The day relenting God,
Hath placed within my hands
A wondrous thing; and God
Be praised. At His command,

Seeking His secret deeds
With tears and toiling breadth
I find thy cunning seeds,
O Million-murdering Death.

I know this little thing
A myriad men will save.
O Death, where is thy sting,
Thy victory, O Grave!

Sonnet tells written by Ronald Ross to his Wife Rosa after his discovery on 20th August 1897 in Secundrabad, India

MATERIALS AND METHODS
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This study was done at Microbiology department, Pramukh swami medical college attached with Shree Krishna hospital, Karamsad. This teaching hospital caters tertiary care to people of Anand, Kheda and Baroda districts. The study was done as a pilot work from December '92 to August '94. This study does not include the data of that period however the basic establishment and preparation foundation was done during this pilot work.

This study includes work done between 1st October '94 to 30th November '97 i.e. of 38 months. This study was undertaken under following six study groups:

[1] Study of all indoor patients who were admitted in the hospital during study period and had proven diagnosis of malaria were included.

[2] All cases of fever coming to out-patient department (OPD) of Shree Krishna Hospital during certain months of year, during both transmission and non transmission season; were examined for presence of malarial parasites. The prevalence of positive smear for malarial parasite was studied by analysing these OPD fever cases. The detail clinical spectrum of non admitted patients were not studied, however the important parasitological and follow up data was noted in each individual cases.

[3] The study for malarial parasite of all blood donors who donated their blood at Gorwala Blood Bank in our institution and Red Cross Blood Bank of Anand was done. The data was analysed to find out asymptomatic blood parasitaemia.
[4] 226 tests of 200 cases of fever were studied as a separate group to evaluate HRP-II antigen based test by Parasight-F test (manufactured by Becton & Dickinson Co.).

[5] Cultivation of *Plasmodium falciparum* parasite was undertaken in a small selected group of 10 patients.

[6] The prevalence of malarial parasite in HIV positive individuals were taken as a separate group. This group was undertaken to find out association of malaria and HIV in our area.

Selection of patients for study group I...

The patients for the first study group were selected by following criteria:

(i) The peripheral smear should show malarial parasite by conventional microscopy.

(ii) If the patient smear was negative on first occasion and any subsequent examination showed any stage of malarial parasites, with clinical manifestations of malaria were included in the study.

(iii) In smear negative cases, if the serological diagnosis was positive, the case was scrutinised for inclusion in the study. If the patient had taken antimalarials and the clinicians were sure of clinical diagnosis of malaria such cases were also included.

(iv) In a smear negative case of a pregnant-post partum patient, positive placental blood smear examination was also included.

Selection of patient for study group II....
All fever cases which came to out patient department (OPD) and clinicians demanded for peripheral smear examination for malarial parasites were taken for this study group. The patients who came to diagnostic rural camps at institution’s out reach programme were also included.

Selection of patients for study group III.

116 blood donors ‘ thick and thin smears from our institution - blood bank and 113 from Red cross blood bank Anand during one of the transmission month i.e. August ‘95 were selected.

Selection of patients for study group IV...[Parasight-F test]

200 cases of pyrexia were selected and it’s detailed evaluation methodology will be discussed seperately.

Selection of patients for study group V [Cultivation of P.falciparum]

(i) Patient who had not taken antimalarials.

(ii) Patient should be an adult patient.

(iii) Patient should have heavy parasitaemia.

Selection of patient for study group VI.

All patients who were HIV positive by two different diagnostic tests were taken for finding out malarial parasite in the blood. This was done irrespective of patient having clinical features of malaria.

METHODOLOGY

All selected patients’ peripheral blood was examined by staining with Giemsa stain. The thick smear was examined for presence of parasites and thin
smear was made for species diagnosis. The method adapted for staining was as per guide lines given by W.H.O.[74].

GIEMSA STAIN: The stock solution of Giemsa stain was prepared from Giemsa powder available commercially. [4]

Giemsa powder ..........3.8g

Glycerol, ...............250 ml

Methyl alcohol ..........250 ml

The stain was prepared best by mixing alcohol and glycerol and then adding gradually small quantities of powder in a porcelain mortar and grinding until most of the powder was dissolved and by leaving the mixture for about a week without filtering, the maximum amount of the stain was absorbed. The prepared stock solution was filtered through Whatman no.1 paper into a brown bottle. And kept in a bottle of hard glass with a close-fitting ground-glass stopper and away from the sunlight.

Dilutions of Giemsa stain: Stock solutions of Giemsa stain was diluted by mixing an appropriate amount of it with phosphate buffer. A buffer solution which gives a pH of 7.2 was prepared as follows:

Potassium dihydrogen phosphate KH2PO4.....0.7g

Disodium hydrogen phosphate Na2HPO4......1.0g
discovery of Malaria parasite in the mosquito by Sir Ronald Ross
Distilled water........ 1 litre.

It was kept in a well-stoppered bottle of neutral glass.

Giemsa staining was done as under:--

[1] Thick and thin smears were prepared from the patient’s blood on clean glass slides and labelled adequately.

[2] From stock solution of Giemsa stain, fresh diluted stain was prepared every time with phosphate buffer pH 7.2. (1 drop stain in 1 ml buffer).

[3] Thick smear was fixed by gentle heat and thin smear was fixed with methanol.

[4] Thick and thin smears were covered with Giemsa stain and kept for 30 minutes. After that slides were washed gently by dipping in a jar containing clean water. Air dried and mounted under oil immersion lens.

Results: Giemsa stain colours the components of blood as follows: erythrocytes, pale red; nuclei of white blood cells, purple with pale purple cytoplasm; eosinophilic granules, bright purple-red; and neutrophilic granules, deep pink-purple. If malaria parasites were present, the cytoplasm stains blue and the nuclear material stains red to purple-red. Schuffner’s dots and other inclusions in the RBC’s will stain red.

In thick film only leucocytes and the parasites were seen. The appearance of the parasites was somewhat altered because of dehaemoglobinization and slow drying in the course of preparation of the film. Thus the young trophozoites appear as incomplete rings or spots of blue.
Thin smear (Giemsa, 1000x) *P. vivax* showing Rings (R), Multiple infection (MI) which is seen in vivax infection and is infrequently observed. It also shows late trophozoites (LT) with Schuffner’s dots and gametocytes.

Thin smear (Giemsa, 1000x) *P. falciparum*, larger rings after 4 hrs of fever.
cytoplasm with a detached red chromatin dot. In the late trophozoites of *P. vivax* the cytoplasm may be fragmented and Schuffner's stippling may be less obvious. The interpretation of the parasites seen in a thick film requires some experience. As mentioned before, the thick film is a time-saving method which reveals even scanty infections within a short period. In doubtful cases repeated blood films were taken every 4 hours and examined. In severe infection with *P. falciparum* such repeated examinations were necessary to assess the response of the parasites to treatment. Accuracy of species diagnosis was essential for good patient management for the selection of the drug.

In each case the peripheral smear examination was done on admission. Then after the peripheral examination was done daily and also as per the demand by the clinicians. Parasite count was done by a simple code from one to four crosses \[75\]. Number of rings per 100 oil immersion fields were counted and average number of rings per one oil immersion fields were also noted.

The peripheral smear examination was done till the smear became negative and then again when patient came for follow up. The patients were asked to come for follow up or any day if the symptoms recurred. In OPD cases all fever cases slides were examined. This was done in the month of July, August, September, October during transmission season and in month of March for the non transmission season.

All the patients who were admitted were analysed in great detail. Their symptomatology, examination and investigations were noted down in prescribed proforma. Various clinical spectrum and it's definition were adapted
from” W.H.O malaria action programme criteria.” This includes definition of severe and complicated malaria, anaemia, hypoglycaemia, acute renal failure and various other terminologies.[90] The patient’s follow-up was done and cases of relapse, recrudescence, resistance were noted. The effects of various drugs on disappearance of parasite was evaluated and in vivo resistant to conventional antimalarials if present were noted. Malaria with cerebral symptoms, neuropsychiatric symptoms, GI manifestations, and renal manifestations were noted. The severity of the clinical disease and parasitological count was correlated. Pregnant and postpartum group was also studied in great detail. The effect of various antimalarials like chloroquine, sulfadoxine-pyrimethamine, quinine with and without tetracycline, mefloquine and artemisinin on disappearance of parasite in the blood was noted. CSF examination was done in patient presented with cerebral and neuropsychiatric symptoms.

Blood culture was done in patients where clinical suspicion of Gram negative septicaemia was present. As per my initial observation, clinicians suspected concomitant septicaemia in more number of cases, however blood culture could not be done in all patients.

One of the research aim was to evaluate the available serodiagnostic test for P. falciparum. First 15 tests were done as a pilot work in whom 5 patients having heavy parasitemia (count >200 rings per oil immersion field) were studied by this strip test. Blood of five normal volunteers were also examined to rule out false positive results. Other 5 cases were taken who had scanty
parasitaemia to see the Parasight-F test results. These 15 tests are not included in the study group. Then after the Parasight-F test was done in all fever cases where clinical suspicion of falciparum malaria was been made by the clinician. To prevent the bias the result of conventional microscopy was not known at the time of doing the test. 5 tests were performed in urine instead of blood. Serial estimation was done in 5 patients. The serological test i.e. parasight-f test was done by the commercially available kit from Becton & Dickinson as follows:

[1] Patients blood was collected in the capillary up to the mark. This heparinised capillary was rolled by the fingers.

[2] In a given tube 3 drops of reagent I was taken.

[3] The capillary blood was transferred to this tube containing reagent I. This reagent I was used to lyse the R.B.C.S

[4] The cap was attached to the tube and one drop was taken in the well.

[5] The given strip was labelled and dipped in the well, after absorption up to the mark, reagent II was added.

[6] Then reagent III was added which act as a washing solution to clear the background of the strip.

[7] Result was read as pink full line below as a test positive, and half pink line above as a control line.

METHOD OF CULTIVATION OF P. FALCIPARUM
In ten patients cultivation was done by following method. This method was modified by us from the W.H.O. macro test to obtain better result in our set up.

[1] 10 ml of venous blood was taken with all aseptic precautions in a sterile test tube. 8 ml of blood was transferred to another test tube to collect autologus serum.

[2] 2 ml blood, under sterile condition was transferred to a sterile flask containing glass beads in the inoculation hood. This was defibrinated by rotation of the flask for 5 minutes.

[3] Medium RPMI-1640 was reconstituted and sterilised by filtration method. 1 ml of medium was transferred to each sterile screw capped bottles. In these bottles, 9 ml sterile double distilled water was added.

[4] Heparin, Gentamicin & Na- bicarbonate was added. pH was checked (7-7.2).

[5] Patient’s blood was added in the above bottles with 5% autologous serum of the same patient for a good growth of the parasites.

[6] This was incubated for 48 hrs. in 10% CO2 jar at 37 degree temperature in incubator.

[7] Within 24 hrs. to 48 hrs. thick and thin smears were prepared and observed for development of mature schizonts.

Before and after using of inoculation hood, it was sterilised by ultra violet light and also cleaned with spirit.

Cultivation of falciparum malarial parasite was tried on various artificial media. Best result which we could obtained was in RPMI 1640 [92] tissue
culture medium. The cultivation was done for 48 hrs. in each cases. Smear was prepared at various time after 24 hrs. to find out growth of blood stages of malarial parasite.