

Characterisation of parasite factor(s) which mediate paroxysms in *Plasmodium vivax* infections

P. vivax infections are characterised by clinical paroxysms, which are episodes of fever with chills and rigors. Plasma collected during paroxysms (PP) was shown to cause aggregation of white blood cells in *in vitro* cultures. This phenomenon is used as an assay to study disease-mediating factors in *P. vivax* infections.

Present study describes the preliminary characterization of the disease - mediating parasite factor(s). PP collected from 6 *P. vivax* patients were pre-treated by (i) heating at 60°C, 80°C and 100°C for 5 minutes (ii) filtration (0.45 µm filters) (iii) centrifugation (180000 xg for 15 minutes) and then tested on cell aggregation assay. Percentage Relative Aggregation Index (% RAI) was calculated for each sample.

AI (Aggregation Index)

$$= \left[\frac{\text{Total no. of white blood cells}}{\text{No. of cell clumps} + \text{no. of single cells}} \right] - 1$$

$$\% \text{RAI} = \left(\frac{\text{AI of test sample}}{\text{AI of PP}} \right) \times 100$$

There was a marked reduction of PP - induced cell aggregation following heating at or above 80°C [% RAI (at 100°C) = 13.51 ± P < 0.05]. The effect was restored by the addition of cytokines to heat inactivated PP (%RAI = 119.58 ± 34.53, P < 0.05). However no such effect was seen when parasite extract was added (%RAI = 42.31 ± 7.84, P > 0.05). Filtration of PP also reduced the cell inducing ability of PP (% RAI = 44.21 ± 14.92, P < 0.05). High-speed centrifugation resulted in the formation of two distinct layers with a thin milky layer at the top. Reconstitution with cytokines and parasite extracts indicated that cytokines are in the bottom layer while the parasite factors were concentrated at the top, which was rich in lipids (Sudan III test).

It is concluded that active parasite factor(s) in PP are heat stable, large molecules (>0.45 µm) that are likely to be lipid in nature.