Research Article

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Atypical Lymphocytes as a Predictor of Dengue Illness among Pediatric Patients Admitted In a Tertiary Institution

Avegail Cardinal MD[1*] and Vincent Joseph Alba MD[2]

[1]*The Medical City, Baguio General Hospital and Medical Center, Philippines.*

[2]*Department of Pediatrics. The Medical City, Pasig City, Philippine Children Medical Center, Philippines.*

***Correspondence:**
Avegail Cardinal, The Medical City, Baguio General Hospital and Medical Center, #9 Quezon Hill Road Baguio City, Philippines, Tel: +639178500995, E-mail: avegailcardinal@gmail.com.

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ABSTRACT

Objective: *We determined the correlation of atypical lymphocyte (AL) in dengue illness and accuracy in predicting dengue infection.*

Statement of the Problem: *Diagnosis of dengue during febrile stage has been challenging. There are several existing diagnostics however most are costly and not available in many tropical countries. Atypical lymphocytes (AL) or reactive lymphocytes are activated non-malignant lymphocyte seen in the peripheral blood smear. There were studies mentioning atypical lymphocytes as an adjunct tool in the diagnosis of dengue infection and could be used as marker of disease severity.*

Methodology: *This is a retrospective case control study of randomly selected pediatric patient admitted in a tertiary institution with confirmed dengue fever cases and other febrile illness (OFI). There were 296 who were able to meet the criteria. CBC results were reviewed on the day of admission and day 1 afebrile. Presence and absence of atypical lymphocytes was noted on each patient.*

Findings: *Significantly more proportion of subjects with dengue illness has atypical lymphocyte than those with Other febrile illness ($p<0.0001$). Of the 155 confirmed cases of dengue, a total of 137 (88.4%) of patients have atypical lymphocyte and 18 (11.6%) found negative. The positive and negative predictive values of atypical lymphocytes were 86.2% and 86.9%, respectively. However no difference was noted when proportion of atypical lymphocyte was compared across dengue severity. Finally, atypical lymphocytes are a significant predictor of dengue fever as derived from logistic regression analysis. The results showed that the risk of a patient with atypical lymphocyte was 41.16 times higher for dengue than those without atypical lymphocyte.*

Conclusion & Significance: *This study shows that the presence of atypical lymphocyte is highly associated with dengue illness. Atypical lymphocyte can be useful in predicting dengue illness. However additional study on the actual quantity of AL is required before the information can be used in usual clinical settings.*

Keywords

Atypical lymphocyte (AL), Reactive lymphocyte, Variant lymphocyte.

Introduction

Diagnosing dengue rapidly at the early stage is at times challenging. There are several existing diagnostic tools for confirming dengue virus infection including detection of the virus, viral nucleic acid, antigens or antibodies. The Virus isolation, nucleic acid or antigen detection can be used to diagnose dengue during the early stage of the disease while serology is the method of choice at the later stage of acute infection [1]. Virus isolation and nucleic acid detection are specific but are also labor-intensive and costly, hence, some clinicians rely more on a high index of suspicion based on clinical symptomatology, especially in the rural setting where some of these tests are not available. Clinical diagnosis alone , on the other

hand are often times difficult since the manifestation of dengue can mimic any flu like symptoms and it is usually confirmed only retrospectively on the basis of serology [2].

There is a need then for a cost-effective diagnostic aid for detecting dengue in the setting where sophisticated dengue tests are not available. Such tool may be the complete blood count (CBC) that is readily available. This study therefore aims to determine whether the presence of atypical lymphocyte together with other CBC components can be used to predict dengue infection and dengue severity.

Dengue fever is the most rapidly extensive vector borne viral disease in the world particularly in tropical and subtropical countries. An estimated 50 million dengue infections occur annually and approximately 2.5 billion people live in dengue endemic countries [3].

In the Philippines, there were reports that dengue became hyperendemic and a leading cause of childhood hospitalization during 1980s [4]. The onset of rainy season brings with it a diverse mosquito-borne disease including dengue fever which creates a serious threat to the health of Filipinos. The Department of health releases preventive measure yearly; however dengue cases continue to increase yearly. The Philippines recorded a significant number of dengue cases during the first six months of 2010. According to the Department of Health, some 29,000 cases were reported and admitted to different hospitals nationwide between January 1 and July 10 2010, compared to around 22,000 during the same period in 2009 (34.7 % increase) [3].

Dengue infection can cause a wide range of symptoms from mild, undifferentiated fever to severe headache, retro-orbital pain, arthralgia and rash, but rarely causing death, fever may last up to seven days. Dengue Haemorrhagic Fever (DHF), a more severe form of dengue, includes hemorrhagic tendencies, thrombocytopenia and plasma leakage. Dengue Shock Syndrome (DSS) includes all the above criteria plus circulatory failure, hypotension for age and low pulse pressure. DHF and DSS are potentially deadly but patients with early diagnosis and appropriate therapy can recover with no sequelae [5].

Early diagnosis is indeed vital for proper treatment and fluid management. Unawareness of the disease may result in more complication as well as losing the patient rapidly. There are several existing diagnostics to support patient management and disease control. The ideal test should be simple, easy to perform, cheap, and able to differentiate recent from acute infections. The Virus isolation and nucleic acid detection are more specific than antibody detection using serologic methods but are also labor-intensive and costly [1].

These tests, however are still not available in many tropical countries. There are even some communities who lack intensive care facilities hence requiring transport of critically ill patient. The short interval between onset of hemorrhage and death, especially in young children, makes rapid medical intervention for DHF or DSS a critical factor for survival. This emphasized the importance of an early indicator for the diagnosis of Dengue hemorrhagic fever or shock [5].

There are studies mentioning atypical lymphocyte as an adjunct tool in diagnosing dengue infection. Atypical lymphocytes or reactive lymphocytes are an activated nonmalignant lymphocyte seen in the peripheral blood smear. It is said to appear in many viral illness like Dengue Fever, Epstein Barr virus, infectious mononucleosis. It plays an important role in immune response and accumulates in areas of inflammation. Reactive lymphocyte shows the different stages of immune responsiveness of B and T lymphocyte s in the peripheral blood and immune system [6]. They act as normal lymphocytes in sites of local inflammation, playing a role in the immune response in a primary cellular immune or helper T-cell response [6].

A study done on naturally infected Thai children showed that subject with DHF have significant alterations in their leukocyte populations during the acute stage of illness. Most prominent were an increase in the percentage and total number of lymphocytes and an increase in the percentage and number of atypical lymphocytes [7]. Hence, atypical lymphocyte could be used as a presumptive diagnostic tool in dengue. Changes in the atypical lymphocytes counts could also be useful markers of the disease activity as the infection progresses [2].

The atypical lymphocyte can be used to predict the severity of dengue infection, since these lymphocytes are found more in patients with dengue hemorrhagic fever and dengue shock syndrome than in patients with simple dengue fever. Previous studies have suggested that events in the peripheral blood, such as dengue viral replication, cytokine expression, and cellular activation/proliferation, are associated with disease severity and outcome [8].

Activation of T lymphocytes and monocytes induces the production of cytokines and chemical mediators like TNF, which in turn cause capillary leakage and may lead to shock. Plasma levels of tumor necrosis factor receptors were higher in children who developed DHF than in those with dengue fever or other non-dengue febrile illnesses and were correlated with the degree of subsequent plasma leakage. These results support the hypothesis that immune activation contributes to the pathogenesis of DHF [9].

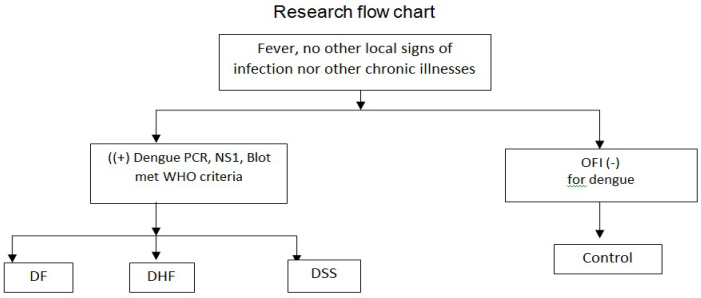
Whether the presence of a typical lymphocyte together with other CBC components can be used to predict dengue infection and dengue severity still needs to be investigated.

Methods

This is a retrospective case control study of pediatric patients admitted with dengue and other non-dengue febrile illnesses from January 2009 to December 2010 at The Medical City. The study was submitted and approved by The Medical City Research Committee Technical Review Board.

Study Population

Randomly selected pediatric patients admitted to The Medical City from January 2009 to December 2010 with final diagnosis of Dengue fever as confirmed by Dengue test such as dengue PCR, Dengue NS1 or Dengue Blot were included in this study. Patients diagnosed with other associated infections such as measles, sinusitis and tonsillitis, were excluded in this study. Patients with anemia, malnutrition, or a history of chronic medical illnesses such as congenital heart disease or thalassemia were also excluded. An equal number of participants diagnosed with other non-dengue febrile illnesses (OFI) were also included for the control group (Figure 1). Controls were obtained from patients admitted with other febrile illnesses resembling dengue infection like systemic viral illness with negative dengue test and with no evidence of bacterial infection. The list of patients meeting the inclusion criteria was obtained from the records section.



Sample size

The sample size was calculated based on an inference about the means using a level of significance 0.05, power of 90% on a previous data showing the mean total lymphocyte in dengue fever 36.4, versus total lymphocyte in dengue like illness 19.2 the sample size calculated is 141 per group [2].

Methodology

Patients admitted at the Medical City Hospital from January 2009 to December 2010 with final diagnosis of Dengue fever who qualified for the criteria was matched with a control group as to age, gender and year of admission. Each chart was then retrieved and reviewed. Demographic characteristics such as age, gender, day of illness upon admission, duration of illness and clinical symptoms was gathered for each patient.

Only subjects with positive serologic dengue test were given the final diagnosis of dengue. A clinical diagnosis of dengue hemorrhagic fever was assigned following WHO clinical definition on the basis of presence of bleeding, thrombocytopenia and plasma leakage.

Bleeding was considered as evidenced by at least one of the following: a positive tourniquet test, petechiae, ecchymosis, or purpura, bleeding from the mucosa, gastrointestinal tract, injection sites or other locations, hematemesis or melena. Thrombocytopenia meant a platelet count of less than or equal to 100,000/mm^3. Evidence of plasma leakage due to increased vascular permeability could include an increase in hematocrit equal or greater than 20% above average for age, sex and population or a drop in the hematocrit following volume-replacement treatment equal to or greater than 20% of baseline ,or signs of plasma leakage such as pleural effusion, ascites, and hypoproteinemia. Dengue shock syndrome was assigned to patients who were able to fulfill the criteria of dengue hemorrhagic fever plus evidence of circulatory failure.

Included in the control groups were subjects diagnosed with other non dengue febrile illness (OFI) and if the serologic test for dengue turned out to be negative and there was no clinical evidence of dengue infection.

Complete blood count results were retrieved from the chart of each qualified subjects during the time of admission and on day 1 afebrile. We also noted if there was presence or absence of atypical lymphocyte.

CBC Collection Method

The CBC at The Medical City was performed on an automated hematology analyzer using well mixed whole blood that is added to a chemical called EDTA to prevent clotting. The machine quantify the number of RBCs, WBCs, and platelets, provide information about their size and shape, measure the hemoglobin content of RBCs, determine the percentage and absolute number of the five white blood cell types, and identify early and abnormal blood cells. Electronic blood cell counting is based upon the principle of impedance.

When the electronic WBC count is abnormal or a cell population is flagged, meaning that one or more of the results is atypical, a manual differential is performed by the senior medical technologist and is checked by the pathology resident. In that case, a wedge smear is prepared. This is done by placing a drop of blood on a glass slide, and using a second slide to pull the blood over the first slide's surface. The smear is air dried, then stained with Wright stain and examined under a microscope. One hundred white cells are counted and identified as neutrophils, lymphocytes, monocytes, eosinophils, or basophils based on the shape and appearance of the nucleus, the color of cytoplasm, and the presence and color of granules. The purpose is to determine if these cells are present in a normal distribution, or if one cell type is increased or decreased. Any atypical or immature cells also are counted.

Data Analysis

Data were encoded and tallied in SPSS version 10 for windows. Descriptive statistics were generated for all variables. For nominal data frequencies and percentages were computed. For numerical data, mean ± SD were generated. Analysis of the different variables was done using the following test statistics:

T-test – used to compare two groups with numerical data.

Mann Whitney U-test – non parametric equivalent of the t-test used to compare two groups with numerical data, compares means instead of medians.

Logistic Regression Analysis

Chi-square test – used to compare/associate nominal data.

Results

Patient population

A total of 296 subjects were included in the study. Table 1 shows the distribution of subjects according to demographic characteristics. Their age ranged from <1 y/o to 18 y/o with a mean of 8.16 years. 155 were classified as Dengue illness while 141 were classified as Other febrile illness as confirmed by dengue serology. The confirmed 155 cases of dengue illness were sub classified into DF 70 (23.6%), DHF 42 (14.2%) and DSS 43 (14.2%) respectively according to WHO criteria. Of the 296 subjects included, more than 50% were positive for atypical lymphocytes.

Characteristics		Frequency (n=296)	Percentage
Age of Children	<1 y/o	12	4.1
	1 – 4 y/o	76	25.7
	5 – 9 y/o	88	29.7
	10 – 14 y/o	74	25.0
	15 – 14 y/o	46	15.5
Mean ± SD = 8.16 ± 5.21			
Sex	Female	144	48.6
	Male	152	51.4
Atypical Lymphocytes	(+)	159	53.7
	(-)	137	46.3
Dengue Severity	DF	70	23.6
	DHF	42	14.2
	DSS	43	14.5
	OFI	141	47.6

Table 1: Demographic Characteristics of Subjects.

Distribution of subject according to Atypical Lymphocytes

Higher number of patient with dengue illness was found having positive for atypical lymphocytes (AL) than other febrile illness (OFI). Table 2 shows the distribution of subjects according to AL and OFI. Of the 155 confirmed cases of dengue a total of 137 (88.4%) of patient had AL and 18 (11.6%) was found negative for AL. On the contrary only 22 (15.6%) of OFI was positive for AL and 119 (84.4%) of the subjects were found negative. The results showed that there was a significant difference on the proportion of AL on subjects with dengue and those with OFI ($p<0.0001$).

The data from table 2 also shows that atypical lymphocyte is a significant predictor of dengue illness as derived from logistic regression analysis. The risk of a patient with AL was 41.16 times higher for dengue than those without AL (odds ratio 41.16, confidence interval 21.08-80.42, p value<0.0001).

Atypical Lymphocytes	DI	OFI	Total
(+)	137 (88.4%) 18	22 (15.6%)	159
(-)	(11.6%)	119 (84.4%)	137
TOTAL	155	141	296

Table 2: Distribution of Subjects According to Atypical Lymphocytes.

Accuracy of Atypical Lymphocyte in predicting dengue infection

The overall predictive value of AL was compared against the reference diagnostic test, Dengue PCR and serology. If the total number of patients with dengue was combined the overall sensitivity was 88.4% while the specificity was 84.4%. The positive and negative predictive values were 86.2% and 86.9% respectively (Table 3).

Outcome	Sensitivity	Specificity	PPV	NPV
DF	88.6	84.4	73.8	93.7
DHF	90.5	84.4	63.3	96.7
DSS	86.0	84.4	62.7	95.2
Dengue Illness (Over-all)	88.4	84.4	86.2	86.9

Table 3: Sensitivities, Specificities, Positive predictive values, Negative predictive values of AL for DF, DHF and DSS.

Distribution of Subjects According to Atypical Lymphocytes and Dengue Severity

A total of 137 (88.4%) of patients with dengue have AL. Among them 62 (45.3%) had DF, 38 (27.5%) had DHF and 37 (27%) had DSS as shown on Table 4. When the proportion of subject positive for AL was compared across dengue severity there was no significant difference seen. The proportion of AL on patients with DHF were elevated compared to dengue fever alone, however this is not significant ($p<1$). Likewise, no significant difference noted when DHF proportion was compared with DSS ($P<0.74$). This study shows that there is no significant difference on AL between DF, DHF and DSS as shown on Table 5.

Atypical Lymphocytes	Dengue Severity			Total
	DF	DHF	DSS	
(+)	62 (88.6%	38 (90.4%)	37 (86.0%)	137 (88.4%)
(-)	8 (11.4%)	4 (9.5%)	6 (14.0%)	18 (11.6%)
TOTAL	70	42	43	155

Table 4: Distribution of Subjects According to Atypical Lymphocytes and Dengue Severity.

	P Value	Significance
DF vs DHF	1.00	Not Significant
DF vs DSS	0.69	Not Significant
DHF vs DSS	0.74	Not Significant

Table 5: P Value Comparing the Proportion of Subjects with AL According to Dengue Severity.

Association of Atypical lymphocytes with other CBC component

The results showed that on admission, there was no significant difference in the platelet count, WBC, hematocrit and differential counts of dengue patients who were positive or negative for AL ($p>0.05$). However, on day 1 afebrile there was a significant difference noted in the WBC, neutrophil count, lymphocytes and platelet counts ($p<0.05$). The WBC and lymphocytes were significantly higher among dengue patients with AL than those without AL. On the other hand, the neutrophil count and platelet count were significantly lower among those with AL than those

dengue patients without AL.

Day CBC taken		Atypical Lymphocytes		P Value
		(+) (n=137)	(-) (n=137)	
Admission	HGB	133.36 ± 14.28	135.38 ± 13.72	0.57 (NS)
	HCT	39.78 ± 4.30	40.44 ± 3.70	0.53 (NS)
	WBC	4.24 ± 2.06	4.26 ± 2.08	0.96 (NS)
	Neutrophil Count	61.62 ± 19.76	69.38 ± 12.58	0.22 (NS)
	Lymphocytes	32.86 ± 19.66 (28)	25.17 ± 11.98 (25)	0.20 (NS)
	Monocytes	4.78 ± 3.58 (4)	13.88 ± 26.92 (5.5)	0.23 (NS)
	Platelet Count	182.09 ± 53.28	189.00 ± 46.88	0.60 (NS)
Day 1afebrile	HGB	137.05 ± 15.17	136.28 ± 15.36	0.84 (NS)
	HCT	41.02 ± 4.44	41.00 ± 4.48	0.98 (NS)
	WBC	4.81 ± 2.48 (4.2)	3.67 ± 1.39 (3.45)	0.03 (S)
	Neutrophil Count	29.91 ± 12.28	43.78 ± 12.75	<0.0001 (S)
	Lymphocytes	62.78 ± 13.00	49.22 ± 13.58	<0.0001 (S)
	Monocytes	5.92 ± 2.58	6.00 ± 2.14	0.90 (NS)
	Platelet Count	114.65 ± 38.68	135.78 ± 48.63	0.03 (S)

Table 6: Comparison of Platelet count, Leukocyte, Hematocrit and differential counts among dengue patients with or without atypical lymphocyte (n=155).

Discussion

Atypical lymphocytes are activated, enlarged lymphocyte in response to stimulation and expressed activation markers on the cell [6]. They are nonmalignant leukocyte seen in the peripheral blood. These are reactive lymphocytes of lymphoid origin and are produced in a variety of disorders usually associated with many viral infections like dengue fever [1].

We examined the complete blood count of randomly selected patient admitted with the diagnosis of dengue illness and other febrile illness upon admission and on day 1 afebrile. We determined whether atypical lymphocytes were associated with dengue fever compared to the control group and how sensitive it was in predicting the illness per se.

Results showed that there was a significant difference on the distribution of atypical lymphocytes of subjects diagnosed with dengue than those with other febrile illness ($p < 0.0001$). Meaning, atypical lymphocytes were significantly found positive among Dengue subjects rather than the control group with other febrile illness.

Furthermore, this study showed that there was no significant difference found on the proportion of atypical lymphocyte in terms of the severity of dengue illness (DF,DHF and DSS) . Result also showed that presence or absence of atypical lymphocyte alone cannot be used to predict the outcome or severity of the disease. This is similar to the study done by Green et al on Peripheral Blood Lymphocytes from Children with Dengue Hemorrhagic Fever at Center for Infectious Disease and Vaccine Research, wherein the mean absolute counts of atypical lymphocytes were markedly elevated in children with DHF and DF compared with controls, but there was no difference between counts in children with DHF and DF [10].

Our results showed that there was no significant difference among CBC counts of patient with or without atypical lymphocytes upon admission. However, on day 1 afebrile there was a significant difference noted in the WBC, neutrophil count, lymphocytes and platelet counts. The WBC and lymphocytes were significantly higher among dengue patients with AL than those without AL, while neutrophils are significantly lower. This means that AL concurrently appear with the other event in the blood count like elevation of typical lymphocyte and decreased in neutrophils and a drop in platelet count on day 1 afebrile . Atypical lymphocyte therefore could be helpful in predicting dengue illness since it appears simultaneously with the decrease in platelet and neutrophils and with the increase of typical lymphocyte.

The decreased WBC and elevated lymphocyte can be attributed to elevated atypical lymphocyte, though part of the limitation of the study is that the actual value of atypical lymphocyte was not quantified. However, in a study done by Wells et al on Kinetics of Peripheral Blood Leukocyte Alterations in Thai Children with Dengue Hemorrhagic Fever, showed that there was a significant increase in total lymphocytes count during acute illness, primarily due to concentrations of atypical lymphocytes [7].

Simmons et al. reported a decrease in the leukocyte count during the illness which was due to a decrease in neutrophils. Halstead and co-workers found that early in the course of illness, patients with either primary or secondary dengue infections exhibited a fall in the leukocyte count associated with a rise in the percentage of lymphocytes.8 Oliveira et al. (2009) have shown that the main hematological findings in dengue included leucopenia (68.3%), thrombocytopenia (66.5%), lymphocytopenia (67.2%), and Atypical lymphocytosis (67%) [11].

Moreover, this study also showed that atypical lymphocytes are significant predictor of dengue fever as derived from logistic regression analysis. The results showed that the risk of a patient with AL was 41.16x higher for dengue than those without AL. Finally, if the total number of patients with dengue was combined, the positive predictive value of AL in detecting dengue was 86.2%. This means that among those with AL, 86.2% were diagnosed to have dengue fever. In the study done by Jampangern et al, they concluded that the atypical lymphocyte and CD19+ cell counts should be considered, besides platelets and white blood cell counts, as a useful diagnostic tool for dengue infection [1].

Conclusion

This study shows that the presence of atypical lymphocyte is

associated with dengue infection. Atypical lymphocyte showed a promise in predicting dengue illness. However, this study showed that there is no significant difference found on the proportion of atypical lymphocyte among the severity of dengue illness. AL concurrently appear with the other event in the blood count like elevation of typical lymphocyte and decreased in neutrophils and a drop in platelet count on day 1 afebrile. Additional study on the actual quantity of atypical lymphocyte is suggested before the information should be used in usual clinical settings.

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