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EARLY TOTAL LEUCOCYTE COUNT AND MONOCYTE COUNT AS MARKERS IN PREDICTING SEVERITY AND OUTCOME OF DENGUE FEVER

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ABSTRACT

Introduction: One of the most widespread viruses that infect humans and are spread by mosquitoes is dengue fever (DF), which is characterized by leucopenia and an increasing haematocrit of 5–10%. Even before thrombocytopenia, which develops after five days of fever, there are hematological alterations associated with dengue fever. **Aims and objectives:** To compare the total leucocyte count and monocyte count taken during the initial 3 days of fever and predicting the severity of dengue as well as predict the duration of hospital stay. **Methodology:** This ambidirectional (prospective and retrospective) longitudinal study was conducted over a period of 3 months in Department of General Medicine, Chettinad hospital and research institute on a total of 60 patients. **Results:** Leukopenia, normal WBC count and leukocytosis was observed in 60%, 33.33% and 6% of the study population respectively. Patients who had a more severe form of dengue showed higher rates of monocytosis. The length of hospital stay and the number of monocytes had a moderately positive and significant connection. Between the length of hospital stay and the total platelet count, a substantial negative connection was found. **Conclusion:** There was no statistically significant difference in leukocyte count or status between the severity and outcome of dengue. By using ROC analysis, monocyte count and percentage within the first three days after fever onset was found to be a more accurate indicator of dengue severity than total leukocyte count.

KEYWORDS:

dengue fever, leucocyte count, monocyte count.

INTRODUCTION

For decades, dengue fever (DF), which is brought on by the dengue virus (DENV) and spread by mosquitoes, has posed a serious threat to public health in India. The growing human population and the expansion of dengue-risk zones offer a significant risk to the disease's expanding trend, especially in tropical and subtropical countries, even though individual mortality and morbidity have decreased with the advent of modern science and medicine. India is one of the more than 100 countries in the tropical and subtropical zone that are constantly dealing with dengue epidemics and outbreaks. The complexity of the virus's vector of transmission and the above described characteristics have caused the illness burden to increase dramatically in recent years. The dengue virus poses a risk of infection to 2.5 billion people worldwide. 390 million cases of dengue are reported each year, putting 500,000 people at risk of experiencing severe dengue sickness symptoms such dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). (Bhat et al., 2013)

In addition to having a very complicated pathophysiology, dengue also poses economic and ecological concerns. A dengue infection might present with anything from shock symptoms to a subclinical illness. The disease's prognosis and result are unparalleled. The majority of patients suffer from subclinical infections. A number of DENV infections progress into more serious conditions such febrile DF, DHF, and DSS. (Guzman and Harris, 2013)

In endemic nations, a great deal of study has been done, particularly on the epidemiology and clinical progression of the disease. Hypothesis about cytokine activation and inflammatory mediators (John et al., 2015), genetics (Coffey et al., 2009), dengue viral strains (Rico-Hesse, 2009), antibody-mediated enhancement (John et al., 2015), and other blood illnesses (Noisakran and Perng, 2008; Tsai et al., 2012). The target cells and process of virus transmission have been the subject of numerous theories, and there is still disagreement on these points. However, several studies have suggested that phagocytic cells and platelet progenitors are viable options. (Halstead et al., 1977; Kou et al., 2008; Scott et al., 1980)

Leucopenia has frequently been reported in dengue along with or even before thrombocytopenia. (Tsai et al., 2017) The DENV virus is thought to target cells that exhibit the monocytosis linked to leukopenia. There has also been discussion of the significance of monocyte count in identifying dengue's evolutionary history. (Kou et al., 2008) The prognosis of the illness can be predicted by evaluating hemodynamic and blood images early in the infection process. (Tsai et al., 2017) This study attempts to evaluate the significance of the leucocyte and monocyte counts in order to predict the severity of dengue and the length of hospital stay.

AIMS AND OBJECTIVES

1. Comparing total leucocyte count and monocyte count taken during the initial 3 days of fever and predicting the severity of dengue.
2. Comparing total leucocyte count and monocyte count taken during initial 3 days of fever and predicting the length of hospital stay

METHODOLOGY

The study was an ambidirectional (prospective and retrospective) longitudinal study conducted over a period of 3 months in the Department of General Medicine, Chettinad hospital and research institute encompassing 60 patients.

Inclusion criteria

- Patients >18 years presenting with complaints of fever and positive for any one or more than one test among NS1Ag, IgM, and IgG .
- Patients >18 years presenting with body ache, headache, retro-orbital pain, low backache, rash, petechiae, bleeding manifestations like bleeding gums, Malena, epistaxis, hematuria with any one or more than one test positive among NS1Ag, IgM, and IgG

Exclusion criteria

- Patients <18 years of age

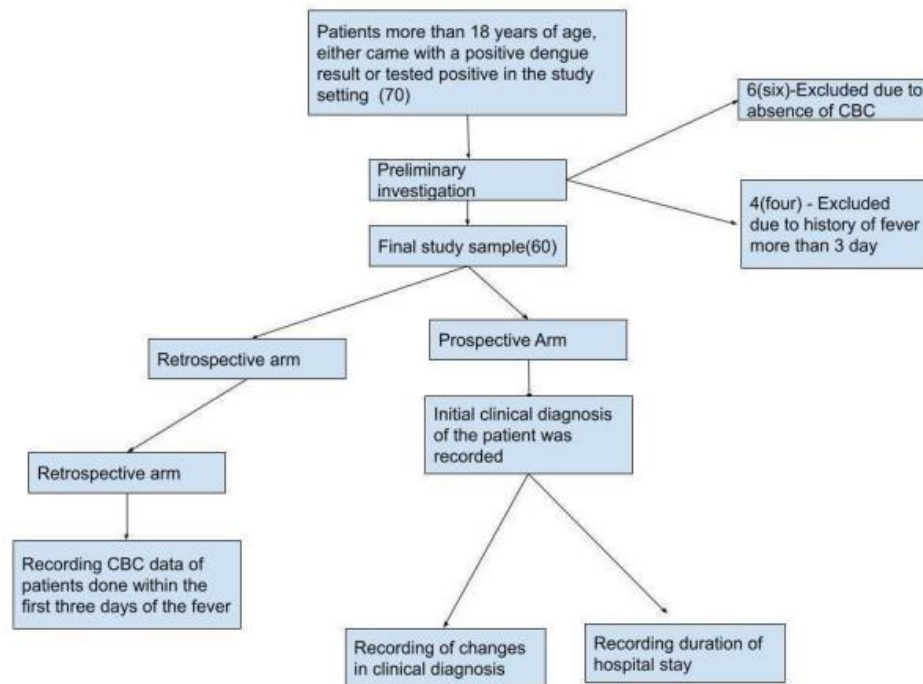
- Patient without a complete blood picture taken within the first three days of fever.
- Patient without proper clinical history.
- Non-consenting patients
- Complaint of fever > 3 days prior to coming to study setting.

Method of Patient recruitment

The research setting being a tertiary health care establishment, patients are referred from other primary and secondary health care establishments. All patients fulfilling the inclusion criteria were recruited in the study. Any patients referred to the study setting with a positive dengue test were also included in the preliminary investigation of the study.

In the initial consultation , the patient's complete blood count was obtained retrospectively throughout the first three days following the onset of fever, and the results were documented. Patients were not included in the trial if they refused to take a CBC or if one was not available. The enlisted patients were monitored until their release, and their progress was documented. The entire amount of time the patient spent in the hospital was also noted. The Ethics Committee provided its approval prior to the initiation of the preliminary study. The research subjects offered their informed consent. The patients were advised that no modifications to the recommended dengue management approach were required by the study.

Figure 1: Schematic representation of patient recruitment for the study



Statistical analysis

Data recording and observation were done using Microsoft excel. Wherever necessary descriptive statistics such as mean, median, proportions, and standard deviation of the data were done. For comparison between different severity outcomes in terms of normally distributed continuous variables independent t-test was used. For comparison between different severity outcomes in terms of ordinal variables and nonparametric variables, the Mann Whitney U test was used. For finding correlations between the variables wherever necessary, spearman's correlation was used. For scatter plots with trend lines and other pictorial representations of data and test findings, Microsoft Excel was used. For constructing a predictive model, the ROC curve was used along with cut-off values and respective sensitivity and 1-specificity. A p-value of less than 0.05 was considered significant for comparative tests. For the aforementioned statistical tests, SPSS version 26.0 was used

RESULTS:

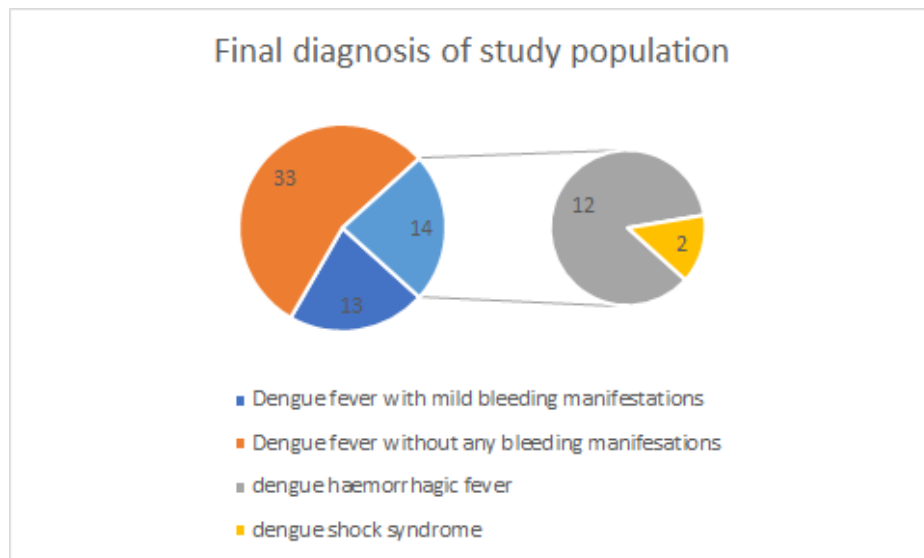
The mean age of the study population who recovered with minimal or moderate dengue (classical dengue fever) was 42.174 ± 13.21 years. The mean age of the study population who progressed into severe dengue illness was 41.786 ± 14.20 years. No significant difference in mean age was observed between these two categories of the study population. (Table 1)

Table 1: Comparison of age and disease severity

	DF	DHF/DSS
Mean age	42.174	41.786
Sd	13.21	14.20
minimum	19	20
maximum	63	62

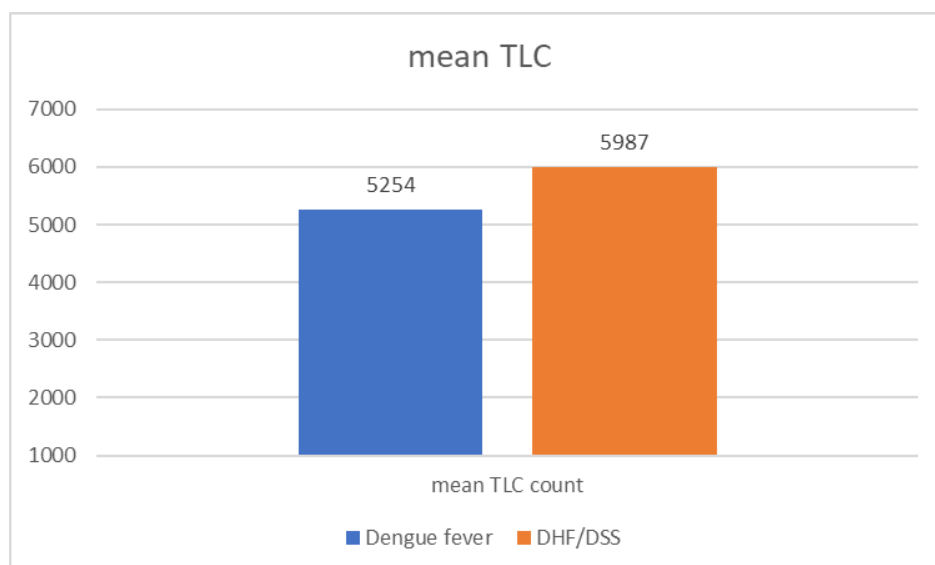
76% of the study population did not progress to a severe form of dengue but 28.2% showed signs of minimal bleeding manifestations. The latter recovered without progressing to severe form of dengue plasma leakage manifestations. 23.3% progressed into a severe form of dengue. (Figure 2)

Figure 2: Final Diagnosis of study population



The mean duration of hospitalization among the patients who recovered from dengue fever and those who progressed into a severe form 5.39 ± 0.07 days and 7.71 ± 0.16 days respectively, the mean difference being statistically significant. The mean value of total leukocyte count among the patients who recovered from dengue and those who progressed into severe dengue was 5254.02 ± 1979 cells per cumm and 5987 ± 2868 cells per cumm respectively which was not statistically significant. (Figure 3)

Figure 3: Mean Total leukocyte count of classical dengue fever and severe dengue

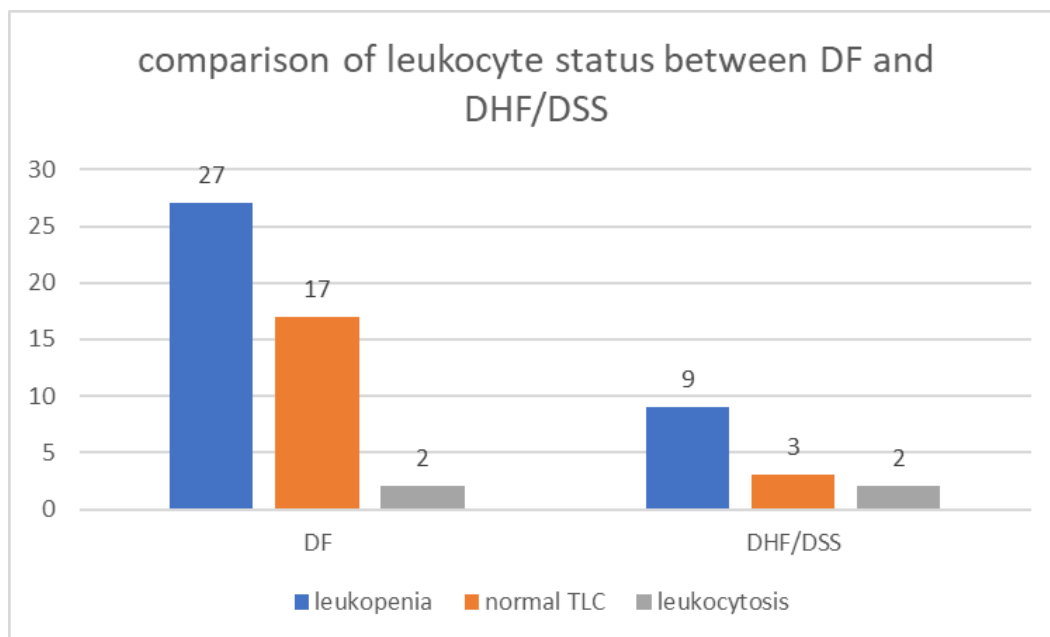


58.7% of the recovered patients had leukopenia while the proportion of leukopenia observed among patients who progressed into severe dengue was 64.3%. 9/12 patients who progressed into severe

dengue (DHF/DSS) had a leukocyte count of less than 4500 per cumm. Normal leukocyte count was observed among 17 patients (37%) and 3 patients (21.4%) in DF and severe dengue respectively.

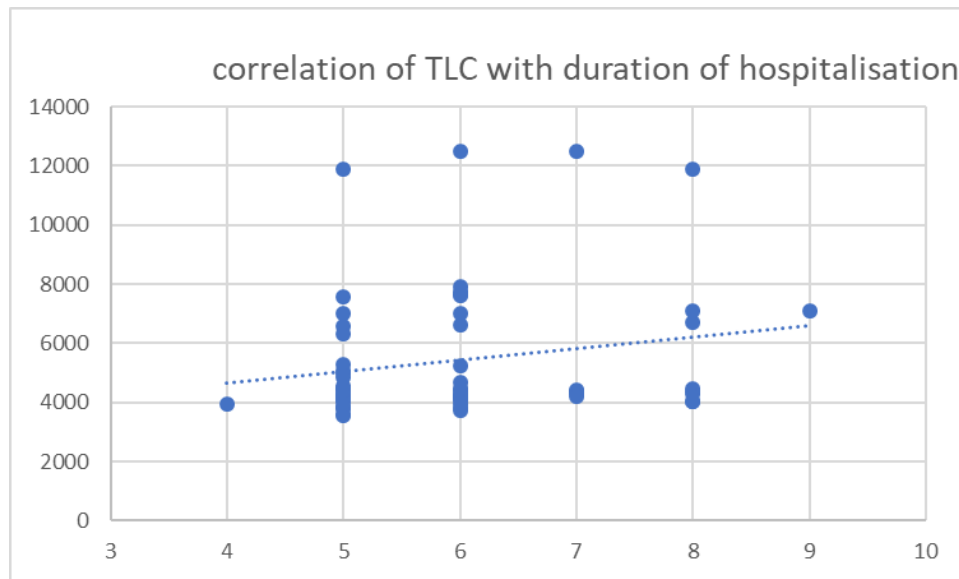
Leukocytosis was observed in the first three days among 2 patients (4.3%) who recovered from dengue fever and among 2 patients (14.3%) who progressed into severe dengue which was insignificant. (Figure 4)

Figure 4: comparison of leukocyte status between DF and severe dengue



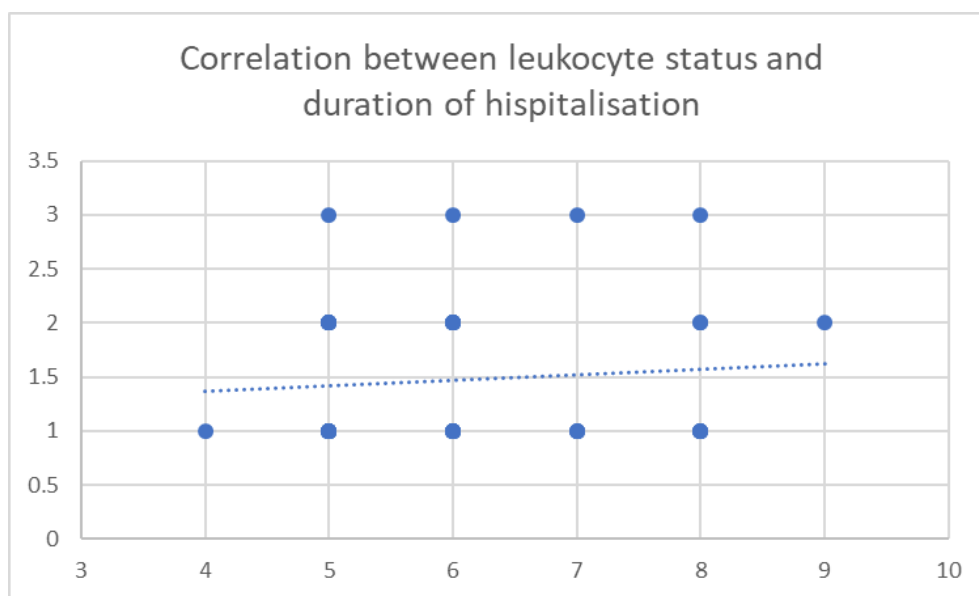
Spearman's correlation test was used to detect a possible impact (if any) of total leukocyte count on the duration of hospitalization. A weak positive correlation with correlation coefficient $r=0.224$ was observed between total leukocyte count as well as leukocyte status and duration of hospitalization, although the value was deemed insignificant as a p-value of 0.085 was observed. (Figure 5 and 6)

Figure 5: correlation of TLC with duration of hospitalization



(X- axis represents days of hospital stay ,Y-axis represents TLC)

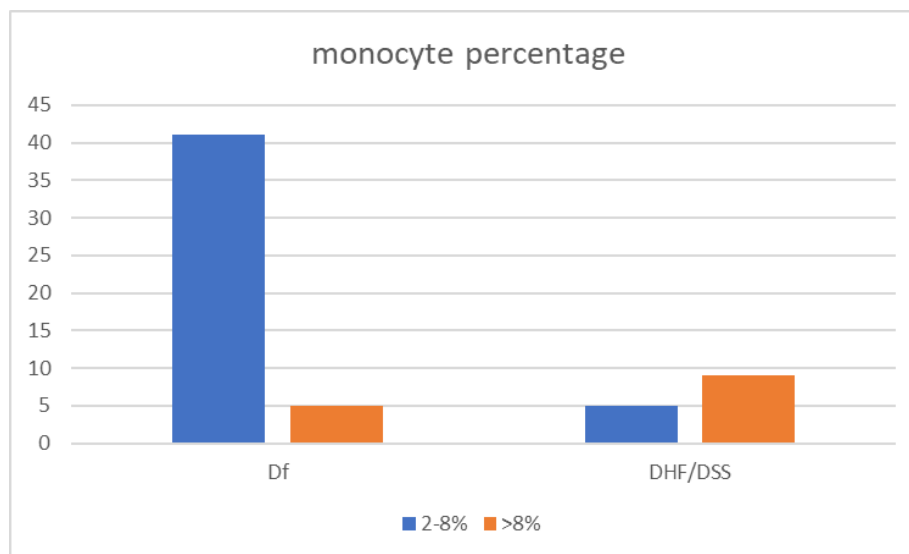
Figure 6: correlation between leukocyte status and duration of hospitalisation



(X-axis represents days of hospital stay ,Y- axis represents leukocyte status)

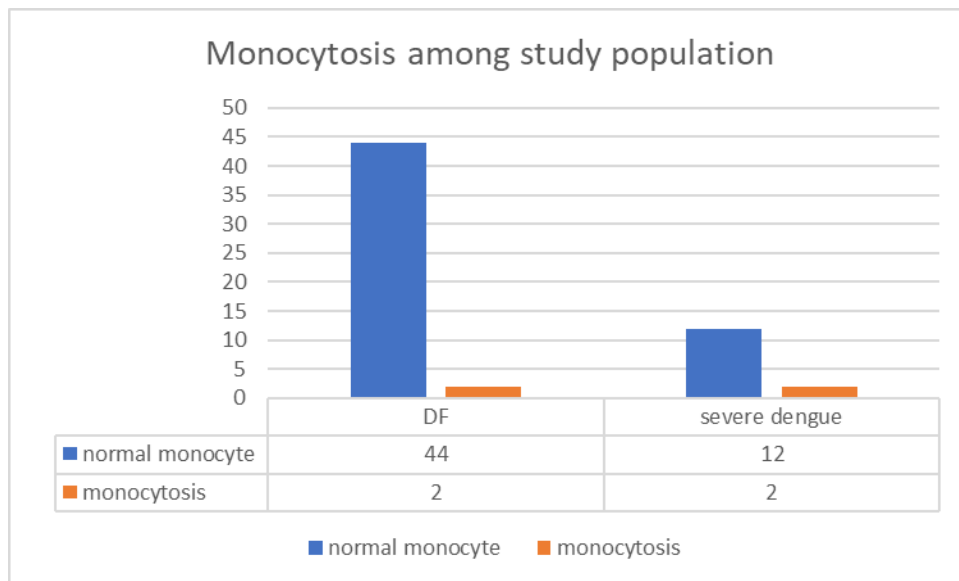
The average monocyte percentage observed within the first three days of onset of fever among recovered patients was $5.58 \pm 2.57\%$ while it was $9.64 \pm 3.65\%$ in those who had progressed into severe dengue, the difference being statistically significant with a p-value of 0.001. A monocyte percentage of more than eight was observed among 10.86% of the recovered patients and 9/14 of the severe dengue cases, the difference of which was statistically significant. (Figure 7)

Figure 7: Bar diagram of monocytes percentage in patients with DF, DHF,DSS.



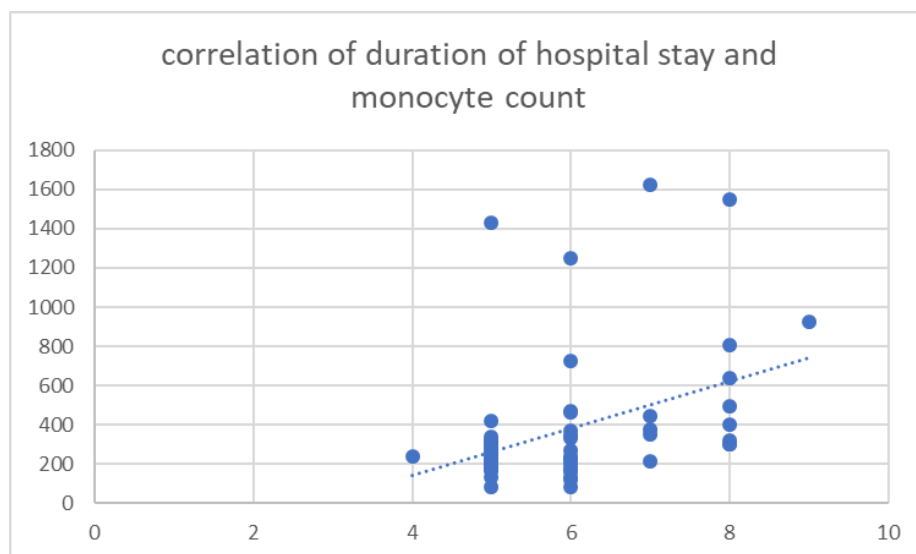
In terms of monocyte count, the mean monocyte count within the first three days of fever onset among patients who progressed into severe dengue was 625 ± 375 cells per cumm while that of the recovered patients was 300 ± 215 cells per cumm, the difference of which was statistically significant. Monocytosis (>950 cells/cumm) was observed among 2/46 of the recovered patients and 16% of severe dengue patients. (Figure 8) This was not statistically significant.

Figure 8: Monocytosis in study population



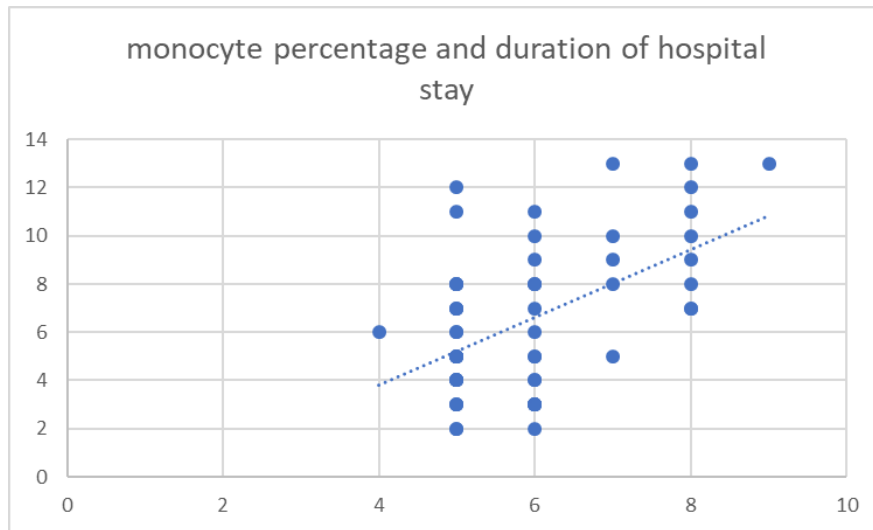
A moderate positive correlation was found between monocyte count and percentage and duration of hospital stay. (Figure 9 and 10).

Figure 9 : scatter plot of monocyte count and duration of hospitalization.



(x-axis represents the duration of hospital stay in days and Y-axis represents the number of cells of monocyte count in cells per cumm)

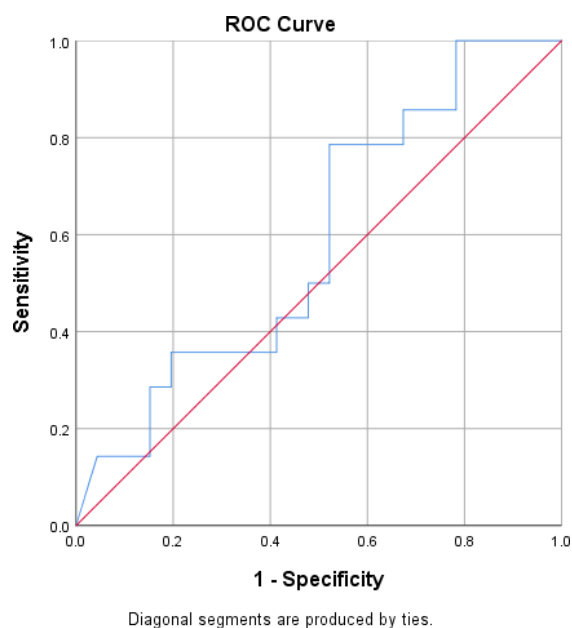
Figure 10: scatter plot of monocyte percentage and duration of hospitalization



(X-axis represents days of hospital stay and Y-axis represents monocyte percentage)

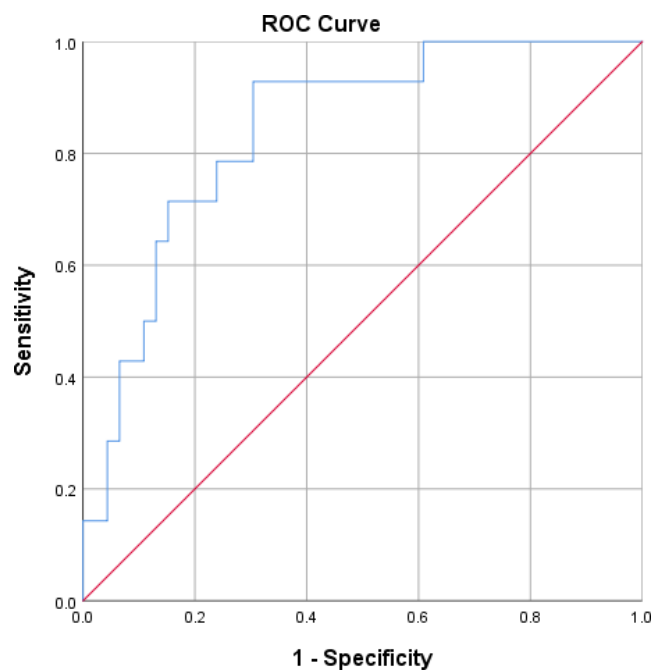
To determine the cut-off value of total leukocyte count for higher severity of dengue outcome, a ROC curve was done (AUC 0.589, p-value 0.319, insignificant). Thus, the total leukocyte count had no discriminatory ability in terms of disease severity in the present study.(Figure 11)

Figure 11: ROC curve for total leukocyte count and disease outcome.



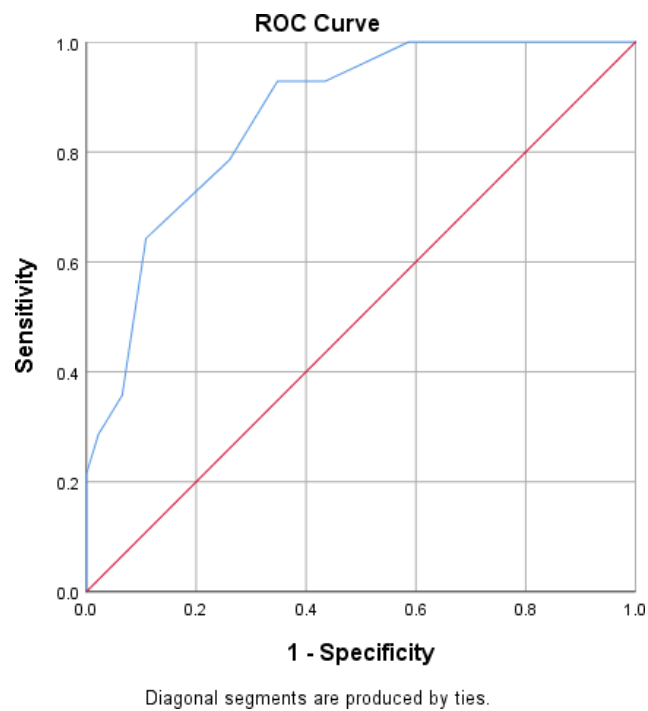
In case of predicting the severity of the dengue outcome ROC curve was performed for monocyte count and disease outcome. (AUC 0.843, 95% CI of 0.736- 0.951, p value< 0.001, highly significant). Thus monocyte count was found to have a discriminatory ability in terms of disease severity in the present study. A monocyte count of 319 was chosen as a cut-off value for severity prediction with a sensitivity of 78.6% and a specificity of 76%. (Figure 12)

Figure 12: ROC curve for monocyte count and disease outcome.



ROC curve was also performed for monocyte percentage and disease outcome (AUC 0.865, 95% CI of 0.767-0.963, P-value <0.001, highly significant). Thus monocyte percentage was found to have a discriminatory ability in terms of disease severity in the present study. A monocyte percentage of 7.5% was chosen as a cutoff value for severity prediction with a sensitivity of 78.6% and a specificity of 74%. (Figure 13)

Figure 13: ROC curve for monocyte percentage and disease outcome



DISCUSSION

The mean duration of hospitalization in the present study was found to be 5.93 ± 1.13 days which was more compared to that observed in a South Indian study (3.4 ± 2.76 days). (Jayanthi and Tulasi, 2016). In a similar study based in a tertiary health care institute, the mean days of hospitalization was observed

to be 5.73 ± 2.75 days, which was comparable to the present study. (Kotwal et al., 2019) In the present study, classical dengue fever, dengue fever with mild bleeding and a severe form of dengue was observed among 58.3%, 21.6% and 23.3% of the study population respectively. These findings were comparable to the dengue classification observed in a similar study based in south India (Malatheesa and Ashwini, 2014). In the aforementioned study, classical dengue fever, dengue fever with mild bleeding and a severe form of dengue was observed among 76.3%, 9.2% and 25% of the study population respectively.

In a study by Ralapanawa et al.,(Ralapanawa et al., 2018) leucopenia was observed in around 70% of the patients. The study found that the average value of WBC among Dengue fever patients who recovered was 4380 cells per cumm compared to 4490 cells per cumm of those patients who progressed into a severe form of dengue (DHF/DSS). The difference in these values between the two groups was deemed not significant statistically. In a study by Eu -Ahsunthornwattana et al,(Eu-Ahsunthornwattana et al., 2008) based in Thailand., people who progressed into severe dengue had a statistically higher leukocyte count compared to mild infection. The average WBC count among mild infection patients was found to be 3050 per cumm compared to 3580 among patients who progressed into grade II or worse of Dengue hemorrhagic fever. The difference in the mean value was deemed to be statistically significant with a p-value of 0.04.

Also, it was found that patients with mild infection who recovered later had a higher proportion of leukopenia on admission compared to patients who developed severe disease. 89% of the patients with mild infection had leukopenia at admission compared to 70% of patients who developed severe disease. In a study based in India by Khandelwal and Khandelwal., (Khandelwal and Khandelwal, 2017) the total WBC count during the initial infection was normal in 66% of the patients, 26% had leukopenia and 8% of the patients had leukocytosis. In a study based on India by Nagaram et al.,(Nagaram et al., 2017) WBC count of more than 11000 cells per cumm was observed significantly higher among patients who deteriorated to severe dengue. Among the patients who developed severe dengue, 48% and 40% had a leukocyte count of > 11000 cells/cumm and 4000-11000 cells/cumm respectively. In contrast, only 14/149 of the non-severe dengue patients had leukocytosis. In a study by Nagaram et al.,(Nagaram

et al., 2017) 47.2% of the study population had a normal WBC count, 45.5% of the patients had leukopenia at the time of admission, and leukocytosis was observed among 5% of the patients. In a study by Khatroth (Khatroth, 2017) leukopenia was observed among 20% of the study population which was far less compared to other findings.

In the present study, leukopenia was observed among 60% of the study population, normal leukocyte count was observed among 33.33% of the study population, and leukocytosis was observed among 6% of the study population. Among patients with leukocytosis, 25% progressed into a severe form of dengue compared to 15% of patients who had normal leukocyte count and progressed to a severe form of dengue. In the present study, no difference in leukocyte count or status was observed between the patients who recovered from dengue fever and those who progressed to severe dengue.

As it can be observed, in terms of early leukocyte status as a predictor of severity outcome in dengue, there are multiple findings that contradict each other. Like in the present study, the difference in the status of early leukocyte count is not significant in other studies like Ralapanawa et al.,(Ralapanawa et al., 2018). The studies in which leukocyte count was higher among patients who progressed to severe dengue are Eu-Ahsunthornwattana et al,(Eu-Ahsunthornwattana et al., 2008), Nagaram et al.,(Nagaram et al., 2017), Azin et al (Azin et al., 2012). On the other hand, studies that suggest a positive outcome with normal leukocyte count are Rasool et al based in Pakistan,(Rasool et al., 2014) and Chaloemwong et al (Chaloemwong et al., 2013). Although the studies contradict each other in terms of leukocyte status and its predictor ability for severe dengue, not all the studies took into account the percentage of atypical lymphocytes.

In a study by Tsai et al.,(Tsai et al., 2017) based in Taiwan, a higher monocyte count was seen on the fifth day of fever among severe dengue patients compared to classical dengue fever patients. In a study by Chaloemwong et al (Chaloemwong et al., 2013) based in China, it was found that there was a significant difference between the monocyte count of patients between the first day of hospital admission to the fourth day. The difference was highly significant with a p-value of 0.001.

In terms of monocyte, all the studies stated above had similar findings. It can be inferred that the higher the monocyte count within the first three days of onset of fever, the higher is the probability of dengue fever to progress into severe dengue. In a study by Kotwal et al (Kotwal et al., 2019), a weak negative correlation with a correlation coefficient of -0.09 was observed between total leukocyte count and duration of hospitalization. The correlation coefficient was found to be statistically not significant with a p-value of 0.81. In the present study, the correlation between total leukocyte count and duration of hospitalization was a very weak positive correlation with an r-value of 0.06 which was found to be not significant.

The study has observed significant findings in terms of monocyte count, no significant findings were observed in terms of leukocyte counts. The observation of the present study coincides with other findings in terms of the monocyte ability to predict the severity of the dengue and its outcome.

CONCLUSION

The outcome and severity of dengue did not significantly differ based on leukocyte count or status. There was no relationship found between the leukocyte count and hospital stay length. By using ROC analysis, monocyte count and percentage within the first three days after fever onset was found to be a more accurate indicator of dengue severity than total leukocyte count.

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