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Concurrent Infection with Dengue and Malaria in Adult Febrile Patients in a Tertiary Care Hospital

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Abstract: ***The study was conducted in a tertiary care hospital over one and a half years, involving 664 patients with acute febrile illness. Prospective testing for Malaria and Dengue was performed using rapid tests, peripheral smear, NS1 antigen, and ELISA antibodies. Additional investigations included CBC, PSMP, LFT, and RFT. The age group 21-30 years had the highest cases (28.76%), with a mean age of 34.16 ±10.12 years. Males (60.54%) were more affected than females (39.46%), with a male-to-female ratio of 1.53:1. Fever (100%) was the predominant symptom, followed by nausea/vomiting (52.25%). Dengue (13.86%) and Malaria (8.89%) were the most diagnosed, with Malaria + Dengue co-infection in 3.91% of cases. Plasmodium Falciparum (44.07%) was the most common malaria type. NS1 antigen (41.26%) was the primary diagnostic method for Dengue. Thrombocytopenia (92.31%) and anemia (100%) were prevalent in co-infection cases. Among 26 patients, 1 (4%) died, while 25 (96%) survived, with some requiring inotropes, oxygen, or hemodialysis.co-infection cases showed that, among 26 patients, 1(4%) died while 25 (96%) survived***

Keywords: Dengue, Malaria, Concurrent Infection

1. Introduction

Concurrent Malaria and Dengue infection is a scenario where both these infections co-exist in a patient at the same time. However, this co infection is not common and is seldom reported. Due to the similar nature of initial symptoms in both infections with overlapping endemicity, misdiagnosis of dual infection as a mono infection is quite possible. There is lack of sufficient information on how concurrent infections affect disease severity and outcome.

Dengue and malaria, both are preventable vector-borne diseases. As we know these infections spread through mosquitoes (Anopheles spp transmitting Malaria and Aedes spp. transmitting Dengue infection) therefore, coexistence of both is very complex and equally important to understand. Also, our country comes in tropical zone and is economically developing, prevalence of these infections is very high. Both the infections have almost similar signs and symptoms but entirely different treatment protocols. Concurrent infection has been rarely reported from Southeast Asia due to different habitats of vectors i.e. Anopheles and Aedes.

Malaria and dengue are common in tropical and sub-tropical areas of the world, causing a high rate of morbidity and mortality especially among children. In 2015, about 212 million people were infected with malaria and 429,000 were estimated to have died globally due to malaria infection. Additionally, more than 390 million people required preventive treatment for dengue and close to 96 million manifested clinical symptoms associated with severe dengue annually. Plasmodium sp. can infect humans and manifest a wide range of signs and symptoms ranging from asymptomatic malaria to severe malaria. Cerebral malaria, hypoglycemia, pulmonary edema, bleeding, acidosis, severe anemia, and acute renal failure were the major complications of severe malaria, which may result in death if no prompt or effective treatments are administered. However, people living in endemic areas of malaria usually show asymptomatic or some non-specific symptoms such as fever, fatigue, chills, and malaise. In the endemic areas of Plasmodium falciparum malaria, children up to 5 years of age had more common cases than older children and adults. This might be due to older children and adults receiving partial immunity from the infection. As mosquitoes are usually present in a tropical country, the co-infection of both malaria and dengue is evident and can cause acute febrile illness among patients. Atypical lymphocytosis, hemoconcentration, and thrombocytopenia are specific markers of dengue infection, which help differentiate the diagnosis of dengue infection from malaria infection. A clear understanding of the epidemiology of malaria during dengue co-infection is essential for informed decisions on appropriate control strategies for dengue and malaria. In addition, we do not know the severity of co-infections when compared to single infections. The outcomes of co-infections are distinct among studies, especially in the selection criteria and diagnostic methods used in each study.

2. Materials and Methods

2.1 Study area

The study was conducted at the Department of Microbiology in a prominent tertiary care hospital located in a metropolitan city. The research spanned over a period of one year and six months, during which data was collected from a sample size of 664 individuals. The sampling method employed was

consecutive sampling, ensuring a systematic and sequential approach to participant selection.

2.2 Study Procedure

Blood sample was Collected from the patients with their consent to take part in the study from ward and ICU. All patients suffering from acute febrile illness suspecting dengue and/or malaria were included. Consents was taken from the patients who fit into inclusion criteria. Data compilation & data entry. Data interpretation and Data analysis.

2.3 Sample Collection

5ml of blood was taken from patients in a plain vacutainer. and EDTA bulb for rapid malaria testing the sample was immediately transported to laboratory detailed history was obtained from each patient for duration and nature of fever, associated symptoms and treatment taken in the patients record form.

Inclusion criteria

a) All febrile patients from ward and ICU.
b) All febrile adult cases
c) All patients should be immunocompetent.

Exclusion Criteria:

a) Pediatric population
b) Patients unwilling to give consent for the study
c) Immunocompromised due to any chronic illness

Sample processing

1) Peripheral blood smear

Thick and thin blood film were prepared on different slide the film was fixed in 100% methanol for fields stain from Hi media Pvt Ltd.

Materials required – Gloves, Lancet or needle, clean Greece free slide, fields stain (A&B), clean cotton swab

Test procedure –

Preparation of thin blood film:

a) A drop of blood not larger than a pin head on a grease free clean slide was taken at distance of about half an inch from the right end.
b) Holding a spreader at an angle of 45 degrees in contact with the drop of blood; then lower it to an angle of 30 degrees and push gently to the left till the blood is exhausted, the film begins to from 'tails' which end near the centre of the slide. The smooth edge spreader of a glass slide used, with the corners cut off at one end, or a coverslip then film is allowed to dry.
c) Fixation of thin blood film; the smear is flooded with pure methyl alcohol for 3 to 5 minutes and allowed to dry

Preparation of thick blood film:

a) A big drop of blood was taken on a slide and spread with the corner of another slide to form an area of half inch square. The thickness of the film was made such as to allow newsprint to be read. The film is dried in a horizontal position and kept covered by a petri dish. It takes about 30 minutes for the smear to dry
b) De hemoglobinization – The film was placed in vertical position in a glass cylinder full of distilled water for 5-10 minutes. When the film becomes white it was taken out and allowed to dry in an upright position
c) Fields staining: The thick film was placed in solution A for few seconds (1-2) or till the hemoglobin was removed and to trace of green coloring left

Similarly, a fixed thin smear was placed in solution A for a few seconds.

They were removed and immediately rinsed gently in a jar of clean water until the stain ceased to flow from the film and the glass of slide was free from stain. They were dipped in solution B for 1-2 seconds. The slides were then raised gently for 2-3 seconds in clean water and dried in vertical position

2) Examination and Report

The slide was examined at 100x magnification under the microscope. Thick smear was evaluated by microbiologist unaware of RDT result a thick smear was considered negative if no parasites were seen in at least 200 fields. (72) since parasites are not evenly distributed in a thick blood film, the film was examined using a scanning process called as 'the farmer ploughing his field' technique-across the film to the opposite edge, a slight lateral move, then back across the film, a slight lateral move, and the process is repeated. This process ensures that complete cross sections of the film are examined.

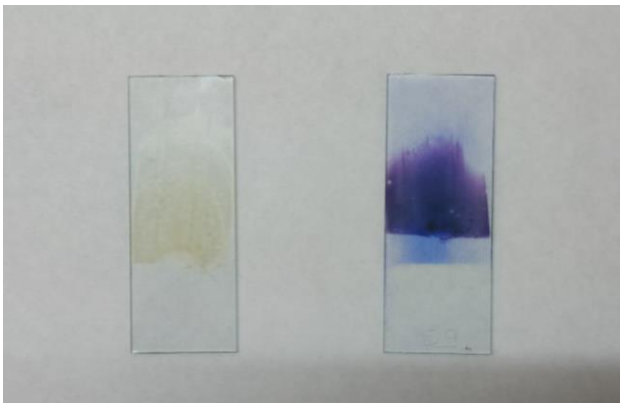

Figure 1: Peripheral smear unstained and stained

For examination of parasite density Field-stained thick blood films were scored by counting the total number of asexual parasites per 200 white blood cells (WBCs) or 500 WBCs for low densities and the parasite index was calculated using the following formula.

(Parasites counted x 8000)/200 = parasite count per microliter of blood thin films were examined for at least 100 fields in not more than 10 minutes Speciation of the parasites was done by examining stained thin films. In thin films the parasites tend to congregate around the 'tail' of the firm, which is much thinner than the rest of the film. Here the 'battlement' technique was used traversing the edge of the tail in short vertical and horizontal tracks.

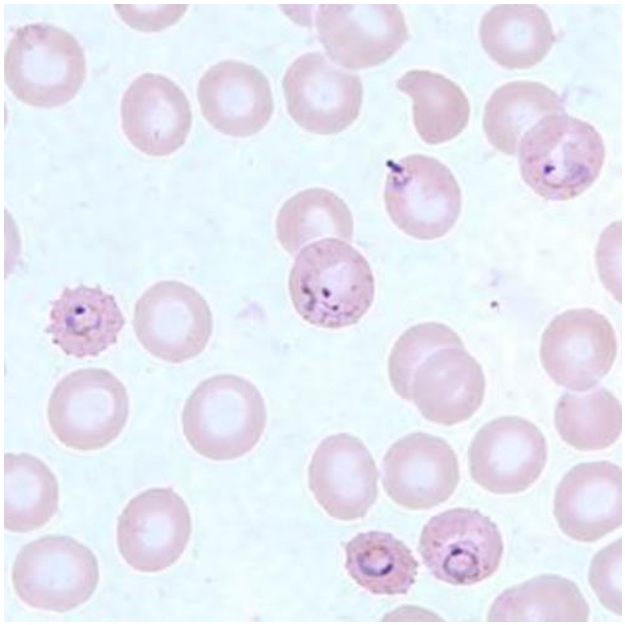

Figure 2: Ring form of Plasmodium vivax in peripheral smear

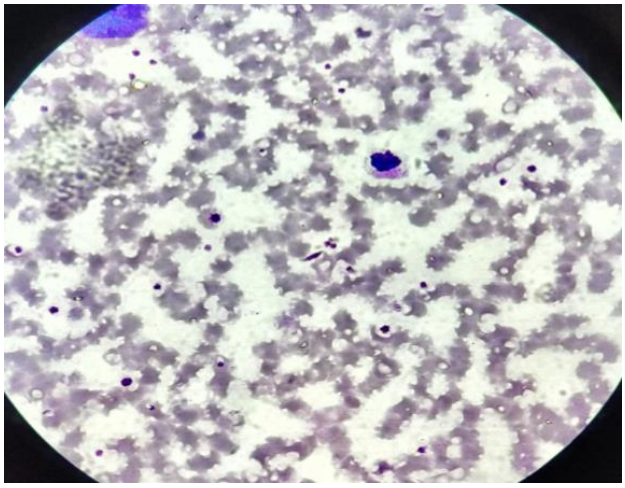

Figure 3: Falciparum gametocyte in peripheral blood smear

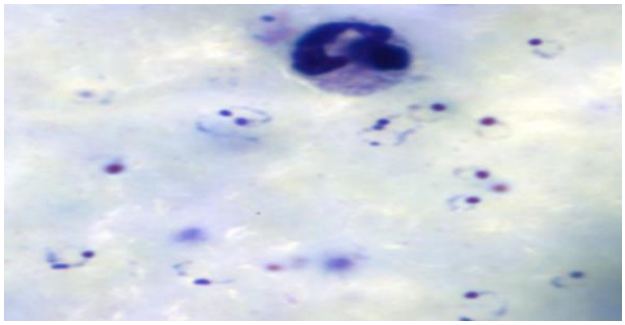

Figure 4: Plasmodium falciparum ring form in peripheral smear

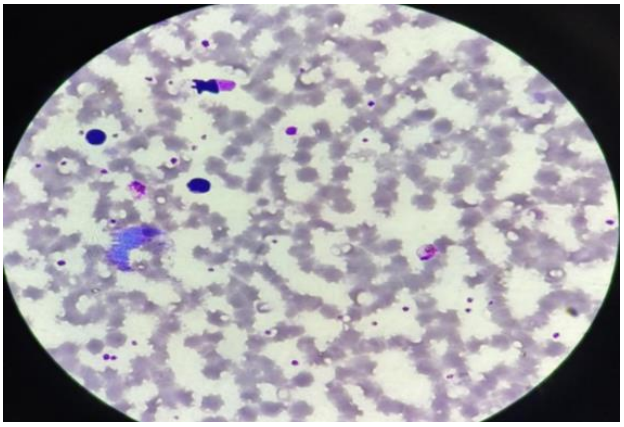

Figure 5: Falciparum Schizonts in peripheral blood smear

3) Malaria Anitgen P.f /P.v Rapid Card Test

Four species of the Plasmodium parasites are responsible for malaria, Infection in human: Falciparum (P.f), Plasmodium Vivax (P.v), P.Ovale (P.o) and P.Malariae (P.m). The Malaria P.f/Pv kit is a rapid test for the detection of P.f malaria, and P.f malaria infections that utilizes the principle of Immunochromatography. This kit is intended for the detection of malaria infection in human blood sample, by using HRP II (Histidine rich protein II) for Plasmodium falciparum and pLDH (plasmodium Lactate Dehydrogenase) for Plasmodium Vivax. As the test sample flows through the membrane of the test device, after addition of the buffer, the anti-pLDH and the anti-P.f HRP-II colored colloidal gold monoclonal antibody conjugates complex with the pLDH and P.f HRP- II, if present in the lysed sample. This complex moves further along the membrane to the test region where it is immobilized by the anti-pLDH and anti-P.f HRP-II monoclonal antibodies coated on the membrane, leading to formation of pink colored lines, which confirms a positive test result.

Materials required – Test Devices, Buffer vial, Droppers, pack insert

Test Procedure -

a) Test device, buffer vial and sample bring to the room temperature.
b) The aluminum package opened and the test device take out.
c) Test used within 20 minutes, after opening the aluminum package
d) Device labeled and placed on horizontal surface
e) 5µl of whole blood added using pipette into sample well of test device. Followed by 3-4 drops (90 -100µl) of buffer into buffer well.
f) Result noted at 20 minutes. (Do not interpret results after 30 minutes).

Figure 6: Malariagen rapid diagnostic kit

Interpretation of test –

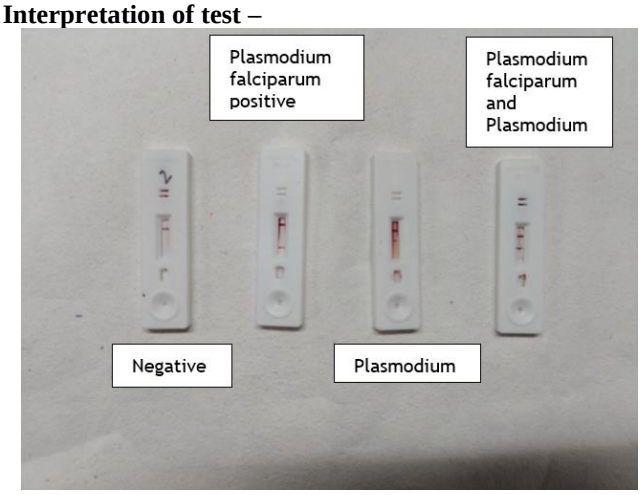

Figure 7: Interpretation of malaria rapid test

a) NEGATIVE: Only one pink/purple line
b) POSITIVE for Falciparum: Pink/Purple lines at C & Pf
c) POSITIVE FOR Plasmodium vivax: Pink/Purple lines at C & Pv
d) POSITIVE for (Plasmodium falciparum & Plasmodium vivax): Pink/Purple lines at C, Pv & Pf S
e) Invalid: If control line does not appear, the test is invalid. In this case, please repeat the test using another device following the test procedure correctly.

4) Dengue Rapid antigen detection tests

The Dengue NS1 Ag Rapid Test is qualitative, membrane-based immunoassay for the detection of NS1 antigen in human serum / plasma. The rapid test membrane is pre-coated with a NS1 specific antibody on the test line region and utilizes a separate Control to assure assay flow and performance. During testing, the test sample is added directly to the sample well followed by 2 drops of buffer. The buffer and sample mix and interact with NS1-specific monoclonal antibodies conjugated to gold nanoparticles. The solution migrates upward on the membrane (via capillary action) to react with the anti-NS1 antibody on the membrane. If NS1 antigen is present, a pink/purple line will appear at the test line region. The pink/purple line at the control line region should always appear if the assay is performed correctly.

Materials required – Dengue NS1 Test Device (Individually pouched), buffer, sample droppers, serum to be tested, product insert

Test procedure –

a) Dengue NS1 Ag Rapid was removed from the foil pouch and assured that all test serum samples are allowed to reach room temperature. Preparation of thick blood film
b) 50µl of serum was added to the well as a pipette (or 2 drops of samples by using provided sample dropper).
c) 2 drops of buffer added in well.
d) Result read after 15 minutes. (Interpretation of results done within 30 minutes)

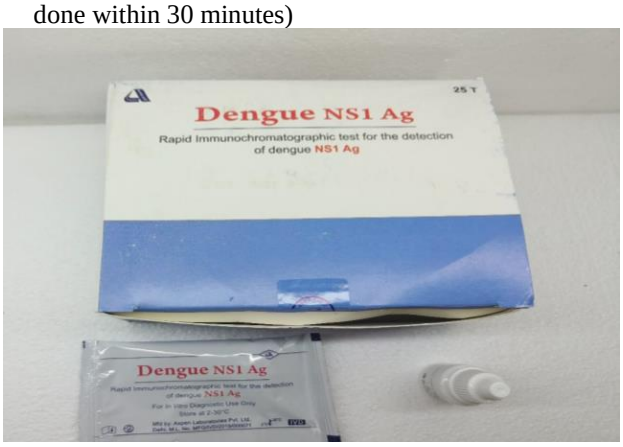

Figure 8: NS1 rapid diagnostic kit

Interpretation of result –

a) Negative: Pink/purple line at C
b) Positive: Pink/purple line at C & T
c) Invalid: No line at C

(If control band does not appear, the test is invalid, in this case, please repeat the test, following the test procedure correctly)

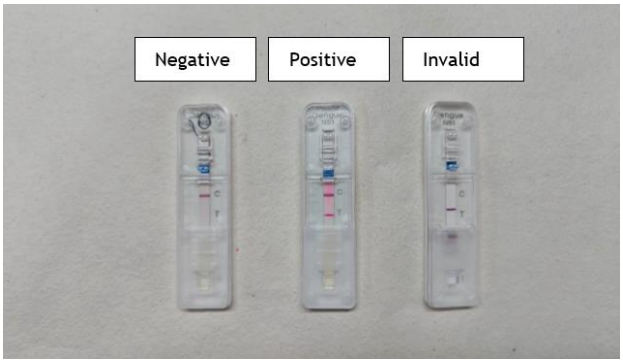

Figure 9: Dengue NS1 rapid diagnostic kit interpretation of results

Sensitivity 98.3% Specificity 99.2%

5) Dengue Rapid antibody detection tests

A number of commercial Rapid Diagnostic Test (RDT) kits for anti-dengue IgM and IgG antibodies are at present commercially available, which produces the results 77 within 15 to 20 minutes.

SD BIOLINE Dengue IgG/IgM WB test device has 3 pre-coated lines on the surface of the device: “G” (dengue IgG test line), “M” (dengue IgM test line) and “C” (control line). The control line on the test device is used for procedural control and should always appear if the test procedure is performed properly and the test reagents of control line are working. When a specimen is added to the sample well, anti-dengue IgGs and IgMs in the specimen will be captured by

the relevant anti-human IgG AND Or anti human IgM immobilized in the two test lines on the test device. Mouse monoclonal anti-dengue ab colloidal gold conjugate will react with dengue virus in the virus pad and forms a complex of antibody antigen which migrates along the length of the test device by capillary cation and generates a colored line.

Materials required: Dengue IgM/IgG Test Device (Individually pouched), Buffer, Sample droppers, Product insert

Test Procedure -

a) Dengue IgG/IgM Ab Rapid was removed from the foil pouch and assured that all test serum samples are allowed to reach room temperature.
b) 10µl of serum was added into the square sample well Using a micropipette marked "S".
c) 4 drops (90-120µl) of assay diluent added to the round assay diluent well.
d) Interpretation of test results done 15-20 minutes

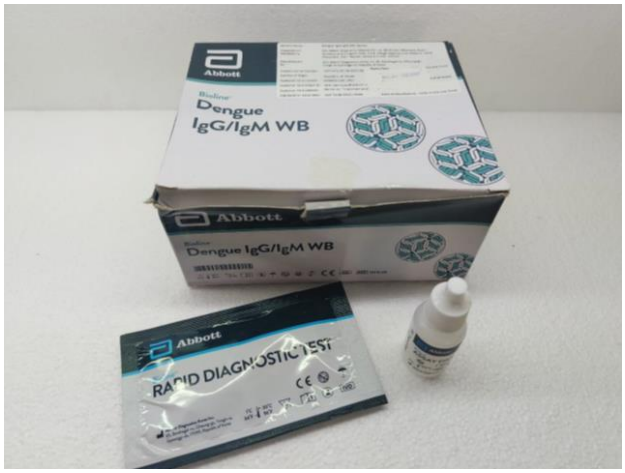

Figure 10: Dengue IgM/IgG antibody rapid kit

Interpretation of test -

a) NEGATIVE: The presence of only the control line ("C") within the result window indicates that no IgG and IgM antibodies were detected. Retest in 3-5 days if dengue infection is suspected.
b) IgM POSITIVE: The presence of two-colored lines ("C" and "M") within the result window, regardless of which band appears first, indicated that the sample is positive for IgM antibodies to dengue virus. This suggests a primary dengue infection
c) IgG POSITIVE FOR Plasmodium vivax: The presence of two-colored lines ("C" and "G") within the result window, regardless of which band appears first, indicated that the sample is positive for IgG antibodies to dengue virus. This suggests a primary dengue infection
d) IgG & IgM Positive: The presence of two-colored lines ("C", "M" and "G") within the result window, regardless of which band appears first, indicated that the sample is positive for both IgM antibodies to dengue virus. This suggests a late primary or early secondary dengue infection.
e) Invalid: If the control line ("C") is not visible within the result window after performing the test, the result is considered invalid. Insufficient specimen volume and incorrect procedural techniques are the most likely reasons for control line failure. Repeat the test using a new test device.

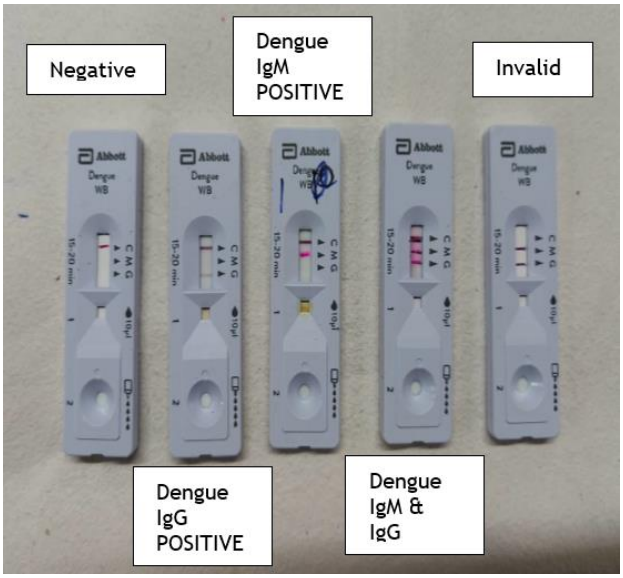

Figure 11: Interpretation of result Dengue IgM/IgG antibody rapid kit

6) ELISA DENGUE NS1

The RecombiLISA Dengue Ag ELISA is a solid phase enzyme-linked immunosorbent assay for the qualitative detection of dengue NS1 antigen (DEN1, 2, 3, 4) in human serum or plasma.

Materials required – RecombiLISA Dengue Ag ELISA, Anti-dengue NS1 Ab coated microwells, HRP-anti-dengue NS1 Conjugate, Dengue NS1 antigen positive control, Dengue NS1 antigen negative control, Dengue NS1 antigen negative control, Sample diluent, TMB substrate A, TMB substrate B, Stop solution

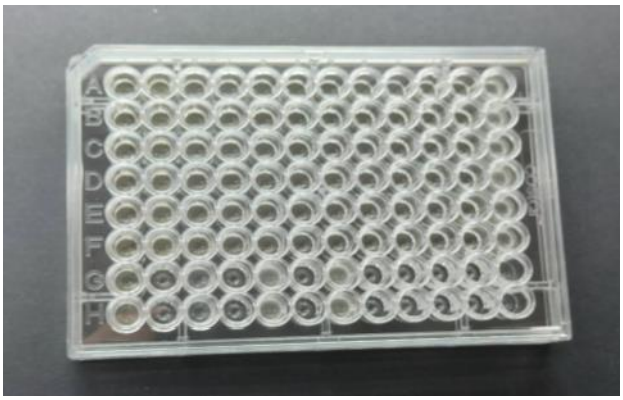

Figure 12: Microtiter plate for Elisa

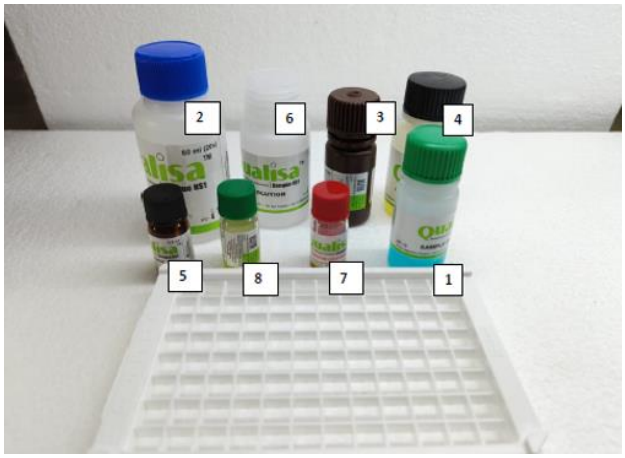

Figure 13: RecombiLISA Dengue NS1 Ag ELISA kit

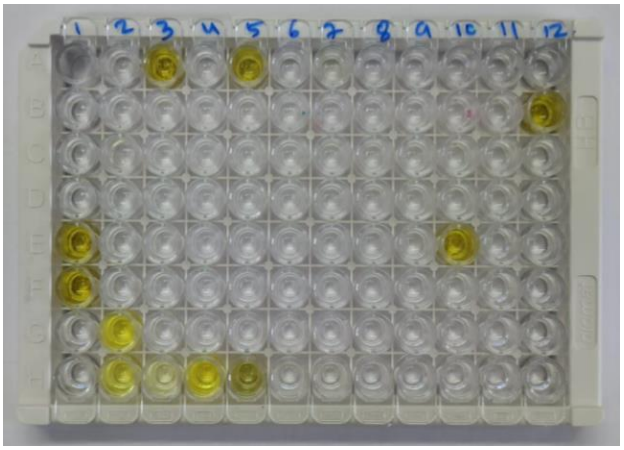

Figure 14: Recombil LISA Dengue Ag ELISA titre plate showing results

Test Procedure -

a) Desired number of strips are removed and secure them in the microwell frame.
b) 50 µL of Sample Diluent added into each Control Well and Test Well, respectively, according to the designation on the ELISA Working Sheet.
c) specimens added to the plate according to the designation on the ELISA
d) we do not add any reagents in Blank Wells
e) Control Wells: 50 µL of Dengue NS1 Positive Control and 50 µL of Dengue NS1 Negative Control added into the designated control wells, respectively.
f) Test Wells: Add 50 µL of test specimens to each test well, respectively.
g) The wells Gently shaken for 20 seconds, and then covered the plate with a sealer.
h) The wells Incubated at 37°C for 60 minutes.
i) Wash Step (Can be performed manually or with automated washing)
j) Automatic washing: (Automatic plate washer was calibrated to ensure efficient washing. Incubation mixture was aspirated from all wells completely. Each well was filled with 350 µL diluted wash buffer and soaked for 20-30 seconds. All wells aspirate completely. Repeated 4 more times.)
k) 100 µL of HRP-anti-dengue NS1 Conjugate into each well was added with the help of pipette except the Blank Well. the microwells shaken to ensure thorough mixing.
l) Incubated at 37°C for 60 minutes.
m) Again, Washed the plate as described in step 5.
n) 50 µL of TMB Substrate A and 50 µL of TMB Substrate B was added into each well including the Blank Well. The microwells shake for 20 seconds to ensure thorough mixing.
o) Incubated at room temperature (20-25°C) in dark for 15 minutes.
p) The reaction was stopped by adding 100 µL Stop Solution into each well. mix for 30 seconds. It is important to make sure that all the blue colour completely changes to a colour yellow.
q) Microplate reader wavelength was set at 450 nm. absorbance (OD) was measured of each well against the Blank Well within 30 minutes after adding Stop Solution. A filter of 620-690 nm can be used as a reference wavelength to optimize the assay results.

Interpretation of Results –

a) Set up the cut-off value

The cut-off value = 0.20 + N
N: Mean OD of the negative control. Use N=0.10 for calculation of the cut-off value if the mean OD is less than 0.10.

b) Calculation of specimen OD ratio

An OD ratio Calculated for each specimen by dividing its OD value by the cut-off value as follows:
Specimen OD ratio = Specimen OD/ Cut-off Value

c) Assay validation

The mean OD value of the positive controls should be > 0.8
The mean OD value of the negative controls should be < 0.2
Check the assay procedure including incubation time and temperature and repeat assay if above criteria is not met.

Interpretation of the results

Specimen OD ratio
Negative < 1.0
Positive ≥ 1.0

1) A negative result indicates that there is no detectable dengue NS1 in the specimen.
2) Specimens with OD ratio > 1.0 are initially considered to be positive by the RecombiLISA Dengue Ag ELISA. Results should be used in conjunction with clinical findings to diagnose dengue infection.
3) Results just below the cut-off value (lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to re-test in duplicate the corresponding specimens when it is applicable). If after retesting the absorbance of one of the duplicates is equal to or greater than the cut-off value, the initial result is repeatable and the specimen is considered to be positive with the RecombiLISA Dengue Ag ELISA, subject to the limitations of the procedure, described below. If after re-testing of a specimen, the absorbance value of the 2 duplicates is less than the cut-off value, the initial result is non-repeatable and the specimen is considered to be negative with the RecombiLISA Dengue Ag ELISA. Non-repeatable reactions are often caused by: Inadequate microwell washing

Contamination of negative specimens
by serum or plasma with a high concentration of NS1 antigen. Contamination of the TMB Substrate by oxidizing agents (bleach, metal ions, etc.) Contamination of the Stop Solution. In the present study field's stain had a sensitivity of 97.72% and a specificity of 100%.

7) Truswell Dengue IgM ELISA Kit
The TRUSTwell Dengue IgM ELISA Kit is a solid-phase, IgM capture enzyme linked immunosorbent assay.
Materials required – Anti-human IgM coated microwells, Lyophilized dengue antigen, HRP-anti-dengue conjugates (100X concentrate), Enzyme diluent, Dengue IgM positive control, Dengue IgM negative control, Sample diluent, Wash buffer (30X concentrate), TMB substrate A, TMB substrate B, Stop solution, ELISA working sheet, Product insert.

Figure 15: Dengue IgM Elisa kit

Assay Procedure -
a) The desired number of strips were removed and secured them in the microwell frame. Resealed un-used strips in Ziploc bag.
b) Specimen added according to the designation on the ELISA Working Sheet: specimen was not added in Blank well
c) Control wells: 100 µL diluted Dengue IgM Positive Control and 100 µL diluted Dengue IgM Negative Control added into the designated control wells.
d) In Test wells 100 µL diluted patient specimen added into each designated test well. The plate was gently rock for 20 seconds, and then cover the plate with the sealer.
e) Then plate Incubate at 37°C for 30 minutes. Plates were washed to remove unbounded materials: Automated washing: Automatic plate washer calibrated to ensure efficient washing. each well fill with 350 µL diluted wash buffer and soak for 20-30 seconds. All well Aspirates completely. And repeated 4 more times. 100 µL prepared antigen working solution(1X) added into each well except the blank well. Gently rock the microwells for 20 seconds to ensure thorough mixing.
f) The wells covered and incubate at 37 °C for 45 minutes.
g) Plate washed 5 times as described in step 4 and 100 µL of HRP conjugate added into each well except the blank
h) Wells Covered and incubated at 37°C for 20 minutes
i) Then wash the plate 5 times as described in step 4.
j) 50 µL TMB Substrate A and 50 µL TMB Substrate B into each well were added, including the Blank Well, microwells Gently rocked for 20 seconds to ensure thorough mixing.
k) Again, Incubated at room temperature (20-25°C) in the dark for 15 minutes.
l) The reaction was Stopped by adding 100 µL Stop Solution to each well, including the Blank Well. Gently rocked for 20 seconds. Pipette the Stop Solution in the same sequence as substrate addition. It is important to make sure that all the blue color completely changes to the color yellow.
m) The microplate reader wavelength Set at 450 nm. Measure the absorbance (OD) of each well against the blank well within 30 minutes after adding Stop Solution. A filter of 620-690 nm can be used as a reference wavelength to optimize the assay result.

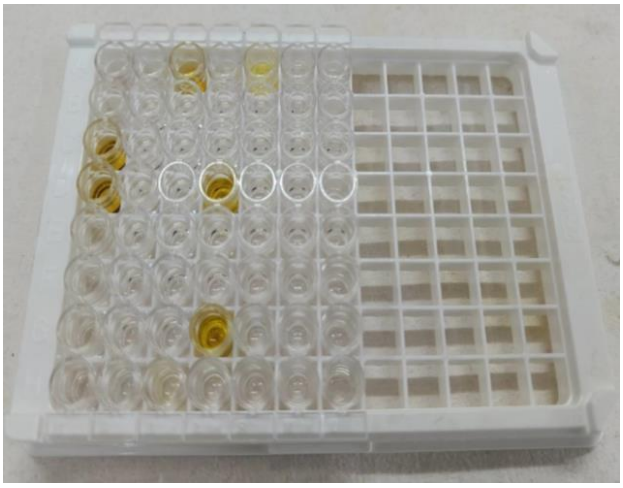

Figure 16: Interpretation of results Dengue Elisa

Interpretation of Results -
a) Set up the cut-off value
The cut-off value = 0.25+NC
NC: Mean OD of the Negative Control.

b) Calculation of specimen OD ratio
Calculate an OD ratio for each specimen by dividing its OD value by the cut-off value as follows:
Specimen OD ratio= Specimen OD / Cut-off Value

c) Assay Validation
The mean OD value of the positive controls should be ≥ 0.50
The mean OD value of the negative controls should be ≤ 0.13
Check the assay procedure including incubation time and temperature and repeat assay if above conditions are not met.

Interpretation of the results
Specimen OD ratio
Negative <1.0
Positive >1.0
a) A negative result indicates that there is no detectable IgM anti-dengue antibody in the specimen.
b) Results just below the cut-off value (lower than 10% of the cut-off value) should be interpreted with caution (it is advisable to re-test in duplicate the corresponding specimens when it is applicable).
c) Specimens with OD ratio ≥ 1.0 are initially considered to be positive by the Dengue IgM ELISA Kit. They should be retested in duplicate before a final interpretation is made. If after re-testing of a specimen, the absorbance value of the 2 duplicates is less than the cut-off value, the initial result is non-repeatable and the

specimen is considered to be negative with the Dengue IgM ELISA Kit.

Non-repeatable reactions are often caused by: Inadequate microwell washing.

Contamination of negative. specimens by serum or plasma with a high antibody titer, Contamination of the substrate solution by oxidizing agents (bleach, metal ions, etc.). Contamination of the Stop Solution. If after retesting the absorbance of one of the duplicates is equal to or greater than the cut-off value, the initial result is repeatable and the specimen is. considered to be positive with the Dengue IgM ELISA Kit, subject to the limitations of the procedure, described below

3. Results

Table 1: Distribution of Patients according to Age

Age Group (Years)	No. of Patients (N=664)	Percentage (%)
<20	76	11.44
21-30	191	28.76
31-40	145	21.84
41-50	104	15.66
51-60	93	14.01
61-70	42	6.33
>70	13	1.96
Total	664	100

Chi-square test = 7.0; df = 5; p> 0.05; Not significant

The above table1 shows age distribution among patients. The maximum numbers of cases were in the age group of 21-30 years191 (28.76%), followed by in 31-40 years 145 (21.84%). The patients ranged from 13 to 75 years and mean age among the distribution of cases was 34.16 ±10.12 years.

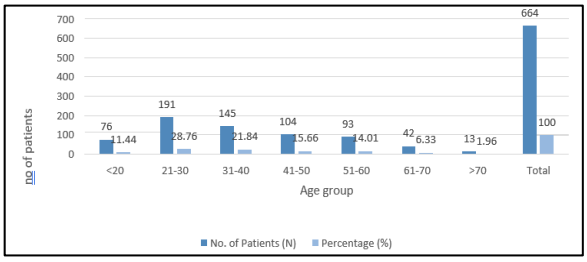

Figure 17: Distribution according to age

Table 2: Distribution of Patients according to Gender

Gender	No. of Patients (N=664)	Percentage (%)
Male	402	60.54
Female	262	39.46
Total	664	100

Chi-square test = 2.273; df = 1; p> 0.05; Not significant
The above table 2 shows sex distribution among patients. Out of 664 cases males 402 (60.54%) were the most affected with febrile illness when compared to females 262(39.46%) and male to female ratio was 1.53:1

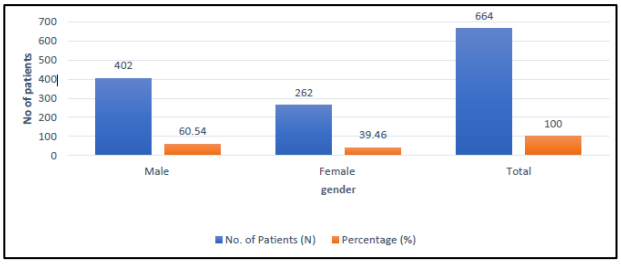

Figure 18: Distribution according to gender

Table 3: Distribution according to Clinical Features*

Clinical Features	No. of Patients (N=664)	Percentage (%)
Fever	664	100
Nausea / Vomiting	347	52.25
Abdominal pain	142	21.38
Hepatosplenomegaly	79	11.89
Headache	56	8.43
Rash	26	3.91
Musculoskeletal pain	21	3.16
Epistaxis	12	1.80
Gum bleeding	11	1.65
GI bleeding	07	1.05
Mucocutaneous bleeding	09	1.35

The maximum numbers of cases were having fever (100%), followed by nausea/ vomiting (52.25%). Abdominal pain was seen in 142 (21.38%) patients, hepatosplenomegaly in 79 (11.89%) patients.
(* Multiple response present)

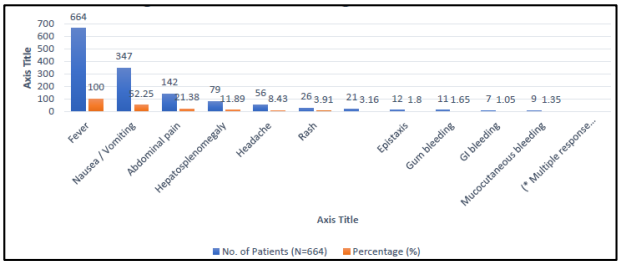

Figure 19: Distribution according to clinical features

Table 4: Peripheral Blood Smear Findings (N=70)

Peripheral blood smear findings	Total samples (N=664). Positive samples (N=70)
Gametocyte	13(18.57%)
Schizonts	27(38.57%)
Ring form	21(30%)
Pigments	09(12.86%)
No findings	594

Amongst slide positive cases schizonts 27(38.57%) were seen predominantly followed by ring form 21(30%), gametocyte13 (18.57%) pigments were seen in few cases 9(12.86%)

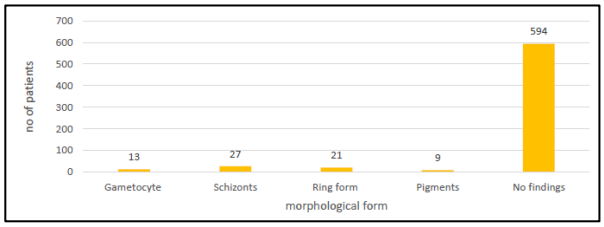

Figure 20: Distribution according to blood smear finding

Table 5: Distribution according to diagnosis: *

Diagnosis	No. of Patients (N=664)	Percentage (%)
Malaria	59	8.89
Dengue	126	18.97
Malaria + Dengue	26	3.91

Chi square test= 20.02; df=2; p<0.01; Highly Significant

The maximum numbers of cases diagnosed as Dengue126 (18.97%), followed by Malaria 59(8.89%) and co-infection of Malaria + Dengue among 26 (3.91%) patients.

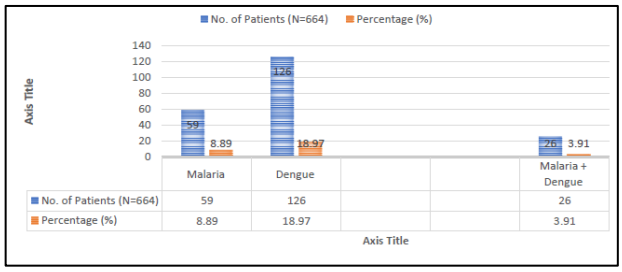

Figure 21: Distribution according to diagnosis

Chi square test= 1.42; df=2; p>0.05; Not Significant

Table 6: Distribution according to type of malaria: *:

Type of malaria	No. of Patients (N=59)	Percentage (%)
Plasmodium Vivax	16	27.12
Plasmodium Falciparum	26	44.07
Mixed	17	28.81

The maximum numbers of cases were diagnosed as Plasmodium Falciparum 26 (44.07%), followed by Mixed Malaria17 (28.81%) and Plasmodium Vivax among 16 (27.12%) patients.

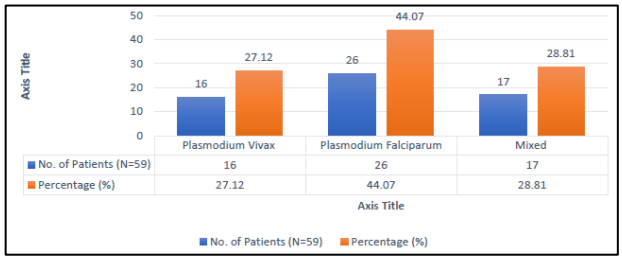

Figure 22: Distribution according to type of Malaria

Table 7: Distribution according to type of Dengue

Type of Dengue	No. of Patients (N=92)	Percentage (%)
Without warning signs	58	63.04
With warning signs	21	22.83
Severe	13	14.13

Chi square test= 17.19; df=2; p<0.01; Highly Significant

The maximum numbers of cases diagnosed as dengue without warning signs 58(63.04%), followed by with warning signs 21(22.83%) and severe dengue among 13 (14.13%) patients.

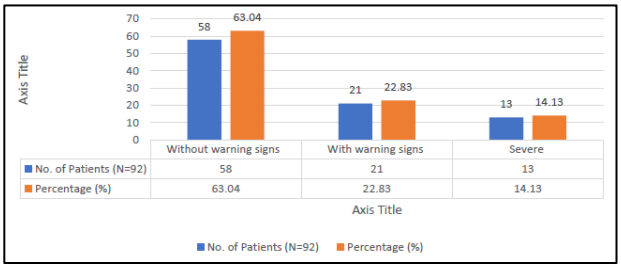

Figure 23: Distribution according to type of Dengue

Table 8: Distribution according to Laboratory Findings

Laboratory Findings		No. of Patients	Percentage (%)
Dengue positive (n=126)	NS1 antigen	52	41.26
	IgM antibody	28	22.22
	NS1 Ag + IgM Ab	33	26.19
Malaria positive (n=59)	PV antigen	16	27.12
	PF antigen	26	44.07
	Both PV and PF antigen	17	28.81

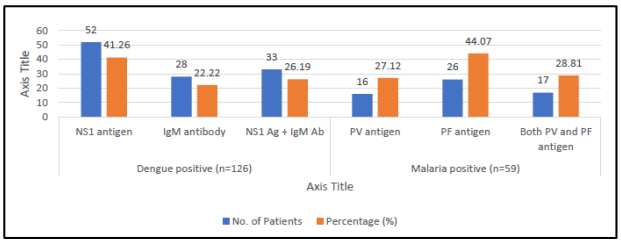

Figure 24: Distribution according to RDT findings

The maximum numbers of cases diagnosed as dengue by NS1 antigen (41.26%), followed by IgM antibody (22.22%) and by dengue NS1 antigen and IgM antibody combined was (26.19%).

Among malaria patients, PF antigen was positive among 26 (44.07%) patients. PV antigen was positive among 16 (27.12%) patients.

Table 9: Malaria and Dengue Co-Infection Cases:

Dengue	P Vivax	Plasmodium falciparum	Mixed	Total
Without warning signs	03 (23.07%)	02 (50%)	00	05 (19.23%)
With warning signs	8 (61%)	03 (33.3%)	02 (66.66)	13 (50%)
Severe	02 (15%)	05(20%)	01 (33.3%)	08 (30%)
Total	13	10	03	26

Chi-square test = 3.425; df = 2; p> 0.05; Not significant

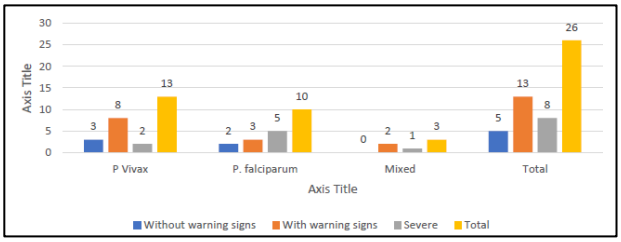

Figure 25: Malaria and Dengue co-infection cases depending on warning signs

Table 10: Laboratory Findings In Malaria Dengue Co-Infection: *

Laboratory Findings	No. of Patients (N=26)	Percentage (%)
Thrombocytopenia (<1,50,000/microL)	24	92.31
Hb <8 mg/dl	26	100
Serum bilirubin >1.2 mg/dL	09	34.62
SGPT >55 IU/L	06	23.08
BUN >20 mg%	02	07.69
Serum Creatinine >1.5 mg/dL	02	07.69

Chi-square test = 2.273; df = 1; p> 0.05; Not significant

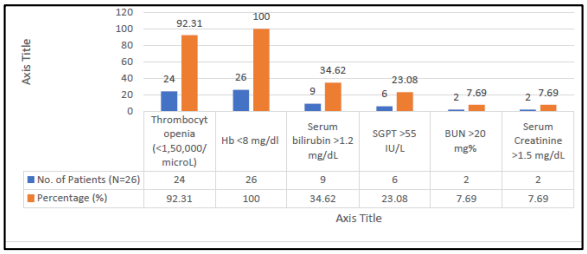

Figure 26: Laboratory findings in malaria dengue co-infection

Table 11: Distribution according to Treatment among Co-Infection Cases

Treatment	No. of Patients (N=26)	Percentage (%)
Need of inotropes	07	26.92
Oxygen requirement	11	42.31
Hemodialysis	03	11.53

Chi square test= 2.48; df=2; p>0.05; Not Significant

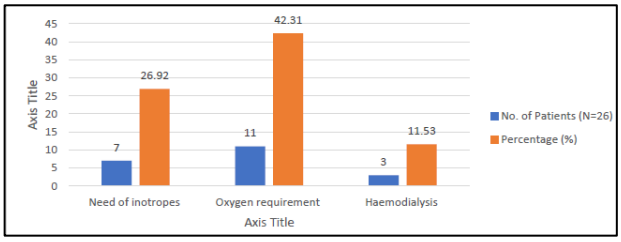

Figure 27: Distribution according to treatment among coinfection

Table 12: Distribution according to Outcome among Mono and Co-Infection Cases

Outcome	malaria n=59	dengue n=92	coinfection n=26
Discharged	58(98%)	89(96%)	25(96%)
Expired and DAMA	1(2%)	3(4%)	1(4%)
Total	59	92	26

Chi square test= *0.004; df=1; p= 0.62; Not Significant
* Yates correction used

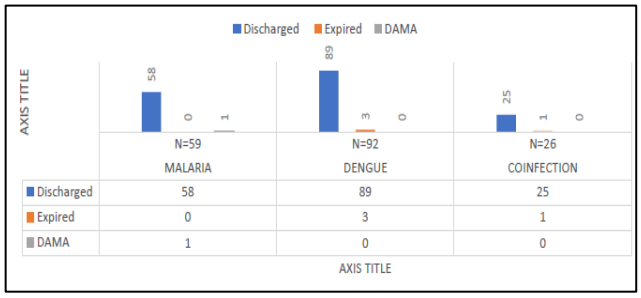

Figure 28: Distribution according to outcome among mono and co-infection cases

4. Discussion

Dengue and malaria, both are preventable vector-borne diseases. Coexistence of both is very important to understand as both have almost similar signs and symptoms but entirely different treatment protocols. Simultaneous presence of both the infections in one individual can easily be missed as detection of any one of them in an acute febrile patient can mask the diagnosis of other.

The present prospective study undertaken to study concurrent infection with Dengue and Malaria in all febrile adult patients in a tertiary care hospital.

A total of 664 sample of adult febrile inpatients attending tertiary care hospital in the time period of December 2020 to JUNE 2022 were examined by using different diagnostic methods mentioned above. The data was collected from patients regarding demographic profile, clinical spectrum, hematological and serological findings and outcomes were assessed.

Demographic details:
In our study the maximum numbers of cases were from the age group of 21-30 years (28.76%), followed by in 31-40 years (21.84%). The patients ranged from 05 to 75 years and mean age among the distribution of cases was 34.16 ±10-12 years.

Sangita Vasava et al in a study on concurrent malaria and dengue infections among febrile patients observed among total of 604 clinically suspected samples the most affected patients were from the age group of 31-60 years (275, 45.53%) and less affected age group was 1-17 years (50, 8.28%).

Distribution of patients according to gender
Out of 664 cases males (60.54%) were the most affected with febrile illness when compared to females (39.46%) and male to female ratio was 1.53:1

Sangita Vasava et al in a study on concurrent malaria and dengue infections among febrile patients observed male patients were more common 61.59% (372), than female 38.41% (232).

Clinical features
The maximum numbers of cases were having fever (100%), followed by nausea/ vomiting (52.25%). Abdominal pain was seen in 142 (21.38%) patients, hepatosplenomegaly in 79 (11.89%) patients.

In research conducted by Dr. Parul D. Shah et al, studied 8364 blood samples were collected in which majority of patients had fever (100%) followed by Hepatomegaly and jaundice (37.3%). This finding was similar to present study.

Peripheral smear
Amongst slide positive cases schizonts 27(38.57%) \were seen predominantly followed by ring form 21(30%), gametocyte13 (18.57%) pigments were seen in few cases 9(12.86%)

In our study, the maximum numbers of cases diagnosed as Dengue126 (18.97%), followed by Malaria 59(8.89%) and co-infection of Malaria + Dengue among 26 (3.91%) patients.

In a study by Parul D. Shah et al on incidence of dengue and malaria co-infection observed among 8364 blood samples, 10.27% (859) patients with fever were tested positive for Dengue, and 5.1% (434) were tested positive for Malaria and only 3.14% (27) cases show concurrent infection with dengue virus and Plasmodium parasites.

Sangita Vasava et al in a study on concurrent malaria and dengue infections among febrile patients observed among in 604 samples, 80 samples were found positive for dengue and 58 samples were positive for malaria. The concurrent infection of both dengue and malaria were 21 (3.47%) found and 445 samples were negative.

The incidence of concurrent infection in other studies has been quite variable and range from 1% in French Guiana 2 to 6% in India and 27% in Pakistan. Classical concepts of malaria occurring in rural areas and dengue in urban areas has been challenged in many reports from different countries due to overlap of mosquito biotypes.

Distribution according to type of Malaria:
In the present study, the maximum numbers of cases were diagnosed as Plasmodium Falciparum 26 (44.07%), followed by Mixed Malaria17 (28.81%) and Plasmodium Vivax among 16 (27.12%) patients. In a study by Parul D. Shah et al on incidence of dengue and malaria co-infection observed among 8364 blood samples, of these, 0.20% (17) were positive for Plasmodium vivax, 0.10% (9) were Plasmodium falciparum and 0.01% (1) case of mixed infection with Plasmodium vivax and Plasmodium falciparum.

Similar study done in French Guiana and Pakistan had shown P vivax in 63.9% and 96.2% cases.10,11 This can be attributed to the species prevalent in a particular geographical region.

Distribution according to type of Dengue
The maximum numbers of cases diagnosed as dengue without warning signs (63.04%), followed by with warning signs (22.83%) and severe dengue among 13 (14.13%) patients.

In a study by Parul D. Shah et al on incidence of dengue and malaria co-infection observed as per recent (2009) WHO classification for dengue cases, out of 859 dengue-positive cases, 50.6% (435) were dengue without warning signs (D), 37.6% (323) were dengue with warning signs (DW) and 11.8% (101) cases were severe dengue (SD).

Distribution according to RDT findings
In the present study, the maximum numbers of cases diagnosed as dengue was by NS1 antigen (41.26%), followed by IgM antibody (22.22%) Among malaria patients, PF antigen was positive among 26 (44.07%) patients. It was observed that 50% of the patients with Malaria had dengue with warning signs followed by severe dengue in 8 (30.8%) patients. Plasmodium falciparum type showed maximum severe dengue cases among 5 (50%) patients.

In a study by Parul D. Shah et al on incidence of dengue and malaria co-infection observed out of total malaria and dengue concurrent infections, 11.11% (3) were dengue without warning signs, 55.55% (15) were DW signs and 33.33% (9) cases were SD cases.

Sangita Vasava et al in a study on concurrent malaria and dengue infections among febrile patients observed out of the 21 with co-infection, 7 (33.33%) were severe dengue cases, 11 (52.38%) showed warning signs of dengue and 3 (14.28%) showed dengue without warning signs with malaria as co-infection.

The laboratory findings:
The laboratory findings among malaria dengue co-infection cases showed anemia among 100% patients, thrombocytopenia among 24 (92.31%) patients, LFT was deranged among 34.62% patients with Serum Creatinine >1.5 mg/dL among 2 (7.69%) patients.

In a study by Parul D. Shah et al on incidence of dengue and malaria co-infection observed majority of patients showing hemoglobin < 12 g/dl in 100% (27) and thrombocytopenia (platelet count < 150,000/cmm) in 96.29% (26) cases.

Sangita Vasava et al in a study on concurrent malaria and dengue infections among febrile patients observed 52.38% had hepatomegaly and jaundice and 23.80% had hemorrhagic manifestation, hemoglobin was < 12 g/dL in all, kidney failure was found in 4.76%, thrombocytopenia (platelet count <150,000/cmm) in 95.23% and condition also more common in Plasmodium vivax infections.

Screening for malaria is essential after clinical and hematological correlation in dengue-positive cases and vice versa. Anemia, thrombocytopenia, altered liver and renal function tests were observed in malaria and dengue co-infection cases with higher number of cases in Plasmodium falciparum infection. Similar findings were found in study done in Pakistan. Deranged liver function was also found in that study. It is interesting to note that hemorrhagic manifestations are uncommon in falciparum malaria whereas in dengue, they are common. As both malaria and dengue can cause thrombocytopenia, it is difficult to decide which one is responsible for the hemorrhagic manifestation. Therefore, malaria with bleeding manifestations is considered as severe malaria and treated accordingly. The biological influence of dengue virus, which affects the endothelium, a major protagonist of severe malaria pathophysiology, on the eventual severity of falciparum malaria, needs to be studied.

Distribution according to Treatment:
In the present study, it was observed that, among 26 patients, 7 (26.92%patients) need support of inotropes while 11 (42.31%) needed oxygen and 3 (11.53%) required hemodialysis.

Distribution according outcome:
The malaria dengue co-infection was seen in 26 cases and among them 1 (4%) expired while 25 (96%) survived.

In a study by Parul D. Shah et al, on incidence of dengue and malaria co-infection observed no mortality was detected in

dengue and malaria co-infection cases.

Sangita Vasava et al in a study on concurrent malaria and dengue infections among febrile patients observed no death was detected in dengue and malaria co-infection cases.

Malaria and dengue must be suspected in febrile patients living in or returning from areas endemic for these infections. If malaria is confirmed first, then dengue should not be ruled out without testing for it. If first dengue is confirmed, then all such cases also should be screened for malaria. All clinicians treating febrile patients in or returning from endemic areas should systematically order examinations for both malaria and dengue diagnoses, even if one or the other is positive

As only hospitalized patients were included in the study, this co-infection incidence does not represent incidence in community or local population. We could not determine the vector load which would have been helpful in determining the concurrent infection in a locality. Other published studies also had similar limitation.

Since malaria and dengue frequently co-exist in the same geographical areas, there are some public health implications. In addition, the clinical outcomes of co-infection were more like dengue mono-infection than malaria mono-infection. Therefore, healthcare workers including physicians, medical technicians, and nurses need to collaborate with each other in order to solve the difficulty of differentiating between both diseases in similar areas. Using clinical outcomes such as fever with typical paroxysm, cerebral malaria, renal failure, and multi-organ failure might rule out patients with co-infection. On the other hand, using bleeding signs might indicate patients with co-infection. Moreover, screening for malaria parasite in patients with dengue infection might help to diagnose patients suspected with coinfection.

Ethical Approval

An ethical clearance was obtained prior to commence of this study.

Conflict of Interest

The authors have declared that no competing interests exist.

Acknowledgments

This work was possible by the constant support and supervision by Anita Ramchandra.

5. Conclusion

In the intricate tapestry of tropical diseases, our study has provided valuable insights into the coexistence of malaria and dengue in geographical regions where their mosquito vectors share common ground. The convergence of these two significant diseases underscores the need for a nuanced understanding of their co-infection dynamics. Our investigation revealed a compelling aspect of concurrent infection: the clinical dominance of dengue fever over malaria. This observation serves as a pivotal guide for clinicians, emphasizing the importance of recognizing and prioritizing the clinical manifestations of dengue in the presence of co-infection. Furthermore, our findings shed light on the heightened risk of Dengue with Warning Signs (DW) and Severe Dengue (SD) in cases of co-infection, particularly when Plasmodium falciparum is involved. This intricate interplay between specific malaria strains and the severity of co-infections adds a layer of complexity to the clinical landscape. In light of these revelations, our study underscores a critical recommendation: the imperative for comprehensive testing of all febrile patients for both malaria and dengue. Neglecting this dual testing approach in cases of concurrent infections may lead to the oversight of one of these diseases, with potential consequences for the patient's health, including the development of severe complications.

Authors' Contributions

This work was carried out under the supervision of Kanchan Joshi and Anita Ramchandran. Praladh Pawar and Pooja Singh designed the study, carried out field and laboratory work, performed the statistical analysis and wrote the manuscript. All of the authors read and approved the final manuscript.

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