ORIGINAL ARTICLE

FREQUENCY OF RAISED SERUM LACTATE DEHYDROGENASE IN PATIENTS WITH JAK2 POSITIVE POLYCYTHAEMIA VERA

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Background: Serum Lactate Dehydrogenase (LDH) is an easily available bio marker used to determine prognosis in various Myeloproliferative Neoplasms (MPN). Its utility in Polycythaemia Vera (PV) is yet to be accessed. The purpose of this study was to determine the frequency of raised serum LDH in patients with JAK2 V617F positive PV patients and its clinic-pathological association. **Methods:** A cross-sectional study using non probability consecutive sampling was conducted at our institute from July 2018 to June 2019. Adult patients of either gender, newly diagnosed with JAK-2 V617F positive PV were included. Patients' demographics, clinical characteristics and baseline CBC and LDH levels were analysed. Stratification was done with regards to age, gender, diabetes mellitus, hypertension, splenomegaly and thrombosis to see the effect of these modifiers on patients with raised LDH by using Chi Square test. *p-value*≤0.05 was considered as significant. **Results:** Forty patients were inducted in the study with male to female ratio of 2:1. Twenty-two (55%) patients had raised LDH levels and showed significant association with diabetes mellitus (p=0.001), splenomegaly (p=0.001) and thrombosis (p=0.018). **Conclusion:** This study observed raised LDH levels in almost half of JAK2 V617F positive PV patient. It warrants a larger scale study and suggests the value of plasma LDH to be used as a future prognostic marker in PV.

Keywords: Serum lactate dehydrogenase; Polycythaemia Vera; Diabetes mellitus; Hypertension; JAK 2 V617F mutation; Myeloproliferative Neoplasm; Pakistan

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INTRODUCTION

Polycythaemia Vera (PV) is an idiopathic chronic clonal disorder of hematopoietic stem cells that belongs to Myeloproliferative Neoplasms (MPN) characterized by proliferation of one or more myeloid lineages.[1] It is caused by acquired mutations; most common is a single nucleotide change, JAK2 V617F expressed within the erythroid progenitors. It promotes EPO independence and hypersensitivity of PV erythroid colonies which increase their proliferation and cause accumulation of erythrocytes.[2] Such mutations occur in almost 95% PV cases.[3] PV has a relatively low occurrence in general population. The incidence per annum shows a wide variation ranging from 0.01 to 2.61/100,000 as reported by various studies.[4,5]

Polycythaemia Vera is characterized clinically by splenomegaly, vasomotor disturbances, pruritus and a risk of progression of disease to acute myeloid leukaemia or myelofibrosis.[6] Arterial and venous thrombosis and haemorrhagic complications in these patients are common and a major cause of mortality.[7] As per the International Working Group for Myeloproliferative Neoplasms Research and Treatment (IWG-MRT) old age, leucocytosis, venous thrombosis and abnormal karyotype are the risk factors recognized for adverse outcome among PV patients.[8]

Current researches have drawn parallels between elevated serum lactate dehydrogenase (LDH) and short survival in leukaemia free Primary Myelofibrosis (PMF) and Essential Thrombocythemia (ET).[9,10] LDH is a routinely measured biological marker which is used to determine prognosis in both non-neoplastic and malignant conditions.[11–13] It is also included as one of the minor criteria for diagnosing PMF.[14] LDH acts under anaerobic conditions by excessively converting pyruvate to lactate which induces the proliferation of oxygenated malignant cells, angiogenesis, and inhibits the innate and adaptive immune responses.[15] The aim of our study was to see if elevated LDH levels can be used as a surrogate marker to assess disease burden and aggression in PV patients due to its low cost and ease of access.

MATERIAL AND METHODS

It was a descriptive cross-sectional study conducted at the department of Haematology, Liaquat National Hospital & Medical College Karachi from Jul 2018 to Jun 2019. The non-probability consecutive technique was employed for sample collection. All adult patients of either gender newly diagnosed with JAK-2 V6175 mutation positive status PV were enrolled. PV was diagnosed as per WHO guidelines as haemoglobin (Hb) >16.5g/dl or haematocrit (HCT)> 0.49 in males and Hb>16.0g/dl or HCT >0.48 in females or increase red cell mass more than 25% above mean normal predicted value, in the absence of other causative factors.[1] Patients

with MPN other than PV and secondary causes of PV such as obesity hypoventilation syndrome, obstructive sleep apnoea, chronic obstructive pulmonary disease (COPD), congenital cyanotic heart disease etc. were excluded. The study was conducted after taking approval from Ethical Review Committee of hospital. Informed consent was taken from patients included in the study. All data was collected by researcher himself; ensuring confidentiality. Data regarding patient's demographics (name, age, gender), base line laboratory findings (Hb, HCT, TLC, PLT and LDH levels) and clinical findings (i.e., diabetes mellitus, hypertension, splenomegaly and any other thrombotic manifestations) were recorded on a proforma (Appendix-A). The effect modifiers and biasness were strictly controlled by inclusion and exclusion criteria. Two ml blood sample was collected in EDTA tube for haematological parameters and determined by Automated Cell Dyne Ruby Counter (Abbott, Diagnostics). 4 ml blood was collected in lithium heparin tube for LDH levels. The samples were centrifuged at 2000rpm for 10 minutes and serum separated which was used for LDH estimation. LDH was performed by DGKC[16] method on Cobas-C501 (Roche Diagnostics) by spectrophotometric assay technique. 480U/L was taken as reference value. Samples with LDH level >480U/L were considered as raised. Data was analyzed using SPSS windows version 22. Mean and standard deviation were computed for quantitative variables like age, Hb, HCT, TLC, PLT and LDH levels. Frequency and percentage were calculated for qualitative variables, i.e., gender, diabetes mellitus, hypertension, splenomegaly and thrombosis. Stratification was done with regards to age, gender, diabetes mellitus, hypertension, splenomegaly and thrombosis to see the effect of these modifiers on outcome (raised level of LDH) by using Chi Square test. *p-value* ≤0.05 was considered as significant.

RESULTS

Among 40 patients included in this study, 27 patients were males and 13 females in a ratio of 2:1. Mean age was 59.20±13.98years. Majority of patients (29/40; 72.5%) were >50 years. Out of 40 patients, HTN was the most common comorbid condition (92.5 %). 52.5% patients had palpable splenomegaly at presentation and 30% had a history of thrombotic event. (22/40; 55%) of patients were found with raised serum LDH as presented in Table-1. Among 22 patients with raised serum LDH, mean haemoglobin was 18.45±1.36 g/dL and mean HCT was 57.12±3.95% as presented in table-2. We found significant association of raised serum LDH with diabetes mellitus (p=0.001), splenomegaly (p=0.001) and thrombosis (p=0.018) among PV patients. (Table-3).

Table-1: Baseline characteristics of study variables

Quantitative Variables	**Mean**	**SD**
Age (years)	59.20	13.98
Haemoglobin (g/dl)	18.15	1.66
Haematocrit (%)	55.14	5.52
Total Leukocyte Count($\times 10^9$/L)	16.71	11.05
Platelet Count($X10^9$/L)	518.48	290.32
Serum LDH (U/L)	438.45	96.30
Qualitative variables	**n**	**%**
Gender		
Male	27	67.5
Female	13	32.5
Hypertension		
Yes	37	92.5
No	3	7.5
Diabetes Mellitus		
Yes	18	45.0
No	22	55.0
Splenomegaly		
Yes	21	52.5
No	19	47.5
Thrombosis		
Yes	12	30
No	28	70
Raised Serum LDH		
Yes	22	55
No	18	45

Table-2: Descriptive statistics of patients with raised LDH (n=22)

	Minimum	**Maximum**	**Mean**	**SD**
Age (years)	28.00	76.00	57.72	15.66
Haemoglobin (g/dl)	14.00	21.20	18.45	1.36
Haematocrit (%)	50.80	65.00	57.12	3.95
Total Leukocyte Count($\times 10^9$/L)	6.40	47.00	17.92	12.94
Platelet Count($X10^9$/L)	187.00	1241.00	515.50	256.99
Serum LDH (U/L)	480.00	564.00	509.09	25.16

Table-3: Stratification of raised LDH level with respect to effect modifiers

Variables	**Raised LDH n(%)**			***p*-value**
Age groups	**Yes (n=22)**	**No (n=18)**	**Total**	
<=50 years	7 (63.6)	4 (36.4)	11	0.499
>50 years	15 (51.7)	14 (48.3)	29	
Gender				
Male	16 (59.3)	11 (40.7)	27	0.435
Female	6 (46.2)	7 (53.8)	13	
Hypertension				
Yes	20 (54.1)	17 (45.9)	37	0.673
No	2 (66.7)	1 (33.3)	3	
Diabetes mellitus				
Yes	16 (88.9)	2 (11.1)	18	0.001
No	6 (27.3)	16 (72.7)	22	
Splenomegaly				
Yes	17 (81)	4 (19)	21	0.001
No	5 (26.3)	14 (73.7)	19	
Thrombosis				
Yes	10 (83.3)	2 (16.7)	12	0.018
No	12 (42.9)	16(57.1)	28	

DISCUSSION

High LDH levels are known to predict a worse overall survival among PV and ET patients.[17] Current researches are focused on finding novel molecular mutations associated with poor survival in such patients.[18] In resource constraint regions where costly investigations are not an option, cheaper tests like LDH are being reconsidered as an alternative prognostic marker.

The major finding of our study was elevated LDH levels seen in 22/40(55%) patients. Although the mean LDH level of our study population was within normal range; those with elevated LDH showed a median value of 509U/L. Comparison with international data shows significant variations in LDH levels. Tefferi *et al* have reported similar figures, i.e., 51% among a cohort of 216 PV cases and values above 1000U/L were associated with high TLC and increased blast counts.[19] An international study conducted in seven centers in three countries showed increased LDH values among 50% patients with PV.[20] High median LDH levels 353 IU/L(normal values: 135–214 IU/l) were seen among 71 patients reported as MPN Unclassifiable by Gianelli *et al*[21] Contrary to these reports, Mazzota S *et al* found no significant association between LDH activity and isoenzyme distributions in patients with PV.[22] Studies from South Asia on this topic are limited. Interestingly an earlier study published from this center showed a higher median LDH level (552.7±309.2) among PV patients; however, percentage of raised LDH levels were not mentioned.[23] Our study population included only JAK2 V617F positive PV cases whereas theirs had both Jak2 positive and negative PV patients, hence the inadvertent inclusion of JAK2 negative pre-fibrotic PMF patients with high LDH levels may be responsible for this ambiguity.

Tendency of thrombosis in MPN including PV is enhanced due to clonally abnormal cells releasing increased mediators of coagulation thus putting these patients at a higher risk of mortality.[24] This fact has been concurred by Bonicelli and Kreher *et al* who reported a prevalence of 21% and 11–39% respectively.[8,25] Multiple studies have quoted higher incidence of arterial thromboses than VTE(16% vs 7.4%, 23.1%vs 4.4%, 18%vs 1.5%).[20,26,27] Although stratification into arterial and venous thromboses was not done by us, we report a higher frequency of thrombo-embolic events (30%) which was statistically significant (*p*-value =0.018). These figures are twice than the ones reported from Pakistan as 11% and 14.3%.[23,28] These results need to be validated as it potentially puts our PV population at a higher risk of adverse events.

We found a higher incidence of enlarged spleen (52.5%) on clinical and or ultrasound examination with 17 patients having elevated LDH levels (p=0.001) Splenomegaly is a common finding seen among PV patients with frequencies ranging between 10–40%.[19,20,23,27,29] This correlation further favours our hypothesis of using LDH as a surrogate marker.

Overall Hypertension was the leading comorbid condition but Diabetes Mellitus was statistically associated with raised LDH (*p*-value=0.001). Literature review shows increase incidence of HTN than diabetes but risk of arterial thrombosis was found to be significantly high (*p*-value =0 .030) in the latter group.[20,30]

CONCLUSION

Twenty two out of 40 JAK-2 positive PV patients were found with elevated serum LDH levels which were significantly associated with thrombo-embolic events at presentation, enlarged spleen and presence of Diabetes Mellitus. These results differ from previously reported findings from this region. This study paves the way for future researches for the possibility of including serum LDH as an independent prognostic marker in JAK2 V617F positive PV patients.

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AUTHORS' CONTRIBUTIONS

SZS collected the data, did literature search and drafted the manuscript. NR been involved in drafting the manuscript, revising it critically, gave final approval and revision of manuscript. MI was involved in statistical data analysis and interpretation. All authors read and approved the final manuscript.

REFERENCES

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, *et al*. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127(20):2391–405.
2. Zivot A, Lipton JM, Narla A, Blanc L. Erythropoiesis: insights into pathophysiology and treatments in 2017. Mol Med 2018;24(1):11.
3. Scott LM, Linda M. The molecular genetics of polycythemia vera. Advances in Genetic Research. 16th ed. New York, NY, United States: Nova Science Publishers, 2016; p.101–22.
4. Titmarsh GJ, Duncombe AS, McMullin MF, O'Rorke M, Mesa R, De Vocht F, *et al*. How common are myeloproliferative neoplasms? A systematic review and meta-analysis. Am J Hematol 2014;89(6):581–7.
5. Orphanet. Polycythemia vera 2019. [Internet]. [cited 2020 Aug]. Available from: https://www.orpha.net/consor/cgi-bin/OC_Exp.php?Expert=729
6. Passamonti F, Rumi E, Pungolino E, Malabarba L, Bertazzoni P, Valentini M, *et al*. Life expectancy and

prognostic factors for survival in patients with polycythemia vera and essential thrombocythemia. Am J Med 2004;117(10):755–61.
7. Stefano V, Za T, Rossi E, Vannucchi AM, Ruggeri M, Elli E, *et al*. Recurrent thrombosis in patients with polycythemia vera and essential thrombocythemia: incidence, risk factors, and effect of treatments. Haematologica 2008;93(3):372–80.
8. Bonicelli G, Abdulkarim K, Mounier M, Johansson P, Rossi C, Jooste V. Leucocytosis and thrombosis at diagnosis are associated with poor survival in polycythaemiavera: a population-based study of 327 patients. Br J Haematol 2013;160 (2):251–4.
9. Shah S, Mudireddy M, Hanson CA, Ketterling RP, Gangat N, Pardanani A, *et al*. Marked elevation of serum lactate dehydrogenase in primary myelofibrosis: clinical and prognostic correlates. Blood Cancer J 2017;7(12):657.
10. Mudireddy M, Barraco D, Hanson CA, Pardanani A, Gangat N, Tefferi A. The prognostic relevance of serum lactate dehydrogenase and mild bone marrow reticulin fibrosis in essential thrombocythemia. Am J Hematol 2017;92(5):454–59.
11. Horvat I, Boban A, Zadro R, Antolic MR, Serventi-Seiwerth R, Roncevic P, *et al*. Influence of Blood Count, Cardiovascular Risks, Inherited Thrombophilia, and JAK2 V617F Burden Allele on Type of Thrombosis in Patients With Philadelphia Chromosome Negative Myeloproliferative Neoplasms. Clin Lymphoma Myeloma Leuk 2019;19(1):53–63.
12. Yadav C, Ahmad A, D'Souza B, Agarwal A, Nandini M, Ashok PK, *et al*. Serum Lactate Dehydrogenase in Non-Hodgkin's Lymphoma: A Prognostic Indicator. Indian J Clin Biochem 2016;31(2):240–2.
13. Chen C, Zhu YH, Huang JA. Clinical evaluation of potential usefulness of serum lactate dehydrogenase level in follow-up of small cell lung cancer. J Cancer Res Ther 2018;14(Suppl):S336–40.
14. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, *et al*. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. Lyon, France: IARC, 2008; p.40–53.
15. Deme D, Telekes A. Prognostic importance of lactate dehydrogenase (LDH) in oncology. Orv Hetil 2017;158(50):1977–88.
16. Roche diagnostics. Cobas-C501 manual (available at https://diagnostics.roche.com/global/en/products/instruments/cobas-c-501.html)
17. Cherry M, Cardenas-Turanzas M, Pham H, Kantarjian H, Cortes J, Pierce S, *et al*. Patients with polycythemia vera and essential thrombocythemia with prior malignancy do not havesignificantly worseoutcome. Leuk Res 2013;37(11):1472–1476.
18. Tang G, Hidalgo Lopez JE, Wang SA, Hu S, Ma J, Pierce S, *et al*. Characteristics and clinical significance of cytogenetic abnormalities in polycythemia vera. Haematologica 2017;102(9):1511–8.
19. Tefferi A, Barraco D, Cerquozzi S, Lasho TL, Hanson CA, Ketterling RP, *et al*. Identification of Serum Lactate Dehydrogenase (LDH) As an Independent Prognostic Biomarker in Polycythemia Vera. Blood 2016;128(22):3111.
20. Tefferi, A, Rumi E, FinazzG , Gisslinger H, Vannucchi AM, Rodeghiero F, *et al*. Survival and prognosis among 1545 patients with contemporary polycythemia vera: an international study. Leukemia 2013;27(9):1874–81.
21. Gianelli U, Cattaneo D, Bossi A, Cortinovis I, Boiocchi L, Liu YC, *et al*. The myeloproliferative neoplasms, unclassifiable: clinical and pathological considerations. Mod Patho 2017;30(2):169–79.
22. Mazzotta S, Guerranti R, Gozzetti A, Bucalossi A, Bocchia M, Sammassimo S, *et al*. Increased serum lactate dehydrogenase isoenzymes in Ph-negative chronic myeloproliferative diseases: a metabolic adaptation? Hematology 2006;11(4):239–44.
23. Sultan S, Irfan SM, Murad S. Clinico-Epidemiological Profile of Patients with Polycythaemia Rubra Vera - a Five Year Experience from a Tertiary Care Center. Asian Pac J Cancer Prev 2016;17(3):1531–3.
24. Barbui T, Finazzi G, Falanga A. Myeloproliferative neoplasms and thrombosis. Blood 2013;122(13):2176–84.
25. Kreher S, Ochsenreither S, Trappe RU, Pabinger I, Bergmann F, Petrides PE, *et al*. Prophylaxis and management of venous thromboembolism in patients with myeloproliferative neoplasms: consensus statement of the Haemostasis Working Party of the German Society of Hematology and Oncology (DGHO), the Austrian Society of Hematology and Oncology (ÖGHO) and Society of Thrombosis and Haemostasis Research. Ann Hematol 2014;93(12):1953–63.
26. Kaifie A, Kirschner M, Wolf D, Maintz C, Hanel M, Gattermann N, *et al*. Bleeding, thrombosis, and anticoagulation in myeloproliferative neoplasms (MPN): analysis from the German SAL-MPN-registry. J Hematol Oncol 2016;9:18.
27. Vaidya R, Gangat N, Jimma T, Finke CM, Lasho TL, Pardanani A, *et al*. Plasma cytokines in polycythemia vera: phenotypic correlates, prognostic relevance, and comparison with myelofibrosis. Am J Hematol 2012;87(11):1003–5.
28. Shaikh MS, Shaikh MU, Adil SN, Khurshid M, Ahmed ZA. Clinico-pathological profile and outcomes of patients with polycythaemiavera, essentialthrombocythaemia and idiopathic myelofibrosis: a tertiary care center experience from southern Pakistan. J Ayub Med Coll Abbottabad 2016;28(2):293–7.
29. Duangnapasatit B, Rattarittamrong E, Rattanathammethee T, Hantrakool S, Chai-Adisaksopha C, Tantiworawit A, *et al*. Clinical manifestations and risk factors for complications of Philadelphia chromosome-negative myeloproliferative neoplasms. Asian Pac J Cancer Prev 2015;16(12):5013–8.
30. Horvat I, Boban A, Zadro R, Antolic MR, Serventi-Seiwerth R, Roncevic P, *et al*. Influence of Blood Count, Cardiovascular Risks, Inherited Thrombophilia, and JAK2 V617F Burden Allele on Type of Thrombosis in Patients With Philadelphia Chromosome Negative Myeloproliferative Neoplasms. Clin Lymphoma Myeloma Leuk 2019;19(1):53–63.

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