International Journal of
Mosquito Research

Shivani Sharma
Post Graduate Student,
Department of Microbiology,
Santosh Deemed to be
University, Ghaziabad, Uttar
Pradesh, India

Dr. Dakshina Bisht
Professor, Department of
Microbiology, Santosh Deemed
to be University, Ghaziabad,
Uttar Pradesh, India

Dr. Ritu Jain
Associate Professor, Department
of Microbiology, Santosh
Deemed to be University,
Ghaziabad, Uttar Pradesh, India

Dr. Juhi Aggarwal
Professor and Head, Department
of Biochemistry, Santosh
Deemed to be University,
Ghaziabad, Uttar Pradesh, India

Dr. Ashutosh Rawat
Professor and Head, Department
of Microbiology, Santosh
Deemed to be University,
Ghaziabad, Uttar Pradesh, India

Distribution and burden of malaria and dengue infections in a tertiary care hospital

Shivani Sharma, Dr. Dakshina Bisht, Dr. Ritu Jain, Dr. Juhi Aggarwal and Dr. Ashutosh Rawat

Abstract
Introduction: Malaria and Dengue are two vector borne diseases having gained a major concerns worldwide, particularly in tropical and subtropical region owing to their recurring outbreaks. Mosquito bite is considered to be the major cause they are transmitted to human by the bite of mosquito and cause different hematological manifestation.
Aim: To compare the laboratory features and outcome of mono-infection or co-infection with both Malaria and Dengue infections.
Materials and Methods: 256 suspected patients were tested for Malaria and Dengue infections. Serological diagnosis of dengue was done using rapid dengue duo kit which detects Ns1, IgM and IgG. Malarial parasite were identified by peripheral blood smear (thick and thin), rapid test were performed by Sure Test Malaria PF/PV HRP2/pLdh Combo kit which detects plasmodium falciparum and plasmodium vivax by using human blood.
Results: Of the 256 cases, most affected age group was 19-35 years (77%) and the less affected age group was >56 years (23%) while male and female ratio is 1:1. Of the 256 cases, 128 were dengue seropositive cases, 2 of which were Malaria positive and 1 case was showed co-infection. We observed that hematological parameters like hemoglobin, platelet count, SGPT and total leucocyte count were significantly higher in both Dengue and Malaria.
Conclusion: This study concluded that all patients presented with the febrile illness should be tested for both Malaria and Dengue. Both presented with similar clinical manifestations which is why specific diagnostic testing along with complete blood count is necessary to confirm the diagnosis and to know the severity of the illness.

Keywords: Malaria, dengue, co-infection, serum glutamic acid test, complete blood count

Introduction

Globally Malaria and Dengue are considered to be the major vector borne diseases causing serious illness especially in tropical and subtropical region because of their recurring outbreaks [1]. Malaria, a mosquito-borne parasitic infectious disease, has been recognized as a significant public threat for a long time where in the year 2020 alone around 241 million cases have been reported [2]. Studies have reported that five different species of protozoal parasite, Plasmodium that includes *P. ovale*, *P. falciparum*, *P. vivax*, *P. malariae*, *P. knowlesi* are carried and spread by female anopheles mosquito [3, 4].

Dengue, also known as break-bone fever caused by dengue virus, is spread by aedes aegypti and to a lesser extent - aedes albopictus mosquito. Four serotypes namely DENV-1, DENV-2, DENV-3, DENV-4 were reported [5]. Worldwide, around 400 million peoples are affected every year with dengue infection [6].

Vector borne diseases share an overlapping epidemic pattern with most cases reported from tropical regions of the world and several studies have been published reporting of co-circulation of malaria and dengue [7, 8] Both diseases can be distinguished on the basis of clinical features like periodic increase and decrease of fever as seen in malaria and hemorrhagic conditions or depletion of platelet count as seen in dengue [9, 10].

Malaria is currently diagnosed by either microscopic inspection of blood smears or Rapid Diagnostic Tests (RDTs).

Corresponding Author:
Shivani Sharma
Post Graduate Student,
Department of Microbiology,
Santosh Deemed to be
University, Ghaziabad, Uttar
Pradesh, India

Dengue fever is currently diagnosed in the laboratory either directly by detecting viral components [non-structural glycoprotein-1 (NS1) antigen] in serum or indirectly by measuring antibodies (IgM). Both can cause life-threatening complications, therefore they must be detected early and precisely to allow for prompt treatment [11].

The purpose of this study was to determine the prevalence of both Malaria and Dengue fever as well as to link the severity of both illnesses to symptoms and hematological indicators.

Materials and Methods

A prospective observational study was carried out in Santosh Medical College and Hospital after obtaining the ethical clearance by Institutional Ethical Committee. A total of 256 samples were collected from clinically suspected cases of Malaria and Dengue infections. A detailed history regarding age, sex, occupation, social status, duration of complaint and clinical history were taken.

4 ml of blood was drawn through venipuncture from suspected samples in EDTA and plain vial was collected. Each sample underwent a thorough laboratory testing for complete blood count (CBC), serum glutamate-pyruvate transaminase (SGPT) measurements. In order to diagnose Malaria, samples were examined for thick and thin peripheral blood smear, stained with Giemsa stain to identify Plasmodium species and simultaneously to a rapid antigen detection test using the Sure Test Malaria PF/PV HRP2/pLDH Combo kit to confirm the diagnosis of Malaria. Diagnosis of Dengue was done by the detection of Ns1 antigen and IgG/IgM antibody by rapid diagnostic tests. (Bioline™ Dengue Duo kit) [12].

Data collection and statistical analysis was done using SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). P = ≤ 0.05 was considered statistically significant.

Results

In the present study 256 cases of clinically diagnosed Malaria and Dengue cases were studied. Total number of patients were 256 and male and female ratio was 1:1. Most affected patients belonged to the age group of 19-35 years (77%) and less affected age group was > 56 years (23%). [Table 1].

Table 1: Distribution of Patients According to Age and Sex

Age (Years)	Sex	
	Male N (%)	Female N (%)
1-18	49(34%)	24(19%)
19-35	44(30%)	49(39%)
36-55	24(19%)	37(29%)
>56	11(17%)	17(13%)
Total- 256	128(50%)	128(50%)

Of the 256 samples, 128 (50%) samples were seropositive by atleast one component (Ns1, IgG, IgM) for dengue and only 1 (0.4%) sample was positive for Malaria. Co-infection of both Malaria and Dengue was found IN only 1 (0.4%) of the 125 samples that were found to be negative. [Table 2].

Table 2: Prevalence of Malaria and Dengue

Name of the disease	Positive N (%)
Dengue	128 (50%)
Malaria	1 (0.4%)
Dengue + Malaria	1 (0.4%)

Of the 128 seropositive Dengue cases, 16 (12.5%) samples were positive for NS1. 8 (6.25%) samples were positive for both NS1 and IgM, 3 (2.3%) samples were positive for both NS1 and IgG while 28 (21.8%) samples were positive for all the three parameters i.e. NS1, IgG and IgM. 6 (4.6%) samples were only IgM positive while 42 (32.8%) samples were IgG positive only and 25 (19.5%) samples were IgG and IgM both positive. [Table 3].

Table 3: Distribution of Seropositive Cases of Dengue (n=128)

Test	Number of Positive Samples	Percentage (%)
Only NS1 positive	16	12.5%
NS1 + IgM positive	8	6.25%
NS1 + IgG positive	3	2.3%
NS1 + IgG + IgM positive	28	21.8%
Only IgM positive	6	4.6%
Only IgG positive	42	32.8%
IgG + IgM positive	25	19.5%
Total	128	

Of the 2 Malaria cases, both were identified as Plasmodium vivax positive and both were males that belonged to the age group category of 36-55 years and > 56 years
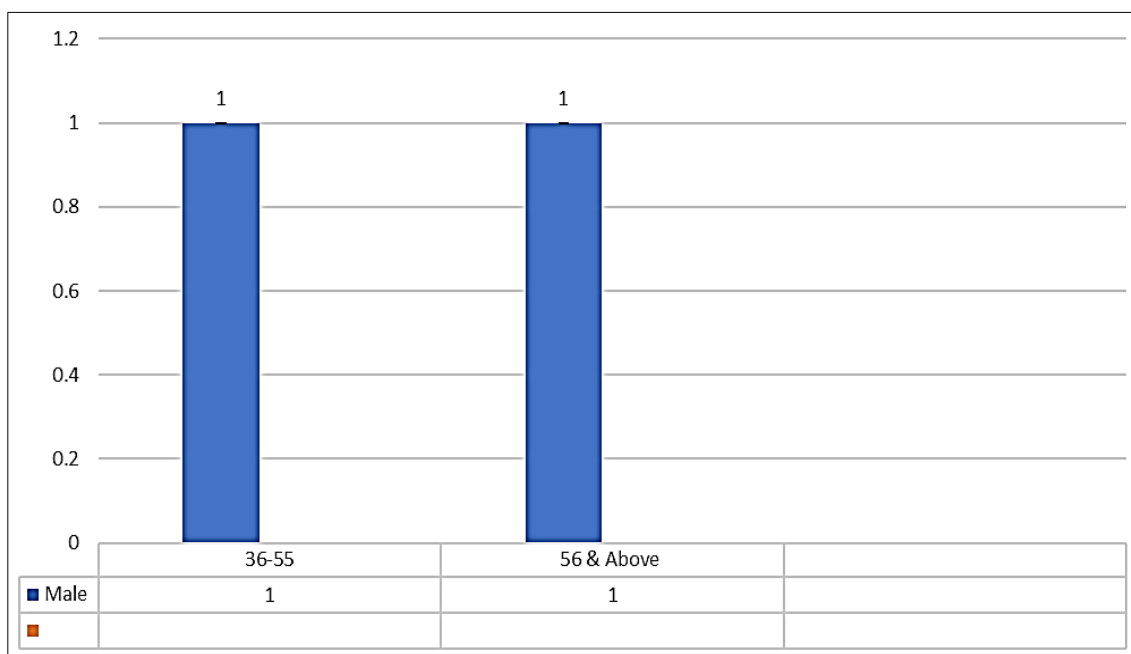
Graph 1: Gender and age wise distribution of positive cases of malaria

On observing the platelet count of seropositive cases, it was noted that of the 128 seropositive cases, 78 (83%) cases had thrombocytopenia (platelet count less than 1,00,000 / µL) and the rest 50 (17%) cases had a platelet count of more than 1,00,000 / µL.

On observing the platelet count of Malaria cases, it was noted that of the 2 positive cases, 1 (50%) case had thrombocytopenia (platelet count less than 1,00,000 / µL) and the rest 1 (50%) case had a platelet count more than 1,00,000 / µL. [Graph 2].
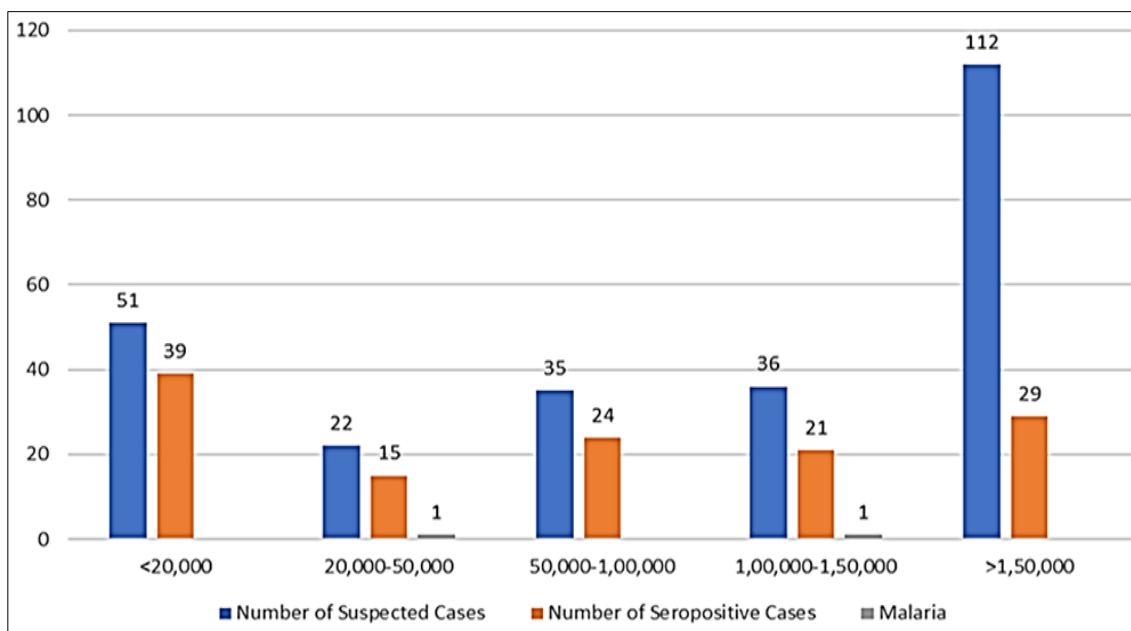
Graph 2: Distribution of Cases Based on Platelet Count

On observing the total leucocyte count of seropositive cases, it was noted that of the 128 positive cases, 48 (37.5%) cases had leukocytopenia (total leucocyte count less than 4,000 per cumm) where as 68 (54%) cases had a leucocyte count in between 4,000– 11,000 per cumm while 12 (9%) cases had leukocytosis (total leucocyte count more than 11,000 per cumm.

On observing the total leucocyte count of Malaria cases, it was noted that of the 2 positive cases, both cases had a leucocyte count in between 4,000-11,000 per cumm. [Graph 3].
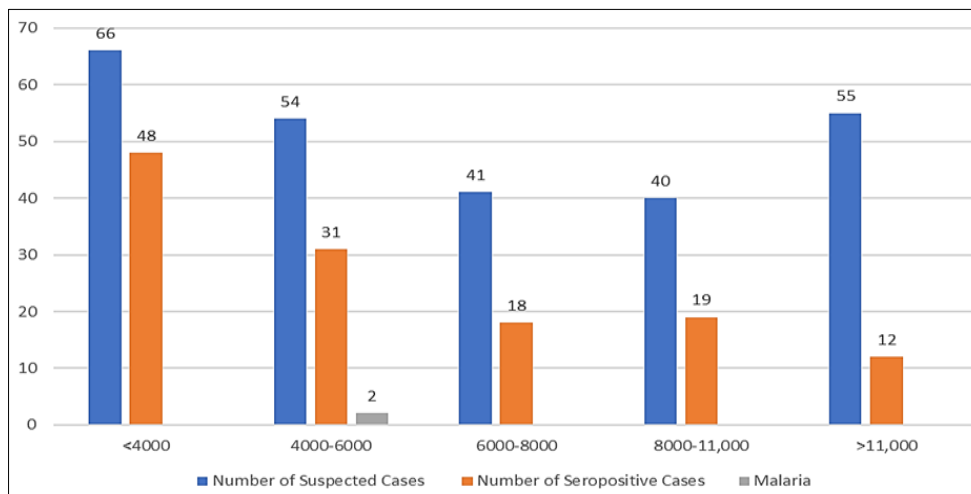
Graph 3: Distribution of Cases Based on Total Leucocyte Count

On observing the SGPT level of seropositive cases, it was observed that of the 128 cases, 92(72%) cases had elevated SGPT level (ALT level more than 35 units / l) while 36 (28%) cases had a SGPT level with in the normal range.

On observing the SGPT level of Malaria cases, it was noted of the 2 positive cases, both cases had elevated SGPT level (ALT level more than 35 units / l) [Graph 4].
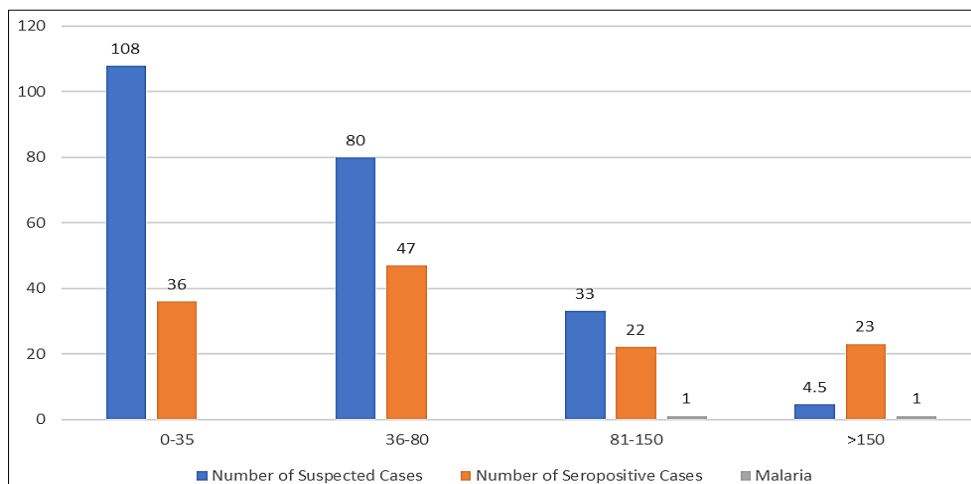
Graph 4: Distribution of Cases Based on SGPT

On observing the hemoglobin level of seropositive cases, it was observed that of the 128 cases, 52 (41%) cases had anaemia (Hb level less than 12 gm / dl) while 76 (59%) cases had hemoglobin level with in the normal range.

On observing the hemoglobin level of Malaria cases, it was noted that of the 2 cases both had anaemia (Hb level less than 12 gm /dl) [Graph 5].
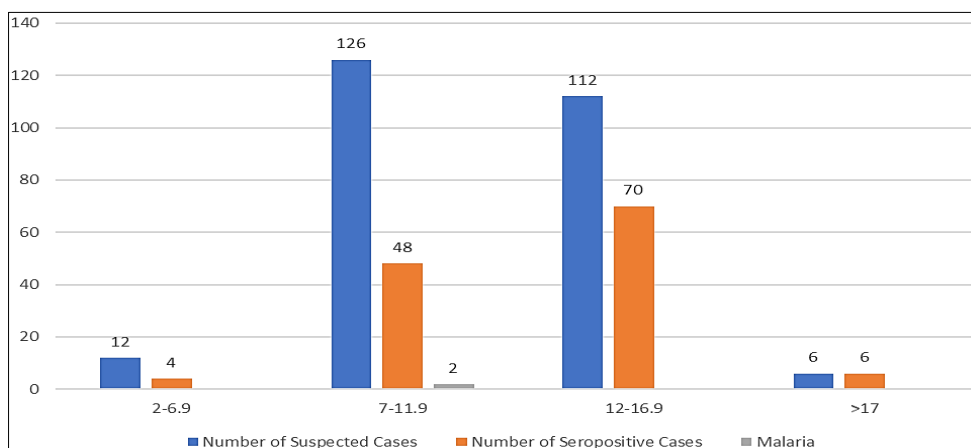
Graph 5: Distribution of Cases Based on Hemoglobin level

Discussion

Malaria and Dengue are arthropod borne diseases spread by mosquito vector found in abundance in tropical regions allover the world. Anopheles is a night biting mosquito and breed in stagnant water [13]. Rapid urbanization without the development of civic infrastructure, constant movement of population for livelihood, monsoon dependent breeding patterns and overlapping habits have lead to co-circulation and co-infection of these pathogens in same population [14].

In the present study a total of 256 cases of Dengue and Malaria were undertaken and it was found that all cases had fever as a common symptom with male and female ratio of 1:1. However one of the common clinical manifestation was thrombocytopenia observed in 42% cases which is analogous to the study of Dost Mohammad khan [2014] where the clinical and statistical relationship [$p<0.05$] was reported between Dengue infection and thrombocytopenia.

Of the total 256 cases, 50% samples were seropositive by at least one component [NS1, IgG, IgM], while Malaria was positive in 1 [0.4%] case and co-infection was found to be observed in only 1 [0.4%] case. Moreover, an interaction between Plasmodium and platelets has been postulated as *P. vivax* within the platelets of patients has been observed under the electron microscopy [16].

Our study showed minor bleeding manifestations due to low platelet count as reported the recently by Cho-Naing17 [2018]. The author in his review based on Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline 18 and reported that there was some evidence of clinical relevance of thrombocytopenia with *P. vivax* malaria leading to bleeding manifestations as more common in this species of Plasmodium.

Recently it has been reported that coinfections with either Malaria and Dengue or any others occurring at the same time is a rare possibility or instance. The above study has also indicated that there are higher percentage of people with febrile symptoms which might have either Malaria/Dengue or Malaria-Dengue coinfection as well. Another study conducted in rural, urban and semiurban areas across the Angul district of Orissa has reported a higher rate of Dengue infection than Malaria infection and the presence of Dengue- Malaria co-infection as common in these localities.

However, in the present study. Of the total 256 suspected cases, considering the pattern of TLC count, leukocytopenia was noted in 26% of cases, whereas leukocytosis was observed in 21% of cases and rest remained within the normal range. Results of the present study was in agreement with the study of Smita Chandra and Harsh Chandra [2013] [21], in which leukocytosis was reported in 18.8% of cases and 11.5% cases to have leukocytopenia with high specificity rate of 90% and low sensitivity [11.3%] for malaria infection.

In the present study, liver enzymes specifically for SGPT in around 148 cases [58%] were found to be raised. Our finding is in concordance with the study done by Huerre MR [22]. [2001], where the author has reported a raise in the levels of aspartate transaminase (AST) around 63-97% of patients, whereas the elevated alanine transaminase (ALT) levels in 45–96% of patients.

In the present study, it was found that of the 138 (54%) out of the total 256 suspected cases were determined to be anaemic cases. Our finding is in agreement with an earlier study by Sangita Devubhai Vasava [23] [2021] regarding evaluation of concurrent Malaria and Dengue infections among febrile patients and it was observed that anaemia was found in 21% of Malaria-Dengue co-infection case, which is quiet significant.

Conclusion

This study concluded that the prevalence of Dengue was higher as compared to Malaria. However, considering the risk of multiple complications, all patient having fever must be diagnosed for both Malaria and Dengue even if co- infection occurs in one patient. Owing that Climate changes with intermittent rain provides a favourable environment for vector breeding, which accounts for the increase in vector borne diseases, prevention from mosquito bite is the way best to save the human life from such life threatening diseases including malaria and dengue. Furthermore, early diagnosis could also be equally important to prevent the patients from other complications.

Conflict of Interest: None.

References

1. Kilpatrick AM, Randolph SE. Drivers, dynamics, and control of emerging vector-borne zoonotic diseases. Lancet. 2012;380:1946-1955. DOI: 10.1016/S0140-6736(12)61151-9.
2. World Health Organization. World Malaria Report 2021. Available at http://www.who.int/malaria/publications/world_malaria_report_2021/report/en/. Accessed on 6 December 2021. Reference no 978-92-4-004050.
3. Miller LH, Ackerman HC, Su XZ, Wellems TE. Malaria biology and disease pathogenesis: Insights for new treatments. Nat Med. 2013;19:156-167. DOI: 10.1038/nm.3073.
4. Barber BE, Rajahram GS, Grigg MJ, William T, Anstey NM. World malaria report: time to acknowledge Plasmodium knowlesi malaria. Malar J. 2017;16:135. DOI: 10.1186/s12936-017-1787-y.
5. Diamond MS, Pierson TC. Molecular insight into dengue virus pathogenesis and its implications for disease control. Cell. 2015;162:488-492. DOI: 10.1016/j.cell.2015.07.005.
6. World Health Organization. http://www.who.int/denguecontrol/epidemiology/en/. Accessed on 19th Oct. 2017.
7. Furuya-Kanamori L, Liang S, Milinovich G, Soares Magalhaes RJ, Clements AC. Co-distribution and co-infection of chikungunya and dengue viruses. BMC Infect Dis. 2016;16:84. DOI: 10.1186/s12879-016-1417-2.
8. Halsey ES, Baldeviano GC, Edgel KA, Vilcarromero S, Sihuincha M. Symptoms and immune markers in Plasmodium/dengue virus co-infection compared with mono-infection with either in Peru. PLoS Negl Trop Dis. 2016;10:e0004646. DOI: 10.1371/journal.pntd.0004646.
9. Hisam A, Mahmood ur R, Khan MB, Kadir E, Azam N. Frequency of co-existence of dengue and malaria in patients presenting with acute febrile illness. J Pak Med Assoc. 2014;64:247-251.
10. Weaver SC, Lecuit M. Chikungunya Virus Infections. N Engl. J Med. 2015;373:94-95.
11. Jain V, Khan A, Garg R, Kashyap VK, Kohli S, Chopra A, *et al*. Discriminating malaria and dengue fever in

epidemic areas: clinical, biochemical, and radiological areas; c2020 Apr 27. https://doi.org/10.1016/j.cegh.2020.04.014.

12. Mohapatra, Manoj, Patra P, Agrawala R. Manifestation and outcome of concurrent malaria and dengue infection. Journal of vector-borne diseases. 2012;49:262-265.

13. Sinka ME, Bangs MJ, Manguin S, Chareonviriyaphap T, Patil AP. The dominant Anopheles vectors of human malaria in the Asia-Pacific region: Occurrence data, distribution maps and bionomic precis. Parasit Vectors. 2011;4:89.

14. Kraemer MU, Sinka ME, Duda KA, Mylne AQ, Shearer FM. The global distribution of the arbovirus vectors Aedes aegypti and Aedes albopictus Elife. 2015;4:e08347.

15. Khan DM, Kuppuswamy K. Evaluation of Thrombocytopenia in dengue infection Along with seasonal variation in rural Melmaruvathur. 2014 Jan;8(1):39-42. Published online 2014 Jan 12. DOI: 10.7860/JCDR/2014/6739.3914.

16. Fajardo LF, Tallent C. Malarial parasites within human platelets. JAMA. 1974;229:1205.

17. Naing C, Whittaker MA. Severe thrombocytopenia in patients with vivax malaria as compared to falciparum malaria: A systemic review and meta-analysis; Infectious diseases of Poverty 7, Article number: 10 (2018) Cite this article 2635 Accesses, 15 Citations.

18. Moher D, Liberati A, Tetzlaff J, Altman DG, Grp P. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med. 2009;6:e1000097.

19. Salam. BMC Public Health 2018: Global Prevalence and Distribution of co-infection of Malaria, Dengue, and Chikungunya: A systemic review. 2018;18:710. http://doi.org/10.1186/s12889-018-5626-2.

20. Rajesh Kumar Rao M. Prevalence of Dengue, Viral and malaria parasitic co-infections in an epidemic district, Angul of Odisha India; An Eco-epidemiological and cross-sectional study for the prospective aspects of public health. Journal of Infection and Public Health. 2016; Jul-Aug;9(4):421-428.

21. Chandra S, Chandra H. Role of Haematological Parameters as an Indicator of Acute Malarial Infection in Uttarakhand State of India. 2013;5(1):e2013009. Published online 2013 Jan 2. DOI: 10.4084/MJHID.2013.009; PMCID: PMC3552839 PMID: 23350022.

22. Huerre MR, Lan NT, Marianneau P, Hue NB, Khun H, Hung NT, *et al*. Liver histopathology and biological correlates in five cases of fatal dengue fever in Vietnamese children. Virchows Archiv. 2001;438:107-115.

23. Vasava SD. Evaluation of Concurrent Malaria and Dengue Infections among Febrile Patients - A Cross-sectional Study. DOI: 10.7860/JCDR/2021/47347.14719.