International Journal of Research in Medical Sciences
Mehta S et al. Int J Res Med Sci. 2017 Dec;5(12):5307-5311
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20175446

Original Research Article

Neutrophil gelatinase-associated lipocalin (NGAL) as a prognostic marker in chronic myeloid Leukemia: an observational study

Shinky Mehta[1], Ravi Rohilla[2], Jyoti Rohila[3]*

[1]Department of Biochemistry, BSA Medical College and Hospital, Rohini, New Delhi, India
[2]Department of Community Medicine, GMCH, Chandigarh, Haryana, India
[3]Department of Anatomy, PGIMS, Rohtak, Haryana, India

Received: 18 September 2017
Accepted: 31 October 2017

***Correspondence:**
Dr. Jyoti Rohila,
E-mail: jotirohilla5671@gmail.com

ABSTRACT

Background: Neutrophil gelatinase-associated lipocalin (NGAL) is a protein which is associated with various inflammatory conditions affecting human tissues, such as those in the respiratory, gastro-enteric and urinary tracts, with a marked increase in the local and systemic expression. Different experimental evidences reveal that NGAL is required for the induction and pathogenesis of chronic myeloid leukemia (CML).
Methods: The present study was conducted in department of Biochemistry in a tertiary care institute of Haryana. 30 cases of CML were included in the study. It was a hospital based observational study which was conducted for one-year duration. Apart from routine biochemical investigations, serum NGAL estimation was done before the initiation of therapy and after 3 months of therapy.
Results: The median age at presentation was 39 years. Male to female ratio was 1.3:1. Weight loss was the most common presentation of patients (53.3%). More than half of the cases occurred in age group of 21-40 years. Serum NGAL was significantly higher in CML patients (358.47±125.65) before treatment as compared to serum NGAL value after treatment (85.03±62.77). In patients who achieved hematological remission, mean serum NGAL levels (62.46 ng/ml±23.72) were statistically lower than mean serum NGAL values in patients who did not achieve remission (231.75 ng/ml±16.7).
Conclusions: The present study concluded that serum NGAL levels can be used as diagnostic and prognostic marker in CML.

Keywords: Chronic myeloid leukemia, Imatinib, Remission

INTRODUCTION

The leukemias are a heterogenous group of diseases characterized by infiltration of blood, bone marrow and other tissues by neoplastic cells of the hematopoietic system. These leukemias are divided into two types-myeloid and lymphoid based upon their origin of cell line and acute and chronic based upon their clinical course.[1] The present study is done on the patients of chronic myeloid leukemia (CML). It is a clonal myeloproliferative disease of primitive hematopoietic stem cells. The median age of diagnosis is 38-40 years.[2]

The Philadelphia (Ph) chromosome is the hallmark cytogenetic abnormality of CML. The Ph chromosome results from reciprocal translocation between chromosome 9 and 22. This genetic alteration results in the formation of a chimeric protein, BCR-ABL.[3] CML evolves in 3 distinct clinical stages: chronic phase, accelerated phase and blast crisis. CML is usually

diagnosed in an asymptomatic phase and progressed to blast crisis within 3 to 5 years through an accelerated phase if not treated.[4] Imatinib mesylate, a tyrosine kinase inhibitor, is now the most effective treatment in Ph chromosome positive patients. Imatinib is now the initial therapy in almost all the patients in all the phases of CML.[5]

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2, siderocalin, uterocalin and 24p3, is a 25 kDa protein belonging to lipocalin superfamily. Various inflammatory conditions affecting human tissues, such as those in the respiratory, gastro-enteric and urinary tracts, are associated with a marked increase in the local and systemic expression of NGAL.[6] In some cases (e.g. during the course of some renal diseases), the evaluation of serum and urinary levels of this protein has been found to be particularly useful as early and specific biomarker of organ damage and clinical prognosis.[7] Also, NGAL actively participates in the processes of growth, development and differentiation of different human tissues as early as in embryonic phases, thus signifying that it also plays an important role in regulation of physiological cell multiplication.[8]

It has been proved in literature that different lipocalins play a key role in proliferation, differentiation and development of human cancers.[9] Different experimental evidences suggest that NGAL is required for induction and pathogenesis of CML. Studies done on the murine homolog of NGAL (24p3/Ngal) suggest that in BCR-ABL positive leukemia, 24p3/Ngal is strongly expressed by the malignant cells.[10,11] Normal hematopoietic cells (but not the BCR-ABL positive leukemia cells) in mice express the receptor for Ngal (called NgalR or 24p3R). The leukemia cells release large quantities of 24p3/Ngal into the bloodstream which acts via its receptor to induce cell death in the normal hematopoietic cells. Since the leukemic cells do not express the receptor, they remain unaffected. This mechanism has been suggested to be responsible for the spread of leukemia cells through the healthy bone marrow.[12-14]

Studies in CML patients have confirmed the findings in mice. Both NGAL mRNA and plasma levels are significantly elevated in patients with CML.[12,15] Further, patients who responded to treatment with Imatinib (a specific inhibitor of BCR-ABL tyrosine kinase) showed a significant decrease in NGAL mRNA.[16]

The present study was conducted to evaluate the status of NGAL in CML patients before and after chemotherapy and also to compare the NGAL values in remission and non-remission CML patients.

METHODS

This study was conducted in a tertiary care centre of Haryana. It was a hospital based observational study. The study was conducted from April 2012 to March 2013 i.e. One-year duration. A total of 30 patients were taken for the study.

The present study was conducted in Department of Biochemistry in collaboration with Department of Medicine (Clinical Haematology unit). Thirty patients of chronic myeloid leukemia were enrolled for study. The diagnosis was made by history, clinical examination, total and differential leukocyte count and cytogenetic studies. CML patients were treated by Imatinib mesylate (400 mg/day) therapy.

Serum NGAL levels were estimated along with routine biochemistry and complete hemogram was performed in newly diagnosed patients before and after chemotherapy. Follow up was done at 3 months. Fasting early morning venous blood sample was taken under all aseptic precautions and serum separated within one hour of collection for NGAL estimation. Samples were stored at -20°C till further analysis.

Figure 1: NGAL ELISA kit.

Serum NGAL was estimated by a commercial Enzyme Linked Immunosorbent Assay kit for human NGAL (Figure 1). The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human neutrophil gelatinase-associated lipocali（NGAL) in samples. The standard density was taken on the horizontal axis, and the optical density value on the vertical axis and standard curve was drawn on graph paper. The corresponding density was found according to the sample optical density value by the sample curve. The result was the sample density.

Statistical analysis

Data was entered in Microsoft Excel spreadsheet and statistical analysis was done by using SPSS (Statistical Package for Social Studies) for Windows version. 20.0. For normally distributed data, mean and standard deviation was calculated. Paired ‘t’ test was used for finding statistical significance of change of parameters (NGAL) before and after chemotherapy. For statistical significance, p value of less than 0.05 ($p<0.05$) was taken.

RESULTS

The mean age at presentation in CML patients was 41.7 years. Median age was 39 years. (Range 28-70 years). There were 17 males and 13 female patients. Male to female ratio was 1.3:1. On distribution according to decades of life, number of patients in group ≤20, 21-40, 41-60 and more than 60 years were 0 (0 male, 0 female), 17 (7 males, 10 females), 10 (7 males, 3 females) and 3 (3 males, 0 female) respectively. Maximum number of patients was in age group of 21-40 years (56.7%).

Table 1: Symptoms of cml patients at time of presentation.

Symptom	No. of patients	(%)
Weight loss	16	53.3
Anemia	3	10
Anorexia	3	10
Lt. upper quadrant pain	7	23.3
Fatigue	7	23.3
Night sweats	5	16.7
Fever	5	16.7

Table 1 shows the incidence of symptoms in patients of CML as observed at the time of presentation. Most common presentation was weight loss, upper quadrant pain, fatigue followed by night sweats and fever.

Table 2: Comparing serum NGAL levels in CML patient's pre-treatment and post-treatment.

Parameter	Pre-treatment (mean ±SD)	Post-treatment (mean ±SD)	p value
Serum NGAL (ng/ml)	358.47±125.65	85.03±62.77	0.001

Serum NGAL value was higher in CML patients before treatment as compared to serum NGAL value after treatment (85.03 ±62.77). This difference was significant. (p=0.001).

Table 3: Comparing serum NGAL levels in CML patients who achieved remission and patients who did not achieve remission.

Parameter	Patients who achieved remission (mean ±SD)	Patients who did not achieve remission (mean ±SD)	p value
Serum NGAL (ng/ml)	62.46±23.72	231.75±16.7	0.001

In patients who achieved hematological remission, mean serum NGAL levels (62.46ng/ml±23.72) were statistically lower than mean serum NGAL values in patients who did not achieve remission (231.75ng/ml ±16.7) (p=0.001).

DISCUSSION

The present study was conducted among 30 patients of chronic myeloid leukemia. CML patients were treated by imatinib (400 mg/day) therapy. Routine biochemistry, complete hemogram, serum NGAL were performed in newly diagnosed patients. Follow up was done at 3 months.

The median age of CML patients in present study at presentation was 39 years (range 28-70 years). In a study done by Manero et al, the median age at presentation of CML was 45-55 years.[4] Golde et al reported that median age at presentation in CML is between 45-65 years.[17] Kantarjian et al reported 48 years as median age of presentation. Sawyers reported 53 years as median age of presentation.[18]

None of the patient was asymptomatic at the time of diagnosis in our study. Wetzler et al also reported that some patients while asymptomatic are diagnosed during health screening tests.[1] Manero et al reported that the incidence of asymptomatic presentation in CML has increased from 15 to 50% approximately in recent time.[4]

Kantarjian et al reported that about 50% patients were asymptomatic at the time of presentation and disease is diagnosed by routine blood tests.[19] Sawyers reported that about 40% of patients are asymptomatic at the time of diagnosis.[18] This difference in presentation of CML patients in our study may be because of advancement of technique of early diagnosis in developed countries by regular health checkup and screening tests.

NGAL levels: Association with treatment outcome

Serum NGAL levels were higher in patients at the time of diagnosis before treatment (358.47ng/ml±125.65). After treatment with imatinib, levels decreased significantly (85.03g/ml±62.77) (p<0.001) (Table 2). 86.67% patients achieved hematological remission whereas 13.33% patients failed to achieve hematological remission after 3 months of therapy.

In patients who achieved hematological remission, mean serum NGAL levels were 62.46ng/ml±23.72 whereas, in patients who did not achieve remission, mean serum NGAL levels were 231.75ng/ml±16.7 (p<0.001) (Table 3).

Druker et al showed that imatinib mesylate is a potent inhibitor of Bcr-Abl tyrosine kinase.[20] In 2003, Kantarjian et al showed high incidence of early complete and major cytogenetic responses by imatinib mesylate therapy in newly diagnosed patients with Philadelphia chromosome-positive CML.[21]

Lin et al demonstrated that NGAL expression in BCR-ABL+ cells require the tyrosine kinase of BCR-ABL. They showed that treatment of cells with imatinib

mesylate, an inhibitor of BCR-ABL tyrosine kinase, greatly reduced NGAL secretion.[14]

Alonci et al conducted a study on 22 patients in chronic phase (9 men, 13 women) and 10 healthy subjects. In all patients receiving imatinib, serum NGAL levels were determined at diagnosis and after a complete molecular response were achieved. They found that median serum NGAL levels in CML patients at diagnosis amounted to 402±181ng/ml, and thus were significantly higher compared to age matched controls (199±108ng/ml) ($p<0.01$).

After imatinib therapy, all patients achieved a complete molecular remission, and NGAL levels decreased (151.70±47ng/ml) ($p<0.01$).[22] In this study, decrease in serum NGAL levels after treatment was 62.26% but in our study, it was 76.27%.[22]

Above findings suggest that NGAL has a role in pathogenesis of CML. So, can be used as diagnostic marker. Since NGAL is significantly correlated with blast percentage, and blasts are consistently present in peripheral blood in CML. So, NGAL can be used as prognostic marker as well in CML. Thus, we conclude that NGAL can be used as diagnostic and prognostic marker in CML.

CONCLUSION

The present study demonstrated that serum NGAL levels were raised in CML. It was observed that serum NGAL levels decreased significantly after chemotherapy with imatinib mesylate for 3 months. Thus, it can be concluded that serum NGAL levels can be used as diagnostic and prognostic marker in CML.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Wetzler M, Bloomfeild CD. Acute and chronic leukemia. In: Fauci AS, Kasper DL, Braunwald E, Hauser SL, Longo DL, Jameson JL, editors. Harrison's Principle of Internal Medicine, 17th ed.New York:McGraw Hill;2008:677-86.
2. National Cancer Registry Programme. Two-year report of the population based cancer registries 1999-2000. New Delhi: Indian Council of Medical Research;2005.
3. Deininger MW, Goldman JM, Melo JV. The molecular biology of chronic myeloid leukemia. Blood. 2000;96:3343-56.
4. Manero GG, Faderl S, O'Brien S, Cortes J, Talpaz M, Kantarjian HM. Chronic myelogenous leukemia: A review and update of therapeutic strategies. Cancer. 2003;98:437-57.
5. Aziz Z, Iqbal J, Akram M, Saeed S. Treatment of chronic myeloid leukemia in the Imatinib Era. Cancer. 2007;109:1138-45.
6. Xu S, Venge P. Lipocalins as biochemical markers of disease. Biochim Biophys Acta. 2000;1482:298-307.
7. Bolignano D, Donato V, Coppolino G, Campo S, Buemi A, Lacquaniti Aet al.Neutrophil Gelatinase-Associated Lipocalin (NGAL) as a marker of kidney damage. Am J Kidney Dis. 2008;52:595-609.
8. Gwira JA, Wei F, Ishibe S, Ueland JM, Barasch J, Cantley LG. Expression of neutrophil gelatinase - associated lipocalin regulates epithelial morphogenesis in vitro. J Biol Chem. 2005;280:7875-82.
9. Bratt T. Lipocalins and cancer. Biochim Biophys Acta. 2000;1482:318-26.
10. Devireddy LR, Gazin C, Zhu X, Green MR. A cell-surface receptor for lipocalin 24p3 selectively mediates apoptosis and iron uptake. Cell. 2005;123:1293-1305.
11. Arlinghaus R, Leng X. Requirement of lipocalin 2 for chronic myeloid leukemia. Leuk Lymphoma. 2008;49:600-3.
12. Leng X, Lin H, Ding T, Wang Y, Wu Y, Klumpp S et al. Lipocalin 2 is required for BCRABL-induced tumorigenesis. Oncogene. 2008;27:6110-19.
13. Hu L, Hittelman W, Lu T, Ji P, Arlinghaus R, Shmulevich I et al. NGAL decreases E-cadherin-mediated cell-cell adhesion and increases cell motility and invasion through Rac1 in colon carcinoma cells. Lab Invest. 2009;89:531-48.
14. Lin H, Monaco G, Sun T, Ling X, Stephens C, Xie S et al. Bcr-Abl mediated suppression of normal hematopoiesis in leukemia. Oncogene. 2005;24:3246-56.
15. Villalva C, Sorel N, Bonnet ML, Guilhot J, Mayeur-Rousse C, Guilhot F, et al. Neutrophil gelatinase-associated lipocalin expression in chronic myeloid leukemia. Leuk Lymphoma. 2008;49:984-88.
16. Owen HC, Roberts SJ, Ahmed SF, Farquharson C. Dexamethasone-induced expression of the glucocorticoid response gene lipocalin 2 in chondrocytes. Am J Physiol Endocrinol Metab. 2008;294:E1023-34.
17. Golde DW, Champlin RE. Chronic myelogenous leukemia: Recent advances. Blood. 1985;65:1039-41.
18. Sawyers CL. Chronic myeloid leukemia. N Eng J Med. 1999;340:1330-40.
19. Kantarjian HM, Deisseroth A, Kurzrock R, Estrov Z, Talpaz M. Chronic myelogenous leukemia: A concise update. Blood. 1993;82:691-703.
20. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. Nat Med. 1996;2:561-66.
21. Kantarjian HM, Cortes JE, O'BrienS. Imatinib mesylate therapy in newly diagnosed patients with Philadelphia chromosome-positive chronic

myelogenous leukemia: high incidence of early complete and major cytogenetic responses. Blood. 2003;101:97-100.
22. Hanai J, Mammoto T, Seth P, Mori K, Karumanchi SA, Barasch J et al. Lipocalin 2 diminishes invasiveness and metastasis of Ras-transformed cells. J Biol Chem. 2005;280:3641-47.

Cite this article as: Mehta S, Rohilla R, Rohilla J. Neutrophil gelatinase-associated lipocalin (NGAL) as a prognostic marker in chronic myeloid Leukemia: an observational study. Int J Res Med Sci 2017;5:5307-11.