ABSTRAK

Mutasi titik gen *pfcrt* pada penderita malaria falciparum umur 1-18 tahun di Kabupaten Ogan Komering Ulu Provinsi Sumatera Selatan

Rosiana A Marbun

Temuan dalam beberapa tahun terakhir menyebutkan bahwa resistensi *P. falciparum* terhadap klorokuin terutama diperankan oleh mutasi pada gen *P. falciparum chloroquine resistance transporter (pfcrt).*Tujuan penelitian ini adalah untuk mengidentifikasi dan mendeskripsikan pola distribusi mutasi titik gen *pfcrt P. falciparum* berdasarkan demografi dan tofografi di Ogan Komering Ulu (OKU) Sumatera Selatan. Penelitian ini merupakan penelitian deskriptif eksploratorik pada anak berumur 1-18 tahun di 3 daerah endemis malaria yang mewakili OKU Sumatera Selatan yaitu Tanjung Lengkayap, Banding Agung, dan Belitang. Tahapannya adalah penentuan *spleen rate*, pengumpulan subyek, diagnosis malaria klinis, pemeriksaan mikroskopis sediaan tebal dan tipis spesimen darah, uji resistensi klorokuin *in vivo*, isolasi DNA, PCR, dan *sequencing*. Nilai *spleen rate* ke 3 daerah tersebut dikelompokan ke dalam kategori hipoendemis malaria. Diperoleh 171 sampel dengan malaria falciparum. Pada uji resistensi klorokuin *in vitro* 25 sampel gagal, dan 146 sampel berikutnya dilanjutkan uji resistensi *in vivo*, didapat 44 sampel yang resisten terhadap klorokuin. Berhasil dilakukan isolasi 168 sampel DNA gen *pfcrt*, PCR, dan *sequencing,* didapatkan mutasi titik pada kodon 74, 75, 76, 97, 220, dan 356 yaitu M74I, N75L, K76T, H97F, A220S, dan I356L. Pola distribusi mutasi titik di Tanjung Lengkayap, Banding Agung, dan Belitang 100% pada kodon 74,75,76, dan 356, sedangkan pada kodon 97 berturut-turut 44%, 46,9%, dan 60%, pada kodon 220 berturut-turut 40,5%, 46,9%, dan 60%. Kesimpulan adalah Distribusi mutasi titik pada kodon 97 dan 220 dijumpai paling banyak di Belitang. Didapatkan mutasi titik lokal baru pada kodon 75 dan 97 gen *pfcrt P. falciparum* belum pernah dijumpai di tempat lain.

Kata kunci: Malaria falciparum, klorokuin, resistensi, mutasi titik gen *pfcrt*

ABSTRACT

Point Mutations of Pfcrt Gene in Patients aged 1-18 years with Falciparum Malaria in Ogan Komering Ulu District in South Sumatra Province

Rosiana A Marbun

The findings in recent years state that the resistance of P. falciparum to chloroquine mainly caused by the mutation in the P. falciparum chloroquine resistance transporter (pfcrt) gene. The objectives of this study were to identify and describe the distribution pattern of point mutations P. falciparum pfcrt gene based on demographics and topography in Ogan Komering Ulu (OKU), South Sumatra. This study was a descriptive exploratory study in children aged 1-18 years in three malaria-endemic areas which represents OKU-South Sumatra, namely the Tanjung Lengkayap, Banding Agung, and Belitang. The stages of this research were the determination of spleen rate, collecting subjects, clinical malaria diagnosis, microscopic examination of thick and thin stocks of blood specimens, test of chloroquine resistance in vivo, DNA isolation, PCR, and sequencing. Based on the value of spleen rate, those 3 regions were grouped into hypoendemic malaria. One hundred seventy-one (171) samples were obtained with falciparum malaria observed by microscopic examination of thin blood supply. Twenty-five (25) samples failed the test of chloroquine resistance in vitro, and the next 146 samples would be proceeded to take resistance test in vivo which, later, it was found that 44 samples were resistant to chloroquine. One hundred sixty-eight (168) samples of pfcrt gene DNA were successfully analyzed by conducting isolation, PCR, and sequencing. As a result, a point mutation was found at codons 74, 75, 76, 97, 220, and 356, namely M74I, N75L, K76T, H97F, A220S, and I356L . The pattern of distribution of point mutations in Tanjung Lengkayap, Banding Agung, and Belitang was 100% in codons 74,75,76, and 356, while at codon 97 was respectively 44%, 46.9%, and 60%, , at codon 220 was respectively 40.5%, 46.9%, and 60%. This research concluded that distribution of point mutations at codons 97 and 220 were mostly found in Belitang. A new local point mutation at codons 75 and 97 of P. falciparum pfcrt gene was found. This has never been found in other places.

Keywords: falciparum malaria, chloroquine, resistance, point mutations of pfcrt gene

ABSTRACT

Point mutation *pfcrt* gene of malaria falciparum patients the group of 1-18 years old in Ogan Komering Ulu districts South Sumatera province

Rosiana A Marbun

The resistance of *Plasmodium* to antimalaria drugs is the most difficult factor to be overcomed because the mutation in *Plasmodium* genome can not be controlled. Some of *P. falciparum* strains are found around the world. The resistance strains survive because they can reduce accumulation of chloroquine in digestive vacuole. The recent studies showed that the resistance of *P. falciparum* to chloroquine is caused by a mutation in *P. falciparum chloroquine resistance transporter* (*pfcrt*) gene.The research aimed to indentify and describe point mutation distribution patterns of *pfcrt* gene based on topography (one aspect of geography) and demography differences.The study was molecular epidemiology with descriptive exploratoric approach of children 1-18 years old patients from 3 endemic malaria areas in South Sumatera:Tanjung Lengkayap OKU Induk is remote area, Banding Agung OKU Selatan is tourism area and Belitang OKU Timur is area with highly population mobilities.The steps of study were spleen rate survey, clinical malaria diagnosis, recruiting subject and collecting blood specimen, microscopic examination chloroquine *in vitro* test, chloroquine *in vivo* test, DNA isolation, PCR and sequencing. The results showed that 3 areas were hypuendemic malaria areas based on the average of spleen rate less than 10%. About 562 blood specimen samples based on clinical malaria diagnosis were defined with microscopic evamination and got 171 (30.4%) samples with falciparum malaria. The group of 5-12 years old were 94 (55%) patients. The history of rigur was dominant in Bamding Agung, 62.7%. Palmar pale dominant in Tanjung Lengkayap 39.9%, also spleen enlargement 20.2%.The history of antimalaria drug therapy 41.7% dominant in Tanjung Lengkayap. The temperature examination results > 37.5^{o}C dominant 58.3% in Belitang. Twenty five samples tried to *in vitro* test three times but the parasite did not growed, so the others 146 samples conducted to *in vivo* test. The results of *in vivo* test were 44 (30.1%) samples resistance to chloroquine. The 168 (98.5%) samples can be isolated of DNA for PCR and sequencing. The puint mutation were found in codons: M74I, N75L, K76T, H97F, A220S, AND I356L. One hundred percent of codons 74, 75, 76, and 356 were mutated. The mutation of codon 97 in Tanjung Lengkayap, Banding Agung, and Belitang 44%, 46.9%, and 60% respectively. The mutation of codon 220 in Tanjung Lengkayap, Banding Agung 40.5%, 46.9%, and 60% respectively. The conclusion: in three areas, the point of mutation was the same in codons 74, 75, 76, and 356, and getting variation at codons 97 and 220. The point mutation of codon 97 and 220 were dominant in Belitang. This study showed that the mutation was *multiple point mutation*. New local point mutation was found at codon N75L and H97F until know never were they find. Jelek

Key words: malaria falciparum, chloroquine, resistance , mutation of *pfcrt* gene

SUMMARY

Point mutation *pfcrt* gene of malaria falciparum patients the group of 1-18 years old in Ogan Komering Ulu districts South Sumatera Province

Malaria is one of the major world health problems with 300-500 million cases and 1.5-3.7 millions death annualy.There were 243 million cases and 863,,000 death and still high in 2008. According to *World Health Organization* (WHO) data, about 56% of world population live in endemic malaria area. In Indonesia, there are 45% of the population live in endemic malaria area also some area in South Sumatera including Ogan Komering Ulu (OKU) districts, *Annual Clinical Malaria Incidence* (AMI) out-side Jawa-Bali were 23.98‰ in 2006. *Annual Clinical Malaria Incidence* in South Sumatera 8.7‰ and 23.48‰ in Oku districts in 2009. Aproximately 61.4% of parasite causing malaria in Indonesia are *P. falciparum* also in OKU districts around 71,64% in 2008.

Chloroquine is a antimalaria agent of 4-amino quinoline group which is schizonrosidal to all kind of *Plasmodium* and also gametosidal to *P. vivax* and *P. malariae*. The drug were delivered to digestive vacuole to inhibit protein synthesis of *P. falciparum*.

P. falciparum resistance to antimalaria drugs are serous problems for selecting antimalaria drugs, patient management, prolong of hospitalized and increasing of mortality. *World Malaria Report* (2005) showed the endemic malaria with chloroquine resistance including South and Central America, West, Central and East Africa, South Asia, Melanesia Islands and Southeast Asia including Indonesia. Data of 1996 showed that *P. falciparum* chloroquine resistance were found in all province of Indonesia with varieties of prevalence. The average of *P. falciparum* chloroquine resistance *in vivo* in Indonesia was 52% and 59% *in vitro*. *P. falciparum* resistance *in vivo* are ability of parasite to survive human host, multiplication and produce of diseases although the treatment

were given properly. *P, falciparum* chloroquine resistance *in vitro* are maximum concentration of chloroquine that have capable to kill 50% parasite (IC_{50}). The mechanism of chloroquine reistance based on fault of chloroquine inhibited heme polymerase synthesis. *P. falciparum* continue to produce hematin to be hemozoin polymer. The strain of *P, falciparum* chloroquine resistance succed reducing chloroquine accumulation in digestive vacuole. The detail of resistance mechanisms remains unclear.

The data from all region of world showed 12 point mutations of *pfcrt* gene related to chloroquine resistance. The mutation could be cause chloroquine induction and spreading of spontaneous mutation from others area. Twelve points mutation caused alteration of amino acid codons: cysteine to serine in codon 72 (C72S), methionine to isoleucine in codon 74 (M74I), asparagine to glutamic acid or aspartic acid in codon 76 (N75E or N75D). lysine to threonine in codon 76 (K76T), histidine to leucine or glutamine in codon 97 (H97L or H97Q), alanine to threonine in codon 144 (A144T), lecine to tyrosine in codon 160 (L160Y), alanine to serine in codon 220 (A220S), glutamine to glutamic acid in codon 271 (Q271E), asparagine to serine in codon 326 (N326S), or aspartic acid (N326D), isoleucine to threonine or leucine in codon 356 (I356T or I356L)), and arginine to isoleucine or threonine in codon 371 (R371I or R371T). The point mutation distribution patterns related to geographic and demographic are still quenstionable.

The researcher was interested in studying about the point mutation patterns in three differences geographic and demographic areas in South Sumatera. The aim of this research is to identify and describe point mutation the distribution of pattern of *pfcrt* gene based on topography (one aspect of geography) and demography differences including remote area, tourism area and highly population mobility area. This molecular epidemiology with descriptive exploratoric approach of children 1-18 years old patients from 3 endemic malaria area in South Sumatera.Tanjung Lengkayap OKU Induk is a remote area, Banding Agung OKU Selatan is a tourism area and Belitang OKU Timur is an area with highly population mobility. The steps of study were spleen rate survey, clinical malaria diagnosis, subject recruitment and blood specimen collection, microscopic

examination, chloroquine *in vitro* test, chloroquine *in vivo* test, DNA isolation, PCR and sequencing.. The results showed that 3 areas were hypoendemic malaria areas based on the average of spleen rate less than 10%. About 562 blood samples based on clinical malaria diagnosis were defined with microscopic examination and got 171 (30.4%) samples with falciparum malaria. The group of 5-12 years old were 94 (55%) patients. The history of rigor was highly found in Banding Agung (62.7%). Palmar pale dominant found in Tanjung Lengkayap (39.3%), also spleen enlargement about 20.2%. The history of anti malaria drug therapy 41.7% found in Tanjung Lengkayap. The temperature examination results > 37.5^{o}C 58.3% found in Belitang. Twenty five sample tried to *in vitro* test three times but the parasite did not growed, so the others 146 samples conducted to *in vivo* test. The results of *in vivo* showed that 44 (30.1%) samples were resintance to chloroquine. The 168 (98.5%) samples can be isolated of DNA for PCR an sequencing. The point mutation were found in codons: M74I, N75L, K76T, H97F, A220S, and I356L. one hundred percent of codons 74, 75, 76, and 356 were mutated. The mutation of codon 97 in Tanjung Lengkayap, Banding Agung, and Belitang 44%, 46.9%, and 60% respectively. The mutation of codon 220 in Tanjung Lengkayap, Banding Agung 40.5%, 46.9%, and 60% respectively. The result showed that in three areas, the pattern of mutation were the same in codons 74, 75, 76, and 356 and getting variation at codons 97 and 220. The point mutation of codon 97 and 220 were dominant in Belitang. This study showed that the mutation was *multiple point mutation*. The new finding or local mutation were found at codons N75L and H97F. Until now never were they find.