Malaria “Mal-air”

It is a world wide distribution disease acute or chronic characterized by fever, anemia & spleenomegaly occurs where anopheles mosquito are present & caused by genus plasmodium, which is host specific.

In IRAQ it is found significantly in the north part of IRAQ.

Animal kingdom

- sub kingdom : protozoa
- sub phylum : Apicomplexa
- class : sporozoea
- genus : plasmodium

41% of the world's population live in areas where malaria is transmitted.
A French army doctor in Algeria observed parasites inside red blood cells of malaria patients and proposed for the first time that a protozoan caused disease.
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Charles Louis Alphonse Laveran

1907 Nobel Prize for Physiology or Medicine!
Around 300-500 million clinical cases of malaria are reported every year, of which more than a million die of severe and complicated cases of malaria.

Malaria ranks third among the major infectious diseases in causing deaths after HIV, pneumococcal acute respiratory infections and tuberculosis, then malaria.

Although malaria has been widely eradicated in many parts of the world, the global number of cases continues to rise. The most important reason for this alarming situation is the rapid spread of malaria parasites that are resistant to anti-malarial drugs.
As you know in the developing world treatable infectious diseases remain big killers.
Species that cause Malaria in man are

**Plasmodium vivax**  “bengin tertian malaria” 48hr

**P falciparum**  “malignant tertian malaria”

**P malariae**  “Qurtan malaria”  72hr

**P ovale**  “mild tertian malaria
ovale malaria”

**General characteristics of genus plasmodium:**

1- All species are parasitic of tissue & blood of their host

2- Have very complicated life cycle: alternation of **sexual** (gametogony & sporogony) & **asexual** (schizogony)

3- No organil of locomotion but at certain stages can move by body flexible or flagella as microgamete
Genus plasmodium requires 2 host to complete their life cycle

-**Vertebrate host**
  Bird, man where asexual cycle takes place (**intermediate host**)

Asexual =**schizogony** (Trophozoite $\rightarrow$ schizont $\rightarrow$ merozoite)

-**Vector host**
  Female Anopheline mosquito where sexual cycle takes place (**final or definite host**)

Sexual=**gametogony** microgamete unites macrogamete $\rightarrow$ zygote

**Sporogony** = zygote $\rightarrow$ oocyst $\rightarrow$ sporozoites
The Malarias:

Plasmodium falciparum
Plasmodium vivax
Plasmodium malariae
Plasmodium ovale
Each disease has a distinct course

“Tertian Malaria”
(P.falciparum, P.ovale and P.vivax)
fever occurs every third day.

“Quartan Malaria”
(P. malariae)
fever occurs every fourth day.
Malarial parasite in man established their 1st foci in non-phagocytic cell of the liver (hepatocyte) = pre erythrocytic cycle before the released into circulating blood to parasitize RBC where erythrocytic cycle established.

**Life cycle**

Infection person → Insect female Anopheline mosquito → Un infected person

Man only reservoir

infected stage is the sporozoite
Malaria

- Malaria parasites are transmitted from one person to another by the female anopheline mosquito.

- The males do not transmit the disease as they feed only on plant juices.

- There are about 380 species of anopheline mosquito, but only 60 or so are able to transmit the parasite.
Life cycle

Vertebrate

- Tissue phase
  - "liver"

- Blood phase

- Erythrocytic cycle (E.C.)
  - Primary E.E.C.
  - Exo-erythrocytic cycle
  - Pre-erythrocytic cycle

- Secondary E.E.C.
  - Para-erythrocytic cycle

Invertebrates

- Female mosquito

- gametogony

- sporogony
Man becomes infected with malaria when female anopheline mosquito introduce the infective stage the **sporozoite** with its saliva during sucking of the blood. These sporozoites are tiny elongated spindle shape about 10-15µ in length with pointed ends. So they enter the man through the skin then they reach the blood & within less than an hour they enter the parenchyma cells of the liver.

The sporozoite surface is covered by a protein called **circumsporozoite protein** which is specifically enters the liver cells not other cells.

In the liver the sporozoite starts primary E.E.C. Primary Exo-erythrocytic (schizogony) pre-Erythrocytic schizogony.
Fig 92: Anopheles Female (Mosquito)
The vector of malaria
Plasmodium sporozoites
Transmission

- sporozoites injected with saliva
- enter circulation
- trapped by liver (receptor-ligand)
Pre.E.E.C.  8 days in \textit{P. Vivax}, 6 days in \textit{P. falciparum}

Sporozoite inside liver cell $\rightarrow$ trophozoites

immature schizont $\rightarrow$ mature schizont

contain 1000 s of merozoites (cryptozoites)

In the liver no clinical manifestation produced by the parasite

\begin{itemize}
  \item Merozoites
    \begin{itemize}
      \item Phagocytized by kupffer cell
      \item Secondary exo-erythro. cycle
        \begin{itemize}
          \item “para-erythro. cycle”
        \end{itemize}
      \item Dormant for indefinite time “hypnozoite” in \textit{P. vivax} & ovale only $\rightarrow$ relapse
      \item Blood erythocytic cycle
    \end{itemize}
\end{itemize}
Hyponozoite Forms

- some Merozoites exhibit delayed replication (i.e., dormant)
- merozoites produced months after initial infection
- only *P. vivax* and *P. ovale*

relapse = hypnozoite  
recrudescence = *P. malariae*
Exoerythrocytic Schizogony

- hepatocyte invasion
- asexual replication
- 6-15 days
- 1000-10,000 merozoites
- no overt pathology
Erythrocytic Cycle (vivax 48 hour)

Inside RBC

Merozoite enter RBC, cytoplasm of RBC ingested by the parasite 

large food vacuole giving the appearance of a ring 
(nucleus at one end) **Ring stage** ,As the trophozoite grows ,vacuole becomes less ,but pigmented granules of hemozoin in the vacuole become apparent

**Hemozoin**

It is the end product of parasite’s digestion of the host’s Hb ,the trophozoite incompletely utilize Hb leaving residues of globin & an iron. **Prophyrin-hematin** which is an insoluble polymer also called malaria pigment (compound of protein +hematin), it has a toxic effect on the body & macrophage, depressing their phagocytes activity

HEMOGLOBIN $\rightarrow$ HEME + GLOBIN (PROTEIN PART)

HEMOZOIN

HEME

HEMOZOIN
Plasmodium vivax. Infected cells show fine stippling of Schüffner’s dots around the edges and the typical heavy chromatin dot.
As the trophozoite grows, the ring enlarges with pseudopodia in all directions; this stage is called the **Amoeboid stage**.

Here in vivax, the infected RBCs enlarged, lose their pink color (pale) and develop a peculiar stippling called "invaginations in the surface of the infected RBC"; this is known as Schuffner's dots.

After 24 hours, the vacuole disappears and nuclear division starts (12-24 nuclei), this is called the **Immature schizont**.

Then, cytoplasmic division results in the mature schizont, which contains a specific number of merozoites in each type, ranging from 12 to 24, usually 16.
Then RBC rupture & releasing merozoites (parasites) + metabolic wastes including hemozoin which responsible for symptoms of malaria.

Merozoites enter new RBC & repeat eryth. cycle (every 48hr).

After an indeterminate number of asexual cycle (eryth. cycle) some merozoites when enter RBC become microgametocyte (male) or macrogametocyte (female).
These gametocytes develop in RBC in the capillary of internal organ (spleen + Bone Marrow) & go to peripheral blood only when become mature (96hr) twice the time of E. schizogony.

The mature gametocyte unless ingested by female anopheline mosq. It will be die & phagocytized.

**Individual who harbor gametocytes in his peripheral blood is called carrier**

When female anopheline mosq. takes erythrocytes containing gametocytes the sexual cycle begin:
Sexual cycle: (gametogony) + sporogony

In the female anoph. mosq. gametocytes develops in to gametes

Macro gametocyte → one macrogamete

Microgametocyte → 6-8 microgamete, by process called exflagellation

**Exflagellation**: the nucleus of microgamete is divided into 6-8 daughter nuclei then axoneme is developed, then the flagella buds with their associated nuclei to outwards → 6-8 microgametes

One micro gametes fertilize macrogamete → zygote → mobile ookinete (in the gut of mosq.)

Ookinete penetrates the gut mucosa of female anoph. mosq. To the hemocoel side (outer side) of the gut → oocyte which contains the sporoblast that divided rapidly to form thousands of sporozoites break out of oocyte → hemocoel → salivary gland → next patient
Sporogony

• occurs in mosquito (9-21 d)
• fusion of micro- and macrogametes
• zygote → ookinete (~24 hr)
• ookinete transverses gut epithelium ('trans-invasion')
Malaria Life Cycle

- Liver stage
  - Sporozoites
  - Mosquito Salivary Gland

- Gametocytes
  - Zygote

- Red Blood Cell Cycle
  - Oocyst
Method of transmission

*sporozoite induced
The infective stage is sporozoite by biting of female anoph. mosq. porter of entry = skin

*trophozoite induced
Blood transfusion especially P. malaria syringe, lab. accident & rarely congenital infection
P. Vivax:

43% of malaria in the world some merozoites remain in the liver hypenozoite so relapse.

Merozoite invades only young RBC (reticulocyte) unable to invade fully mature RBC, black people have got natural resistance to P. vivax infection, because merozoite enter RBC through receptor which is the Duffy blood group protein a Ag fy a, fy b.

Black people usually with no such Ag fyo.

Infected RBC in P. vivax enlarge, pale, with schuffner’s dots, (fine dots).

Schizont in E. cycle with 12-24 hr usually 16 merozoits.

E. cycle with 48 hr periodicity = tertian malaria.
**P. Falciparum** = malignant tert. m.

50% of human malaria most virulent, 90% of mortality of malaria greater killer of humanity in tropical zone

No relapse (no hypnozoites)

**Invade RBC at any age even reticulocyte so much higher parasitemia than other types (25% of RBC infected)**

Soon after invasion of RBC the trophozoite produce protein that are deposite in the eryth. surface membrane in the deformation called **knobs**, these protein bind to certain glyco-protein on the post capillary venular endothelium this binding cause sequestration of the infected RBCs so stick to venular endothelium, also those RBCs stick to normal RBC → thrombosis

Gametocyte don’t produce these knobs so gametocyte infected RBCs don’t stick
So in the peripheral blood only the early ring stages & gametocyte are seen in *P. falciparum*. 

Ring stage in *P. falciparum* are smallest than other species, multiple infection of the same RBC is common, rings with binucleated (2 chromatin dots) also present may be division of the ring.

RBC with Maurer's dots larger than the fine schiiffner’s dots of *P. vivax*.

These dots for transport of nutrient Amoeboid & schizont (8-32 merozoites), not seen in the peripheral blood but in the capillaries of the internal organ (spleen, B.M.).

Gametocytes are crescent in shape.
A, B, C: Multiply infected red blood cells with appliqué forms in thin blood smears
D: Signet ring form.
E: Double chromatin dot
F: A thick blood smear showing many ring forms of P. falciparum (X 1000)
A, B, C, D: Gametocytes of P. falciparum in thin blood smears.
**P. Malariae** 7%

Quatrain malaria (paroxysm every 72 hr), amoeboid band form schizont 6-12 (9) merozoites in a rosette shape, infect only old (aging) RBC so low parasitemia

Can live in blood up to 50 years so important in blood transfusion, transmission & cause recrudescence but no relapse

**P. Ovale**

Mild tertian malaria, oval malaria

Rarest type, RBC oval in shape schuffner’s dots appear earlier, larger more numerous than vivax, schizont 6-12 (9)
P. malariae

- ring form
- early band form
- band form
- early schizont
- mature schizont
- female gametocyte
- male gametocyte
P. ovale

- young ring
- older ring
- comet form
- trophozoite
- young schizont
- schizont
- mature schizont
- female gametocyte
- male gametocyte
Pathogenesis of malaria

Major clinical manifestation is due to
1- Host inflammatory response which produce chills & fever
2- Anemia due to enormous destruction of RBC

*Severity depends on species of malaria, most serious one is *P. falciparum*

*The fever in malaria is stimulated by the waste products of parasites which are released after lyses of RBSs, which includes mainly malarial toxins “hemozoin or malarial pigments” into circulation trigger = TNF (tumor necrosis factor) \(\rightarrow\) fever
Fever in malaria is *intermittent*.

- **P. vivax**: benign tertian malaria
  - 48 hr
- **P. falciparum**: malignant tertian malaria
- **P. oval**: oval malaria
  - 72 hr
- **P. malaria**: Quarter malaria
  - 72 hr
Anemia:
-Malaria causes destruction both infected & non-infected RBCs
-Inability to recycle iron bounded in hemozoin
-Defective bone marrow response
-Spleen removes infected & non-infected RBCs from blood, due to TNF toxicity
Anemia may cause Jaundice
Age

- No age limit
- Pregnant women and children are most likely to get it.
- People from non-malaria zones are at much higher risk than natives when they are in malaria zones.
Clinical feature of malaria:

Incubation period in P. Vivax = 9-12 days (no symptoms)

Then the main clinical manifestation in a typical case of malaria started which include febrile paroxysms followed by anemia & splenomegaly.

Febrile paroxysms:

- each paroxysms shows 3 stages

1-Cold stages (last 20-60 min. usually 1/2hr)
Chill, felling of intense cold, although temp. 40°C, shivering

2-Hot stages (last 2-4hr)
Fever, 40-41°C, headache, mild delirium

3-Sweating stages (last 2-3 hr)
Perspiration

The total duration of febrile cycle ≈ 6-8 hr, these paroxysms synchronies with the eryth. shizogony.
In tertian fever the paroxysms recurs every 48 hr, while in quarter malaria (P. malaria) recurs every 72 hr, after rupture of RBCs. When paroxysm is over after 8 hr, patient feels tired & goes to sleep for a while, & then feels fairly well until next paroxysm.

**Anemia:** After few paroxysms anemia occurs microcytic, hypochronic type.

**Splenomegaly:** One of important physical sign “spleenic index.”

In P. falciparum there is serious complications due to clotting of capillary of affected organ, circulatory stasis & hypoxia:
- Cerebral malaria: (convolution, coma, death)
- Pulmonary edema
- Algid malaria: rapid development of shock (adrenal involvement)
- Septicemia & toxemia
Black water fever :-

Only in *P. falciparum*, acute Massive lyses of RBCs, high level of Hb & its products → renal insufficiently & renal failure

Hb & its products in urine → dark (black) urine

Usually occur in patient who is taking inadequate or irregular treatment with quinine drug → may produce anti quinine Ab → auto immune hemolytic anemia → auto Ab to drug or to *P. Falciparum*

Administration of steroids is often helpful in treatment of this hemolytic crises
Relapse

Back into disease months or years after apparent curve occurs only in P. Vivax & oval (activation of hypnozoites in the liver) may be due to:

- Lowered Ab titer
- Genetic variation of the parasite

Symptoms of relapse usually less severe than the primary attack

Recrudescence:

In P. malariae, renewal of clinical manifestation after month& year, without re-exposure, because of persistence of the parasite in blood at too low level to be detected & produce symptoms, such parasitemia persist for years until sudden increase malarial symptoms

In P. falciparum, if the patient survive remission naturally or with treatment the parasite completely disappear from the blood cure
Each disease has a distinct course.

P. ovale and P. vivax can cause chronic malaria, reappearing after months or years due to latent parasites in liver.
Diagnosis of malaria:
- Depend to some extent on clinical manifestation of the disease
- Demonstration of the parasite on stained smears of the peripheral blood

Microscopically examination of blood film is the most important diagnostic procedures

thin for species identification
thick for quick diagnosis

Blood film done:

Just before or at beginning of paroxysms

- Visualizing the parasite after staining by fluorescent dye
- PCR
- Dip-stick method for detection of material Ag
- Serological tests C.F.T. precipitation test
Examination of a thick blood film should be the first step since this has the advantage of concentrating the parasites by 20 fold in comparison to a thin film, although the parasites may appear distorted making species identification difficult. If parasites are seen then the species should be confirmed by the examination of a thin film. Ideally blood should be collected when the patient's temperature is rising.

Preparation of thick and thin blood films :

Thick films:- place a drop of blood in the middle of a clean microscope slide and with the corner of a second slide spread the drop until it is about 10-15mm in diameter. The thickness should be such that it is just possible to see news print through it. Thin films are made in the standard manner. Allow the films to dry, do not leave on the bench in a laboratory which is not fly proofed otherwise the film will be eaten.
Immunity to malaria is specific, strain & variant specific

Genetic resistance to malaria
1- Black people (Duffy blood group) natural resistance to P. vivax
2- Sickle cell anemia
   - fauvism
   - Abnormal Hb
   - Thalassemia

Treatment of malaria:
- Anti malarial drugs
  - Alkaloid: quinine, chloroquine, primequine
  - Chloroquine prevent digestion of Hb so no hemozoin, it is not effective in exo-eryth. schizgy
  - Primequine kill hypnozoint so prevent relapse
In resistant malaria: mefloquine, fansidar
Genetic Resistance

- Sickle celled anemia
- Codominant trait (Allele “A” and “B”)
  - AA have sickle celled anemia
  - AB have both types of cells
- Sickle cells don’t support species of *Plasmodium* well.
- Resistance to infection
**Prophylaxis :-**

Vaccine : difficult (different stages , changing their Ag )

Travelers to endemic take chloroquine 2 weeks before & contain to 6 weeks after leaving , followed 2 weeks primaquine

**Control :-**

Mosquito control (netting , window screen , destruction area of mosquito life cycle using predators (fishes) insecticides DDT

*Drug resistance malaria & mosquito resistance so malaria will be with us as long in there is people*