehingga memungkinkan terciptanya kombinasi genetik. Kepulauan Maluku Tenggara merupakan salah satu daerah endemik malaria dan sebagai daerah terisolasi. Pemberian anti malaria di daerah ini misal primaquin sebagai bahan oksidan tanpa dilakukan uji saring defisiensi G6PD terlebih dahulu, akan menimbulkan gejala klinik yang tidak diinginkan.

Sebagian besar defisiensi G6PD tidak menunjukkan gejala klinis, sehingga pemahaman mengenai akibat-akibat yang mungkin timbul pada penderita defisiensi G6PD yang terpapar bahan oksidan masih belum sepenuhnya dipahami, oleh sebab itu kepentingan diagnosis dini belum sepenuhnya disadari. Penderita G6PD tidak menyadari kalau pemberian bahan oksidan akan menimbulkan gejala klinik misal anemia hemolitik, hepatitis yang berulang, keguguran dan lain-lain.

Penelitian ini mempunyai tujuan untuk menganalisis mutasi gen defisiensi G6PD di Surabaya dan Maluku Tenggara antara lain pulau Kur, Tanimbar, Babar dan Romang Penelitian ini adalah penelitian eksploratif observasional laboratorik, ditinjau dari paradigma biologi molekuler dan genetika populasi. Metode untuk menentukan sebagai penderita defisiensi G6PD di Surabaya dengan di kepulauan Maluku Tenggara berbeda. Di Surabaya sampel berasal dari Rumah Sakit Katolik St Vincentius A Paulo pemeriksaan aktivitas G6PD menggunakan Randox Kit dengan persyaratan bahwa aktivitas G6PD kurang dari 60% dari normal adalah defisiensi G6PD. Di Maluku Tenggara sampel berasal dari orang yang datang berobat secara cuma-cuma di Bakti Sosial Surya Baskara TNI AL Indonesia ke LXII, pada pemeriksaan *Formazan Ring Test* dengan diameter kurang dari 7 mm dinyatakan defisiensi G6PD. Dari sampel darah diambil DNA dengan menggunakan metode *simple rapid genomic DNA.* Konformasi mutasi dilakukan dengan metode PCR, pertama untuk mendapatkan target DNA dan kedua untuk deteksi mutan gen G6PD menggunakan metode MPTP (*Multiplex PCR using Multiple Tandem forward Primers and a Common reverse primer*) pada ekson 5, 6, 9, 11 dan 12 sesuai anjuran Shirakawa.

Secara singkat beberapa aspek telah dipelajari dalam penelitian ini sebagai berikut:

1. Tujuh jenis mutan G6PD di Surabaya pada penelitian ini semua tergolong mutasi titik antara lain :
   a) 4 kasus G6PD Vanua Lava terjadi mutasi pada urutan nukleotida 383, dari timin menjadi sitosin (T 383 C), sehingga asam amino kodon 128 terjadi perubahan dari leusin menjadi prolin (L 128 P)
   b) 1 kasus G6PD Viangchan terjadi mutasi pada urutan nukleotida 871 dari guanin menjadi adenin (G871A), sehingga asam amino kodon 291 terjadi perubahan dari valin menjadi metionin (V291M)
   c) 5 kasus G6PD Chatham terjadi mutasi pada urutan nukleotida 1003 yaitu dari guanin menjadi adenin (G1003A), asam amino kodon 335 terjadi perubahan dari alanin menjadi treonin (A 335 T)
   d) 2 kasus G6PD Union terjadi mutasi pada urutan nukleotida 1360 yaitu substitusi dari sitosin menjadi timin (C 1360 T), asam amino kodon 454 terjadi perubahan arginin menjadi sistein (R 454 C)
   d) 7 kasus G6PD Canton terjadi mutasi pada urutan nukleotida 1376 dari guanin menjadi timin (G 1360 T), terjadi perubahan asam amino kodon 459 dari arginin menjadi leusin (R 495 L).
   e) 6 kasus G6PD Kaiping terjadi substitusi pada urutan nukleotida 1388 dari guanin menjadi adenin (G 1388 A), sehingga asam amino kodon 463 terjadi perubahan dari arginin menjadi histidin (R 463 H)
   f) 2 kasus G6PD Silent terjadi substitusi pada urutan nukleotida ke 1311 dari sitosin menjadi timin (C 1311 T), tetapi tidak terjadi perubahan asam amino tirosin pada kodon 437

2. Tiga jenis mutan G6PD di kepulauan Maluku Tenggara yang ditemukan di penelitian ini juga tergolong mutasi titik antara lain : 5 kasus G6PD Vanua Lava di pulau Tanimbardan 1 kasus di pulau Romang, 1 kasus G6PD Kaiping di pulau Romang dan 2 kasus G6PD Chatham di pulau Babar. Di pulau Kur tidak didapatkan mutan G6PD

3. Sampel defisiensi G6PD hasil negatif pada pemeriksaan MPTP adalah : 5 sampel dari 31 kasus defisiensi G6D di Surabaya dengan pemeriksaan Random Kit test positif, tetapi negatif pada pemeriksaan MPTP dan 2 kasus dengan G6PD Silent.. Di Maluku Tenggara didapatkan 3 sampel dari 12 kasus defisiensi G6PD didapatkan positif dengan pemeriksaan Formazan ring tetst, tetapi negatif pada pemeriksaan MPTP Hal ini dapat terjadi karena didapatkan nilai positif palsu pada uji saring *Formazan* atau ada mutasi gen G6PD tetapi tidak terdeteksi melalui metode Shirakawa. Oleh karena itu perlu diteliti lebih lanjut untuk menentukan mutan G6PD dengan metode lain, tidak terlupakan penggunaan sekuensing.

4. Diversitas genetik .
   Mutan gen G6PD di Surabaya ditemukan ada 7 jenis. Hal ini dapat disebabkan letak kawasan yang terbuka dengan mobilitas penduduk yang tinggi serta heterogenik dan adanya proses *genetic flow*. Kepulauan Maluku Tenggara karena letak geografis yang terisolasi oleh laut, adanya *Founder effect* dan *genetic drift* ditemukan hanya 3 jenis mutan gen G6PD

5. Defisiensi G6PD dan keguguran.
   Sembilan (9) kasus defisiensi G6PD didapatkan pada sampel wanita dengan riwayat keguguran di Surabaya yaitu: 3 kasus G6PD Canton, 3 kasus G6PD Kaiping, 1 kasus G6PD Vanua Lava dan 2 kasus dengan hasil negatif pada MPTP.

6. Prevalensi G6PD.
   Sebagian besar gejala klinis defisiensi G6PD adalah asimptomatik. Gejala klinis akan nampak bila penderita defisiensi G6PD terpapar senyawa oksidan antara lain anemia hemolitik, hepatitis yang berulang, keguguran yang berulang dan sebagainya, maka perlu dilakukan uji saring G6PD pada populasi di Indonesia terutama daerah yang diperkirakan terdapat defisiensi G6PD. Prevalensi defisiensi G6PD di beberapa daerah di Indonesia prosentasinya bervariasi, menentukan diagnosis awal sangat menguntung kan bagi penderita defisiensi G6PD.

SUMMARY

G6PD DEFICIENCY RESULTING FROM GENETIC MUTATION IN SURABAYA AND SOUTH-EAST MALUKU

Suhartati

G6PD deficiency is an X-linked recessive hereditary metabolic disorder caused by G6PD gene mutation. This mutation may caused defects on location of either substrate or co-enzyme binding sites thus causing defects in the enzyme's catalytic mechanism.

Indonesia shows differences in population diversities. Area like Surabaya and surroundings is open area, while malaria is a relatively rare, whereas South-East Maluku islands are isolated areas where malaria is highly endemic, in which the use of primaquine as an anti-malarial drug may cause problem due to its oxidant properties. Coastal areas like Surabaya and surroundings are areas where many people of different ethnicity live together and intermarriage thus offering the possibility of greater diversity as compared to isolated areas like South-East Maluku islands.

Most people suffering from G6PD are asymptomatic. They do not realize that they are prone to suffering from various disorders when they take oxidants such as certain drugs or foodstuffs. These disorders manifest itself as hemolytic anemia, repeated hepatitis, repeated miscarriages etc. That way an early diagnosis would be of advantage to these people.

The purpose of this study is to analyze G6PD mutation diversity in Surabaya and in small isolated islands in South-East Maluku : Babar, Tanimbar, Kur and Romang islands. This study is an exploratory, observational, laboratory study using molecular biology and population genetic approaches. The screening method used in Surabaya and South-East Maluku were differs. In Surabaya suspected patients were referred by physicians attending patients in the St Vincentius A Paulo Catholic Hospital Surabaya. These patients were screening using Randox Kit to determine the activity of G6PD, taking a cut-off value of less then 60% as indicating G6PD deficiency, whereas in South-East Maluku, G6PD deficiency were determined using the Formazan Ring Test. In this study, genomic DNA was obtained using a simple genomic DNA extraction method. The extracted DNA was then amplified by a two stages PCR to amplify certain exons of the G6PD gene. The first PCR is just to obtain sufficient target DNA which is then followed by a second PCR called Multiplex PCR using multiple Tandem forward Primers and common reverse primer (MPTP) to amplify exon 5, 6, 9, 11 and 12 of the G6PD gene according to Shirakawa.

Following are the results of this study:

Samples from Surabaya:

Sevens mutants were found, all were point mutation. These are:

1. Four cases of G6PD Vanua Lava, showing a change from T to C at nucleotide 383 (T 383 C) causing a substitution of amino acid 128 from leusin to prolin (L 128 P)

2. A single G6PD Viangchan, showing a change from G to A at nucleotide 871(G 871 A) resulting in a substitution of amino acid 291 from valine to methioine (V 291 M)
3. Five cases of Chatham, showing a change from G to A at nucleotide 1003 (G 1003 A) leading to a change of amino acid 335 from alanine to threonin (A 335 T)
4. Two cases of G6PD Union, showing a change from C to T at nucleotide 1360 (C 1360 T) resulting in a substitution of amino acid 454 from arginine to cysteine (R 454 C)
5. Seven cases of G6PD Canton, showing a change from G to T at nucleotide 1376 (G 1376 T) leading to a change substitution of amino acid 459 from arginine to leucine (R 459 L)
6. Four cases of G6PD Kaiping, showing a change from G to A at nucleotide 1388 (G 1388 A) leading to a change substitution of amino acid 463 from arginine to histidine (R 463 H)
7. A change of C to T at nucleotide 1311 (C 1311 T) which is a G6PD Silent mutation not causing a change in amino acid position 437, which tyrosine (Y)

Samples from South-East Maluku :

Three different mutants were found :

1. G6PD Chatham, 2 cases in Babar island
2. G6PD Vanua Lava , 5 cases in Tanimbar and 1 case in Romang island
3. G6PD Kaiping , a single case in Romang island

No G6PD mutant was found on Kur island

Undetected mutants:

Five samples from Surabaya tested positive with the Random Kit test but negative with MPTP, whereas in South-East Maluku, three tested positive with Formazan Ring test but negative with MPTP. The nature of these samples, which can be either false positives or mutants undetectable by the MPTP method need be further investigated using other available methods including sequencing if necessary.

Genetic diversity

Genetic diversity in Surabaya is much greater than that found in South-East Maluku islands. The greater diversity in Surabaya is probably due to genetic flow, and genetic drift whereas the much lower diversity in South-East Maluku is probably due to genetic drift and founder effect.

G6PD deficiency and repeated miscarriages

G6PD deficiency may be one cause of repeated miscarriages hitherto less well-known. In this study nine G6PD deficiency cases was found in women with a history of repeated miscarriages in Surabaya; 3 cases of G6PD Canton, 3 cases of G6PD Kaiping, one single case of G6PD Vanua Lava and 2 cases of G6PD deficiency tested negative with MPTP.

G6PD prevalence

G6PD deficiency is more or less prevalent in certain areas in Indonesia especially in open regions such as in the big cities with a population mixed. In view of the asymptomatic nature of the disorders, G6PD deficiency tests are recommended in special cases where G6PD deficiency is suspect.

ABSTRACT

G6PD DEFICIENCY RESULTING FROM GENETIC MUTATION IN SURABAYA AND SOUTH-EAST MALUKU

Suhartati

Glucose 6 phosphate dehydrogenase (G6PD) deficiency was studied in open Surabaya region and small isolated islands of South-East Maluku, where malaria is highly endemic. Seventy-six blood samples were collected from Surabaya and 298 samples from Kur, Tanimbar, Babar and Romang islands in South-East Maluku. The Surabaya samples were screened using Randox Kit to determine the activity of G6PD, taking a cut-off value of less than 60% as indicating G6PD deficiency, whereas in South-East Maluku G6PD deficiency was determined using the Formazan Ring test. Thirty one of Surabaya cases tested positive with the Randox Kit test, 26 of which tested positive with MPTP, whereas 12 of the South-East Maluku cases tested positive with the Formazan Ring Test, 9 of which tested positive with MPTP test.

In this study, genomic DNA from blood samples were extracted using simple rapid genomic DNA method. The extracted DNA was then used to amplify exon 5, 6, 9, 11, and 12 of the G6PD genes to detect mutants using Multiplex PCR with Multiple Tandem forward Primers and a common reverse primer (MPTP) technique according to Shirakawa. Seven different G6PD mutants were found in Surabaya: 4 cases of Vanua Lava, a single G6PD Viangchan cases, 5 cases of G6PD Chatham, 2 cases of G6PD Union, 7 cases of Canton , 6 cases of Kaiping and 2 cases of Silent mutation. Double mutants were also found, all involving silent mutations: Canton + Silent, Vanua Lava + Silent, and Viangchan + Silent, one case each. Three different mutants were found in South-East Maluku : Chatham, 2 cases in Babar island; Vanua Lava, 5 cases in Tanimbar and 1 case in Romang island and a single Kaiping case in Romang island. No G6PD mutant was found on Kur Island. Five samples from Surabaya tested positive with the Random Kit test, but negative with MPTP, whereas in South-East Maluku, three tested positive with Formazan Ring test but negative with MPTP. The nature of these samples, which can be either false positives or mutants undetectable by the MPTP method and two Surabaya cases with a Silent mutation when tested with MPTP need further investigation using other available methods including sequencing if necessary.

Nine cases of G6PD deficiency were found in women with a history of miscarriages in Surabaya: 3 cases of Canton, 3 cases of Kaiping, one single case of Vanua Lava and 2 tested negative with MPTP.

Fewer G6PD mutants were found in isolated islands of South-East Maluku as compared to that found in the open region of Surabaya. The mutant diversity in Surabaya is probably due to genetic flow and genetic drift whereas that in South-East Maluku to founder effect and genetic drift.

G6PD deficiency is usually asymptomatic. Symptoms will only appear if those persons ingest oxidants such as certain drugs or foodstuffs. The disorders manifest itself as hemolytic anemia, repeated hepatitis, repeated miscarriages etc. Since G6PD deficiency is more or less prevalent in Indonesia, an early diagnosis would be of advantage, so that people with G6PD deficiency could avoid ingesting oxidants

Keywords: G6PD deficiency, MPTP, Formazan Ring test, genetic flow, genetic drift, founder effect.