

## PLATELET COUNT IN SEROPOSITIVE AND SERONEGATIVE DENGUE CASES IN RAICHUR DISTRICT

Inder Raj Itagi<sup>1</sup>, Ramesh B. H<sup>2</sup>, Ambika H. K<sup>3</sup>

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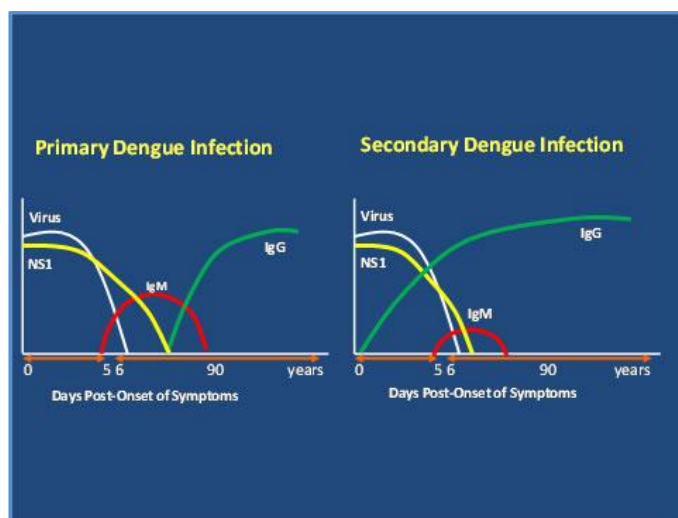
**ABSTRACT:** Dengue Fever is caused by Dengue Viruses (4 Serotypes) by the bites of aedes aegypti mosquito. Laboratory findings in dengue cases show leucopenia and thrombocytopenia which is mild in nature. In this study we have made an attempt to compare platelet count in seropositive and seronegative dengue cases in and around Raichur District. **AIMS AND OBJECTIVES:** To compare the platelet count in seropositive and seronegative dengue fever patients. **MATERIALS AND METHODS USED:** Automated cell counter (SYSMEX-5PART). **Specimen:** Blood or serum in a red top tube. Acute and convalescent specimens do not need be sent together. **Collection:** KHEL Serology kit with the yellow (red) top blood tubes or any other red topped, clot separator blood tubes. **Volume:** 2 cc (ml.) of centrifuged serum or plasma. **Storage:** On ice or in refrigerator (not in a freezer) until it is delivered to CDC Dengue Branch. Any specimens stored greater than a month prior to arrival at CDC will not be tested. Timing of collection for serology: Acute- obtained up to 5 days after onset of symptoms; convalescent- 6 or more days after the onset of symptoms. Test results are normally available 3 days (PCR) to 1 week (serology) after specimen receipt. During periods of a severe dengue epidemic it may be necessary to prioritize testing based on the severity of disease. Any severe case that is hospitalized should be indicated on the form. **Type of Study:** PROSPECTIVE (COHORT STUDY) **Duration of Study:** 6 months (Dec-2011 to May 2012). **Study Site:** RAICHUR DISTRICT IN KARNATAKA.

**KEYWORDS:** Dengue fever, seropositive cases, sero negative cases, platelet count.

**INTRODUCTION:** The classical clinical presentation of Dengue virus infection includes fever, joint pains, headaches and rashes (petechial or maculopapular rash) usually involving the limbs and trunks are the characteristic symptoms of the disease. In addition, thrombocytopenia is more marked and develops earlier in cases with a fatal outcome<sup>1</sup> (Fausiet al., 2008). However many other types of clinical presentations have been observed. They are neurological, hepatic, cutaneous and gastrointestinal. Among neurological manifestations encephalopathy, acute motor weakness, seizures, neuritis, hypokalemic paralysis are common<sup>2, 3,4,5</sup> (Misra et al., 2006; Verma et al., 2012; Kumar et al., 2008; Verma et al., 2011). In the hepatic involvement acute liver failure, hepatic encephalopathy, hepatomegaly and jaundice are commonly seen<sup>6,7,8,9</sup> (Deepak et al., 2006; Vinodh et al., 2005; Giri et al., 2008; Jhamb et al., 2011). In the gastrointestinal (GI) system lower GI bleeding and acute inflammatory colitis is seen<sup>10</sup> (Rama et al., 2006). Hemophagocytic syndrome includes bone marrow hemophagocytosis associated with nasal bleeding and pancytopenia.<sup>11,12,13</sup> (Jain et al., 2008; Ray et al., 2011; Kapdiet al., 2012).

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**IMMUNOLOGICAL RESPONSE TO DENGUE INFECTION:** The acquired immune response following a dengue infection consists of the production of IgM and IgG antibodies primarily directed against the virus envelope proteins. The immune response varies depending on whether the individual has a primary (first dengue or other flavi virus infection) versus a secondary (had dengue or other flavi virus infection in past) dengue infection. In general, diagnosis of dengue is dependent on the phase of the infection. The general timeline of a primary infection from virus isolation or identification, to IgM detection followed by IgG detection is as follows:



**Fig. 1**

A primary dengue infection is characterized by a slow and low titer antibody response. IgM antibody is the first immunoglobulin isotype to appear. Anti-dengue IgG is detectable at low titer at the end of the first week of illness, and slowly increases. In contrast, during a secondary infection, antibody titers rise extremely rapidly and antibody reacts broadly with many flavi viruses. High levels of IgG are detectable even in the acute phase and they rise dramatically over the preceding two weeks. The kinetics of the IgM response is more variable. IgM levels are significantly lower in secondary dengue infections and thus some anti-dengue IgM false-negative reactions are observed during secondary infections. According to the Pan American Health Organization (PAHO) guidelines 80% of all dengue cases have detectable IgM antibody by day five of illness, and 93-99% of cases have detectable IgM by day six to ten of illness, which may then remain detectable for over 90 days.

MAC-ELISA has become an important tool for routine dengue diagnosis, MAC-ELISA has a sensitivity and specificity of approximately 90% and 98%, respectively but only when used five or more days after onset of fever (i.e., in convalescent phase). Different formats such as capture ELISA capture ultra-micro ELISA, dot-ELISA, AuBioDOT IgM capture and dipsticks have been developed. Serum, blood on filter paper, and saliva (but not urine) are useful for IgM detection if samples are taken in convalescent phase of illness (Vasquez et al., 2006). A variety of different commercial kits are available with variable sensitivity and specificity. Dengue diagnosis becomes even more challenging because dengue IgM antibodies also cross-react to some extent with other

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flavi viruses such as JEV- Japanese encephalitis virus, SLE- St. Louis encephalitis virus, WNV- West Nile virus and YFV- Yellow fever virus.

**CASE DEFINITION OF DENGUE FEVER:** DF is most commonly an acute febrile illness defined by the presence of fever and two or more of the following, retro-orbital or ocular pain, headache, rash, myalgia, arthralgia, leukopenia, or hemorrhagic manifestations (e.g., positive tourniquet test, petechiae; purpura/ecchymosis; epistaxis; gum bleeding; blood in vomitus, urine, or stool; or vaginal bleeding) but not meeting the case definition of dengue hemorrhagic fever. Anorexia, nausea, abdominal pain, and persistent vomiting may also occur but are not the case-defining criteria.

## **DENGUE HEMORRHAGIC FEVER (DHF) – CLINICAL DESCRIPTION FOR SURVEILLANCE:**

**DHF is characterized by all of the following:**

- Fever lasting from 2-7 days,
- Evidence of hemorrhagic manifestation or a positive tourniquet test,
- Thrombocytopenia ( $<100,000$  cells per  $\text{mm}^3$ ),
- Evidence of plasma leakage shown by hemo concentration (an increase in hematocrit  $>20\%$  above average for age or a decrease in hematocrit  $>20\%$  of baseline following fluid replacement therapy), OR pleural effusion, or ascites or hypoproteinemia.

## **Dengue Shock Syndrome (DSS) – Clinical Description for Surveillance:**

DSS has all of criteria for DHF plus circulatory failure as evidenced by:

- Rapid and weak pulse and narrow pulse pressure ( $<20\text{mm Hg}$ ), OR
- Age-specific hypotension and cold, clammy skin and restlessness.

## **Laboratory Criteria for Case Classification of DF, DHF and DSS:**

### **Confirmatory tests:**

- Isolation of a dengue virus from or demonstration of specific arboviral antigen or genomic sequences in tissue, blood, cerebrospinal fluid (CSF), or other body fluid by polymerase chain reaction (PCR) test, immunofluorescence or immunohistochemistry, OR
- Seroconversion from negative for dengue virus-specific serum Immunoglobulin M (IgM) antibody in an acute phase ( $>5$  days after the onset of symptoms) specimen to positive for dengue-specific serum IgM antibodies in a convalescent-phase specimen collected  $>5$  days after symptom onset, OR
- Demonstration of a  $>4$ -fold rise in reciprocal Immunoglobulin G (IgG) antibody titer or Hemagglutination inhibition titer to dengue virus antigens in paired acute and convalescent serum samples, OR
- Demonstration of a  $>4$ -fold rise in PRNT (plaque reduction neutralization test) end point titer (as expressed by the reciprocal of the last serum dilution showing a 90% reduction in plaque counts compared to the virus infected control) between dengue viruses and other flaviviruses tested in a convalescent serum sample, OR

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- Virus-specific immunoglobulin M (IgM) antibodies demonstrated in CSF. Isolation of dengue virus from serum and/or autopsy tissue samples, or Kansas Disease Investigation Guidelines Version 01/2010 Dengue Fever, Page 2.
- Demonstration of a > 4-fold rise or fall in reciprocal immunoglobulin G (IgG) or immunoglobulin M (IgM) antibody titers to one or more dengue virus antigens in paired serum samples, OR
- Demonstration of dengue virus antigen in autopsy tissue or serum samples by immunohistochemistry or by viral nucleic acid detection.

## **Presumptive/Probable:**

- Dengue-specific IgM antibodies present in serum with a P/N ratio >2.

## **Case Classification of DF, DHF and DSS:**

**Confirmed:** A clinically compatible case of DF, DHF, or DSS with confirmatory laboratory results.

**Probable:** A clinically compatible case of DF, DHF, or DSS with laboratory results indicative of presumptive infection.

**Suspected:** A clinically compatible case of DF, DHF or DSS that is epidemiologically linked to a confirmed case (For reporting, report "Dengue shock syndrome" cases as "Dengue hemorrhagic fever" recording in Symptoms on the Investigation tab the clinical syndrome: "Dengue hemorrhagic fever/Dengue shock syndrome" and in "Other symptoms?" that hypotension or a narrow pulse pressure was present.)

**LABORATORY ANALYSIS:** Dengue viruses have sufficient antigenic similarity to other flavivirus, including yellow fever virus, Japanese encephalitis virus, and West Nile virus, that previous infection or vaccination may raise cross-reactive serum antibodies and false positive results. PRNT can often resolve cross-reactive serum antibodies in this situation and identify the infecting virus. However, high-titered cross-reactive antibody levels produced from multiple previous flavivirus infections cannot be resolved by PRNT.

Only a small proportion of the US population has evidence of previous flavivirus infection (or vaccination) so that cross-reactive flavivirus antibodies should not be a significant limitation to dengue diagnosis among most US travellers. Among US residents, most testing for dengue is done through private clinical laboratories using IgM or IgG detection techniques. To assist health departments in the interpretation of commonly performed serology testing, Table VII-B-2 for test interpretation is available on the Council of State and Territorial Epidemiologists website: [www.cste.org/ps2009/09-ID-19.pdf](http://www.cste.org/ps2009/09-ID-19.pdf) CDC Dengue Branch, San Juan, Puerto Rico will provide dengue testing free of cost to submitting physicians, state and private laboratories if certain criteria are met. Kansas Health and Environmental Laboratory (KHEL) can assist with the coordination of sample transport. Call KDHE Bureau of Surveillance and Epidemiology (BSE) at 877-427-7317 before sending any samples to KHEL.

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## The criteria for testing at the CDC include:

- A clinical diagnosis of suspected dengue based on an acute febrile picture accompanied by headache, retro-orbital pain, body pain, often a rash, and other variable symptoms that can include obvious or mild hemorrhagic manifestations or hemo concentration, shock or coma.

## Kansas Disease Investigation Guidelines:

### Version 01/2010 Dengue Fever, Page 3:

- A complete, clear Dengue Case Investigation Report with the following \*:
- Complete name, age and sex of patient.
- Home address.
- Date of onset of symptoms.
- Date that sample was obtained.
- Complete name and mailing address of physician, laboratory, clinic or hospital that result should be sent to.
- Samples with the above-mentioned information missing, or written with illegible handwriting or with more than a month from date of sample collection to date of arrival at CDC, will not be analyzed.

**DISCUSSION:** In our study of 200 patients it's found that platelet counts are markedly decrease in seropositive patients than in seronegative patients.

PLATELET COUNT	NO. OF PATIENTS
<20000cells/dl	01
20000-50000cells/dl	11
50000-100000cells/dl	27
100000-150000cells/dl	29
150000-400000cells/dl	109
>400000cells/dl	23

In table: 1 it is seen that 39 patients out of 200 falls below the 100000/microlitre range which is the standard demarcation for thrombocytopenia. Out of 39 patients 1 patient was below the 20000 mark. 11 patients were in 20000-50000 range and 27 patients were in the 50000-100000 range. Most of the patients (109) however were in the normal range of 150000-400000range.

PLATELET COUNT	SERONEGATIVE PATIENTS	SEROPOSITIVE PATIENTS
<20000cells/dl	00	01
20000-50000cells/dl	07	04
50000-100000cells/dl	10	17
100000-150000cells/dl	19	10
150000-400000cells/dl	94	15
>400000cells/dl	21	02

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In table no.2 above, the platelet count has been tabulated for seropositive and seronegative patients. It is clearly seen that platelet count is markedly low in seropositive patients compared to seronegative patients. 1 Seropositive patient lies below the 20000cells/dl mark. 04seropositivepatients in the range of 20000-50000cells/dl. 17 seropositive patients are in the range of 50000-100000cells/dl. 10 seropositive patients are in the range of 100000-150000 cells/dl. 15 seropositive patients are in the range of 150000-400000cells/dl. Only 02 seropositive patients are more than 400000cells/dl. Compared to 15 seropositive patients in the 150000-400000 range there are 94 seronegative patients n compared to 17 seropositive patients in the 50000-100000 range there are only 10 seronegative patients indicating there's marked fall in the platelet count of seropositive patients than seronegative patients.

SEROLOGICAL MARKERS POSITIVE	NO. OF PATIENTS
IgG	26
IgM	03
Ns1Ag	23
IgG+IgM	05
Ns1Ag+IgM	03
IgG+IgM+Ns1Ag	02

In table no.3 above our study has segregated patients based on the serological results. It is found that 26 Cases are IgG POSITIVE, 03 Cases are IgM POSITIVE,23 Cases are Ns1Ag POSITIVE,05 Cases are IgG & IgM POSITIVE,03 Cases are Ns1Ag & IgM POSITIVE and 02 Cases are Ns1Ag, IgM & IgG POSITIVE.

**CONCLUSION:** Thus in this study above, it is found that platelet counts of seropositive patients fall in the thrombocytopenia range.

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## **AUTHORS:**

1. Inder Raj Itagi
2. Ramesh B. K.
3. Ambika H. K.

## **PARTICULARS OF CONTRIBUTORS:**

1. Associate Professor, Department of Pathology, Raichur Institute of Medical Sciences & Pathlabs, Raichur.
2. Professor & HOD, Department of Pathology, Raichur Institute of Medical Sciences & Pathlabs, Raichur.
3. Tutor, Department of Pathology, Raichur Institute of Medical Sciences & Pathlabs, Raichur.

## **NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:**

Dr. Inder Raj Itagi,  
Associate Professor,  
Department of Pathology,  
Raichur Institute of Medical Sciences,  
Raichur-584102.  
E-mail: drinderrajitagi@gmail.com

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