

<http://www.pjbs.org>

PJBS

ISSN 1028-8880

**Pakistan
Journal of Biological Sciences**

ANSI*net*

Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Fibrin Degradation Products in Hyperleucocytic Chronic Myeloid Leukaemia

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Abstract: This study is aimed at finding out whether or not activation of the coagulation system and fibrin deposition occurs in Chronic Myeloid Leukaemia (CML) patients with Hyperleucocytic Syndromes (HLS). A total of 14 CML patients with HLS were evaluated with respect to white cell count, presence of symptoms and signs of HLS and plasma D-dimer levels. Equal number of sex and age matched CML patients without HLS were similarly evaluated. Patients with HLS had a significantly ($p < 0.05$) higher mean white cell count of $510 \times 10^9 \text{ L}^{-1}$ as compared to their counterparts who did not present with HLS and had a mean white cell count of $120 \times 10^9 \text{ L}^{-1}$. All CML patients without HLS had normal levels of D-dimer with a mean value of 87 ng mL^{-1} . However, among the CML patients with HLS, 78.6% had elevated D-dimer levels with a mean value of 325 ng mL^{-1} , while the remaining 21.4% had normal D-dimer levels with a mean value of 95 ng mL^{-1} . This data was interpreted to indicate that in the majority of cases of CML with HLS, microvascular endothelial damage and fibrin deposition occur and contribute to the microvascular blockade initiated by leucostasis. There is therefore, the need to investigate a possible beneficial role of low dose anticoagulation in mitigating secondary fibrin deposition and minimizing tissue infarction and sensory organ damage in such patients.

Key words: Chronic myeloid leukaemia, hyperleucocytic syndrome, D-dimer

INTRODUCTION

Chronic Myeloid leukaemia (CML) is a hematopoietic stem cell disorder that is characterized by myeloid leucocytosis, anemia, normal or elevated platelet count and massive splenomegaly (Lichtman, 1991a; Durosini, 1998). The hematopoietic stem cells acquire a reciprocal translocation between chromosome numbers 9 and 22 in over 90% of patients resulting in the characteristic shortening of the long arms of one of the chromosomes number 22 that is referred to as the Philadelphia chromosome (Lichtman, 1991a; Durosini, 1998). The Philadelphia translocation results in the formation of the chimeric BCR-ABL gene that encodes the protein p210 with a greater tyrosine kinase activity than that of a normal ABL gene product (Goldman, 2001). It is thought that the BCR-ABL gene might cause leukaemic transformation by opposing cellular apoptosis and impeding programmed cell death in the target hematopoietic stem cell leading to myeloid leucocytosis (Goldman, 2001). Patients are usually diagnosed in the chronic phase of the disease with a leucocyte count of between $50\text{-}200 \times 10^9 \text{ L}^{-1}$ with a full spectrum of cells in the

myeloid series, ranging from blasts to mature neutrophils with myelocytes and neutrophils predominating (Goldman, 2001). However, about 15% of CML patients present with leucocyte count above $300 \times 10^9 \text{ L}^{-1}$ that causes leuco-occlusion and impairment of the micro-circulation of the central nervous system, special sensory organs and penis, resulting in hyperleucocytic syndromes that usually manifest as blindness, deafness, or priapism (Lichtman and Rowe, 1982; Lichtman *et al.*, 1987). Hyperleucocytic syndromes usually respond to the rapid decrease in white cell count by a combination of leucapheresis and chemotherapy, but residual irreversible damage may persist and lead to permanent functional impairment (Onwukeme and Lot, 1993; Mackie and Collins, 1974). Leucostasis in small blood vessels resulting in consequent reduction in blood flow that eventually causes tissue hypoxia and organ damage has been identified as the fundamental pathological process in patients with hyperleucocytic syndromes (Mackie and Collins, 1974; McCarthy, 1985; Lichtman, 1991b). However, we hypothesize that leucostasis has the potential to damage the endothelial cells of the microvasculature. If vascular endothelial damage

occurs, it will lead to exposure of sub-endothelial structures and collagen, which will result in activation of intrinsic coagulation pathway, fibrin deposition and degradation with a concomitant rise in fibrin degradation products including the D-dimer fragments (Evatt *et al.*, 1992; Brozovic, 1991; Brozovic and Mackie, 1991). In this research we studied the levels of D-dimer fragments in CML patients with hyperleucocytic syndromes with the aim of finding out whether or not activation of the coagulation system and fibrin deposition occurred in such patients as seen at the University of Maiduguri Teaching Hospital, North East Nigeria.

MATERIALS AND METHODS

A total of 14 CML patients with hyperleucocytic syndromes who presented at the Department of Hematology of the University of Maiduguri Teaching Hospital (1996-2004) were evaluated at diagnosis with respect to the presenting white cell count, presence of symptoms and signs of hyperleucocytic syndromes and plasma D-dimer levels. Equal number (14) of sex and age matched CML patients who presented without hyperleucocytic syndromes were similarly evaluated in parallel for comparison.

The diagnosis of chronic phase CML was in all cases carried out based on clinical and laboratory features of the disease at presentation, which is characterized by massive splenomegaly, myeloid leucocytosis in excess of $50 \times 10^9 \text{ L}^{-1}$ and a full spectrum of myeloid precursors but with the proportion of blasts and promyelocytes of less than 10% in blood and marrow, anemia, normal or elevated platelet count, low neutrophil alkaline phosphatase and hypercellular bone marrow with myeloid hyperplasia (Durosinmi, 1998).

D-dimer Assay Technique: The tests were performed in all cases using rapid latex agglutination assay on glass slides. Commercial kit [Dade Diagnostics, USA] containing D-dimer monoclonal antibodies coupled to latex beads (0.25 mL) is mixed with the test plasma (0.1 mL) in each case and agglutination is read macroscopically after 3 min on the recommendations of commercial manufacturers of the D-dimer kits and as described in standard methodology (Brozovic and Mackie, 1991). The tests were performed using plasma in serial doubling dilutions and the results were calculated in ng mL^{-1} and normal D-dimer level was defined as less than 200 ng mL^{-1} , while levels of 200 ng mL^{-1} or greater were considered elevated (Brozovic and Mackie, 1991).

The student t. test was used to determine statistical significance and $p < 0.05$ was taken as significant.

Table 1: Clinical status and white cell count of CML patients

Clinical status	No. of patients	White cell count ($\times 10^9 \text{ L}^{-1}$)	
		Range	Mean
With HLS*	14	310-630	510
Without HLS*	14	98-250	120
Total	28	98-630	215

*HLS = Hyper Leucocytic Syndrome

Table 2: Clinical features among CML patients with hyperleucocytic syndromes

Clinical features	No. of patients
Hearing impairment	7 (50%)
Visual impairment	3 (21.4%)
Hearing and visual impairment	2 (14.3%)
Priapism	2 (14.3%)
Total	14 (100%)

Table 3: Pattern of D-dimer levels among CML patients with and without hyperleucocytic syndromes

Clinical status	No. of patients with normal D-dimer levels	No. of patients with elevated D-dimer levels
With HLS* (n = 14)	3 (21.4%)	11 (78.6%)
Without HLS* (n = 14)	14 (100%)	0 (0%)

*HLS = Hyper Leucocytic Syndrome, (n) = No. of patients in each category

RESULTS

Overall, the CML patients had a mean white cell count of $215 \times 10^9 \text{ L}^{-1}$ (range $98-630 \times 10^9 \text{ L}^{-1}$) as shown in Table 1. Patients with hyperleucocytic syndromes had a significantly ($p < 0.05$) higher mean white cell count of $510 \times 10^9 \text{ L}^{-1}$ (range: $310-630 \times 10^9 \text{ L}^{-1}$) as compared to their counterparts who did not present with the syndrome and had a mean white cell count of $120 \times 10^9 \text{ L}^{-1}$ (range: $98-250 \times 10^9 \text{ L}^{-1}$).

Out of the 14 patients with hyperleucocytic syndromes, 7 (50%) presented with hearing impairment, 3 (21.4%) presented with visual impairment, 2 (14.3%) presented with both hearing and visual impairment and 2 (14.3%) presented with priapism as shown in Table 2.

All 14 cases that presented without hyperleucocytic syndromes had normal levels of D-dimer with a mean value of 87 ng mL^{-1} . However, among the 14 cases with hyperleucocytic syndromes, 11 (78.6%) cases had elevated D-dimer levels with a mean value of 325 ng mL^{-1} , while the remaining 3 (21.4%) cases had normal D-dimer levels with a mean value of 95 ng mL^{-1} . The pattern of D-dimer level variation among patient categories is shown in Table 3.

DISCUSSION

The higher mean white cell count associated with hyperleucocytic syndromes in this study (Table 1) was an expected finding that was consistent with the fundamental role of high white cell counts in the pathogenesis of the

syndromes (Lichtman and Rowe, 1982). In the western countries only about 15% of CML patients present with hyperleucocytic syndromes (Lichtman and Rowe, 1982). However, previous reports showed that the proportion of CML patients who presented with hyperleucocytic syndromes was about 20-25% in Nigeria because most patients came to the hospital with late presentations after having tried a number of unorthodox therapeutic alternatives (Essien, 1976; Onwukeme *et al.*, 1987). The majority of our CML patients with hyperleucocytic syndromes presented with hearing impairment (Table 2), as was the case in earlier studies conducted in Nigerian patients (Onwukeme *et al.*, 1987).

The pattern of D-dimer levels found among CML patients revealed contrasting features between patients who presented with hyperleucocytic syndromes and those who did not present with the syndromes (Table 3). All of the patients without hyperleucocytic syndromes had normal levels of D-dimer. This result was an expected finding and consistent with the fact that such patients generally had white cell count that was lower than $300 \times 10^9 \text{ L}^{-1}$ (Table 1), a level below which leucostasis and microvascular blockade do not occur (Lichtman *et al.*, 1987). Under these conditions, vascular endothelial damage would not occur to cause any contact activation of the coagulation system and fibrin deposition, hence the finding of normal levels of D-dimer among such patients. In contradistinction from these results, CML patients who presented with hyperleucocytic syndromes had white cell counts that were higher than $300 \times 10^9 \text{ L}^{-1}$ (Table 1), a level above which leucostasis and microvascular blockade usually occur (Lichtman *et al.*, 1987). However, the D-dimer levels were elevated in only 78.6% of such patients, while the remaining 21.4% had normal levels of D-dimer (Table 3). This pattern of results would suggest that the clinical manifestations of hyperleucocytic syndromes in the 21.4% of patients in whom the D-dimer levels were normal were attributable to hypoxia due leucostasis and microvascular blockade that was not severe enough to cause microvascular endothelial damage to warrant contact activation of coagulation and fibrin deposition. Nonetheless, the majority (78.6%) of CML patients with hyperleucocytic syndromes had elevated D-dimer levels (Table 3). This data was interpreted to imply that the clinical manifestations of hyperleucocytic syndromes in such patients were attributable to hypoxia due to leucostasis and microvascular blockade that was sufficient enough to cause endothelial damage and exposure of sub-endothelial structures and collagen, which would result in activation of the intrinsic

coagulation pathway and microvascular deposition of fibrin (Hutton, 1989). Further more, the tissue injuries associated with hypoxia would invariably trigger inflammatory reactions within the tissues and the microvascular endothelial cells respond to inflammatory mediators by producing procoagulant tissue factor III that facilitates activation of the coagulation cascade via the extrinsic pathway leading to further microvascular deposition of fibrin, which is then subsequently broken down by secondary fibrinolysis into fibrin degradation products including the D-dimer fragments (Hutton, 1989; Bevilaqua *et al.*, 1984; Rijkins *et al.*, 1982). In fact similar findings of elevated D-dimer levels were reported to occur in sickle cell disease as a result of vascular endothelial damage caused by sickled erythrocytes during crisis (Ahmed *et al.*, 2002). Fibrin deposition is obviously undesirable in hyperleucocytic syndromes since it would aggravate the pre-existing microvascular blockade initiated by leucostasis and cause more tissue infarction and sensory organ damage.

CONCLUSIONS

Hyperleucocytic syndromes in CML patients were associated with elevated levels of D-dimer in majority of cases. These changes were interpreted to be the result of microvascular endothelial damage leading to activation of coagulation factors and fibrin deposition. Although leucostasis was the primary initiator of microvascular blockade in hyperleucocytic syndromes, our data suggest that fibrin deposition occurred secondarily and aggravated the microvascular blockade in majority of cases. There is therefore, the need to investigate a possible beneficial role of low dose anticoagulation in mitigating secondary fibrin deposition and minimizing tissue infarction and sensory organ damage in such patients.

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