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Please read this section first

The HPCSA and the Med Tech Society have confirmed that this clinical case study, plus your routine review of your EQA reports from Thistle QA, should be documented as a "Journal Club" activity. This means that you must record those attending for CEU purposes. Thistle will not issue a certificate to cover these activities, nor send out "correct" answers to the CEU questions at the end of this case study.

The Thistle QA CEU No is: **MT-2015/009.**

Each attendee should claim THREE CEU points for completing this Quality Control Journal Club exercise, and retain a copy of the relevant Thistle QA Participation Certificate as proof of registration on a Thistle QA EQA.

DIFFERENTIAL SLIDES LEGEND

CYCLE 46 SLIDE 1 – FEBRUARY 2015

MALARIA MIXED INFECTION

Malaria is an important cause of morbidity and mortality. Malaria is caused by the parasite Plasmodium, and is transmitted by the Anopheles mosquitoes. Plasmodium parasites have five species affecting humans. These are Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae and Plasmodium knowlesi. These species cause approximately 225 million infections and over 600 000 deaths per year. Among them, P. falciparum is the most prevalent and common malaria species worldwide, especially in Africa. It causes the most severe form of the disease and is responsible for over 90% deaths.

Malaria can occur in single and mixed infections. Mixed infections involving more than 1 species of Plasmodium may occur in high endemic areas of multiple circulating malarial species. In these cases, clinical differentiation and decision making will be important, as mixed infection can easily lead to misdiagnosis. Occasionally, morphologic features do not permit distinction between P. falciparum and other Plasmodium species. In such cases, patients from a P. falciparum endemic area should be presumed to have P. falciparum infection and be treated accordingly.

In patients from Southeast Asia, the possibility of P. knowlesi infection should also be considered. This species frequently causes hyper-parasitaemia and the infection tends to be more severe than infections with other non P. falciparum and should be treated as P. falciparum infection. P. falciparum is resistant to chloroquine treatment except in Haiti, the Dominican Republic, parts of Central America, and parts of the Middle East. Resistance is rare in P. vivax infection, and P. ovale & malariae remain sensitive to chloroquine. Primaquine is required in the treatment of P. ovale and P. vivax infection in order to eliminate the hypnozoites (liver phase).

Clinical diagnosis of Plasmodium species by microscopy is not precise. However, it is still the basis of therapeutic care and plays a key role in the diagnosis of febrile patients in malaria endemic areas. Laboratories in malaria endemic areas needs accurate and precise diagnosis of mono-infection and mixed species infections in order to assure proper treatment decision. This helps to prevent the advent of drug resistant parasite population. Accurate and effective diagnosis is the only way of assuring rational treatment and therapy. Microscopic observation of P. falciparum infection is influenced by its parasite density. Parasite density of infections by non-falciparum Plasmodium species is usually low compared to P. falciparum. Therefore, other Plasmodium species are easily missed, particularly in the absence of symptoms. Moreover, in mixed infections, the background of large numbers of P. falciparum parasites makes the observation difficult to differentiate other species.

Mixed species infection can not only complicate diagnosis, but also alter the severity and morbidity of the disease. Co-existence of P. falciparum and P. vivax in a single human host suppress each other. P. falciparum can suppress P. vivax parasitaemia by interspecies inhibition. Severity of malaria, in mixed species infection, depends

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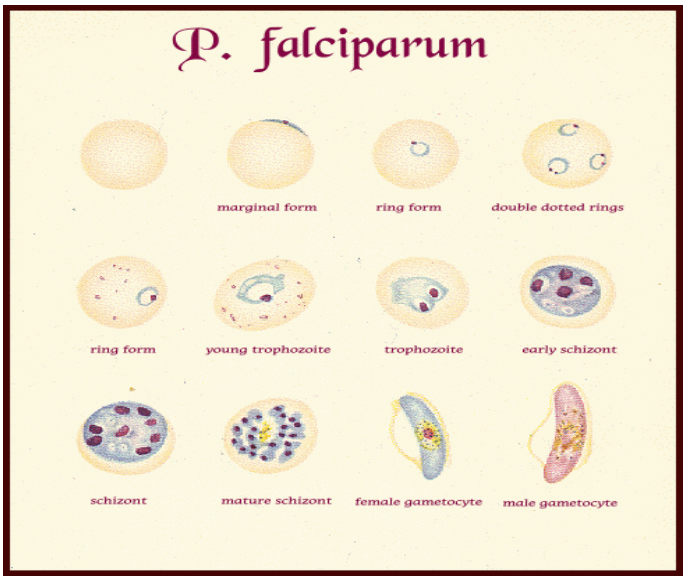
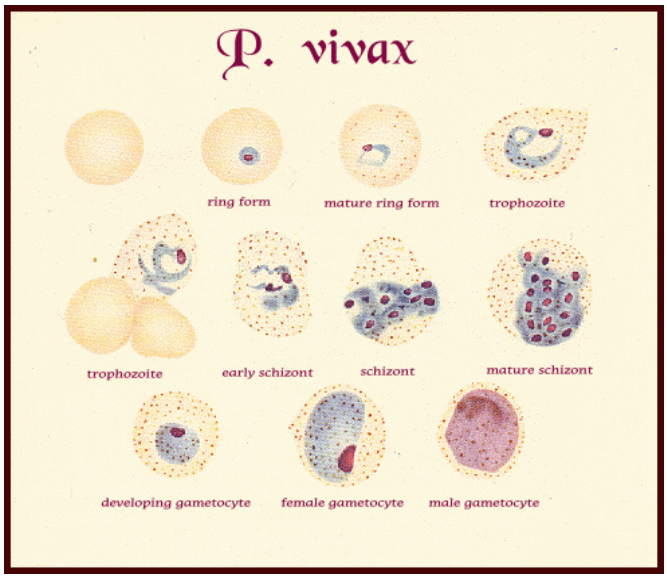
on whether it is a P. falciparum or a P. vivax super infection. P. vivax super infection over an existing P. falciparum infection leads to the rise of P. falciparum parasitaemia and causes severe malaria. In contrast, P. falciparum super infection over an existing P. vivax infection reduces P. falciparum parasitaemia. Therefore, it prevents the development of severe malaria.

Mixed infection incidence is less than the prevalence of individual species and it is also seasonal. Malaria transmission occurs more in wet season than dry season. The seasonal variation is due to the spread of various mosquito species in wet season. The spread of various mosquitoes in malaria areas causes high incidence of both single and mixed infections in wet season than dry season. Relatively high prevalence of individual species occurs in malaria areas where Anopheles mosquito population is high, but the prevalence level of mixed infection is usually less compared to single infection prevalence because mixed infection occurs either by simultaneous inoculation of different Plasmodium species at a time or inoculation of Plasmodium species at different times on a single host. Simultaneous inoculation rarely occurs, therefore, the rate of mixed infection is less than the rate of single infection. If mixed-species malaria is misdiagnosed as a single P. vivax infection, treatment of P. vivax increases P. falciparum parasitaemia. Mixed-species infections increase the possibility of anti-malarial drug resistance. Hence, a drug-resistant population of Plasmodium parasites will emerge. Therefore, accurate diagnosis or species identification of mixed-species malaria is critical for therapeutic decisions. It helps to manage the selection, dose, and timing of anti-malarial drugs. Mistreatment of single or multiple species have serious clinical consequences.





Diagnostic Points

1) Red Cells are normal.
2) Rings appear fine and delicate and there may be several in one cell.
3) Some rings may have two chromatin dots.
4) Presence of marginal or applique forms.
5) It is unusual to see developing forms in peripheral blood films.
6) Gametocytes have a characteristic crescent shape appearance. However, they do not usually appear in the blood for the first four weeks of infection.
7) Maurer's dots may be present

1) Red cells containing parasites are enlarged.
2) Schuffner's dots are frequently present the red cells as shown above
3) The mature ring forms tend to be large and coarse.
4) Developing forms are frequently present.

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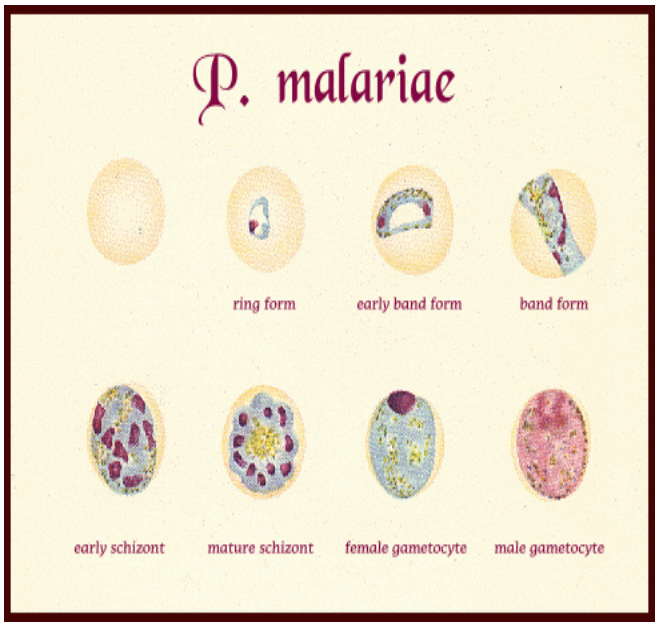

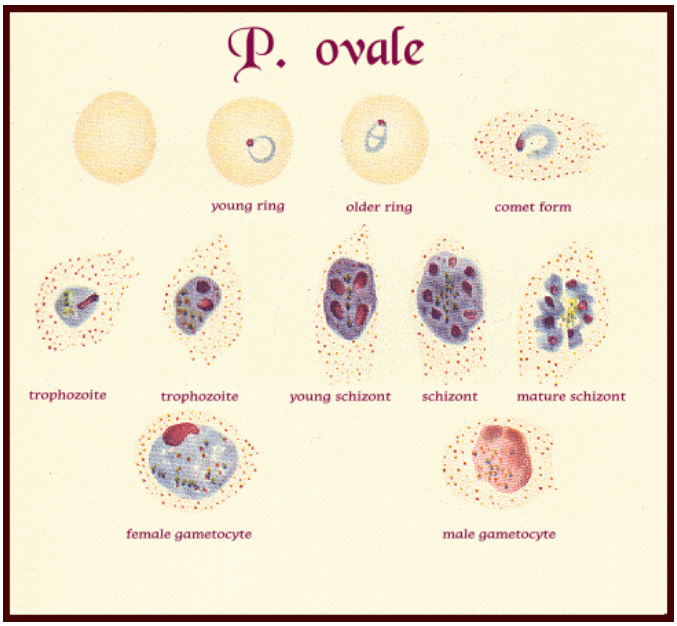

Diagnostic Points

1) Ring forms may have a squarish appearance.
2) Band forms are a characteristic of this species.
3) Mature schizonts may have a typical daisy head appearance with up to ten merozoites.
4) Red cells are **not** enlarged
5) Chromatin dot may be on the inner surface of the ring.

1) Red cells enlarged.
2) Rings large and coarse.
3) Comet forms common (top right).
4) Schuffner's dots, when present, may be prominent.
5) Mature schizonts similar to those of P. malariae but larger and coarser.

Treatment

The following is a summary of general recommendations for the treatment of malaria:

- P. falciparum malaria - Quinine-based therapy is with quinine (or quinidine) sulfate plus doxycycline or clindamycin or pyrimethamine-sulfadoxine; alternative therapies are artemether-lumefantrine, atovaquone-proguanil, or mefloquine
- P. falciparum malaria with known chloroquine susceptibility (only a few areas in Central America and the Middle East) - Chloroquine
- P. vivax, P. ovale malaria - Chloroquine plus primaquine;
- P. malariae malaria - Chloroquine

References

1. Malaria: Emilio V Perez-Jorge, MD, FACP; Chief Editor: Michael Stuart Bronze, MD
2. Detection of mixed infection level of Plasmodium falciparum *and* Plasmodium vivax by SYBR Green I-based real-time PCR: Addimas Tajebe, Gabriel Magoma, Ulugeta Aemero and Francis Kimani

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Questions

1. Discuss the diagnostic points of the four most common species of Plasmodium.
2. Discuss the general recommendation for the treatment of malaria.
3. What other diagnostic tests can be used to confirm the diagnosis of malaria?
