International Journal of Science and Healthcare Research
Vol.3; Issue: 4; Oct.-Dec. 2018
Website: www.ijshr.com
Original Research Article *ISSN: 2455-7587*

Correlations of NGAL with Age, Haemoglobin and Platelet Counts in Chronic Myeloid Leukemia Cases and Controls

Dr. Shinky Mehta[1], Dr. Asha Kumari[2], Dr. Deepika Dalal[3], Dr. Rajesh Kumar[4]

[1]Tutor, Department of Biochemistry, Dr. B R Ambedkar College, Rohini
[2]Assistant Professor, Department of Biochemistry, SHKM, GMC, Mewat
[3]Demonstrator, Department of Biochemistry, PGIMS, Rohtak
[4]Demonstrator, Department of Biochemistry, Kalpana Chawla Medical College, Karnal

Corresponding Author: Dr. Asha Kumari

ABSTRACT

NGAL is one of upcoming markers in diagnosis and management of kidney diseases at an early stage. It has been correlated with haematological malignancies like CML, polycythemia vera and essential thrombocytopenia also. This study was conducted to find NGAL levels in CML cases and controls and find correlation if any with age, haemoglobin and platelet counts.

Aims and objectives:

To estimate the levels of NGAL in 30 CML cases and 30 healthy controls.

To assess the correlation between NGAL level with Age, Hemoglobin and Platelet count of cases before and after chemotherapy.

Materials and methods: 30 chronic myeloid leukaemia patients along with 30 healthy age and sex matched controls were enrolled for the study. Chemotherapy given consisted of imatinib (400 mg/day). Complete haemogram, routine biochemistry and Serum NGAL were estimated in all subjects. Serum NGAL was estimated by a commercial Enzyme Linked Immunosorbent Assay kit for human NGAL.

Results and observations: Serum NGAL was significantly higher in CML patients (358.47 ± 125.65) as compared to serum NGAL in controls (44.5 ± 15.65) (p=0.001). 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) (p=0.001). No correlation was observed in age of controls and NGAL levels while a very weak positive correlation was found between age of cases and NGAL values (r = 0.32 post treatment and r = 0.26 pre treatment). It is also positively correlated with platelet count and this correlation is statistically significant (r=0.951, p=0.001). There is a weakly positive correlation between NGAL and hemoglobin (r=0.40, p=0.028).

Conclusion: NGAL is a potential biomarker for not only kidney diseases but chronic myeloid leukaemia. It is positively correlated with Platelet count of cases and weakly correlated with hemoglobin levels.

Key words: NGAL, hemoglobin, Platelet count, CML, chronic myeloid leukaemia, biomarker.

INTRODUCTION

Neutrophil glucosaminidase-associated lipocalin, also referred as lipocalin 2, 24p3, oncogene 24p3, p25, migration stimulating factor inhibitor (MSFI), human neutrophil lipocalin (HNL), α1-microglobulin related protein, siderocalin, or uterocalin. It belongs to Lipocalin family including glycoproteins. [1,2] Encoded by a gene at chromosome locus 9q34.11, NGAL is a 198 amino acid glycoprotein. [3]

NGAL has been recently highlighted as a biomarker for acute kidney diseases. Other functions of NGAL are searched in latest studies. Crystallographic studies gave evidence of role of NGAL in iron metabolism. Goetz et al demonstrated that NGAL interferes with siderophore-mediated iron uptake by bacteria. [4] NGAL can be endocytose alone or with iron-binding siderophores, accordingly increasing or decreasing intracellular Iron. [5]

NGAL levels are used as a marker for numerous malignancies like Chronic Myeloid leukaemia, polycythemia vera and

essential thrombocytopenia. [6] Studies done on the murine homolog of NGAL (24p3/Ngal) suggest that in leukemias, particularly those where the cells expresses BCR-ABL, 24p3/Ngal is strongly expressed by the malignant cells. [7]

In many studies, no correlation was noted between NGAL expression in the blood and any other hematologic parameters. [8]

This study was planned to assess the NGAL levels in CML cases and healthy controls and find the correlation between NGAL levels with age, haemoglobin and platelet counts.

Aims and objectives:

To estimate the levels of NGAL in 30 CML cases and 30 healthy controls.

To assess the correlation between NGAL level with Age, Hemoglobin and Platelet count of cases before and after chemotherapy.

MATERIALS AND METHODS

This case control study was carried in Department of Biochemistry with Department of Medicine, PGIMS, Rohtak over a period of 1 year. 30 Chronic myeloid leukaemia patients along with 30 healthy age and sex matched controls were enrolled for the study. Diagnosis was made on the basis of history, clinical examination, total and differential leukocyte count, and cytogenetic studies. Chemotherapy given consisted of imatinib (400 mg/day). Complete haemogram, routine biochemistry and Serum NGAL were estimated in all subjects. Follow up of cases was done at interval of 3 months.

Sample collection and storage:

Venous blood samples were collected aseptically from fasting subjects in purple and red capped vacutainers for the investigations. Serum was separated and stored at -20°C till further analysis.

Estimation of serum NGAL:

Serum NGAL was estimated by a commercial Enzyme Linked Immunosorbent Assay kit for human NGAL. [9] The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human neutrophil gelatinase-associated lipocalin（NGAL）in samples.

Statistical analysis:

Microsoft excel was used for analysis of data. Paired ‘t’ test was used for finding statistical significance of change of parameters (NGAL) before and after chemotherapy. For comparison between cases and controls, independent ‘t’ test was used. Correlation coefficient was calculated between NGAL levels and age, haemoglobin and platelet counts of cases.

OBSERVATIONS AND 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 male and 13 female patients. Male to female ratio was 1.3:1. No correlation was observed in age of controls and NGAL levels while a very weak positive correlation was found between age of cases and NGAL values ($r = 0.32$ post treatment and $r = 0.26$ pre treatment).

Serum NGAL was significantly higher in CML patients (358.47 ± 125.65) as compared to serum NGAL in controls (44.5 ± 15.65) ($p=0.001$). 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) ($p=0.001$).

There is a weakly positive correlation between NGAL and hemoglobin ($r=0.40$, $p=0.028$). NGAL is positively correlated with TLC and this correlation is statistically significant ($r=0.964$, $p=0.001$). It is also positively correlated with platelet count and this correlation is statistically significant ($r=0.951$, $p=0.001$). NGAL is also positively correlated with blast percentage and this correlation is also statistically significant ($r=0.945$, $p=0.001$).

DISCUSSIONS

Thirty patients of chronic myeloid leukemia and thirty age and sex matched healthy controls were taken up for study. The diagnosis was made by history, clinical

examination, total and differential leukocyte count, and cytogenetic studies. CML patients were treated by imatinib (400 mg/day) therapy. Routine biochemistry, complete hemogram, serum NGAL were performed in newly diagnosed patients and in thirty age and sex matched healthy controls. Follow up was done at 3 months in Department of Medicine and Biochemistry.

The patients belonged to various age groups ranging from 28-70 years. Most of the patients (90%) were of age between 21-60 years. It is similar to study done by Kantarjian et al. [10] Sawyers found that CML affects all age groups including children. [11]

The median age of CML patients in present study at presentation was 39 years (range 28-70 years) which was in accordance with previous studies. [12, 13, 10, 11] No correlation was observed in age of controls and NGAL levels while a very weak positive correlation was found between age of cases and NGAL values (r = 0.32 post treatment and r = 0.26 pre treatment).

Anemia was detected in 3 (10%) patients at the time of presentation. The mean Hb of patients at presentation was 11.34 g% ± 1.17 (range 8-12.9 g%). Most of the patients (90%) had hemoglobin >10 g/dL at the time of presentation. There is a weakly positive correlation between NGAL and hemoglobin (r=0.40, p=0.028) in the current study. Manero et al. observed anemia in 20% of patients. [12] Kantarjian et al. reported median hemoglobin level in chronic phase of CML as 12.3 g% and anemia (Hb<10 g%) in only 3% of patients. [10] Since in our study, all patients presented in chronic phase, our finding is comparable with previous literature.

In present study, patients presented with platelet count in range of 20 X 10^9 – 230 X 10^9. 16 patients (53.3%) of CML had normal platelet count at the time of presentation and 14 patients (46.7%) presented with thrombocytosis. Mean platelet count at time of presentation was 367±124.395. It is also positively correlated with platelet count and this correlation is statistically significant (r=0.951, p=0.001). Wetzler and Bloomfield reported that platelet count is almost always elevated at the time of diagnosis. [16] Manero et al. reported that thrombocytosis is observed at presentation in 30-50% of patients with CML. [12] Kantarjian et al. reported the median count as 375x10 ^{9}/L with thrombocytosis in 40% of patients. [10] Our finding is in accordance with previous studies in literature.

In present study serum NGAL levels were significantly increased in CML patients (358.47 ng/ml ± 125.65) as compared to controls (44.5 ng/ml ± 15.65) (p=0.001).After treatment, serum NGAL values decreased (85.03 ng/ml ± 62.77) (p=0.001).

In 2011 Alonci et al. found that median serum NGAL levels in CML patients at diagnosis (402 ± 181 ng/ml) were significantly higher compared to age matched controls (199 ± 108 ng/ml) (p<0.01). [14] In2005, Lin et al. demonstrated that NGAL expression in BCR-ABL+ cells requires 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. [15]

In 2011 Alonci et al. conducted a study on 22 patients in chronic phase (9 men, 13 women) and 10 healthy subjects. None of the patients and control subjects had symptoms of active infections, inflammatory diseases or kidney failure. The eligibility criteria included chronic-phase CML at diagnosis and no prior therapy for CML. Imatinib was administered orally 400 mg daily. 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±181 ng/ml, and thus were significantly higher compared to age matched controls (199±108 ng/ml) (p<0.01). After imatinib therapy, all patients achieved a complete molecular remission, and NGAL

levels decreased (151.70±47 ng/ml) ($p<0.01$). [14] In this study, decrease in serum NGAL levels after treatment was 62.26% [14] but in our study, it was 76.27%.

CONCLUSION

NGAL is a potential biomarker for not only kidney diseases but chronic myeloid leukaemia. It is positively correlated with Platelet count of cases and weakly correlated with hemoglobin levels.

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How to cite this article: Mehta S, Kumari A, Dalal D et.al. Correlations of NGAL with age, haemoglobin and platelet counts in chronic myeloid leukemia cases and controls. International Journal of Science & Healthcare Research. 2018; 3(4): 279-282.

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