ORIGINAL ARTICLE

CHANGING HAEMATOLOGICAL PARAMETERS IN DENGUE VIRAL INFECTIONS

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Background: Dengue Fever is the most common arboviral disease in the world, and presents cyclically in tropical and subtropical regions of the world. The four serotypes of dengue virus, 1, 2, 3, and 4, form an antigenic subgroup of the flaviviruses (Group B arboviruses). Transmission to humans of any of these serotypes initiates a spectrum of host responses, from in apparent to severe and sometimes lethal infections. Complete Blood count (CBC) is an important part of the diagnostic workup of patients. Comparison of various finding in CBC including peripheral smear can help the physician in better management of the patient. **Material and Methods:** This cross sectional study was carried out on a series of suspected patients of Dengue viral infection reporting in Ittefaq Hospital (Trust). All were investigated for serological markers of acute infection. **Results** Out of 341 acute cases 166 (48.7%) were confirmed by IgM against Dengue virus. IgG anti-dengue was used on 200 suspected re-infected patients. Seventy-one (39.5%) were positive and 118 (59%) were negative. Among 245 confirmed dengue fever patients 43 (17.6%) were considered having dengue hemorrhagic fever on the basis of lab and clinical findings. Raised haematocrit, Leukopenia with relative Lymphocytosis and presence atypical lymphocytes along with plasmacytoid cells was consistent finding at presentation in both the patterns of disease, i.e., Dengue Haemorrhagic fever (DHF) and Dengue fever (DF). **Conclusion:** Changes in relative percentage of cells appear with improvement in the symptoms and recovery from the disease. These findings indicate that in the course of the disease, there are major shifts within cellular component of blood.

Keywords: Dengue, Complete Blood count, atypical lymphocyte

INTRODUCTION

Dengue viruses are a major cause of morbidity and mortality among tropical and subtropical areas of the world. The four serotypes of dengue virus, 1, 2, 3, and 4, form an antigenic subgroup of the flaviviruses (Group B arboviruses).[1] Transmission to humans of any of these serotypes initiates a spectrum of host responses, from in-apparent to severe and sometimes lethal infections. The most severe host response is dengue hemorrhagic fever (DHF), in which patients present with fever, hemorrhagic diathesis, hypotension, thrombocytopenia, and increased vascular permeability.[2] Some of these patients develop vascular collapse, i.e., dengue shock syndrome, which may be fatal if not adequately treated. The occurrence of the severe aspects of dengue infection correlates in most cases with the patient's immunological status. Although, dengue shock syndrome may occur in primary infections, the majority of cases occur in patients with pre-existing serum antibody to dengue viruses.[3]

Pre-existing antibody appears to have multiple roles in the pathogenesis of severe dengue infections. It has been demonstrated that infection of peripheral blood leukocytes by dengue viruses in vitro is markedly enhanced by the presence of non-neutralizing dengue antibody and that dengue virus may be isolated from the peripheral blood leukocytes of naturally infected patients.[4]

Early diagnosis of dengue is important for provision of specific care which ensures marked reduction in the morbidity of the disease itself. This study focused on clinical presentation and haematological indicators serially during acute stage and in convalescence. Thrombocytopenia, raised haematocrit, lymphocytosis especially atypical lymphocytosis and neutropenia are the consistent findings.[5]

The objective of this study was to determine the changing haematological indicators during dengue viral infections.

MATERIAL AND METHODS

A total 341 suspected cases of dengue fever were admitted in Ittefaq Hospital (Trust) Lahore during the epidemic, i.e., 22nd October to 4th December 2010 were included in the study and were subjected to serological tests including antibody detection of dengue specific IgM and IgG with ELISA method.[6]

All the confirmed patients of Dengue viral infection underwent detailed of clinical examination and haematological findings in the patients were recorded. Patients were examined at least once daily during their stay in the hospital. Along with the samples were for

serological studies, 3 cc of blood samples were drawn in EDTA for CBC at the time of admission and convalescent samples were taken approximately 7 and 10 days later.

Complete Blood count (CBC) was carried out in auto cell analysers, i.e., Sysmex KX 21/Sysmex XT. A blood smear was stained with Giemsa stain, and a differential count was performed to determine the percentages of normal lymphocytes, atypical lymphocytes, plasma cell/plasmacytoid cell, neutrophil, eosinophil, and monocytes. Platelet counts were recorded in each individual and the results were verified on slide. Each determination was done in duplicate at the time of admission, days 7 and 10. For statistical analyses, Student's t test was used, and $p<0.01$ was considered necessary to obtain significance.[7]

RESULTS

Out of 341 acute cases, 166 (48.7%) were confirmed by using dengue specific IgM kit, 27 (7.9 %) patients fell in Gray zone and 148 (43.4) were declared as negative, having no viral antibody after 6 days of on set of symptoms. IgG dengue specific kit was used for 200 cases segregated on suspicion of suffering from dengue re-infection 79 (39.5 %) were Positive, 3 (1.5%) in Gray zone and 118 (59%) were negative after 6 days of on set of symptoms. Male to female ratio found was 1.25:1 and most of the patients were adults 82% while patients below the age of 15 years were 18%.

Most of the patients in this study revealed typical sign and symptoms of dengue viral infection. Among them fever was the most common presenting symptom in (98%) patients followed by headache in (81%), myalagia in (74%), Anorexia in (63%), Malaise in (47%), skin rash in (41%), pruritis in (14.9%) and retro-orbital pain in (10.8%) cases.

Among these 166 confirmed dengue fever patients (44) 18% were labelled as DHF cases on the basis of IgM+, TLC <3.0, Platelets <100 and none of the patient showed sign and symptoms of DSS like hypotension, shock etc during their illness or hospital stay. Male to female ratio was (1:08) and child to adult ratio observed was (1:4.5)

Analysis of the Blood samples taken for CBC on the day of admission, on 7th and 10th day were recorded and up on analysis of the date following findings were observed. Leucopenia with white cell count less than 3,000/cmm was observed in (56.6%). Atypical/activated lymphocytes were seen in 93% of patients. Plasmacytoid lymphocytes were seen in 5% of above mentioned group. In some of the patients atypical lymphocytes was the first finding and thrombocytopenia was found in (62.6%) cases.

During the acute illness there was a significant increase in both the percentage and the concentration of total lymphocytes, which was due to a marked increase in both the percentage and the number of atypical lymphocytes, whereas normal lymphocytes were essentially unchanged (Table-1).

There was essentially no change in monocyte percentage and number, but during day 15 of convalescence a transient increase in the total number of leukocytes due to an increase in both the percentage and the absolute number of granulocytes was observed.

The absolute number, but not the percentage, of basophils increased during convalescence. Noteworthy finding was that a marked increase during convalescence in both the percentage and the concentration of eosinophils was observed. Thus, the major changes noted in the leukocytes were a marked increase in atypical lymphocytes during the acute stage of DHF and the appearance of increasing numbers of eosinophils during the convalescent stages of the illness (Table-2).

When the data were analyzed to compare dengue with and without shock, no differences were found in the mean percentages or concentration values for either the leukocyte differentials or the lymphocyte subpopulation determinations. A raise haematocrit of more than 20% was seen in 46 (26%) patients. Partial thromboplastin time (PTT) was significantly prolonged in 42 (25%) patients, whereas prothrombin time was found to be prolonged in 40 (24%) patients (Table-3).

Table-1: Changes in the relative percentages of peripheral leukocytes during acute and convalescent phases of DHF and DF (Mean%±SEM)

Phase of illness	Total WBC ($10\times^9$/l)	Total lymphocytes	Atypical Lymphocytes	Neutrophils	Eosinophils	Monocytes	Platelets $10\times^9$/l
On Admission	1.2±1.8	53.6±5.2	10.5±2.9	40.7±5.4	0.5±0.3	3.0±0.7	55±15
Day=7	2.5±2.2	39.1±3.2 ($p<0.001$)	1.1±0.3 ($p<0.001$)	53.4±3.1 ($p<0.01$)	3.7±1 ($p<0.01$)	3.4±0.6	85±20
Day=10	9.2±2.6	45.4±3.0 ($p<0.01$)	0.6±0.2 ($p<0.001$)	43.4±2.9	6.3±0.85 ($p<0.001$)	7.9±0.7	100±30

Numbers within parentheses indicate the p-value for convalescence phase in comparison with acute illness

Table-2: Changes in the Concentration of peripheral Lymphocytes during acute and Convalescent phase of DF

Phase of illness	Total WBC ($10\times^9$/l)	Total Lymphocytes	Normal Lymphocytes	Atypical Lymphocytes	Neutrophils	Eosinophils	Monocytes	Platelets $10\times^9$/l
On Admission	1.2±1.8	94±148	572±31	188±11	200±80	26±8	22±5	25±5
Day=7	2.5±2.2 ($p<0.01$)	1200±270 ($p<0.01$)	890±324	81±33 ($p<0.001$)	910±111 ($p<0.01$)	45±110 ($p<0.01$)	55±12	60±20
Day=10	9.2±2.6 ($p<0.01$)	2760±178 ($p<0.01$)	2200±300	15± 8 ($p<0.001$)	5000±290	35±0.8 ($p<0.001$)	22±17	100±70

Table-3: Coagulation profile patterns in patients of Dengue viral infection

Test	Mean	Remarks	
PT (sec)	37	<14 (n=126, 76%)	>14 (n=40, 24%)
APTT (sec)	47.5	<35 (n=124, 75%)	>35 (n=42, 25%)

DISCUSSION

A range of findings in peripheral blood is seen mostly during dengue viral infections. Heamo-concentration and raised haematocrit are well known findings in patient of Dengue haemorrhagic fever.[7] A total decrease in the leukocyte count during the illness is mainly due to a decrease in granulocytes, i.e., neutrophils. Presence of atypical lymphocytes with activated lymphocytes and even plasma cells along with thrombocytopenia is reported consistently along with other laboratory findings.[8]

A number of studies have shown that early in the course of illness, patients with either primary or secondary dengue infections exhibit a fall in the leukocyte count associated with a rise in the percentage of lymphocytes and this finding is in parallel to marrow suppression during acute phase.[9,10] Most prominently increase in the percentage and total number of lymphocytes and an increase in the percentage and number of atypical lymphocytes.[11]

Atypical lymphocytes are frequently seen in a variety of viral illnesses, including infectious mononucleosis, herpes, rubella, influenza and viral hepatitis. The exact function of atypical lymphocytes is unclear, they incorporate increased amounts of [3H] thymidine into deoxyribonucleic acid and are similar in appearance to lymphocytes which undergo blast transformation after stimulation with mitogens (such as phytohemagglutinin).[12]

Thus, it is possible that atypical lymphocytes represent a response to non-specific viral stimulation or to specific viral antigens due to recognition followed by transformation.[11] The potential importance of this in DHF stems rom studies which have demonstrated that the dengue virus *in vitro* invades and replicates poorly in resting lymphocytes but well in stimulated transformed B lymphoblast cells.[13] We observed a marked increase in concentration of Plamacytoid Lymphocytes and even Plasma cell in five of our patients. Similar findings have been reported by John Gawoski and Winnie Ooi[14] and it mainly represents that serum immunoglobulin production is enhanced during dengue viral infection, these are mostly against the specific serotype and obviously not protective to the infections caused by other serotypes.[15]

It has been suggested that the atypical lymphocytes in secondary dengue infection can be representative of augmented immune response in an attempt to control the spread of dengue virus-infected cells.[16] Differences between the samples at the time of admission and collected later during convalescence were also found in the concentrations of eosinophils. Similar findings have been reported for other viral infections, where, in response to inflammation during the acute phase, eosinophil concentrations fell. During convalescence, the eosinophil concentrations rise to normal or even more.[17]

Eosinophilia during the convalescent phase of dengue fever has been reported consistently in a number of studies.[18,19] An increase in the total number of basophils is also seen in the convalescent period. The increase in basophils may be due to element of recovery from the bone marrow suppression during convalescence.[9]

The most significant laboratory abnormality seen in our patients was thrombocytopenia, as observed in other studies.[20] This is thought to be due to depression of bone marrow observed in acute stage of dengue virus infection.[9] Other explanations are direct infection of the megakaryocytes by virus leading to increased destruction of the platelets or the presence of antibodies directed against the platelets.[21] Thrombocytopenia may result from by destruction of peripheral platelet or bone marrow megakaryocytes by viruses which consequently reduce the platelet production.[22] Haemorrhagic manifestations are very common with severe thrombocytopenia and severity of haemorrhagic tendency correlates with the platelet counts.[11]

Coagulopathy is also frequent in most patients with dengue fever. In our study, prolongation of aPTT was quite common, whereas PT was normal in majority of our patients (76%) where as the samples labelled as having prolonged timing were (24%), similar results have been reported in other studies.[23,24]

CONCLUSION

Peripheral blood parameters are very helpful for disease monitoring and can be useful in prediction of prognosis. These indicators, if rightly and timely assessed can be of value for better care of complicated cases.

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