［課程−２］

審査の結果の要旨

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This research attempted to clarify how change of drug treatment policy in Myanmar, a country with increasing prevalence of artemisinin resistance and the greatest burden of malaria in the Greater Mekhong Sub-region (GMS) particularly dramatic increase in artemisinin usage, affected population dynamics of *P. falciparum*. And the following results were got:

1. Previous in vivo studies in Myanmar showed that frequency of treatment failure by chloroquine reached a peak in the period from early 1980s to mid-1990s. After that, the frequency of resistance decreased to about 25-30% in the early 2000s. However, decrease trend of chloroquine resistance was not supported from the present analysis of *pfcrt* genotyping. Resistant mutation (K76T) was observed in almost all parasites in 2002-2005 and it became fixed in 2013.

2. In contrast, disappearance of *pfmdr1* N86Y mutation which is also associated with chloroquine resistance was observed in 2013 from 23.7% in 2002-2005. The introduction of artemisinin+lumifantrine may have played some roles in this observation. These results support the idea that the N86 allele may be advantageous in the presence of lumefantrine pressure. Therefore, wide-spread usage of lumefantrine as an ACTs (Artemisinin based combination therapies) regimen in Myanmar would have induced the significant decrease of *pfmdr1* N86Y mutation. Selection of the K76 allele in *pfcrt* was also reported after exposure to artemether +lumefantrine in Africa, suggesting the possibility that *pfcrt* K76 allele might also be selected in Myanmar in the future.

3. Similar to chloroquine resistance, predominance of highly mutant *dhfr* and *dhps* alleles in 2002-5 as well as in 2013 suggests that there is no sign of recovery of SP sensitivity. Previous molecular epidemiological studies in Myanmar and neighboring countries showed similar findings; triple and quadruple mutant in *dhfr* (CIRNI and CIRNL) and triple mutant in *dhps* (AGEAA and SGEGA) continued to be predominant after SP withdrawal.

4. In this present study, a significant shift of the predominant triple mutant *dhps* haplotype from AGEAA to SGEGA was found after the implementation of ACTs. Similar findings were reported after the drug policy change from SP to ACTs in Tanzania and Kenya. So far, no clear mechanism that can explain this finding has been described. However, SGEGA may be more fit than the other *dhps* AGEAA in the presence of artemisinin.

5. Haplotyping of six microsatellite markers flanking *pfcrt* in the present analysis revealed that chloroquine-resistant isolates in Myanmar share a common resistant lineage with resistant isolates in the other GMS countries. Similar findings were obtained for SP resistance.

6. By contrast, a different evolutionary pattern is probable in artemisinin resistance. In the initial report of *kelch 13*, the C580Y mutation was characterized as the responsible mutation for artemisinin resistance in the natural parasite population. Shortly thereafter, various alleles in *kelch 13* have been evidenced to confer artemisinin resistance, suggesting that *kelch 13* mutants independently arose multiple times within GMS. In the present study, however, no parasite was found to harbor the known artemisinin-resistance related mutations in both 2002-2005 and 2013. Rather, in 2013, the most predominant allele was a novel Y511H mutation with a prevalence of 14.8%. Accumulated evidence have shown that nearly all *kelch 13* mutations observed at significant frequencies in the endemic areas were associated with delayed parasite clearance after artemisinin treatment. Therefore, the observed high prevalence of Y511H mutation is most likely due to selection under increasing artemisinin pressure.

7. These molecular epidemiological observations regarding the dynamics of molecular markers for anti-malarial resistance after the introduction of ACTs in Myanmar will provide an insight into future policy making for implementing a strategy to prevent and control artemisinin resistance, initially in the Mekong subregion, expanding worldwide and utilising multi-drug regimens with different ACTs. This research is award of a Doctor’s degree.