EFFECT OF VERAPAMIL IN MALARIA

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MARCH 2007

CERTIFICATE

This is to certify that this dissertation entitled **"EFFECT OF VERAPAMIL IN MALARIA"** is a bonafide record of the research work done by **Dr. K. LATHA** in the Department of Pharmacology, Stanley Medical College, Chennai-600 001 during the period between 2004 –2007

I also certify that this dissertation is the result of the independent work done by the candidate.

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DECLARATION

I solemnly declare that this dissertation "**EFFECT OF VERAPAMIL IN MALARIA"** was done by me in the Department of Pharmacology, Govt. Stanley Medical College and Hospital, Chennai, under the guidance and supervision of Prof**. Dr. A. Ruckmani, M.D., D.D.,** Former Professor and Head, Department of Pharmacology, Govt. Stanley Medical College, Chennai-600 001.

This dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of degree of **M.D. Branch VI Pharmacology** examinations to be held in March 2007.

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CONTENTS

INTRODUCTION

Malaria, one of the commonest parasitic diseases of the world, is prevalent throughout the tropics and subtropics[1]. It is the most important devastating [2] parasitic disease of humans, affecting more than 1 billion people and causing about 1 to 3 million deaths worldwide each year.

HISTORY

Malaria (or ague [3]—as it was called earlier) has been known from antiquity. Seasonal intermittent fevers with chills and shivering, recorded in religious and medical texts of ancient Indian, Chinese and Assyrian civilizations, are believed to be due to malaria. Charaka and Susrutha have described the disease and have mentioned its association with mosquitoes.

Hippocrates as early in 5th century BC gave a detailed account of the disease. The relationship between the disease and stagnant water, swamps and marshy land was recognized and measures to control the disease by effective drainage were practiced in Rome and Greece in the 6th century AD.

The name ‘malaria’ (mal-bad, aria-air) was given in the 18th century in Italy. Malaria was then believed to be due to mal-air or bad air. “Paludism”- is another name for malaria. “Palus” in Latin means marsh. The recent demonstration of a specific parasitic antigen in Egyptian mummies probably indicates that malaria was present thousands of years ago.

Alphone Laveran, a French army surgeon in Algeria, discovered the causative organism of malaria in a patient in 1880. In 1886, Golgi in Italy described the asexual development of the parasite in RBCs, which came to be called the Golgi cycle.

Romanowsky, from Russia in 1891, developed a method of staining malarial parasite present in blood films. Three different malarial parasites infecting man namely P.vivax, P. malariae, P.falciparum were described in Italy between 1886 and 1890. The 4th species P. ovale was identified only in 1922. The mode of transmission of the disease was established in 1897, when RONALD ROSS, in Secunderabad, India identified the developing stages of malarial parasites in mosquitoes. This led to various measures for the control and possible eradication of malaria by mosquito control. Both Ross (1902) and Laveran (1907) won the Nobel Prize for their discoveries.

EPIDEMIOLOGY

GLOBAL

Malaria due to P.falciparum and P. ovale is primarily a disease of tropics, whereas malaria due to P. malaria occurs extensively in the subtropics and temperate zones. Vivax malaria is more commonly seen both in tropics and subtropics than all other types of malaria [4].

The incidence of malaria worldwide is estimated to be 300-500 million clinical cases every year, of which 90% occurs in sub Saharan Africa. Malaria is reported to cause 1.1 to 2.7 million deaths worldwide each year, of which about 1 million are children under the age of 5 years.

Malaria has been eliminated from North America, Europe and Russia. But P. vivax infection is common in Central America. The prevalence of P. vivax and P. falciparum is equal in South America. The incidence of malaria in USA in the year 1999 was 1666. In UK it was 1600 – 2500 and the deaths due to malaria was 9 –15. In Australia the incidence was 54.

The incidence is on the increase in both the Urban and periurban areas of South Asia. Military conflicts and civil unrest, along with unfavorable ecological changes, have greatly contributed to malaria epidemics, as large number of unprotected, non- immune and physically weakened refugees move into malaria endemic areas. Such population movements contribute to new malaria outbreaks and make epidemic prone areas more explosive.

All member countries of SEAR (except Maldives) have endemic areas in where, about 90% of the native population reside. There were over 23.7 million estimated cases of malaria in the region during 1998. In spite of the increasing frequency of drug resistant P.falciparum, the estimated numbers of deaths decreased because of the introduction of the new anti malarial drugs.

INDIA

In India, with the implementation of Modified Plan of Operation (MPO) in 1977, the upsurge of malaria dropped down from 6.74 million cases in 1976 to 2.1 million cases [5] in 1984. During the year 2001, there were 0.98 million reported cases of malaria in the country out of which 0.46 million were P.falciparum cases. 431 deaths were reported during the same period. A decline of 3.42% in total malaria cases and 11.09% in P.falciparum incidence were

recorded in the country as compared to the corresponding period in the year 2000.

Northeastern states contribute 8.5 to 11% of total malaria cases and 13 to 15% of total malaria mortality in the country. Among the northeastern states, Assam reports maximum followed by Arunachal Pradesh, Tripura, and Meghalaya. During 2001, maximum deaths were reported from Assam followed by Mizoram and Tripura.

Hence in order to bring down the incidence and morbidity in the country, the national malaria eradication followed by the control programmes were implemented.

MALARIA PROGRAMMES

I. National anti – malaria programme

National malaria control programme (NMCP) was launched in India in April 1953. In this programme, indoor spraying of DDT was carried out (1g/sqm of surface area) twice a year in endemic areas where spleen rate was 10%. The results of the programme was highly successful in that the incidence of malaria declined from 75 million cases in 1953 to 2 million cases in 1958. Encouraged by this result the Ministry of health changed the strategy from control to eradication, and launched National malaria eradication programme (NMEP) in 1958.

Considering the resurgence of malaria, a Modified plan of operation (MPO) to control malaria was launched and put in to operation from April 1977. It has the following objectives:

1. To prevent deaths and reduce morbidity due to malaria

2. To maintain agricultural and industrial production by undertaking intensive antimalarial measures in such areas to consolidate the gains so far achieved.

Flexibility in the policies according to the epidemiological situation and local conditions is an essential feature in this programme.

II. Malaria control through primary health care

This new approach to control malaria was approved by WHO in 1978. In 1994, due to resurgence of malaria the Government of India evolved Malaria action programme (MAP) and guidelines were distributed to all the states for prediction, early detection and effective management to malaria outbreaks at district level.

The antimalarial activities have been intensified with additional inputs in 100 selected districts of the states including Tamilnadu with 'Enhanced malaria control project' with the support of World Bank in September 1997.

In 1999, the Government of India decided to drop the term "National malaria eradication programme" and renamed it as "National anti-malaria programme".

In spite these control measures and effective antimalarial treatment there is still significant incidence and prevalence of malaria, which is mainly due to treatment failure and vector resistance. One of the main causes for treatment failure is the development of resistance by the malarial parasites to many of the antimalarial drugs especially to chloroquine.

NEWER DRUGS IN MALARIA

Hence management of drug resistance is an urgent need to decrease the incidence of malaria. Several commonly used drugs such as verapamil [6-16] chlorpheniramine maleate [7], tricyclic antidepressants [7], desipramine [7,9,14,17], cyproheptadine [7], chlorpromazine [14] have been shown to reverse the resistance of P.falciparum to chloroquine in vitro. Mostly the antihypertensive, Verapamil is proved as an effective chemosensitizer. Thus although the first line antimalarial drug chloroquine appears to lose its importance in most part of the world, this inexpensive, rapid acting well tolerated antimalarial may be still used with the same efficacy by combining it with effective resistance reversers.

Therefore if chloroquine is combined with anyone of the above resistance reversing drugs, the efficacy of chloroquine is expected to be retained, in resistant malaria. Verapamil is reported to reverse chloroquine resistance by several in vitro studies. Whether verapamil will have such action when used clinically? Will verapamil facilitate the action of chloroquine in sensitive malaria and bring out a synergistic effect?

Thus, in order to find out the effect of verapamil in malaria irrespective of the sensitivity status, the present study was under taken.

AIM OF THE STUDY

The aim of the study was to find out

1. The effect of Verapamil in malaria
2. Whether it has any facilitatory action when given along with Chloroquine and
3. Whether it is safe.

REVIEW OF LITERATURE

Malaria occurs in all the countries extending 40 degree South to 60 degree North. The tropical zone is the endemic home of all malarial parasites. All the malarial parasites belong to the genus Plasmodium, and there are 4 different species that transmit malaria to humans.

TYPES OF MALARIAL PARASITES

1. P. malariae (Laveran- 1881) & (Grassi & Feletti-1890)

   This is the parasite of quartan malaria. Laveran first studied this species and gave the name, which is still retained.

2. P. vivax (Grassi & Feletti-1890)

   This is the parasite of benign tertian or vivax malaria. The specific name “vivax” is derived from the Latin word ‘vivere’ which means - to live and indicates the movement.

3. P. falciparum (Welch- 1897)

   This is the parasite of malignant tertian or falciparum malaria. The specific name “falciparum” from Latin (falx- a sickle) is derived from its sickle shaped gametocytes.

. 4. P. ovale (Stephens- 1922)

   The parasite will cause ovale tertian malaria. Its name is derived from its oval shape and also the shape of the infected RBCs which is rendered oval

LIFECYCLE OF MALARIAL PARASITES

They pass through 2 different hosts [18]

1. Man – The parasites residing inside the RBC and the liver cell reproduce by asexual method. Hence man is the intermediate host of the malarial parasite.

2. Female anophelene mosquito – For the initiation of the mosquito cycle, sexual forms (male, female gametocytes) are first developed inside the human host. These are then transferred to their insect host where they develop further and are transformed to sporozoites. These sporozoites are infective to man. On account of this sexual method of reproduction, mosquito is the definitive host of the malarial parasites.

ASEXUAL CYCLE IN HUMANS

Human cycle starts with the introduction of sporozoites by the bite of an infected anophelene mosquito. It comprises of the following stages

1. Pre erythrocytic schizogony

Sporozoites undergo a developmental change inside the parenchymal cells of the liver in the man and get transformed into schizonts. This phase consists of only one generation of pre- erythrocytic schizont. It lasts for

- 8 days in P. vivax
- 6 days in P. falciparum
- 9 days in P. ovale
- 15 days in P. malariae

2. **Erythrocytic schizogony**

During this phase, the parasite resides inside the RBC and develops through the stages of trophozoite, schizont and merozoite. Once the merozoites mature, they are released into the circulation. The release of merozoites, their destruction and the release of toxin are responsible for the clinical attacks of malaria. Each cycle lasts for

- 48 hours in P. vivax, P. ovale, P. falciparum
- 72 hours in P. malariae

3. **Gametogony**

After the parasites have undergone erythrocytic schizogony for a certain period, some of the merozoites develop in to gametocytes, which are capable of sexual function after leaving the human host. They develop inside the RBCs of the capillaries of spleen and bone marrow. Only the mature gametocytes are found in the peripheral blood. The maturation completes in about 96 hours. Gametocytes do not cause any febrile reaction in the human host. The individual who harbors the gametocytes is known as a “carrier”

4. **Exo- erythrocytic schizogony**

After the release of merozoites into the blood the initial tissue phase disappears completely in P.falciparum, where as in P. vivax, P. ovale and in P. malariae it persists in the form of a local liver cycle. The persistence of this late tissue phase is described as exo erythrocytic schizogony, which is responsible for the relapse of P. vivax, P. ovale

and P. malariae malaria. In the absence of fresh infection this phase forms the source of asexual parasites.

SEXUAL CYCLE IN THE MOSQUITOES

The sexual cycle of malarial parasite first starts from human host, by the formation of gametocytes. A female anopheles during its blood meal from an infected person ingests both the sexual and asexual forms of parasite. But it is only the mature sexual forms, which are capable of development, remains and the rest die immediately. It has been estimated that in order to infect a mosquito the blood of a human carrier must contain at least 12 gametocytes per cu.mm of blood. And the number of female gametocytes must be in excess of the number of male gametocytes.

The gametocytes mature inside the stomach of the mosquito. They are ready then for fertilization. By a process called chemotaxis, one of the male gamete attaches with the female gamete. After fusion, the zygote is formed. This develops in 20 min to 2 hours after the blood meal. Next the zygote is transformed into a spherical mass surrounded by a cyst wall. This is called oocyst. As the oocyst matures, it develops into a large number of sickle shaped bodies known as sporozoites.

After full maturation and after the 10^{th} day of the infection the oocyst ruptures, releasing sporozoites in the body cavity of the mosquito. The sporozoites are distributed to various organs and tissues of the mosquito. They have a special predilection towards the salivary glands and ultimately reach a maximum concentration in salivary ducts. The mosquito at this stage is capable

of transmitting the infection to man. A single bite of mosquito is sufficient for this purpose. Different species of malarial parasites can develop in the same mosquito and this can give rise to mixed infection in man.

EVOLUTION OF KNOWLEDGE OF MALARIA [18]

1847-49 --- Meckel & Virchow, first found out the presence of black pigment in organs with malarial infections

1880 --- Laveran discovered the malarial parasite in an unstained preparation of fresh blood

1883 --- Marchiafava used methylene blue for staining of malarial parasite

1885 --- Golgi demonstrated erythrocyte schizogony in benign tertian malaria (Golgi cycle)

1891 --- Romanowsky introduced the staining methods of malarial parasite

1898 --- Ross worked out mosquito cycle with parasite of human malaria

1934 --- Tissue phase of malarial parasite demonstrated in avian malaria

1949 --- Shortt pre erythrocytic schizogony of P.falciparum in liver cells

1954 --- Granham et al discovered the preerthrocytic schizogony of P. ovale

TYPES OF MALARIA

Depending upon the developmental stage in which the parasite is transmitted.

1. Sporozoites induced malaria

Injection of an emulsion of salivary secretion of the mosquitoes containing the sporozoites will induce infection.

2. Trophozoite induced malaria

a) Transfusion malaria [19.]

When infected patients (latent infection) are used as donors, malaria develops after blood transfusion.

b) Congenital malaria [5]

Transmission of infection to the foetus in utero through some placental defects is called as congenital malaria.

c) Malaria in drug addicts

Seen in drug addicts who share syringes.

d) Therapeutic malaria

Malarial infection is artificially induced for the treatment of neuro syphilis.

SPREAD OF MALARIA

A human-to-human cycle transmitted by mosquito (human carrier)

HUMAN CARRIER

↓

MOSQUITO

↓

HUMAN

The factors responsible for the spread of malaria include the following [3]

1. Source of malarial parasite (the presence of a gametocyte carrier)
2. Existence of a suitable anophelene vector
3. A susceptible person

If this cycle or chain can be broken at any point, the occurrence of malaria can be prevented.

PATHOGENICITY

Each species causes a characteristic fever based on which the disease is designated as follows

P. vivax --- Benign tertian malaria (vivax malaria). The fever recurs every third day since the cycle is 48 hrs.

P. malariae --- Quartan malaria. The fever recurs every fourth day since the cycle is 72 hrs.

P falciparum --- Malignant tertian malaria (falciparum malaria). The fever recurs every third day. It is also responsible for pernicious malaria and Black water fever. . fever. 'Malignant' because it is the most severe form of malaria and can be fatal.

P. ovale --- Ovale tertian malaria. The fever recurs every third day since the cycle is 48 hrs.

INCUBATION PERIOD

The period between the introduction of sporozoites into the blood stream by the mosquito and the onset of symptoms is called the incubation period, which varies with different species as follows [18]

P. vivax & P. ovale --- 10 to 17 days (average 15 days)

P. falciparum --- 8 to 12 days

P. malariae --- 21 to 28 days (can be 30 – 60 days)

CLINICAL FEATURES[19,20]

In a typical case, there are series of febrile paroxysms, followed by anemia and splenic enlargement.

1. **Febrile paroxysms**[18] – it has 3 stages
    a. Cold stage – Lasts for 20 min to 1 hour
    b. Hot stage - Lasts for 1 to 4 hours
    c. Sweating stage – Lasts for 2 to 3 hours

Total duration of febrile cycle is from 6 to 10 hours. (Varies with species of plasmodia)

Febrile paroxysms synchronize with the erythrocytic schizogony of the malarial parasite.

a. With a 48 hrs cycle - Fever recurs every 3rd day (Tertian fever)
b. With a 72 hrs cycle - Fever recurs every 4th day (Quartan fever)
c. Fever recurs at intervals of 24 hrs (Quotidian periodicity)

2. **Anaemia**

After a paroxysm, anaemia of a normocytic hypochromic type develops as a result of breaking down the RBCs during destruction of the parasites.

3. **Splenomegaly**

Enlargement of spleen is an important physical sign in malaria. In primary cases, the enlargement is so slight as to escape detection by palpation. After some paroxysms, and usually by 2nd week, it is definitely enlarged and palpable.

COMPLICATIONS

1. Cerebral malaria

It is the characteristic and ominous feature of falciparum malaria. It manifests as symmetric encephalopathy. Coma persists for more than 30 minutes after generalized convulsions. It is 20% fatal in adults and 15% in children.

2. Acidemia/ Acidosis

The arterial pH is less than 7.25 (or) Plasma bicarbonate level is less than 15mmol/lit and venous lactate level is more than 5mmol/lit. It will manifests as labored deep breathing.

3. Hypoglycemia

It is due to failure of hepatic gluconeogenesis and increase in the consumption of glucose by both the host and the malarial parasites. It is seen in children and pregnant women.

4. Renal failure

It is common in adults. It is related to erythrocyte sequestration interfering with renal microcirculatory flow and metabolism. Manifests as urine output less than 400 ml in adults and there will be no improvement with rehydration. Serum creatinine will be more than 3mg/dl.

5. Pulmonary odema

It is non- cardiogenic pulmonary edema. It is aggravated by over hydration. Mortality is more than 80%.

6. Hypo tension / Shock

Systolic BP is less than 80mmhg in adults, core / skin temperature difference will be more than 10^0 C.

7. Disseminated intravascular coagulation

Bleeding from the gums, nose and gastrointestinal tract.

8. Convulsions

There can be more than two generalized seizures / day.

9. Jaundice

Mild haemolytic jaundice can be seen in infection with falciparum malaria. Serum bilirubin level is more than 3 mg/ dl. It is commonly seen in children.

10. Anaemia

Due to the destruction of RBCs severe normocytic normochrmic anemia can occur. Hemoglobin level of less than 5 mg / dl can be seen.

PATHOLOGY OF MALARIA [18]

There is hyperplasia of reticuloendothelial system resulting from increased activity in order to deal with the plasmodia and their products. (Haemozoin, toxins etc.) The haemozoin pigments are always found within the cells of the reticulo endothelial system

Parasitised erythrocytes fill the lumen of the capillaries of the internal organs. This is particularly seen in P.falciparum infection as the schizogony is mainly completed in the internal organs. Vascular changes consist of congestion and dilatation of sinusoidal vessels. Perivascular haemorrhages,

resulting from the damage to the capillary epithelium are seen in falciparum malaria.

The spleen functions as a filter, removing the parasite as well as the product of their schizogony from the blood stream. The parasites are found in abundance in all stages of development in spleen, in all the forms of malaria, particularly in falciparum malaria. The organ is moderately enlarged. Malarial parasites and haemazoin pigments are actively phagocytosed in the spleen by macrophages, mainly by the cells of Billroth cords (Red pulp). The colour varies from slate-grey to black, depending on the amount of pigmentation.

Liver is uniformly enlarged. The colour varies from chocolate-red to slate-grey or black depending upon the state of congestion and the amount of haemozoin pigment. The Kupffer's cells are increased in number and their cytoplasms are filled with haemozoin pigment. The parenchymal cells of the liver lying in the central zone show fatty degeneration, atrophy and necrosis (centri-lobular necrosis).

In acute cases, the marrow of the long bones undergoes very little change. In chronic cases, the upper and lower thirds of the long bones are reddish brown in colour. The vascular cellular tissue gradually replaces the yellow fatty marrow. Hyperplasia of reticulo endothelial cells which are laden with the haemozoin pigment and an erythroblastic reaction of the normoblastic type with some depression of myeloblastic activity.

CLINICAL PATHOLOGY

Changes in the blood [19]

- Haemolytic anaemia (Normocytic, hypochromic type)
- Leucocyte count is increased (with recurring paroxysms leucopoenia established)
- Decreased neutrophils
- Increased monocytes
- Increased Serum Gamma globulin
- Increased ESR
- Elevated C- reactive protein, and other acute phase proteins

CHRONIC EFFECTS OF MALARIA [19,21,36]

1. Tropical splenomegaly syndrome

Some young adults in zones endemic for P. falciparum malaria develop marked chronic splenomegaly. The spleen may enlarge over years to the weight of 3 to 4 kg, resulting in severe hypersplenism.

2. Quartan malaria Nephrotic syndrome

This pattern of nephrotic syndrome in children in Sub-Saharan Africa have proliferative or sclerosing glomerulonephritis. A characteristic histological type is associated with endemic P.malariae infection, and immunoflurescence shows P. malariae antigen in the glomeruli.

3. Malaria and Malignant lymphoma

There is epidemiological evidence that high grade non- Burkitt's, non-Hodgkins malignant lymphomas over represented in areas of Africa where P. falciparum malaria is holo endemic.

LAB DIAGNOSIS

I. DEMONSTRATION OF THE PARASITE [22]

a. Examination of Thick and thin smear

Microscopic demonstration of parasites in the blood smears, is the definitive method of diagnosis of malaria. Examination of both thin and thick smears of blood is recommended. Thin film is useful for identifying the specific species and thick film is to detect the presence of the parasite.

Specimen

Blood film should be prepared directly from capillary blood. In case of EDTA anticoagulated blood, smears are to be made within an hour of collection of blood. Best time to take blood films is the midway between paroxysm of chills and fever. (When greatest number of intracellular organisms is present)

Thin smear

Wipe off the 1st drop of blood. Next drop of blood is touched with a clean dry glass slide near its end. The blood is spread evenly and thinly with the edge of a spreader slide. The film should stop before it reaches the edge of the slide. The thin, feathered end should occupy the central area of the slide. Thin smears are one blood cell in thickness. The film is dried in air. From this thin layer, the size of RBCs, and extra cellular forms can be easily visualized[23]

Thick smear

2 – 3 drops of capillary blood are directly placed from the finger prick to other end of the same slide as for thin smear and with the corner of another slide produce a square or circular patch of about 10mm diameter. Continuous stirring for 30 sec is necessary to prevent the formation of fibrin strands, which may obscure the plasmodia after staining. The optimum thickness of thick film is that which will just allow printed letters to be read through it.

Staining

Both the films are air-dried. The thick film is dehaemoglobinised by placing the film in distilled water. It is kept in a vertical position in a glass cylinder for 5 – 10 min, and then dried in air in upright position. Both the films are stained with Leishman's or Giemsa stain. Other stains like Wright's or Fildes stain or JSB (Jaswanth Singh and Bhattacharjee) stain may also be used.

Examination

All asexual erythrocytic stages, as well as gametocytes can be seen in peripheral blood in P. vivax, P. ovale and P. malariae infection, but in P.falciparum infection, only the ring form and gametocytes can be seen.

Quantitation of the parasites

The approximate number of parasites / thick film field (100*objectives) is as follows:

+ - 1 – 10 parasites /100 thick film field

++ - 11 – 100 parasite/ 100 thick film field

+++ - 1 – 10 parasite / thick film field

++++ - More than 10 parasites / thick film field

b. Quantitative buffy coat [24-34]

Parasites can be concentrated by micro centrifugation using glass capillary tube and closely filling plastic insert. (QBC malaria blood tubes available from Becton Dickinson, - USA [23])

QBC tube is specially prepared glass haematocrit tube, precoated internally with acridine orange stain and potassium oxalate.

A volume of 55 – 65 micro liters of blood is collected from finger, ear and heal puncture and centrifuged at 12000 rpm for 5 min. RBCs containing malarial parasites are less dense than normal RBCs and concentrate just below the leucocytes at the top of the erythrocytic column.[22]

The parasite contains DNA, but the mature RBCs do not contain DNA & RNA. Parasite DNA is detected by acridine orange stain and appear as bright specks of light among the non- fluorescing erythrocytes when the QBC malaria tubes are rotated under a special type of lens (Paralens UV microscope adapter, with a 60 X oil immersion objective [27] attached to an ordinary light microscope), almost all the plasmodia in the blood sample can be visualized.

Advantages

A negative test can be reported within a minute and positive result within few minutes. It is an easy method, reliable [24], faster (within 15 – 30 min), sensitive, and is as good as a thick film [25].

It is a qualitative screening test for species identification [31] and accurate. QBC paralens can perform a greater variety of tests, including Chlamydia, Filaria, Anti Nuclear Antibody (ANA), and Cyto MegaloVirus (CMV) etc.

Quick and accurate blood cell count with 2 part differential count can be done. It gives very accurate platelet count for monitoring dengue patients.

Disadvantages

Quantitation of parasitemia relies on a subjective grading system. Expense is high. The events of blood filled tubes breaking or leaking in the centrifuge machine are other drawbacks [24.]

Equipment required and QBC tubes are expensive, unaffordable to public health services and small lab in developing countries [27.]

Howell- jolly bodies, artifacts such as cell debris and bacterial contamination may give false positive results.

The tubes do not remain readable for more than a few days, and hence cannot be kept for record purpose.

Examination

Due to acridine orange, the malarial parasite stains green (DNA, RNA nucleus) and orange (RNA, cytoplasm). The tube is examined in the region between the RBCs and granulocytes and within the granulocytes and mononuclear cell layer, where parasites are most abundant [24.]

An attempt was made to estimate the relative quantity of parasites in the specimens, using the Plus system.

+ - 1 parasite/ QBC field

++ - 1 – 10 parasite/ QBC field

+++ - 11 – 100 parasite/ QBC field

++++ - 100 parasites/ QBC field

QBC was found to be 100% sensitive for both P. vivax and P.falciparum and 100% specific for P.falciparum and 95% specific for P. vivax [28]

In some occasions, when the parasitemia is high, gametocytes, schizonts and trophozoites were widely dispersed within the entire span of the blood column, so it is necessary to scan through the whole blood column before giving a final report of a QBC test [29-34.]

c. Micro concentration technique

Blood is collected in microhematocrit tube and centrifuged at high speed. The sediment is mixed with normal serum and smear is prepared. Although the positivity rate is increased by this method, there is change in morphology of the parasite.

II. IMMUNODIAGNOSIS

Serology cannot match the sensitivity of microscopic detection of malarial parasite, but assist the diagnosis of mild infection. These antibody tests include indirect fluorescent antibody (IFA) test, indirect

haemagglutination assay (IHA), ELISA and RIA (Radio Immuno Assay). They are useful for epidemiological purpose.

III. NEWER DIAGNOSTIC METHODS

a. Rapid and Simple stick test

This test is based on detection of monoclonal antibody against P.falciparum histidine rich protein 2 antigens (HRP-2). This test is based on antigen capture and has been incorporated in a dipstick format. Antibodies specific for P.falciparum, HRP – 2 are immobilized in the dipstick (test strip) when dipped in the blood. A solid pink line indicates positive test. This is very useful in treatment and diagnosis of drug resistant P.falciparum.

b. Use of fluorescent dye

Benzothiocarboxypurine (Fluorescent dye) intensely stains nucleic acid of malarial parasites after penetrating RBCs. The dye does not stain nuclei of WBCs. This is useful in field lab for mass screening, because of rapid staining and evaluation, but requires a fluorescent microscope.

c. Detection of parasitic antigen

Detection of malarial antigen by Radio Immuno Assay (RIA), DNA probes and Polymerase Chain Reaction (PCR) is under evaluation. PCR detects P.falciparum and P. vivax nucleic acids. In suspected mixed malarial infections, PCR detection system has got its importance undoubtedly.

DRUGS USED IN MALARIA[35]

The drugs used in malaria act at different stages of development of the malarial parasites. Depending upon the stage, they can prevent the attacks (prophylaxis), relieve the signs and symptoms (clinical curatives) or prevent relapses (radical curatives).

1. Drugs used for causal prophylaxis (True prophylaxis)

They are given in the pre- erythrocytic phase in the liver, which is the cause of malarial infection and clinical attacks.

- Proguanil

2. Drugs used for clinical and suppressive cure (Clinical curatives)

They act on the parasites in the erythrocytic stage. They are used to terminate the episode of malarial fever produced by the erythrocytic schizonts and alleviate the clinical symptoms and signs.

Fast acting - Chloroquine, Quinine, Mefloquine, Atovaquone, Artemisinin
Slow acting - Antimalarial antifolates and antibiotic compounds

3. Drugs used to eradicate the parasites (Radical curatives)

These drugs act in the exoerythrocytic stage and when given together with a clinical curative, will achieve total eradication of the parasites from the patients, thereby prevent the relapse.

- Primaquine

4. Drugs used for preventing transmission

They eliminate the male and female gametes of Plasmodia formed in the patient's blood.

a. From human to the vector

   Chloroquine, Quinine – For P. vivax, P. ovale, P. malariae

   Primaquine – P.falciparum

b. From vector to humans

   Not yet known

PHARMACOLOGY OF DRUGS USED IN MALARIA

Antimalarial drugs are used to prevent and treat clinical attacks of malaria. Some drugs completely eradicate the parasites from the patient's body and reduce the human reservoir of infection. They attack the parasite at its various stages of life cycle in the human host. Antimalarials that act on erythrocytic schizogony are called erythrocytic schizonticides. Those that act on pre erythrocytic and exoerythrocytic stages in the liver are called tissue schizonticides. The drugs that kill gametocytes in blood are called as gametocides.

Quinine

Quinine was the first drug given for malaria since 1633. A powder of Cinchona "given as a beverage, cures the fevers and tertians" was used in South America.

In 1640, Quinine was used in Europe for the treatment of fever. In 1820 it was isolated from cinchona powder and the extract was used for malaria [2.] It gets concentrated in the acidic vacuoles of the blood schizonts and causes pigment changes. It will inhibit polymerization of heme to hemazoin. Free heme or heme- quinine complex damages parasite membrane and kills it.

Quinine was the first drug used for falciparum malaria especially in severe cases. It can be given in the dose of 600 mg three times a day for three days. It is a rapidly acting drug. Half- life is about 11 hrs. It is effective against blood schizonticide. It is also a gametocidal drug. It is used in severe falciparum malaria and in babesiosis. It can produce adverse effects such as cinchonism, hypersensitivity reactions, hypoglycemia, hypotension, and black water fever.

Chloroquine

It was discovered in 1934, and named Resochin. The antimalarial research was done in United States during World War II, and by the year 1943 it was synthesized and tested for its activity in humans. This is discussed in detail later.

Mefloquine

It is a fluorinated 4 – quinoline methanol compound. It is given by oral route (There is no parentral preparation for this drug). Peak plasma concentration of mefloquine is within 18 hrs. It is highly protein bound. It is slowly excreted via feces for 20 days. It is a strong erythrocytic schizonticide for P.falciparum and for P. vivax but not for severe infections. Epigastric pain,

headache, arrhythmia, bradycardia are the common side effects. It is safe in children and pregnant women. It is used in both the treatment of falciparum and vivax infection and for chemoprophylaxis. For falciparum infection it is given in the dose of 25mg/kg. It is splitted into 2-3 doses on the same day. In prophylaxis, it can be given in the dose of 5mg /kg /week.

Amodiaquine

It is a 4 aminoquinoline with a similar mode of action as chloroquine. It is more active against resistant isolates of P.falciparum. It can be given in the dose of 25-35 mg /kg over three days.

Primaquine

It is an 8- aminoquinoline used for hypnozoites of P. vivax and the gametocytes of P.falciparum. It is well absorbed and cleared by hepatic clearance. Elimination half-life is about 17 hours. Nausea, vomiting and abdominal discomfort is common with higher doses. The principal toxicity of primaquine is haemolysis in patients with G6PD deficiency. It is used in radical cure of relapsing malaria. It is given in the dose of 15mg (0.25mg/kg/day) for two weeks.

Proguanil/ Chlorproguanil

These are the safest of all malarial drugs. They act by dihydro folate reductase (DHFR) inhibition. They are well absorbed orally. Parent compounds are eliminated very slowly. Mouth ulcers and hair loss are the common side effects. They are used as prophylactic drugs. It is given in the dose of 200mg/ day.

Pyrimethamine

It is also a dihydrofolate redutase inhibitor. It is used widely in combination with long acting sulfonamides like sulfadoxine and sulfalene. It inhibits the development of mature trophozoite stage of asexual parasite, in addition to having pre- erythrocytic and sporontocidal activities. It is well absorbed and is eliminated over several days (half life is 3 days) allowing single dose treatment. It is given as 1.25-mg /kg single oral dose.

Halofantrine

It is a 9-phenanthrene methanol. It is more potent than quinine and mefloquine. It is poorly absorbed. Fatty food increases its absorption. It is extensively distributed and cleared largely by hepatic biotransformation. The elimination half-life is 1-3 days. Vomiting, dizziness, weakness and dysphoria are the common side effects. It is used for the treatment of uncomplicated multidrug resistant falciparum malaria. The dose is 8mg/kg three times a day and repeated one week later in non- immune patients [36].

Qinghaosu

It is also known as artemisinin. It is sesquiterpene lactone peroxide extracted from the leaves of shrub Artemesia annua. Two derivatives are widely used, the oil soluble methyl ether, artemether and the water-soluble hemisuccinate derivative artesunate. These are rapidly acting of all known anti malarials. They also have a broad window of anti malarial effect against ring forms to the mature trophozoites. They produce rapid parasite clearance and are very safe. Arteether is a very similar compound to artemether. It is the oil

soluble ethyl ether given by intramuscular injection. Artelinic acid [36] is a water-soluble second-generation compound under development. They are well absorbed and have rapid elimination with half-lives varying from minutes (artesunate) to hours (arteether and artemether).

Antibacterials with antimalarial activity

1. Sulphonamides / Sulphones

They will inhibit plasmodial folate synthesis by competing with the enzyme dihydrofolate reductase. They are usually combined with pyrimethamine or the antimalarial biguanides.

2. Tetracyclines

They are consistently active against all the species of malaria.

3. Macrolides

They are active in vitro. Azithromycin has been evaluated as prophylaxis and Clindamycin has proved effective in the treatment of falciparum malaria.

4. Rifampicin

It is a weak antimalarial in vivo. It acts very slowly hence combined with rapidly acting agents.

5. Fluoroquinolones

They have some antimalarial activity, but not yet proved.

CHLOROQUINE

History

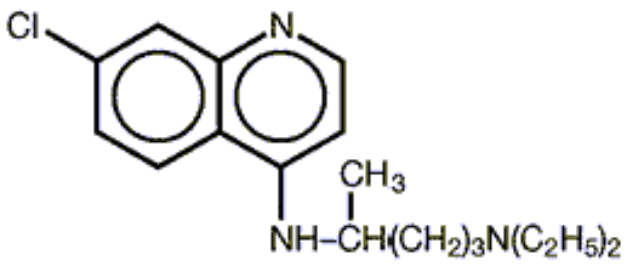
It is the drug of choice for both treatment and chemoprophylaxis of malaria since 1940 [37.] It is one of the large series of 4 aminoquinolines, investigated as part of the extensive programme of antimalarial research in United States during World War II. Beginning in 1943, thousands of these compounds were synthesized and tested for their activity. It was synthesized and studied under the name of Resochin by Germans as early as 1934.

Chemistry

It closely resembles the obsolete 8 aminoquinoline antimalarials, Pamaquine and pentaquine. It contains the same side chain as quinacrine, but differs from this antimalarial in having a quinoline instead of an acridine nucleus and in lacking the methoxy moiety.

The d, l, forms of chloroquine have equal efficacy in duck malaria, but the “d” isomer is less toxic than “l” isomer in mammals.

The chlorine atom attached to position 7 of the quinoline ring confers the greatest antimalarial in human malaria [2.]



PHARMACOLOGICAL EFFECTS

Antimalarial actions

It is highly effective against erythrocytic forms of P. vivax, P. ovale, P. malariae and chloroquine sensitive strains of P.falciparum. It exerts activity against gametocytes of the first three plasmodial species but not against those of P.falciparum. The drug has no activity against latent tissue forms of P. vivax or P. ovale and cannot eradicate infections with these species. Chloroquine is not active against parasites in the liver.

Other actions [35]

Chloroquine has direct toxic action against trophozoites of E. histolytica, and it reaches high concentration in the liver, hence used to treat hepatic amoebiasis.

Chloroquine and Hydroxy chloroquine have been used to treat a variety of chronic diseases, because both the alkaloids concentrate in lysosomes and have anti-inflammatory properties. Hence used in Rheumatoid arthritis, Systemic Lupus Erythematosis, Discoid lupus, sarcoidosis, photosensitivity diseases such as porphyria cutanea tarda and severe polymorphous light eruptions.

Mechanism of action

Malarial parasites survive by digesting hemoglobin in the acidic food vacuoles of host RBCs, a process that generates free radicals and heme (Ferriprotoporphyrin IX) as highly reactive by-products.

After nucleation aided by histidine rich proteins and lipids, heme polymerizes into an insoluble unreactive malarial pigment termed haemozoin.

Quinoline blood schizonticides are weak bases and they get concentrated in the acidic food vacuoles of susceptible plasmodia. They increase the pH of the food vacuoles, prevent the oxidation of heme to hemazoin, and disrupt its non- enzymatic polymerization to haemozoin. Hence heme remains inactivated and kills the parasites by causing oxidative damage to parasite membranes.

Pharmacokinetics

Chloroquine is well absorbed from gastrointestinal tract, and rapidly from intramuscular and subcutaneous routes. Drug distributes slowly into a very large apparent volume (over 100 – 1000 l /kg) of distribution. This is due to extensive sequestration of the drug in tissues like liver, spleen, kidney, lung, and melanin containing tissues and to a lesser extent brain and spinal cord.

It moderately binds (60%) with plasma proteins and undergoes biotransformation via the hepatic cytochrome P450 system to 2 active metabolites – Desethyl chloroquine and Bisdesethylchloroquine. These metabolites reach concentration in plasma to 40% & 10% of that of chloroquine respectively.

Excreted in urine with initial half-life of 3 to 5 days, but terminal elimination half-life is 2 months. The renal clearance of chloroquine is about half of its systemic clearance. Unchanged chloroquine and its metabolites account for more than 50% and 25% of urinary drug products respectively. The renal excretion of both the products is increased by acidification of the urine.

Chloroquine is given mainly orally. It is also given either by slow intravenous infusion or small divided doses by subcutaneous or intramuscular routes. It is safer when given orally, because the rate of distribution and absorption are more closely matched. Peak plasma level is achieved in about 3 – 5 hrs after oral route.

It is more potent and less toxic and it needs to be given only once weekly as a suppressive agent compared to quinine. Chloroquine is known to cause vomiting. But when given along with meals, vomiting will be reduced significantly. It is safe in pregnant women and children [38.]

DOSAGE OF CHLOROQUINE [39,40]

Dose of chloroquine

Age in years	**As base (Each 250 mg tablets contain 150 mg base & each 5 ml suspension contains 50 ml base)**			
	1 st dose	**2 nd dose**	**3 rd dose**	**4 th dose**
0-1	75 mg	75 mg	37.5 mg	37.5 mg
1-5	150 mg	150 mg	75 mg	75 mg
5-9	300 mg	150 mg	150 mg	150 mg
9-14	450 mg	225 mg	225 mg	225 mg
>14	600 mg	300 mg	300 mg	300 mg

The first dose is given as a loading dose followed by second dose after 8 hours, third dose on second day and third dose on fourth day.

TOXICITY & SIDE EFFECTS

If taken in proper dose chloroquine is an extraordinarily safe drug.

1. In therapeutic doses

Oral route – vomiting, headache, visual disturbances, and urticaria.

On prolonged treatment -

Headache, blurring of vision, diplopia, confusion, convulsions, lichenoid skin eruptions, bleaching of hair, widening of QRS interval, and T wave abnormalities.

2. Acute chloroquine toxicity

Frequently encountered with high doses administered too rapidly by parenteral routes. Toxic manifestations are,

CVS - Hypotensive shock, vasodilatation, suppressed myocardial function, cardiac arrhythmias, and eventual cardiac arrest.

CNS - Confusion, convulsion and coma

3. High daily doses (> 250mg) **for non- malarial conditions**

When used for the treatment of diseases other than malaria, results in irreversible retinopathy and ototoxicity.

PRECAUTIONS & CONTRAINDICATIONS

It is not used in persons with epilepsy, myasthenia gravis because it itself can induce epileptic attacks.

Given cautiously in hepatic, severe gastrointestinal, neurological or blood disorders.

Can cause hemolysis in patients with G6PD deficiency.

Gold derivatives and Phenylbutazone should be avoided along with chloroquine since all the three can produce dermatitis.

It should not be combined with mefloquine to avoid convulsions, and with amiodarone and halofantriene to avoid ventricular arrhythmias.

RESISTANCE

Resistance to chloroquine is common among strains of P.falciparum, but occurs also for P. vivax in many parts of the world [40,42]

The resistance is reported to be caused by relatively reduced levels of chloroquine in food vacuoles due to difference in plasmodial uptake and transport of chloroquine. The resistant strains develop the ability to cause efflux of chloroquine, so that the preventive action of chloroquine on heme metabolism is inhibited.

2 homologous mdr genes (multi drug resistant genes) P.fmdr.1 & p.fmdr.2 in plasmodium falciparum have been identified [2.]

CURRENT TREATMENT[19]

When a patient from an endemic area presents with fever suggestive of malaria, smear should be prepared to confirm the diagnosis and identify the species of infecting parasites. Patients with severe malaria or those unable to

take oral therapy should receive parentral antimalarial therapy. Chloroquine is the treatment of choice for the “benign malaria” (P. vivax, P. ovale, P. malariae).

Treatment of uncomplicated malaria

1. Infections due to P. vivax, P. ovale, P. malariae, and sensitive strains of P.falciparum should be treated with oral chloroquine.

- Chloroquine (base) 600 mg stat followed 8 hours later by 300 mg, and after that 300mg once daily for days 2 & 3. (Total dose of 25 mg of base/kg).

Or

- Amodiaquine (base) 600 mg followed by 200 mg (base) on the day one after 8 hours and, 400 mg once a day for days 2 & 3.

Or

- Quinine (salt) 300 mg, 6 tablets daily for three days, followed by 4 tablets daily for the next 5-10 days

2. Chloroquine resistant falciparum can be treated with,

- Sulfadoxine/ Pyrimethamine (25/1.25mg/kg) 3 tablets (single dose) followed by quinine 600 mg, orally three times a day for 2 days.

Or

- Quinine 600mg three times a day & Tetracycline (250 mg four times a day for 7 days or Doxycycline. (Tetracyline and Doxycycline cannot be given to pregnant women and to children less than 8 years of age)

Or

- Mefloquine 750 mg orally, repeated after 6 hours (given in a total dose of 25mg / kg)

Or

- Mefloquine & Artesunate, or Artemether (4mg /kg /day for 3 days)

Or

- Artemether & Lumefantrine

Or

- Atovaquone & Proguanil

All the drugs are well tolerated, and given in 3 days regimen.

3. To eradicate persistent liver stages and prevent relapse

- Primaquine (0.3 mg base /kg /day, 15 mg base, adult dose) for 14 days to patients with P. vivax, P. ovale infections after tests for G6PD deficiency have proved negative. If there is mild G6PD deficiency, Primaquine should be given in the dose of 0.6 mg base /kg (45 mg maximum) once weekly for 8 weeks.

Treatment for severe malaria

1. Chloroquine sensitive P. falciparum cases

- Chloroquine 10 mg base/ kg infused intravenously at a constant rate over 8 hours followed by 15 mg base/ kg over 24 hrs.

2. Chloroquine resistant P.falciparum

- Quinine is the most widely used drug for the treatment of severe malaria worldwide

- Quinine dihydrochloride 7mg salt/ kg infused over 30 minutes followed by 10 mg /kg over 4 hrs. Maintenance dose – 10 mg salt /kg infused over 2-8 hrs at 8 hours intervals.

Or

- Artemether 3.2 mg/kg stat intramuscularly followed by 1.6 mg/kg at 24 hour intervals

Or

- Artesunate (2.4 mg/kg stat intravenously or intramuscularly followed by 1.2 mg /kg at 12 and 24 hours then 1mg /kg daily)

Other supportive measures like anti histamines, antipyretics, analgesics, antiemetics, and anti ulcer treatment is given according to the individual patients need.

TREATMENT FAILURE

Many factors contribute to treatment failure, including incorrect dosing regimen, drug interactions, poor or erratic absorption and drug resistance [8]. They also contribute to the development and intensification of true drug resistance through increasing the likelihood of exposure of parasites to sub optimal drug levels.

RESISTANCE

The resistance can be of two types.

a. Drug resistance
b. Vector resistance

DRUG RESISTANCE

The WHO developed the following grading system to describe the relative degrees of resistance of strains of P. falciparum to chloroquine [41.]

1. R I resistance is defined as clearance of asexual parasitemia, followed by recrudescence
2. R II resistance is defined as marked reduction of asexual parasitemia, but no clearance.
3. R III resistance is defined as no marked reduction in parasitemia.

Biological influences on resistance [36,39]

1. Factors that decrease immune system in clearing parasite (malnutrition, patients on anti HIV treatment, immunosuppressed state etc)
2. Certain combination of drug resistant parasites and vector species enhance resistance.
3. Many antimalarial drugs in current usage are closely related chemically and development of resistance to one facilitates development of resistant to others.
4. Genetic plasticity allows the parasite to rapidly adapt to a new drug.
5. Using drugs with long half life (because of prolonged elimination periods)

Pharmacological influence on resistance

1. Over all drug pressure
2. Inadequate drug intake (poor compliance or inappropriate dosing regimens)
3. Reliance on presumptive treatment can facilitate.

MECHANISM OF DRUG RESISTANCE

Resistance appears to occur through spontaneous mutations that confer reduced sensitivity to a given drug or class of drugs. Resistance is more common with falciparum malaria.

It is believed that resistance of P.falciparum to chloroquine is related to an increased capacity for the parasite to expel chloroquine at a rate that does not allow chloroquine to reach levels required for inhibition of heam polymerization. This chloroquine efflux occurs at a rate 40-50 times faster among resistant parasites than sensitive ones [36]. This efflux mechanism is similar to that of found in multidrug resistant mammalian tumour cells, and is mediated by an ATP requiring transmembrane pump, P.glycoprotein [10,15,36,37]. Chloroquine resistance in P.falciparum is associated with multiple unlinked mutations in the digestive vacuole transmembrane protein PfCRT [6]

Specific gene mutations encoding for resistance to both Dihydropteroate synthase and Dihydrofolate reductase have been associated with varying degrees of resistance to antifolate combination drugs. P. vivax is intrinsically relatively insensitive to these drugs [36.]

Single point mutation in cytochrome – b gene causes resistance to atovaquine [14.] Factors that have contributed for antimalarial drug resistance include vector and parasite biology, pharmacokinetic properties of the drugs, and economic status of the patient.

TO REDUCE THE INCIDENCE OF RESISTANCE

1. Reducing the over all drug pressure
2. Improving the prescribing, follow up and patient compliance.
3. Combination therapy [19]

To overcome drug resistance, many new antimalarial drugs have been discovered by adopting several strategies ranging from, [7]

1. Minor modification of existing agents to the design of novel agents that acts against new targets.
2. Combining previously effective drugs with compounds that reverse parasite resistance offers another approach to chemotherapy.
3. Combining 2 or more drugs with different modes of action

VECTOR RESISTANCE

Female anopheles mosquito transmits malaria. This is usual and common mode of transmission. Over 60 species of mosquitoes act as vectors. Out of them 45 are present in India. The important vectors in India include An. Culcifacies, An. Stephensi, An. phillippiensis, An. minimus, An. fluviatilis, An. sundaicus, An. maculatus and An. annularis. An. subpicters has also been

incriminated as vector in certain areas. The vectors of major importance are An. Culcifacies in rural areas and An. Stephensi in urban areas. [22]

Vector control

Vectors can be controlled in 2 ways. Using Larvicides, which acts against Larva (Paris green, pyrethrum, Organochlorides (not safe) and organophosphorous (safe) compounds), and Imagocides, which acts against adult mosquito [36] (DDT (2,2 bis – (p-chlorophenyl)- 1,1,1- trichloroethane), chlorinated hydrocarbons, gamma benzene hexachloride (gamma HCH), dieldrin, Pyrethrins, pyrethroids, Anticholinesterases organophosphorous compounds).

Resistance

Malathion and fenithrothion are organophosphates insecticides, which are being used with increasing frequency for malaria control following the development of vector resistance to DDT [3]

Because of resistance to the newer larvicidals, the old method such as oiling the collections of standing water or dusting them with Paris green is used.

PROPHYLAXIS

Personal protection against malaria includes [19,36]

1. Suitable clothing of exposed skin surfaces.
2. Application of permethrin or delmethrin to clothing or the use of insect repellants containing Diethyl toluamide (DEET) (10-35%)

3. Insecticide impregnated (Permethrin, delamethrin) bed nets & other materials

4. Houses can be mosquito proofed by housing wire mesh grilles over windows, and designed in such a way as to discourage mosquito ingress.

Administration of chemoprophylaxis to high- risk groups like [19]

- Pregnant women traveling to endemic areas.

- Children between the ages of 3 months to 4 years in areas where malaria causes high childhood mortality.

- Children born to non-immune mothers in endemic areas and

- Travelers who are going to an endemic areas.

Anti malarial prophylaxis should be started from at least 1 week before departure and continued till 4 weeks after the traveler has left the endemic area except if atovaquine- proguanil or primaquine has been taken can be discontinued 1 week after departure from the endemic areas.

The drugs used are [19]

Drug	Use	Adult dose	Child dose
Mefloquine	In areas where chloroquine resistant malaria is reported	228 mg base (250 mg of salt) Orally, once a week	< 15 kg- 4.6mg base/kg (5 mg of salt) 15-19 kg- ¼ tab /week 20-30 kg- 1/2 tab /week 31- 45 kg- 3/4 tab /week > 45kg 1tab /week
Doxycycline	Alternative to mefloquine or atovaquine-proguanil	100mg orally, once/day	> 8 years age-2 mg /kg per day Maximum dose 100 mg /day
Atovaquine-proguanil	Alternative to mefloquine or doxycycline	250/100mg orally, once/day	11- 20 kg - 62.5 mg /25 mg 21-30 kg- 125 mg/50mg 31-40kg-187.mg / 75mg > 40 kg- 250 /100mg
Chloroquine	Used in areas where chloroquine – resistant malaria has not reported	300 mg of base orally, once a week	5 mg/kg of base orally, once a week. Maximum dose 300 mg base
Proguanil	Along with chloroquine, as alternate to mefloquine or doxycycline	200 mg orally, once daily with weekly chloroquine	< 2 years- 50 mg /day 2-6 years- 100mg /day 7-10 years- 150mg /day > 10 years- 200mg /day
Primaquine	For travelers only after testing for G6PD deficiency, post exposure prevention for relapsing malaria or prophylaxis	Post exposure- 15mg base (26.3mg of salt) once daily for 14 days Prophylaxis- 30mg of base daily	0.3mg base /kg (0.5mg of salt/kg) orally, once a day for 14 days

USE OF VERAPAMIL IN MALARIA

Many drugs have been shown to reverse the resistance of P.falciparum to chloroquine in vitro. Among them the antihypertensive, Verapamil is proved as an effective chemosensitizer.

In 1987, Martin et al has reported about the reversal of chloroquine resistance in P.falciparum by verapamil [8.] Here the chloroquine resistance was reversed in vitro and in animal models by co- administering with verapamil.

In 1988, another study done by Bitoni et al has revealed reversal of chloroquine resistance by verapamil and desipramine [17.]

In 1990, Watt.g et al proved that verapamil and desipramine caused a dose dependant increase in the accumulation of chloroquine within human and mouse hepatocytes [10.]

In 1991, Ohsawa k, et al has shown the reversal of chloroquine resistance by verapamil, in human and rodent malarial parasites. They proved the ultra structural changes (swelling of the food vacuoles) associated with reversal of chloroquine resistance by verapamil in P.chabaudi [42.]

In 1994, Bray PG, et al proved the relationship of global chloroquine transport and reversal of resistance in P.falciparum [16.]

In 1995, Martiney et al proved that verapamil increases the net uptake and cytotoxicity of structurally diverse hydrophobic molecules in many multidrug resistant mammalian cell lines. The resistance reversal mechanism may be an increase in the amount of chloroquine present in erythrocytes

infected with the infected parasites. In short time incubations verapamil was found to increase net chloroquine accumulation in erythrocytes with both chloroquine sensitive and resistant organisms. It was also found that verapamil is also toxic [11] for trophozoites.

In 1996, Rabinovich et al revealed the reversing action by in vivo experiments using P. berghei model, resistant to chloroquine [13.]

In 1998, Alovelande J described the interaction of two calcium channel blockers, verapamil and fantofarone in reversing chloroquine resistance in P.falciparum [21.]

In 1998, Jean bikii et al investigated the assessment of three in vitro tests and in vivo test for chloroquine resistance in P.falciparum clinical isolates [14.]

In 1999, Kaminsky R. et al investigated mutidrug – resistant and as well as susceptible Trypanasoma brucei brucei. Verapamil showed antitrypanosomal activity against the both [43.]

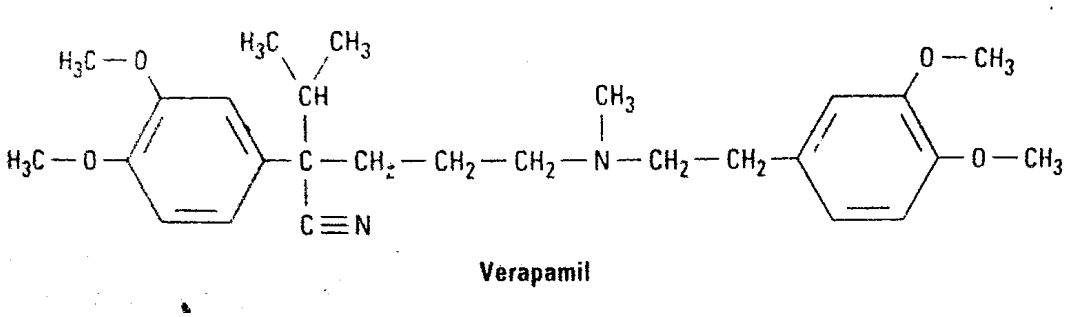
In 2003, David C warhurst showed the polymorphism in the P.falciparum chloroquine – resistance transporter protein, links verapamil enhancement of chloroquine sensitivity with the clinical efficacy of amodiaquine[15.] It was demonstrated that inhibition of calcium influx during cell activation by blocking voltage regulated Ca channels.

All these studies indicate that verapamil would be effective in chloroquine resistant malaria.

VERAPAMIL

It is the first drug used clinically as a calcium channel blocker. Hass and Hartfelter reported in 1962 that verapamil has a coronary vasodilator effect and possess negative inotropic and chronotropic effect. In 1967 it was proved that negative inotropic effect was due to inhibition of excitation contraction coupling and the mechanism involved is the reduction of movement of calcium into cardiac myocytes.

Chemistry



Verapamil

It belongs to a phenylalakylamine group of calcium channel blocker. It is prescribed as a racemate "l" verapamil, which is more potent Ca^{2+} channel blocker than "d" verapamil.

Pharmacokinetics

Peak effect of verapamil occurs within 15 min of its intravenous administration. With oral therapy "l" verapamil under goes more extensive first pass metabolism. For this reason, a given concentration of verapamil prolongs PR interval to a greater extent when the drug is administered intravenously.

Verapamil can cause gastro esophageal reflex. Constipation can occur with oral verapamil [22]. 70 – 98% bound to plasma proteins.

Its elimination half-life is widely variable. N- methylation of verapamil results in production of norverapamil, which is biologically active, but much less potent than parent compound. Half-life is 10 hrs. In patients with hepatic disease the half-life increases to 14 – 16 hrs because of increased volume of distribution. It can be given in the dose of 80- 160 mg orally for every 8 hours.

Verapamil competitively blocks the drug transporter, 'p' glycoprotein [10,15,36.37]. Both the renal and hepatic disposition of digoxin occurs via the transporter. So verapamil inhibits the elimination of digoxin and other drugs that are cleared from the body by P – glycoprotein.

Pharmacodynamics

Verapamil binds with the transmembrane segment 6 of domain IV in $alpha_{1.}$subunit of L type calcium channel. The drug acts from the inner side of the membrane and bind more effectively to channels in depolarizing membrane. Binding of the drug reduces the frequency of opening in response to depolarization. It results in marked decrease in transmembrane calcium current associated with smooth muscle with a long lasting relaxation.

It is a potent coronary vasodilator; it suppresses cardiac contractility, suppresses the SA node automaticity, suppresses AV node conduction, and reduces the myocardial oxygen demand.

It lowers BP by relaxing arteriolar smooth muscle and decreasing peripheral vascular resistance. As a consequence of a decrease in peripheral

vascular resistance it evokes baroreceptor mediated sympathetic discharge. But tachycardia induced by this drug is minimal or sometimes absent, because of the direct chronotropic effect.

Precautions & Contraindications

Re-entrant Supra ventricular tachycardia (SVT) and nodal arrhythmias (Wolf Parkinson White Syndrome [35]) are susceptible to verapamil, but it should not be used because of risk of increased ventricular rate due to reflex sympathetic stimulation and reduction of effective refractory period (ERP) of the bypass tract in some cases.

Intravenous verapamil with beta-blocker is contraindicated, because it has increased propensity for AV block or severe depression of ventricular function.

In patients with ventricular tachycardia, intravenous injection of verapamil has precipitated ventricular fibrillation and therefore contraindicated. It is not recommended for digitalis toxicity, because additive A-V block can occur. It is contraindicated in partial heart block and sick sinus syndrome.

Uses

1. Paroxysmal supra ventricular tachycardia
2. To control ventricular rate in Atrial fibrillation and Atrial flutter.
3. In hypertrophic cardiomyopathy it will improve the left ventricular out flow obstruction and symptoms of the patients.
4. Prophylaxis of migraine headaches.

METHODOLOGY

STUDY CENTRE

The study was conducted in the Department of Medicine, Government Stanley Medical College Hospital, Chennai.

STUDY DESIGN

Prospective, Randomized double blind control clinical study.

STUDY PERIOD

From July 2005 to December 2005

ETHICAL COMMITTEE APPROVAL

Study was conducted only after getting approval of the institutional Ethics committee. A copy of the approval is enclosed in the Annexure I

SELECTION OF PATIENTS

INCLUSION CRITERIA

Patients who fulfilled the following criteria were included in the study.

- Patients diagnosed to have malaria by QBC method
- Both the sexes
- 15 – 60 years age

EXCLUSION CRITERIA

Patients who had any of the following were excluded from the study.

- Children < 15 years
- Pregnant or lactating mothers
- Those with known cardiac diseases (Hypertension, Coronary artery diseases, Bradycardia and conduction defects)
- Diabetes and other associated illnesses.

METHODOLOGY

Sixty patients with QBC positive malaria were enrolled in the study. All the 60 patients were admitted in the Medicine ward. They were well informed about the study. Written consent was obtained from all of them.

Patients name, age, sex, residence, income, and locality were noted. Then the detailed history about the fever (duration, chills, rigor, whether continuous or intermittent), vomiting, abdominal pain, headache, and loss of appetite were recorded.

General and systemic examinations were done. Temperature, pulse rate, blood pressure were recorded 4^{th} hourly. And the patient was examined for anemia, Jaundice, splenomegaly and hepatomegaly.

After the clinical examination the following lab investigations were done.

1. Hematological investigations:

- Hemoglobin
- Total Count
- Differential Count
- Erythrocyte Sedimentation Rate
- Peripheral smear for Malarial Parasite

2. Urine examination

- For Red Blood Cells (RBCs) along with the routine examination.

3. Other investigations

- Electrocardiogram (ECG)
- Ophthalmic examination.

A copy of the proforma is enclosed in Annexure II

TREATMENT

After the pre-trial investigations each patient was randomly allocated to either one of the 2 groups namely A & B.

Patient in-group A received the standard chloroquine therapy (600mg as first dose and after 8 hours 300mg, followed by 300 mg after 24 hours then another 300 mg after 48hours) along with symptomatic treatment.

Patient in-group B received in addition to chloroquine, 40mg of verapamil orally 2 hours after the administration of chloroquine. The drugs were given to the patient by the investigator personally. Since verapamil is an anti hypertensive drug, blood pressure and pulse rate were recorded 4^{th} hourly.

Each patient was examined 4th hourly for improvement in signs & symptoms (fever, chills, rigor, blood pressure, vomiting and abdominal pain) using the under mentioned parameters. 12th hourly QBC was done to know the rate of clearance of parasitemia.

EVALUATION

The following evaluating parameters were recorded.

1. Time taken for the body temperature to become normal.
2. Disappearance of symptoms like chills and rigor.
3. Time taken for clearance of parasitemia.
4. Reduction in splenomegaly.
5. In addition, all the patients were subjected to the same pre-trial investigations and any change in the results was suitably analyzed.

RESULTS

The double blind randomized control trial was undertaken to study the effect of verapamil in facilitating the action of chloroquine in malaria. Efficacy of the treatment was analyzed by using the parameters. The results were analyzed by Pearson Chi square test and Student t test and the $P< 0.01$ was considered significant.

It was observed from the above study that verapamil has a beneficial effect in malaria. Though both the groups got clinically cleared of malaria, the patients who were given verapamil had an easier relief and very faster clearance of parasitemia. All the patients who were included in the study completed their study. There were no dropouts. No one had vomiting or visual disturbances. And patients who received verapamil did not have any significant bradycardia or hypotension.

Table 1 COMPARISON OF DEMOGRAPHIC PROFILE

S. No	Character	Control group		Study group	
		Mean	SD	Mean	SD
1	Age	26.40	12.05	23.23	9.93
2	Sex	24 Male (80%) 6 Female (20%)		27 Male (90%) 3 Female (10%)	
3	Residence	18 From Slum (60%) 12 From other areas (40%)		14 From Slum (46.7%) 16 From other areas (53.3%)	

The mean age in control group was 26 years and in study group was 23 years. Among the 60 patients enrolled in the study, 51 were males and 9 were female patients. Even in random allocation the number of male patients who entered the study was high in both the groups. The number of patients from slum and from other areas was equally distributed. There was no statistical significance in the residence

Table 2 COMPARISONS OF CLINICAL FEATURES

		Group			
		Control (30)		Study (30)	
		Number of patients	%	Number of patients	%
Fever (F)	Present	30	100.0%	30	100.0%
F+ Rigor (R)	Absent	3	10.0%		
	Present	27	90.0%	30	100.0%
Vomiting (V)	Absent	16	53.3%	10	33.3%
	Present	14	46.7%	20	66.7%
F+R+V	Absent	16	53.3%	10	33.3%
	Present	14	46.7%	20	66.7%
Headache	Absent	19	63.3%	19	63.3%
	Present	11	36.7%	11	36.7%
Abdominal pain	Absent	24	80.0%	22	73.3%
	Present	6	20.0%	8	26.7%
Loss of appetite	Absent	25	83.3%	20	66.7%
	Present	5	16.7%	10	33.3%
Anemia	Absent	2	6.7%	6	20.0%
	Present	28	93.3%	24	80.0%
Splenic enlargement	Absent	15	50.0%	16	53.3%
	Present	15	50.0%	14	46.7%
Liver enlargement	Absent	24	96.0%	28	93.3%
	Present	1	4.0%	2	6.7%

The above table shows various clinical features of the control and the study group.

Out of 60 patients studied, in the control group 46.7% of patients and 66.7% patients in the study group were complaining of vomiting. The difference was statistically not significant.

Fever, rigor and vomiting were also complained by 46.7% in control group and 66.7% by study group, which was also statistically not significant.

Headache was present for 36.7% in both the groups. Abdominal pain was present for 20% in control group and 26.7% in study group, which was statistically not significant. Loss of appetite was present in 16.7% of control group and 33.3% of study group.

Anaemia was present in 93.3% of control group and 80% in study group. Spleen was enlarged in 50% of control group and 46.7% in study group. The difference was statistically not significant. Liver was enlarged in 4% of the control and 6.7% in study group.

Table 3 PRE TRIAL INVESTIGATIONS

	Group	N	Mean	Std. Deviation
Hemoglobin	Control	30	9.9536	1.11272
	Study	30	10.5286	.88270
Total count	Control	30	8126.6667	1191.32882
	Study	30	7103.3333	1062.36020
Polymorphs	Control	30	51.5667	4.74657
	Study	30	48.5000	5.02236
Lymphocytes	Control	30	46.1333	4.73966
	Study	30	47.8000	4.15559
Monocytes	Control	30	2.7000	.95231
	Study	30	2.6333	.88992
Eosinophills	Control	30	1.6000	1.03724
	Study	30	2.8667	1.43198
ESR1/2	Control	30	3.1667	1.17688
	Study	30	3.0000	.90972
ESR1	Control	30	6.3333	2.35377
	Study	30	6.0000	1.81944

The above table shows the Pre-trial investigations of both the control and study group.

Before the start of the treatment all the patients under went pre trial laboratory investigations like hemoglobin, total count, differential count and erythrocytic sedimentation rate. The mean Hemoglobin for control group was 9.9 and in the study group it was 10.5. There was no statistical significance between the two values. Total count in control group was 8126.6 and in study group it was 7103.3, here also there was no statistical significance.

Polymorphs, in control group were 51.5 and in study group was 48.5. Lymphocytes, in control group were 46.1 and in study group was 47.8. Monocytes, in control group were 2.7 and in study group 2.6. Eosinophills in control group was 1.6 and in study group it was 2.8. There was no statistical significance in the differential count in both the groups. ESR in half an hour is 3.1 in control group and 3 in study group. ESR in one hour is 6.3 in control group and 6 in study group. Here also there is no statistical significance in the difference between the two groups.

Table 4 REDUCTION IN TEMPERATURE

Time taken for normalization of temperature (in hrs)	Group			
	Control		Study	
	Number of patients	%	Number of patients	%
12	9	30.0	28	93.3
24	18	85.7	2	100.0
36	3	100.0		
48	0			

The above table shows reduction in temperature in the control and the study group.

Temperature was reduced to normal in 30% of the patients' in-group A, whereas 93.3% in group B within 12 hrs of drug administration, which is found to be statistically significant.

At the end of 24 hrs, temperature was reduced in 85.7% of patients in control group whereas 100% reduction in patients in study group (Fig.1).

Reduction in temperature is an important clinical sign denoting improvement. Within the first 12 hours of drug administration, only 30% of patients in the control group had shown reduction in temperature, whereas 85.7% in the study group had fall in temperature.

Table 5 REDUCTION IN CHILLS & RIGOR

Time taken for normalization of chills and rigor (in hrs)	Group			
	Control		Study	
	Number of patients	%	Number of patients	%
12	9	30.0	28	93.3
24	16	76.2	2	100.0
36	5	100.0		
48				

The above table shows the reduction in chills and rigor in the control and study group.

Chills and rigor was controlled in 30% of the control group with in 12 hrs whereas in the study group 93% had relief, which is statistically highly significant. (P=0.001)

Chills and rigor was controlled in 76% of the patients in control group within 24 hrs whereas it was 100% in study group (Fig.2).

Table 6 PARASITEMIA CLEARANCE

Time taken for parasitemia clearance (in hours, by QBC method)	Group			
	Control		Study	
	Number of patients	%	Number of patients	%
12	3	10.0	18	60.0
24	16	59.3	11	91.7
36	8	72.7	1	100.0
48	3	100.0		

The above table shows clearance of parasitemia by QBC method in the control and the study group.

The clearance of parasitemia by QBC was 10% with control group within 12 hours whereas 60% in study group, which is statistically highly significant. (P = 0.001)

By 24 hrs, it was 60% with control group and 92% with verapamil group. It is statistically highly significant. By 36 hrs 73% of the control group and 100% of the study group were cleared of parasites (Fig.3).

The study has shown that, all the patients in the study group treated with chloroquine and verapamil had complete relief of signs and symptoms within 36 hours. The duration in temperature, relief from chills and rigor and the clearance of parasitemia were significantly earlier than the control group.

All the patients in the control group had relief from symptoms and signs by 48 hours but the rate of clearance was found to be slower than the study group.

The difference between the two groups was found to be statistically significant indicating that varepamil has facilitated the action of chloroquine.

Table 7 POST TRIAL INVESTIGATIONS

	Group	**N**	**Mean**	**Std. Deviation**
Hemoglobin	Control	30	9.7238	1.11354
	Study	30	10.5151	.87525
Total count	Control	30	9214.4500	1185.35822
	Study	30	7600.4853	1075.35869
Polymorphs	Control	30	49.5847	4.78569
	Study	30	50.4153	5.14524
Lymphocytes	Control	30	48.4583	4.79874
	Study	30	45.4200	4.16584
Monocytes	Control	30	2.7124	.87431
	Study	30	2.6752	.92452
Eosinophills	Control	30	1.4658	1.25844
	Study	30	2.2547	1.25848
ESR1/2	Control	30	3.1547	1.45878
	Study	30	3.2540	.924582
ESR1	Control	30	6.4751	2.87574
	Study	30	6.2541	1.95784

The above table shows the Post-trial investigations done in both control and study group.

After the treatment all the patients under went post trial laboratory investigations like hemoglobin, total count, differential count and erythrocytic sedimentation rate. The mean Hemoglobin for control group was 9.7 and in the study group it was 10.5. There was no statistical significance between the two values. Total count in control group was 9214.4 and in study group it was 7600.5, here also there was no statistical significance.

Polymorphs, in control group were 49.5 and in study group was 50.4. Lymphocytes, in control group were 48.4 and in study group was 45.4. Monocytes, in control group were 2.7 and in study group 2.7. Eosinophills in control group was 1.4 and in study group it was 2.2. There was no statistical significance in the differential count in both the groups. ESR in half an hour is 3.1 in control group and 3.2 in study group. ESR in one hour is 6.4 in control group and 6.2 in study group. Here also there is no statistical significance in the difference between the two groups.

The difference between the control group and the study group was not statistically significant in both pre trial and post trial laboratory investigations.

DISCUSSION

Among the febrile diseases, though easily diagnosable and treatable, malaria still remains a challenging disease of the fatality associated with cerebral malaria and the development of resistance by malarial parasites to most of the antimalarial drugs.

Followed by aminoquinolines, (Chloroquine and its derivatives) several other groups of drugs have been introduced (Mefloquine, Halofantrine, Artemisinin derivatives etc). Among the available drugs, chloroquine is still the effective drug in sensitive malaria. But resistance is common and widespread between P.falciparum. High-grade resistance was reported as early in 1978.

Resistance among P. vivax was reported in 2000 from Chennai, Madhyapradesh and Mumbai [35]. Subsequent to the development of resistance to chloroquine, several alternative drugs have been used.

Mefloquine is effective against both chloroquine sensitive and resistant malaria. However like chloroquine, relapses occur in vivax malaria and resistance to mefloquine among P. falciparum has been reported from Thailand, Cambodia and Myanmar[35]. Though resistance to mefloquine has not been widely reported from India (except in Gujarat), due to its long half-life the possibility of selection of resistance strains is high. Resistance to mefloquine confers cross-resistance to quinine and halofantrine also.

Quinine is an erythrocytic schizonticide effective in most chloroquine and multidrug resistant P. falciparum malaria. Though effective in terminating the acute attacks of malaria it may not prevent recrudescence. Moreover

toxicity of quinine is very high. Cinchonism can occur even after single therapeutic dose in sensitive individuals.

Pyrimethamine- Sulfonamide combination has supra additive effect and highly effective in P. falciparum. But efficacy against P. vivax is low. It is contraindicated in infants and those who are allergic to sulfonamide. Though resistance to this combination has not been reported in India, resistance is high in South East Asia, South America and Southern Africa.

The Artemisinin compounds are potent and rapid blood schizonticides. Three derivatives are available in India. They are Artemether, Artesunate and Arteether. Arteether has been developed in India. They are active against both the sensitive and resistant P. falciparum. They produce quicker parasitemia clearance than chloroquine. But their duration of action is short and recrudescence rate is high when they are used alone. Hence artemisinin compounds are combined with drugs like mefloquine to prevent recrudescence.

Halofantrine is effective against resistant strains of both P. vivax and falciparum. Its schizonticidal activity and its potential to cause cardiac toxicity, halofantrine should be used cautiously even in therapeutic dose.

Thus, though alternative drugs are available for the treatment of chloroquine resistant malaria, resistance to these drugs is also emerging and multidrug resistant malaria is prevalent in many parts of the world.

Whenever organisms become resistant to the available drugs, two modalities of treatment can be followed.

1. To select a new drug, if new drug is not available

2. To use the existing drug along with another drug which has the property of reversing the resistance.

As chloroquine which has been effective for more than fifty years, has recently developed resistance and when drugs which can reverse chloroquine resistance have been reported it would be economical to use chloroquine along with any one of these resistance reversers rather than finding out a new drug which would be very expensive and time consuming.

Hence it would be appropriate to study the effect of chloroquine resistant reversers. The calcium channel blocker verapamil has been reported to be an effective resistant reverser by many in-vitro studies [6-16]. But its effect in sensitive malaria is unknown. By its mechanism of preventing the efflux of chloroquine from the parasite by inhibiting the P-glycoprotein [10,15,36,37] (A multidrug resistant protein, which increases the efflux of the drug) or inhibition of Calcium channel (which is necessary for drug influx), whether it would facilitate and prolong the action of chloroquine? Therefore, in order to find out its effect in both sensitive and resistant malaria the present study was undertaken.

Sixty patients were included in the study. Both the control (Group A) and the test group (Group B) had equal number of thirty patients. All the patients were found to belong to lower and lower middle class family.

The mean age in control group was 26 years and in study group 23 years. Even though malaria is common in all the age group, our study group had only young adults. Among the 60 patients enrolled in the study, 51 were males and 9 were female patients. Even in random allocation the number of male patients who entered the study was high in both the groups. The male preponderance may be due to the outdoor sleeping habit of male workers.

46.7% of patients in the control group and 66.7% patients in the study group complained of vomiting before treatment. Vomiting is attributed to high concentration of parasites and toxemia. After the treatment was started the number of patients with vomiting was reduced which may be due to the clearance of parasitemia and toxemia. Very few patients complained of vomiting even after treatment, which could be due to the drug effect. In the study group vomiting was relieved within 12 hours compared to the control group.

Similarly fever and rigor subsided 12 hours earlier in the study group. It is due to early clearance of parasitemia.

Loss of appetite was present in 16.7% of control group and 33.3% of study group, which improved within 36 hours of the treatment.

Reduction in temperature is an important clinical sign denoting improvement. It was reduced to normal in all the patients in-group A within 36 hours, whereas in-group B within 24 hrs of drug administration (12 hours earlier than the control group) which is found to be statistically very significant.

All the patients in the control group had relief from symptoms and signs by 48 hours but the rate of clearance was found to be slower than the study group.

Thus, the study has shown that, all the patients in the study group treated with chloroquine and verapamil had complete relief of signs and symptoms within 36 hours. The duration of temperature, relief from chills and rigor and the clearance of parasitemia were significantly earlier (12 hours) than the control group.

The difference between the two groups was found to be statistically significant indicating that verapamil has facilitated the action of chloroquine.

Though it is reported that in malaria there will be increased leucocyte count, increased monocytes [19], and increased ESR, decreased neutrophils, this picture was not seen in this study. Anemia was found in 93.3% in control group and 80% in study group. The increased incidence of anemia could be due to the extensive destruction of RBCs in patients treated with chloroquine alone.

Verapamil a calcium channel blocker, is known to cause bradycardia, hypo tension and constipation due to its inhibitory effect on heart and gastrointestinal tract. But none of the patients suffered from hypotension or bradycardia. When verapamil is used either as an antihypertansive or antiarrhythmic, the duration of therapy is long, whereas in malaria it is used only for three days and the maximum dosage administered was only 160 mgm. Hence at this dosage verapamil has not produced any side effect and found to be very safe. The number of days of stay in the hospital was reduced to two

because of the early relief of symptoms. This has reduced the cost on hospitalization and helped the patients resume duty at an early date.

The beneficial effect of verapamil is as discussed earlier due to its action on chloroquine efflux leading to increased concentration of chloroquine in the parasite causing early death of the parasites resulting in early relief of signs and symptoms.

To summarize, the present study has shown that verapamil has a definite beneficial effect in malaria when used along with chloroquine.

SUMMARY

Among the protozoal diseases malaria is the most devastating one and cerebral malaria can be fatal if not treated promptly. Several antimalarial drugs from the older quinine to the recent artemesinin are available today. Among them chloroquine is still the most commonly used and an effective antimalarial drug. But resistance to chloroquine developed by the malarial parasites posses a great problem in treating malaria. Hence prevention of development of such resistance is mandatory to prevent the malarial morbidity.

Recent studies have shown that drugs such as verapamil, chlorpheniramine maleate, desipramine, etc reverse chloroquine resistance. Among these, verapamil has been widely reported as an effective resistance reverser by in vitro studies. But in vivo study establishing the efficacy of verapamil has not been carried out. Hence with an aim of finding out whether verapamil will be synergistic when given with chloroquine in all cases of malaria, a randomized controlled clinical study was conducted.

The study has revealed that verapamil is synergistic when given with chloroquine by producing early reduction in temperature, early clearance of parasitemia and other constitutional symptoms. As the dose administered is very small (160 mg total, which can be a single antihypertensive dose) no untoward effects occurred in any of the patients.

Hence this drug can be safely used along with chloroquine in malaria for early recovery and to shorten hospitalization as well as reduce the cost of therapy.

CONCLUSION

It can be concluded from this study that,

1. Verapamil is effective in malaria.
2. Verapamil has a synergistic action when given with chloroquine in malaria
3. It is safe
4. Hence it can be used along with chloroquine both in sensitive and resistant malaria for better efficacy and early recovery.

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EFFECT OF VERAPAMIL IN MALARIA

Dissertation Submitted to

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

in partial fulfillment of the regulations

for the award of the degree of

M.D. (Pharmacology)

BRANCH – VI

GOVT. STANLEY MEDICAL COLLEGE & HOSPITAL

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI, INDIA.

MARCH 2007

CERTIFICATE

This is to certify that this dissertation entitled **"EFFECT OF VERAPAMIL IN MALARIA"** is a bonafide record of the research work done by **Dr. K. LATHA** in the Department of Pharmacology, Stanley Medical College, Chennai-600 001 during the period between 2004 –2007

I also certify that this dissertation is the result of the independent work done by the candidate.

DEAN
Stanley Medical College and Hospital
Chennai-600 001.

PROFESSOR
Head of the Department of Pharmacology,
Stanley Medical College,
Chennai-600 001.

DECLARATION

I solemnly declare that this dissertation "**EFFECT OF VERAPAMIL IN MALARIA"** was done by me in the Department of Pharmacology, Govt. Stanley Medical College and Hospital, Chennai, under the guidance and supervision of Prof**. Dr. A. Ruckmani, M.D., D.D.,** Former Professor and Head, Department of Pharmacology, Govt. Stanley Medical College, Chennai-600 001.

This dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai in partial fulfillment of the University regulations for the award of degree of **M.D. Branch VI Pharmacology** examinations to be held in March 2007.

Place: Chennai.

Date:

Dr. K. LATHA

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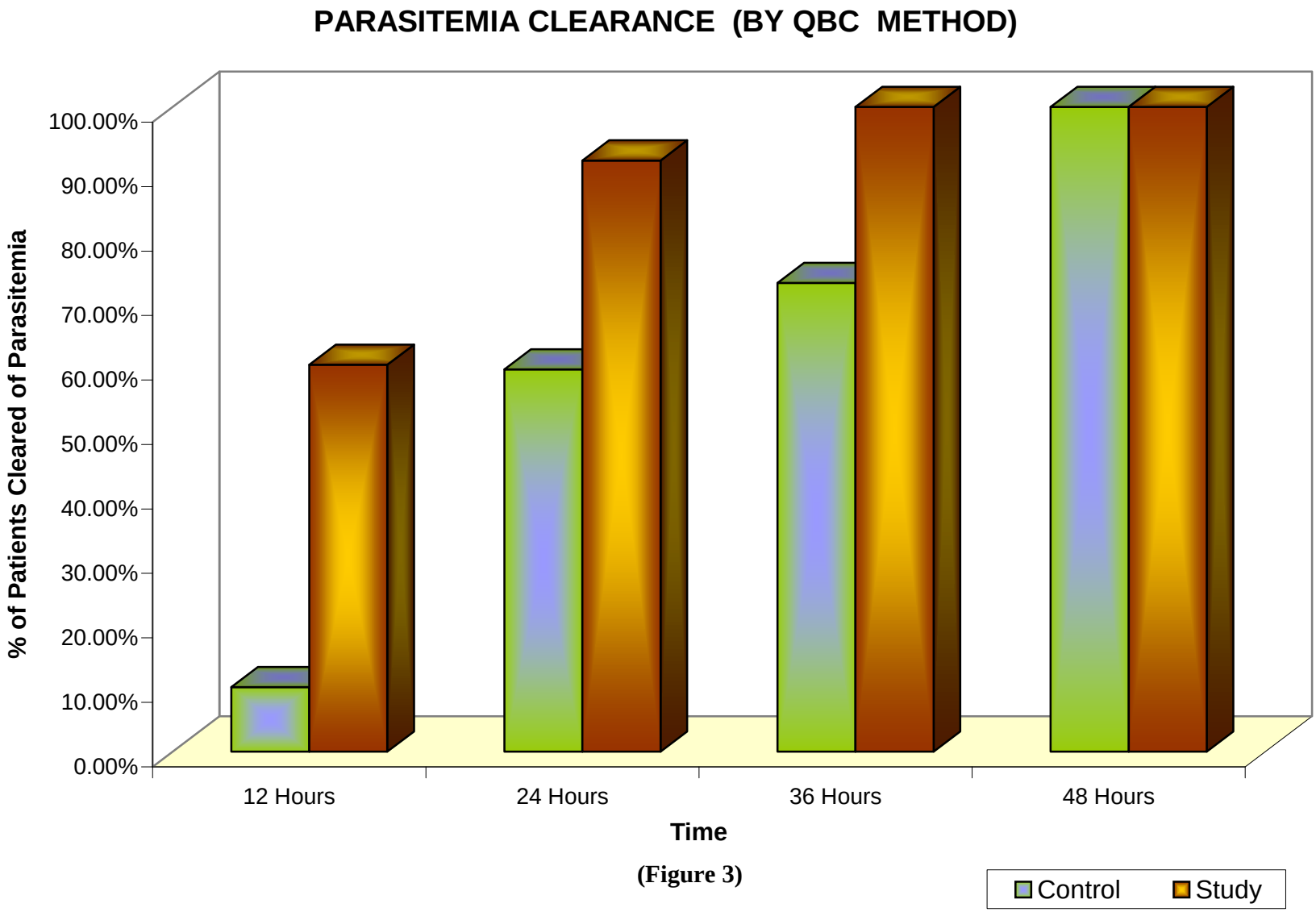
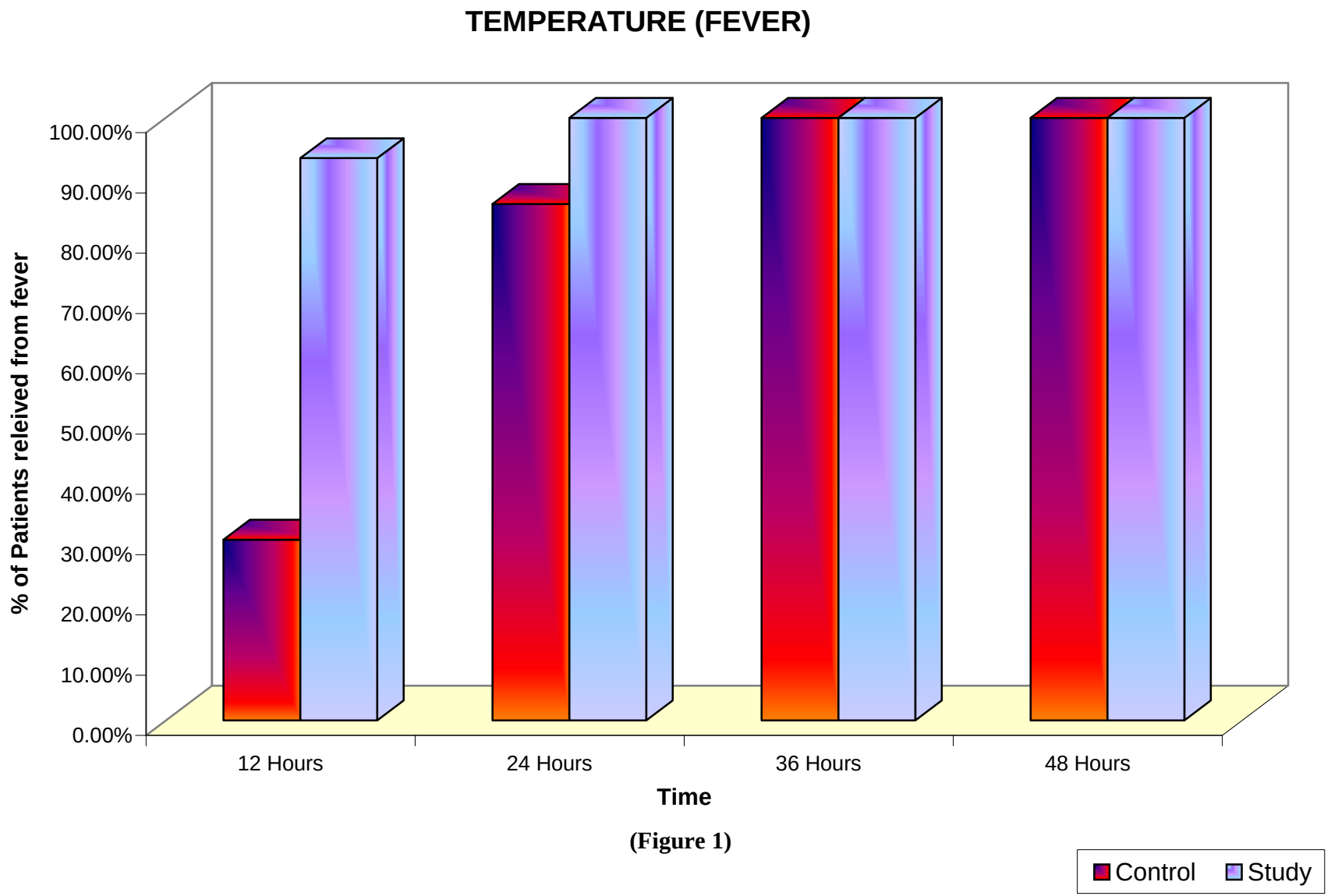
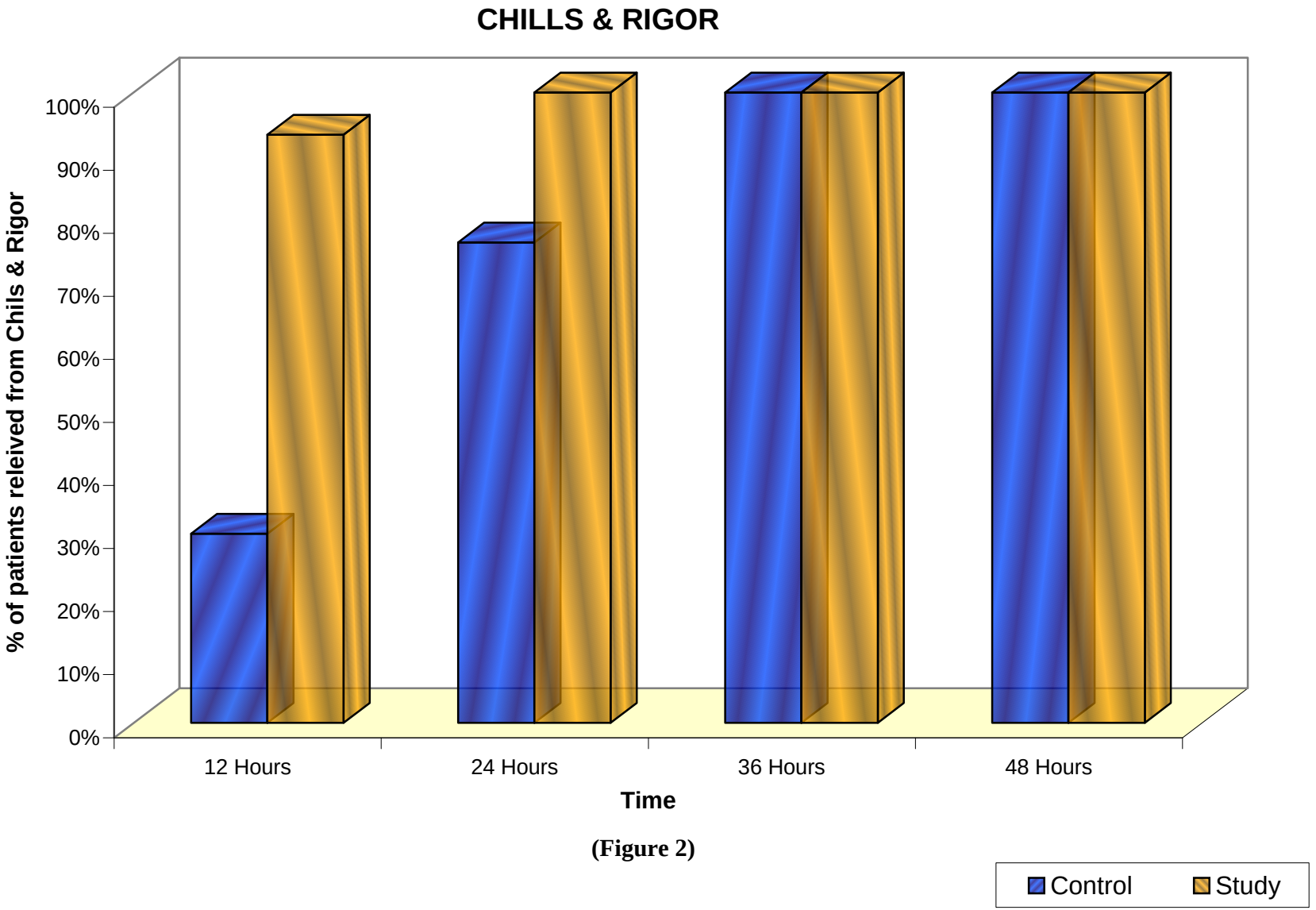
CONTENTS


PARASITEMIA CLEARANCE (BY QBC METHOD)
% of Patients Cleared of Parasitemia
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
12 Hours
24 Hours
36 Hours
48 Hours
Time
Control
Study

(Figure 3)


TEMPERATURE (FEVER)
% of Patients releived from fever
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
12 Hours
24 Hours
36 Hours
48 Hours
Time
Control
Study

(Figure 1)


CHILLS & RIGOR
% of patients releived from Chils & Rigor
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
12 Hours
24 Hours
36 Hours
48 Hours
Time
Control
Study

(Figure 2)

12 Hours	10.00%	60.00%
24 Hours	59.30%	91.70%
36 Hours	72.70%	100.00%
48 Hours	100%	100%

12 Hours	30.00%	93.30%
24 Hours	85.70%	100.00%
36 Hours	100.00%	100.00%
48 Hours	100%	100.00%

12 Hours	30%	93.30%			
24 Hours	76.20%	100%			
36 Hours	100%	100%			
48 Hours	100%	100%			

		group			
		control		study	
		n	%	n	%
Chi+Rig12	no	9	30.00%	28	93.30%
	yes	21	70.00%	2	6.70%
Chi+Rig24	no	16	76.20%	2	100.00%
	yes	5	23.80%		
Chi+Rig36	no	5	100.00%		
Chi+Rig48	.				

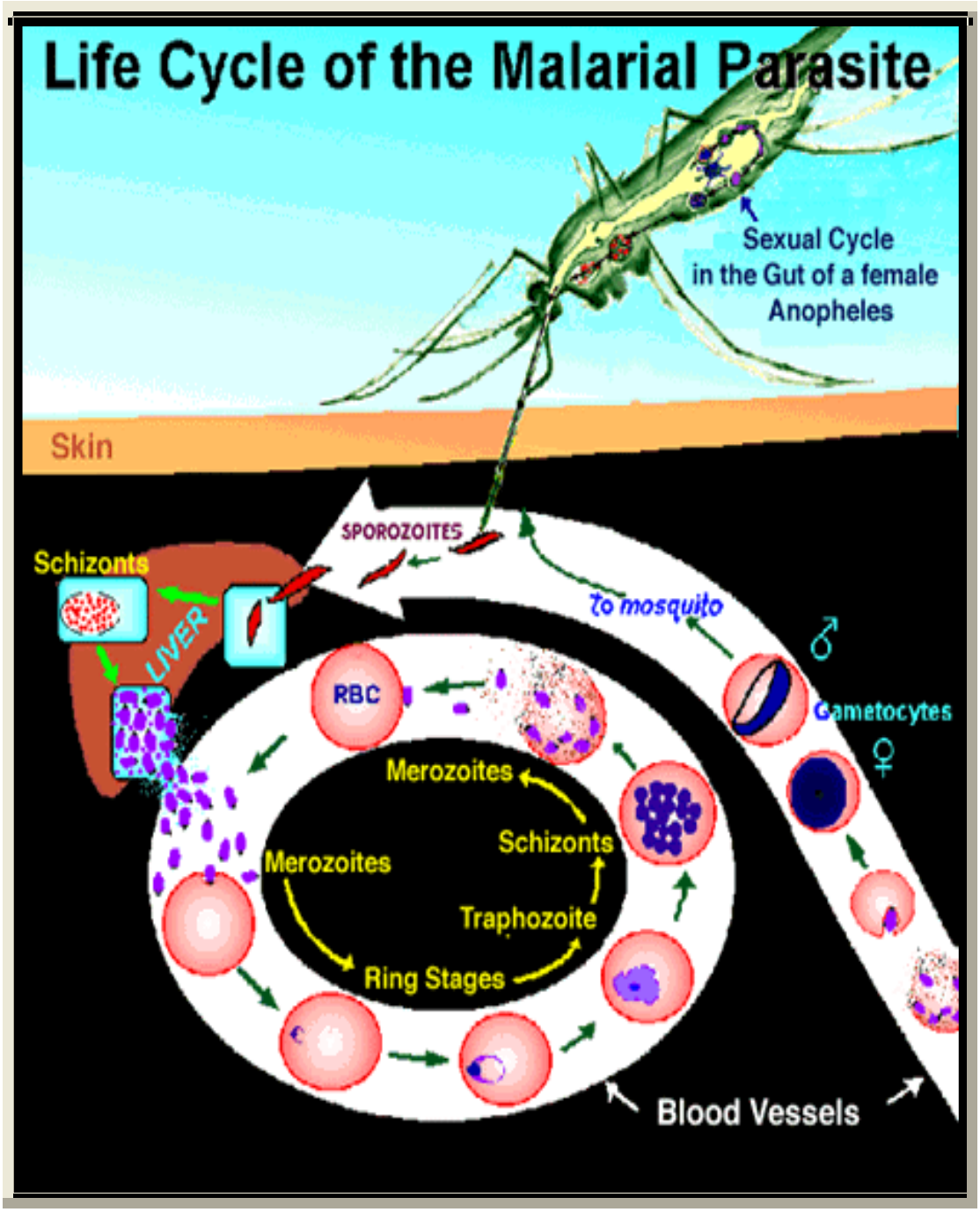
The QBC Tube

« Platelets

« Lymphocytes/ monocytes

« Granulocytes

« Red Blood Cells


Life Cycle of the Malarial Parasite
Sexual Cycle
in the Gut of a female
Anopheles
Skin
SPOROZOITES
Schizonts
LIVER
To mosquito
♂
RBC
Gametocytes
♀
Merozoites
Schizonts
Merozoites
Traphozoite
Ring Stages
Blood Vessels

THE QBC TEST

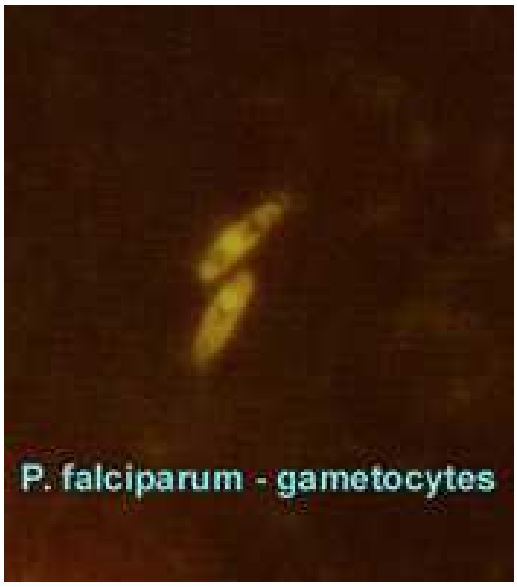

P. vivax - trophozoites

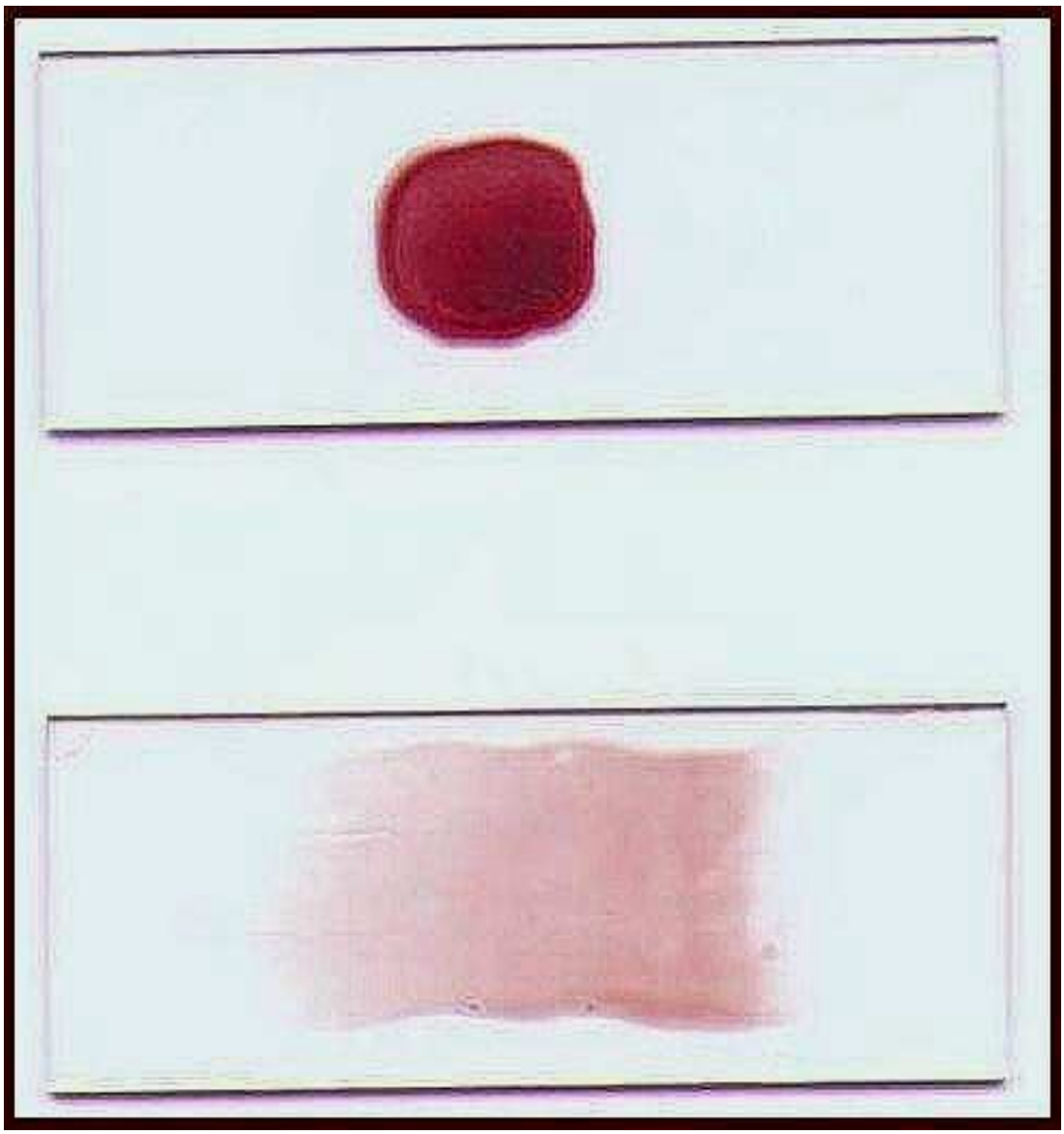

P. vivax - rings(+) and schizont (arrow)


P. vivax - gametocyte


P. falciparum - single ring


P. falciparum - double rings


P. falciparum - gametocytes



Thick and Thin Smears

The following Clinical Trial which was presented at the Ethical Committee meeting held at the Dean's Chamber, Stanley Medical College, on 14-6-2005, by the Department of Pharmacology is approved by the Ethical Committee.

Study Title : Study of effect of Verapamil in Malaria

Members	Signature
1.Prof. T.Raveendran	
2.Prof. V.Rajalakshmi	
3.Prof. Ruckmani	
4.Prof.S.Nadarajan	
5.Prof. Jayaraman	
6.Prof. P.R.Thenmozhivalli	
7.Prof. V. Jayanthi	
8.Tmt. G.Vatsala	
9.Thiru Sugumar	
10.Lawyer	(B. REKHA)

PROFORMA FOR THE STUDY OF EFFECT OF VERAPAMIL IN MALARIA

GROUP A/B

NAME :
AGE :
SEX :
RESIDENCE :

INCOME :
LOCALITY :

PRESENT HISTORY :

ASSOSIATED ILLNESS :

PERSONAL HISTORY :

GENERAL EXAMINATION :

LOCAL EXAMINATION :

INVESTIGATIONS :

SERIAL NUMBER	INVESTIGATIONS	PRE TRIAL	POST TRIAL		
1	HAEMOGLOBIN				
2	TOTAL COUNT				
3	DIFFERENTIAL COUNT				
4	ESR				
5	ECG				
6	VISION				
7	URINE-RBC				
8	QBC	POSITIVE/NEGATIVE	**TIME TAKEN FOR CLEARANCE**		
			12 hrs	24 hrs	48 hrs
9	PERIPHERAL SMEAR				

EVALUATION :

1) Time taken for the reduction in body temperature :
2) Time taken for disappearance of chills & rigor :
3) Time taken for clearance of parasitemia :
4) Spleen enlargement :
5) Investigations- change in post trial :

RESULT :