Study on the Changes in Common Laboratory Parameters in Dengue Patients with Secondary Bacterial Infection

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Abstract

Secondary bacterial infection can adversely affect the clinical course of primary viral infection in dengue fever. Time consuming microbiological culture confirmations and false negative culture results delay the timely initiation of antibiotics. The objective of this study was to describe the variation in the common laboratory parameters during the illness, identify criteria that may predict secondary bacterial infection before microbial culture confirmation, identify the common organisms causing secondary bacterial infections and their antibiotic sensitivity patterns. A retrospective case-control study was carried out at the Dengue Management Unit of the National Institute of Infectious Diseases, Sri Lanka. Patients treated with antibiotics upon suspicion or culture confirmation of secondary bacterial infections and patients not treated with antibiotics were selected for the study. Total WBC count, absolute neutrophil count, platelet count, HCT (Hematocrit), CRP (C Reactive Protein) and average body temperature were recorded. Independent sample t-test was used to analyze the data. Study results showed that most positive microbial cultures were detected on the 6th and 7th days of illness. Staphylococcus, Streptococcus, and non-speciated Gram-positive cocci were the commonly isolated organisms. Patients with confirmed secondary bacterial infection had significantly higher neutrophil count (3.32±0.35x103/µL, $p<0.05$) and lower platelet counts (49.33±7.66x103/µL, $p<0.05$) on the 6th and 5th day of illness respectively. The average body temperature was significantly higher in the study group than the control from the fever day 3 onwards ($p<0.05$). The study subjects were more likely to have significantly lower HCT values (38.62±1.12%, $p<0.05$) than their controls on the third and fourth days of fever. Overall, the present study suggests that bacterial co-infection increases the mortality rate in dengue patients. The variation in absolute neutrophil count, platelet count, and body temperature pattern compared to the illness day can be used along for early prediction of secondary bacterial infection.

Keywords: dengue, bacterial infection, antibiotics, platelet count, HCT

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1. Introduction

Dengue is one of the arboviral, mosquito borne diseases in humans. Around 3.6 billion people from 125 tropical and subtropical countries suffer from developing dengue infection [Gubler, 2012] and about 22,000 deaths are reported annually around the world [Khurram, 2014]. Clinical characteristics of this disease vary from a simple febrile illness to life-threatening complications such as hemorrhagic fever and shock [Hassan, 2016]. It is shown that a bacterial co-infection adversely affects the clinical course of viral infection [See, 2013]. In dengue patients, secondary bacterial infection / bacterial co-infection is confirmed using conventional microbiological methods such as culture and serological techniques. However, microbial cultures can be falsely negative and delays in the initiation of antibiotics awaiting culture confirmation or empirical coverage by multiple antibiotics may worsen the clinical outcomes. The objective of this study was to describe the variation in the common laboratory parameters during the clinical course of the illness, identify criteria that may predict secondary bacterial infection before culture confirmation, identify the common organisms causing secondary bacterial infections and their antibiotic sensitivity patterns.

2. Methodology

A retrospective case-control study was carried out with 206 subjects at the Dengue Management Unit of the National Institute of Infectious Diseases (Infectious Disease Hospital / IDH), Angoda, Sri Lanka.

The study group consisted of 103 patients, including 57 males and 46 females who received antibiotics upon clinical suspicion of having secondary bacterial infection by the clinical team. The mean age of this group of patients was 32 (±3.18) years. As 35 of them had positive microbial cultures during their stay in the hospital, the study group was further divided into 2 groups: (1) Confirmed Group (n = 35) which includes the patients who were confirmed as infected with bacterial infection through a microbial culture using the biological samples such as blood, urine, canula tip and sputum and received antibiotics. (2) Suspected Group (n = 68) which includes the dengue patients who were clinically suspected to be infected with secondary bacterial infection without any positive microbial culture tests and received antibiotics. Further, the control group (n=103) was selected from age and gender matched patients who were not treated with antibiotics during the study period.

Parameters of laboratory investigations including total white blood cell (WBC) count, absolute Neutrophil count, Platelet count, Hematocrit (HCT), C - Reactive Protein (CRP) and body temperature were recorded. The independent sample t-test was used to find the variations of each parameter in both case and control groups during the illness.

The first day the patients reported as the onset of fever is considered as the “fever day 1/ illness day 1”. The subsequent days were calculated accordingly.

3. Results

Among the total study subjects of 206, 28% (58 patients) had positive dengue NS1/IgM results. Positive microbial cultures were detected in 21 blood samples, 6 urine samples, 4 each from cannula-tip and sputum samples, 1 each from endotracheal tube secretions and peritoneal dialysis catheter samples. Staphylococcus, Streptococcus, and non-speciated gram-positive cocci were the most commonly identified organisms in the sample cultures (Figure 1).

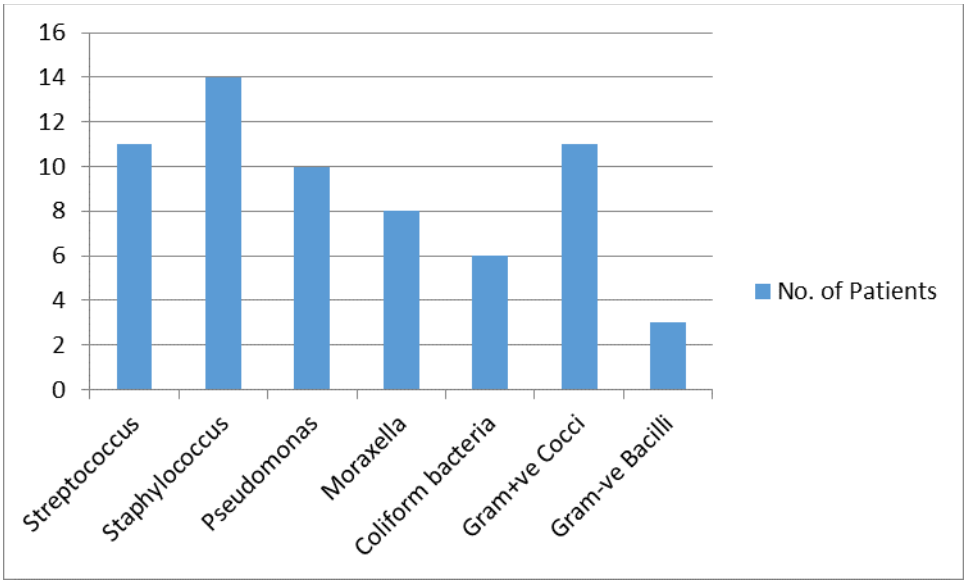

Figure 1: Types of microorganisms found in confirmed group which includes, dengue patients with a secondary bacterial infection following their microbial culture test.

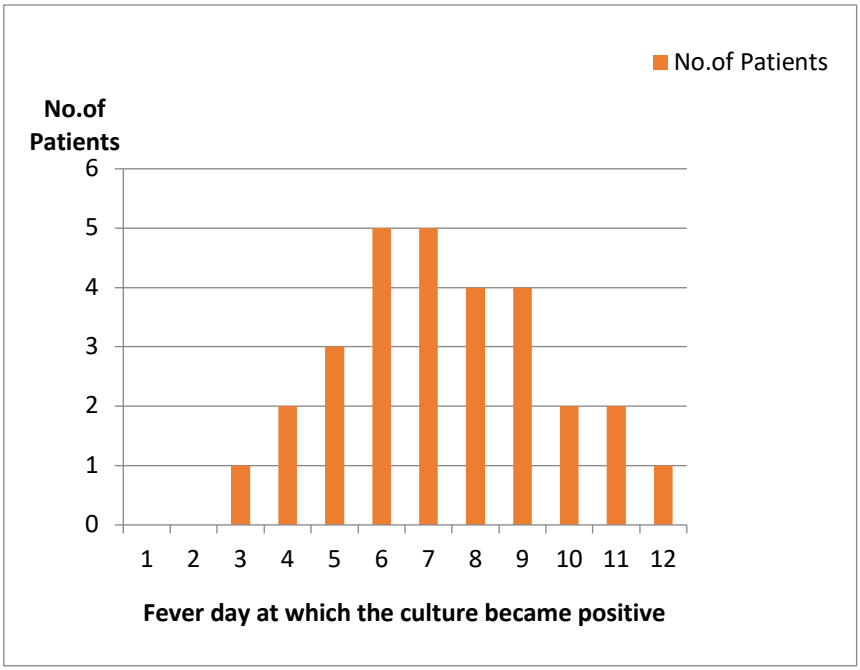

Figure 2: Culture positive samples against the day of fever

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The microbial cultures were positive between day 3 to day 12. The majority was detected from the samples taken on the 6th and 7th days of fever (Figure 2). The patients with confirmed secondary bacterial infection had significantly higher total WBC counts on the 7th and 8th days from the onset of fever (Table 1, Figure 3) and significantly higher absolute neutrophil count ($P<0.05$) on the 6th day of fever (Table 1, Figure 4). However, the patients confirmed with secondary infection had significantly lower platelet counts on the 5th day of illness (Table 1, Figure 5).

Table 1: Variations of different parameters with fever days. * Indicates significance.

Parameters	Groups	Day of fever											
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Total WBC count x10^3 (cells/mm^3)	Control	4.99±0.23	4.53±0.36	3.92±0.19	3.54±0.20	3.73±0.20	4.83±0.27	5.85±0.29	6.17±0.43	6.43±0.88	-	-	-
	Suspected	4.2	4.14±0.32	3.61±0.25	3.00±0.29	2.76±0.23	3.09±0.22	4.54±0.34	5.63±0.44	6.05±1.00	-	-	-
	p-value$^{\$}$	-	0.4276	0.3264	0.1279	0.0020*	1.2e-06*	0.0043*	0.379	0.7798	-	-	-
	Confirmed	7.07±1.60	5.59±1.04	4.96±0.83	3.88±0.66	3.76±0.48	5.71±0.59	8.37±0.86	9.59±1.28	9.89±1.43	10.12±0.81	10.35±1.32	11.2±1.13
	p-value $^{\pi}$	0.3236	0.3647	0.2383	0.625	0.9446	0.178	0.0087*	0.0160*	0.0504	-	-	-
Absolute Neutrophil count x10^3 (cells/mm^3)	Control	4.16±0.24	3.50±0.40	2.40±0.17	1.80±0.17	1.54±0.11	1.39±0.11	1.40±0.11	1.38±0.14	1.22±0.31	-	-	-
	Suspected	3.22±0.06	3.39±0.29	2.65±0.22	1.94±0.26	1.49±0.11	1.55±0.12	2.09±0.20	2.57±0.29	2.7±0.42	-	-	-
	p-value$^{\$}$	0.0153*	0.6793	0.314	0.6641	0.7412	0.3436	0.0030*	0.0005*	0.0262*	-	-	-
	Confirmed	5.55±1.22	4.80±0.96	3.89±0.77	2.37±0.42	2.17±0.34	3.32±0.35	5.17±0.70	6.08±1.10	5.83±1.07	5.88±0.87	6.05±1.29	7.08±1.17
	p-value $^{\pi}$	0.3696	0.2435	0.079	0.221	0.0935	7.9e-06*	9.8e-06*	0.0002*	0.0004*	-	-	-
Platelet count x10^3 (cells/mm^3)	Control	159.8±10.22	134.7±7.85	114.1±5.99	92.3±4.24	72.7±4.06	61.2±3.74	55±3.64	65±4.99	69.8±10.51	-	-	-
	Suspected	197	144.15±8.47	118.5±5.02	93.30±4.99	67.19±4.63	49.11±4.01	39.7±3.38	53.34±4.85	67.5±14.35	-	-	-
	p-value$^{\$}$	-	0.4185	0.5756	0.8814	0.3706	0.0291*	0.0024*	0.0993	0.0899	-	-	-
	Confirmed	217.33±32.36	137.75±16.86	113.28±9.52	71.43±9.57	49.33±7.66	44.90±8.37	50.39±8.35	80.32±11.4	109.05±16.47	133.31±23.74	121.6±29.37	181.25±51.19

	p-value ᵠ	0.2107	0.8716	0.9416	0.055	0.0100*	0.0825	0.6125	0.2238	0.0563	-	-	-
Changes in average body temperature (°F)	Control	101.4±1.4	101.1±0.52	100.6±0.28	100±0.24	99.8±0.20	98.9±0.13	98.5±0.06	98.4±0.04	98.4±1E-14	-	-	-
	Suspected	102.5±0.5	102.11±0.41	101.8±0.299	101.2±0.19	101.3±0.20	100.5±0.17	99.4±0.17	98.9±0.20	98.9±0.5	-	-	-
	p-value$	0.5732	0.1451	0.0044*	0.0003*	2e-07*	5e-11*	1e-5*	0.0189*	0.1752	-	-	-
	Confirmed	102.55±0.64	102.3±0.48	101.9±0.26	101.4±0.40	101.1±0.34	100.5±0.33	100.4±0.34	99.9±0.32	99.6±0.31	100.3±0.47	99.2±0.25	99.1±0.24
	p-value ᵠ	0.5577	0.1169	0.002*	0.0049*	5e-05*	5e-06*	0.0001*	0.0015*	-	-	-	-
Hematocrit (%)	Control	37.99±0.69	41.52±1.25	42.36±0.70	42.05±0.54	41.21±0.46	41.25±0.43	41.27±0.48	40.17±0.57	40.15±1.08	-	-	-
	Suspected	27	39.75±1.62	39.44±0.62	39.44±0.64	40.02±0.67	40.67±0.74	40.29±0.73	39.13±0.90	39±1.86	-	-	-
	p-value$	-	0.397	0.0022*	0.0022*	0.1443	0.5022	0.2603	0.3321	0.608	-	-	-
	Confirmed	36.8±1.74	39.2±1.63	38.62±1.12	39.95±0.96	39.76±1.05	40.66±1.03	40.42±1.23	38.49±0.90	37.93±1.06	36.12±1.13	36.38±1.79	34.78±2.38
	p-value ᵠ	0.5773	0.2762	0.0078*	0.0081*	0.2121	0.6007	0.5222	0.1211	0.1634	-	-	-

*Statistically significant by Paired t-Test

$ p-value: p value was calculated between control and suspected groups

ᵠ p-value: p value was calculated between control and confirmed groups

SD: Standard deviation

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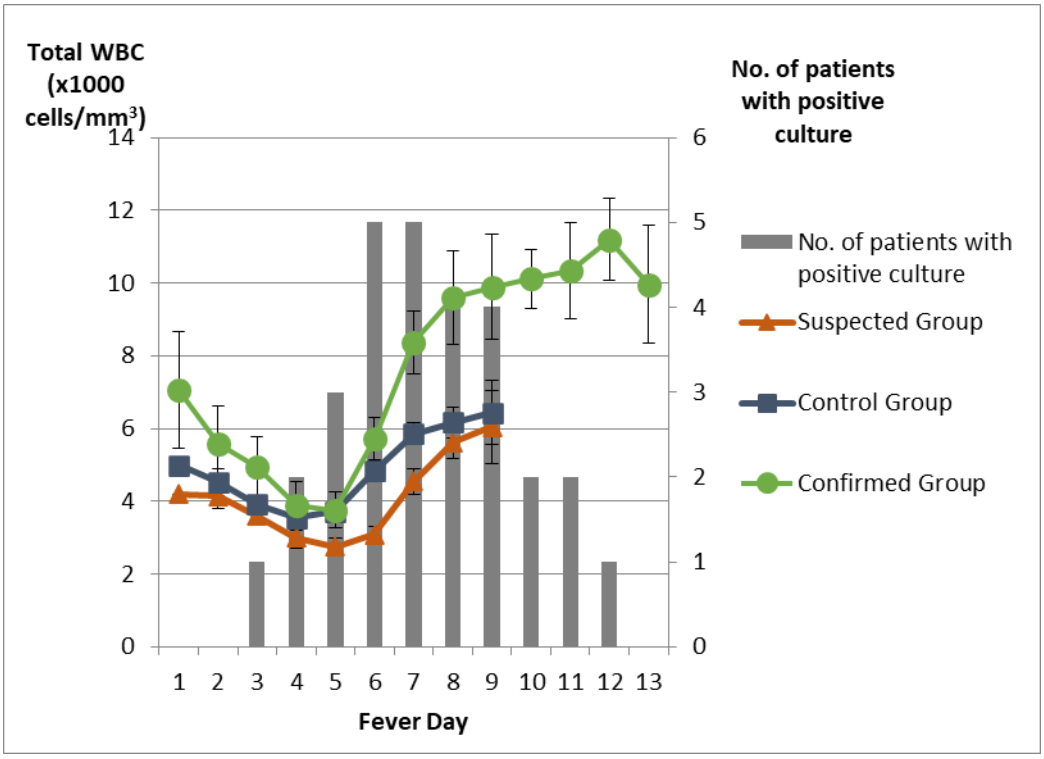

Figure 3: Variation in total WBC count compared with the illness day shows how the WBC count varies throughout the illness.

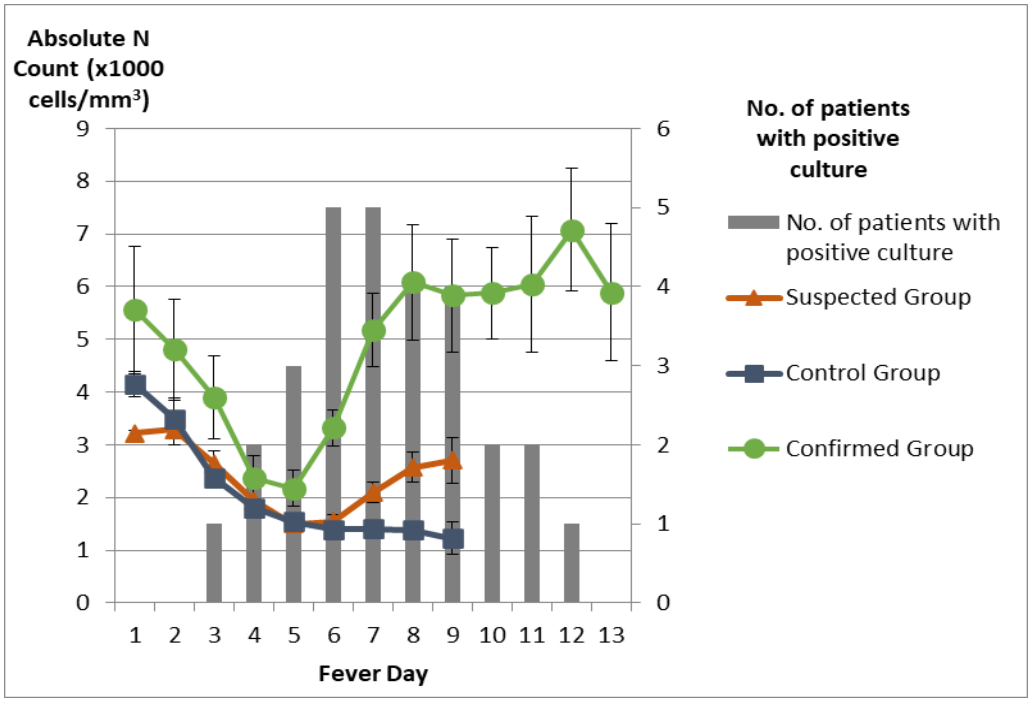

Figure 4: Variation in the absolute neutrophil count compared with the fever day describes how the neutrophil count varies throughout the illness.

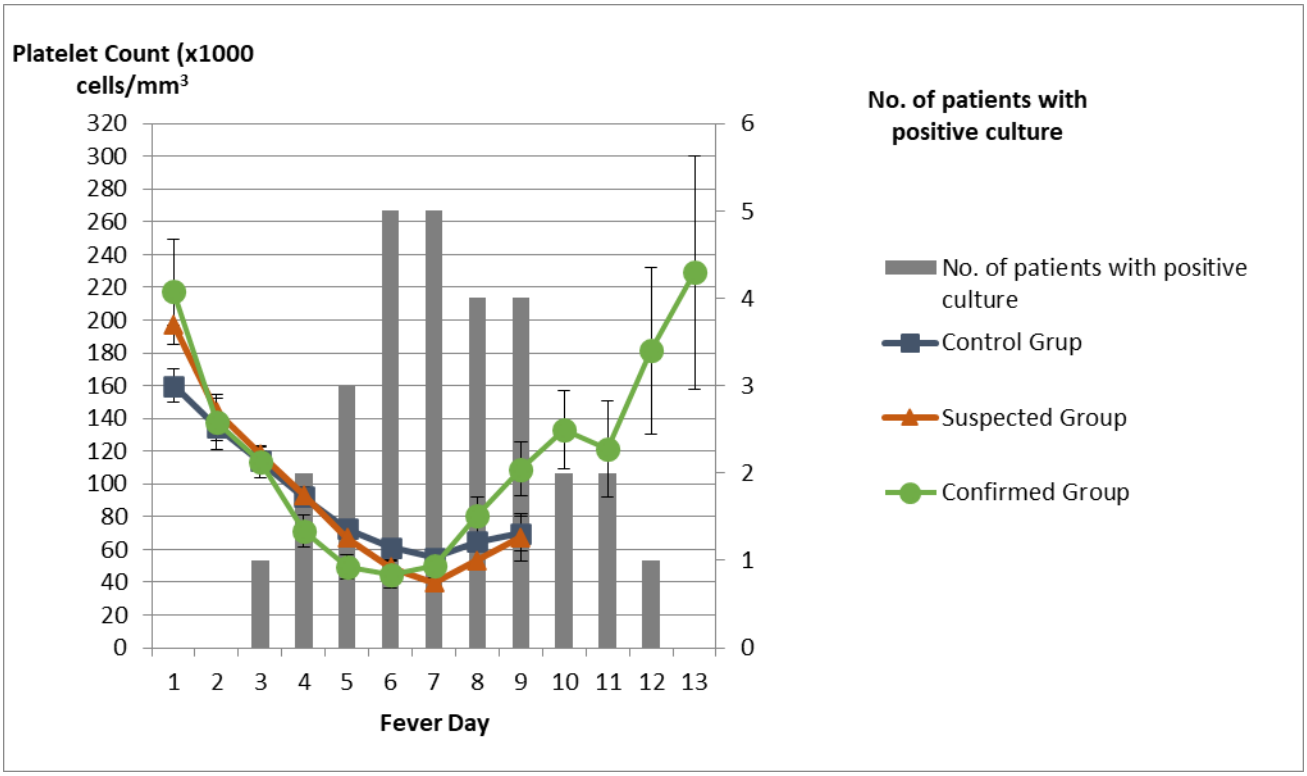

Figure 5: Variation in total platelet count compared with fever day

Average of the maximum body temperature recorded during the day in both confirmed and suspected secondary infection groups were significantly higher than the control group from the fever day 3 onwards (Table 1, Figure 6). Confirmed and suspected secondary infection group subjects were more likely to have lower hematocrit (HCT)/ Packed cell volume (PCV) values than their controls (Table 1, Figure 7).

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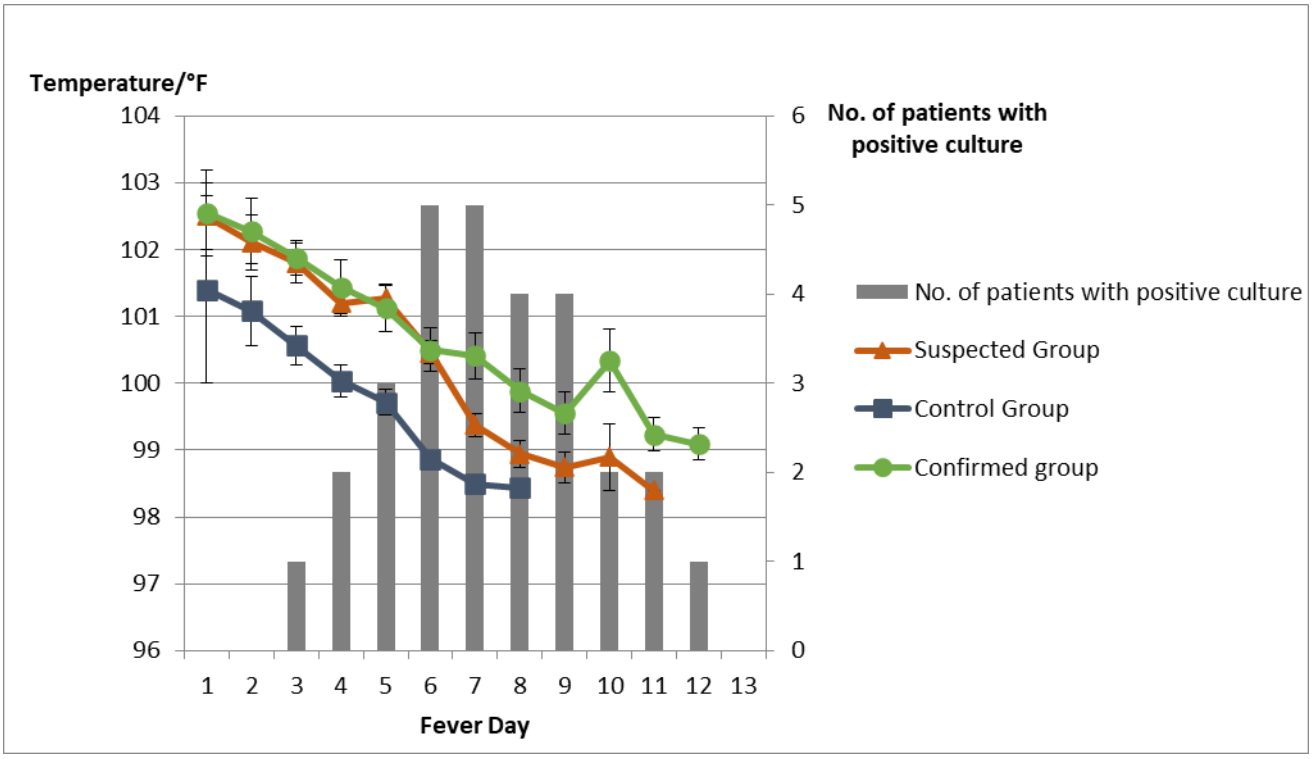

Figure 6: Variation in the average of the highest body temperature recorded during the day compared with fever day shows the higher body temperature in study groups than the control group from the first day of illness.

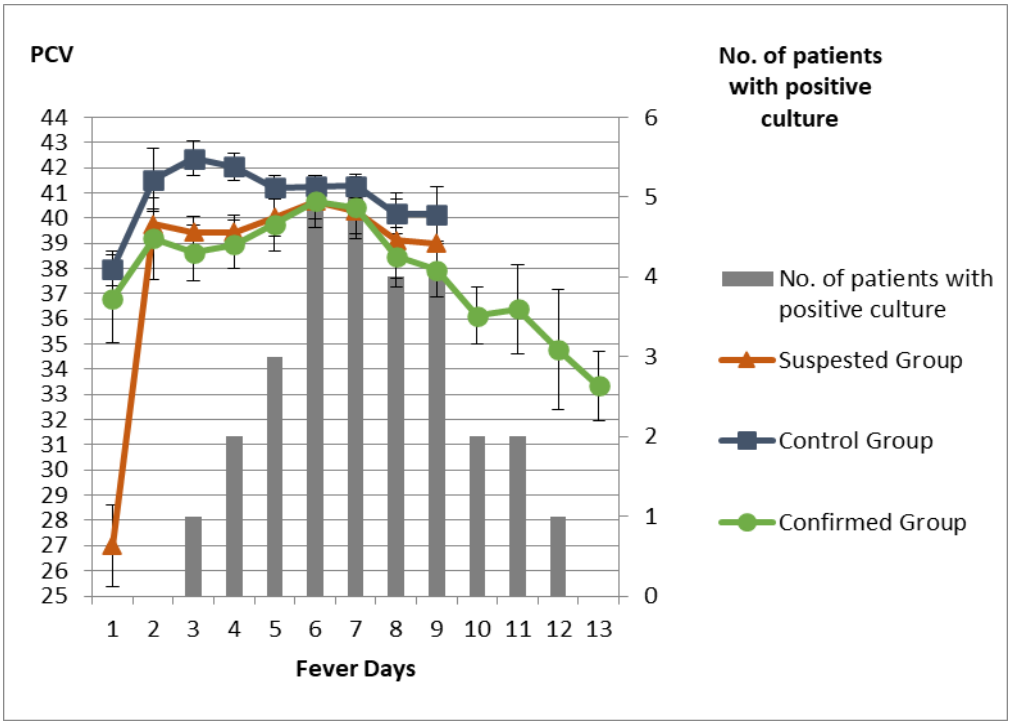

Figure 7: Variation in hematocrit (PCV) compared with the fever day shows lower PCV values in study groups compared to their control group.

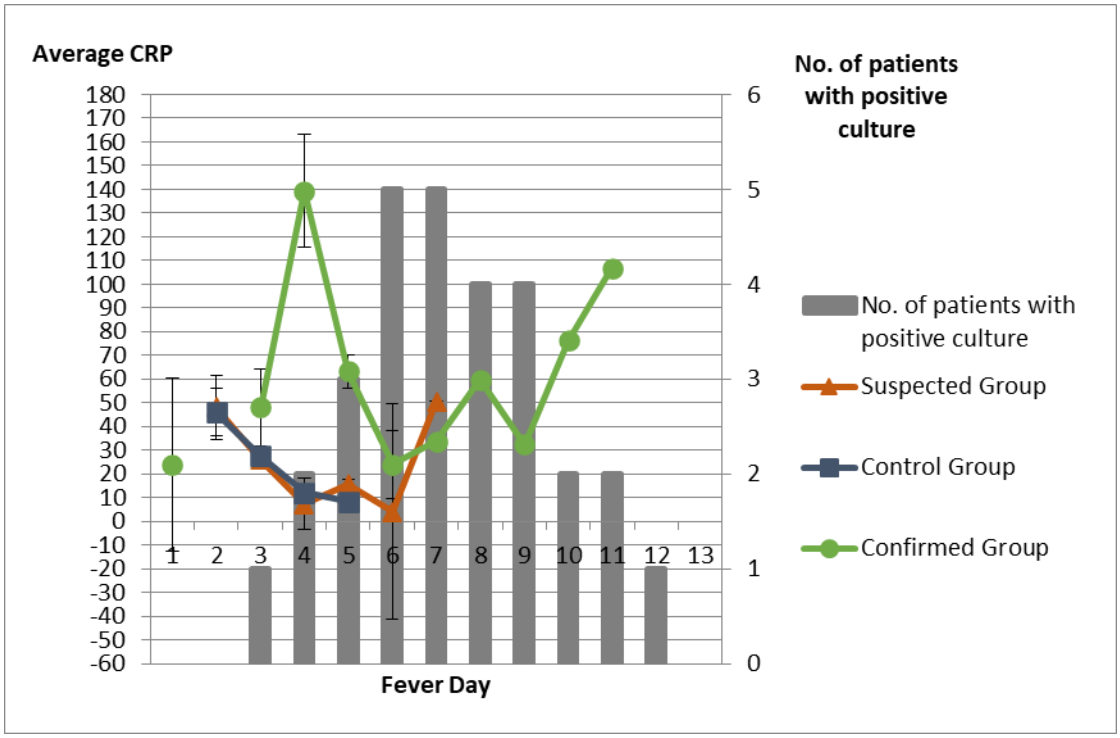

Figure 8: Variation in CRP compared with fever day shows the changes in CRP throughout the illness.

C- reactive protein (CRP) of the patients with confirmed secondary infection was higher than the control group, from the third day of fever onwards (Figure 8). Although the significance of the results is doubtful as a result of the limited number of data gathered.

Considering the antibiotic sensitivity pattern of the sample cultures, Staphylococcus spp. was susceptible to vancomycin, teicoplanin and clindamycin whereas Streptococcus spp. was susceptible to teicoplanin. (Figure 9)

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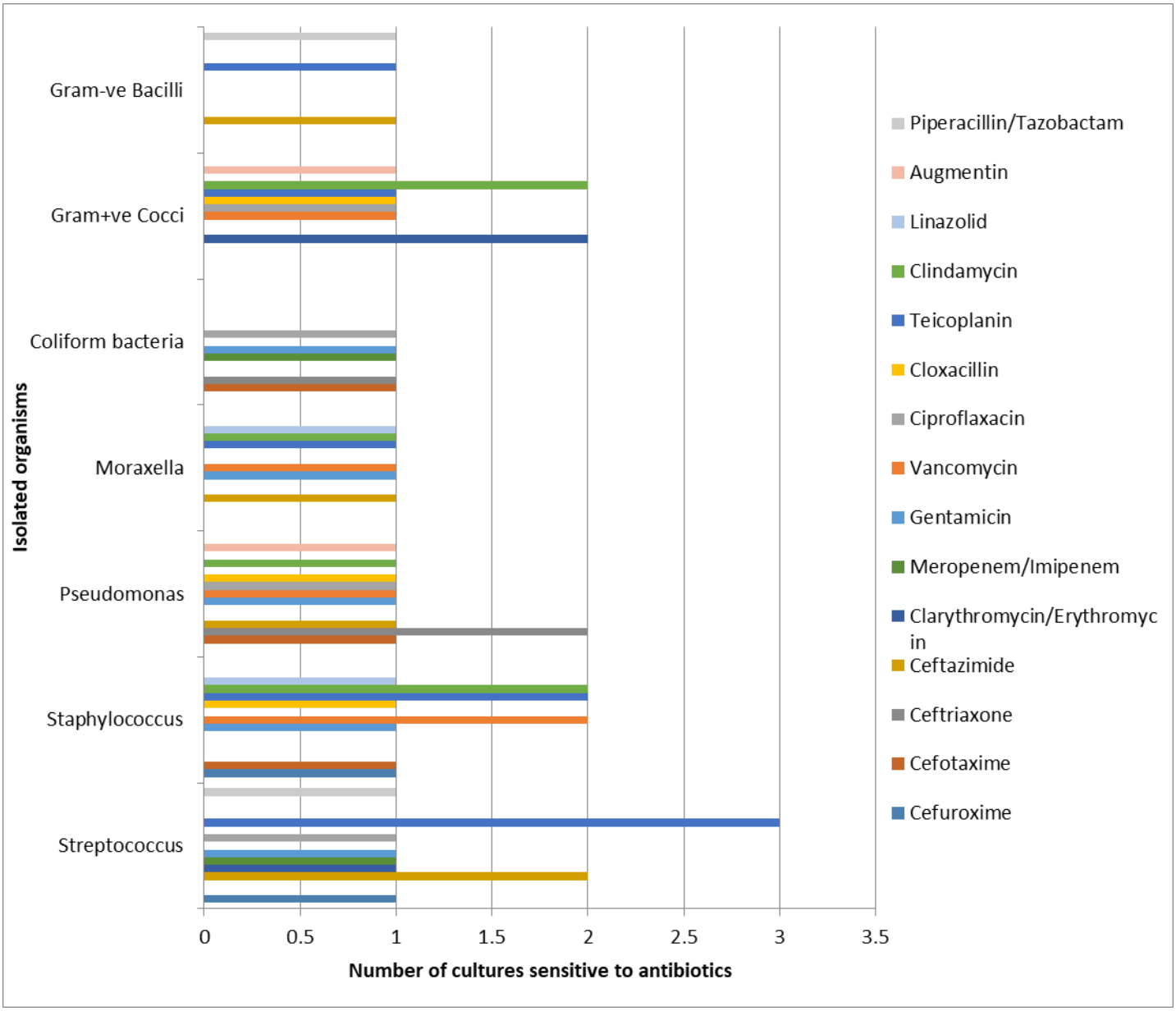

Figure 9: Antibiotic sensitivity patterns of the bacterial cultures

During the period of data collection, 18 subjects of the confirmed group and 3 subjects of the suspected group required intensive care unit (ICU) admission and out of which 04 of the confirmed group died during the ICU stay. In comparison, none of the controls required ICU admission nor had case fatalities.

4. Discussion

Low total WBC counts, and absolute Neutrophil counts were seen on days 4-5 of the illness as shown in the study (Figure 3 and 4) may make the patient more susceptible to bacterial infections with reduced cell-mediated immunity. This is likely the reason that most positive cultures were detected in the immediately following days, illness days 6-7, of illness (figure 2). A previous study suggested that

leucocyte infection with the dengue virus may make the dengue patient more susceptible to bacterial infections [Lahiri, 2008].

The significantly increased neutrophil count and decreased platelet counts of the confirmed secondary infection group compared to the control group on the 6th and 5th day of fever respectively in the current study is supported by the former research which revealed that patients with dengue and secondary bacterial infection are more likely to have higher neutrophil count and lower platelet counts compared to dengue patients without bacterial infections [Thein, 2015].

The higher average temperature in both confirmed and suspected secondary infection groups compared to the control group may be used as a predictor to identify the susceptible patients for secondary bacterial infections (figure 6). Another group of researchers has revealed that the dengue patients with secondary bacterial infection showed higher HCT change (≥20%) and lower serum albumin levels than dengue patients without secondary bacterial infection [Thein, 2015]. The findings in the present study regarding the temperature and HCT are in parallel with the findings of an earlier study [See, 2013].

In a previous study, it was shown that the predominant bacterial strain responsible for the secondary infections in dengue patients was Staphylococcus aureus and other isolates include Salmonella typhi, Escherichia coli, Klebsiella pneumonia, Streptococcus sp., Pseudomonas aeruginosa and Acinetobacter baumannii.[Thein, 2015]. The present study reveals that the predominant strains isolated were Staphylococcus, Streptococcus and non-speciated Gram-positive cocci. Other isolates include Pseudomonas, Moraxella, coliform bacteria, and non-speciated Gram-negative bacilli.

The patients with secondary bacterial co-infection are at risk of ICU stay or death compared to dengue patients without bacterial co-infection. A case study conducted by a group of researchers explained that a bacterial co-infection in dengue patients has associated with life-threatening issues and therefore, it is advisable to be attentive to unusual signs of dengue fever which may be an indication of a concomitant bacterial infection [Araujo, 2001].

5. Conclusions

The secondary bacterial infections may occur on the 4th-5th day of illness in dengue patients due to their being more susceptible with low absolute neutrophil counts. A significantly higher total WBC count on the 7th and 8th days from the onset of fever, a significantly higher absolute neutrophil count on the 6th day of fever, a significantly lower platelet count on the 5th day of illness and the body temperature pattern can be used to predict the bacterial co-infection in dengue. These parameters can be used as a guide for the timely initiation of antibiotics and taking necessary microbial cultures for confirmation of infection and detection of sensitivity patterns. Further research into the optimization of a clinical prediction tool using these parameters would offer the clinician confidence in decision making. This approach will prevent the delays in the initiation of antibiotics, reduce complications and improve the clinical outcome of dengue patients with bacterial co-infections.

Limitations

Although all subjects taken into the study were clinically treated as having dengue viral infection, only 28% had serological confirmation of having dengue fever due to a lack of resources.

Patients with co-morbid illnesses such as Diabetes, hypertension were not excluded. The weight of the patients was not recorded in the case notes of all the subjects, therefore was not taken into consideration.

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The complication rate of the cohort of patients is far higher than the actual due to active inclusion of suspected or confirmed secondary bacterial infections as the study sample and only selecting the same number of samples from the uncomplicated illness group as the control. The actual number of patients with dengue-related complications compared to the total number of admissions to the dengue treatment unit was low.

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