The Correlations between *JAK2*^{V617F} Mutational Status and Serum Levels of Folate and B12 in a Group of Patients with Chronic Myeloproliferative Neoplasms

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ABSTRACT:

BACKGROUND:

The Philadelphia-negative classical chronic myeloproliferative neoplasms are characterized by proliferation of one or more cell lines in the bone marrow and increased number of mature and immature cells in the peripheral blood. These myeloproliferative neoplasms include; polycythemia vera, essential thrombocythemia and idiopathic myelofibrosis, in which *JAK2*^{V617F} is by far the most prevalent mutation. Because of the rapid turnover of cells in these neoplasms, derangement in the serum levels of vitamin B12 and folate is expected.

OBJECTIVE:

To assess the possible correlation of serum B12 and folate levels with *JAK2*^{V617F} mutation in patients with polycythemia vera, essential thrombocythemia and idiopathic myelofibrosis.

PATIENTS AND METHODS:

This case-control study was conducted from December 2012 to December 2013, and enrolled 54 patients, diagnosed as polycythemia vera (36), essential thrombocythemia (6) and idiopathic myelofibrosis (12), attending Medical City, Baghdad Teaching Hospital. Twenty healthy volunteers were included as a control group. *JAK2*^{V617F} mutation status had been reviewed at time of sampling. The following investigations were done: automated complete blood counts; serum B12 and folate were measured using the electrochemiluminescence immunoassay.

RESULTS:

The mean levels of serum B12 and folate were much higher in myeloproliferative neoplasm patients than control group (p-value of 0.028 and 0.004 respectively).

Serum B12 level showed significant difference between polycythemia vera patients with positive *JAK2*^{V617F} and negative mutation (p= 0.04), while no significant difference was found for serum folate level (p= 0.630). Insignificant differences were also found for idiopathic myelofibrosis patients (B12, p= 0.140; and serum folate, p= 0.098), and essential thrombocythemia patients (B12, p= 0.133; and serum folate, p= 0.800).

Correlations of serum folate and B12 with hematocrit, white blood cell count, absolute neutrophil count, platelet count, mean cell volume, and mean cell hemoglobin in myeloproliferative neoplasm patients: significant correlations were only found in PV patients between B12 and total white blood cell count, absolute neutrophil count and platelet count (p= 0.009, 0.012 and 0.002 respectively).

CONCLUSION:

There is derangement in the levels of serum B12 in patients with polycythemia vera in relation to *JAK2*^{V617F} mutation, thus we may propose that this mutation may have a possible impact on polycythemia vera patients, in particular, reflected by higher levels of serum B12 through the associated increase in absolute neutrophil count.

KEYWORDS: myeloproliferative neoplasms, B12, Folate, *JAK2*^{V617F} mutation.

INTRODUCTION:

Myeloproliferative neoplasms (MPNs) are group of closely related clonal disorders of hemopoiesis characterized by proliferation of one or more cell lines in the bone marrow and increased number of mature and immature cells in the peripheral blood. The Philadelphia-negative MPNs classically include three disorders; Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Idiopathic Myelofibrosis (IMF).[1,2]

Since the discovery of *JAK2*^{V617F} mutation the understanding of the pathogenesis of these disorders has been greatly revolutionized. *JAK2*^{V617F} is by far the most prevalent mutation in Philadelphia-negative classical MPNs; it occurs in about 95% of patients with PV, in 55%

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of patients with ET and in 65% of patients with IMF.[3,4]

Because of the rapid turnover of cells in those disorders derangement in the levels of vitamin B12 and folate is expected. Normal to high serum vitamin B12 concentrations have often been reported in many studies,[5] on the other hand deficiency of folic acid, and vitamin B12, alone or in combination, have been also reported in association with MPNs[6] where a true deficiency of B12 can be masked by an elevated serum level. In fact some studies have reported that measurement of homocysteine levels is more accurate in assessing folate or B12 deficiency in patients with MPN.[7]

AIM OF THE STUDY:

The current study aims to assess the possible correlation of serum B12 and folate levels with *JAK2*^{V617F} mutation in patients with PV, ET and IMF.

PATIENTS, MATERIALS AND METHODS:

This case-control study was conducted during the period from December 2012 till December 2013; the study enrolled 54 MPN patients attending the Hematology outpatient clinic of Baghdad Teaching Hospital, Medical City in Baghdad. Patients with PV (36) and IMF (12) were diagnosed according to the criteria of British Committee for Standardization in Haematology (BCSH),[8] and 6 patients were diagnosed as ET according to the Polycythemia Vera Study Group (PVSG) criteria.[9] In addition; 20 sex and age-matched healthy volunteers were included as a control group. All clinical and laboratory data of patients had been reviewed at time of sampling including *JAK2*^{V617F} mutation ratio.

From each patient 5 ml of blood was aspirated and collected into K_2EDTA-containing tubes for complete blood counts, and in a plain tube for serum B12 and folate. Serum was separated immediately and stored at -80°C till testing within 3 weeks.

Serum B12 and folate were measured using the electrochemiluminescence immunoassay of Cobas e411 autoanalyzer. The results were determined via a calibration curve which was instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode. Serum B12 normal range according to the manufacturer's instruction was 243–894 pg/ml and for serum folate was 4.6–18.7 ng/ml.

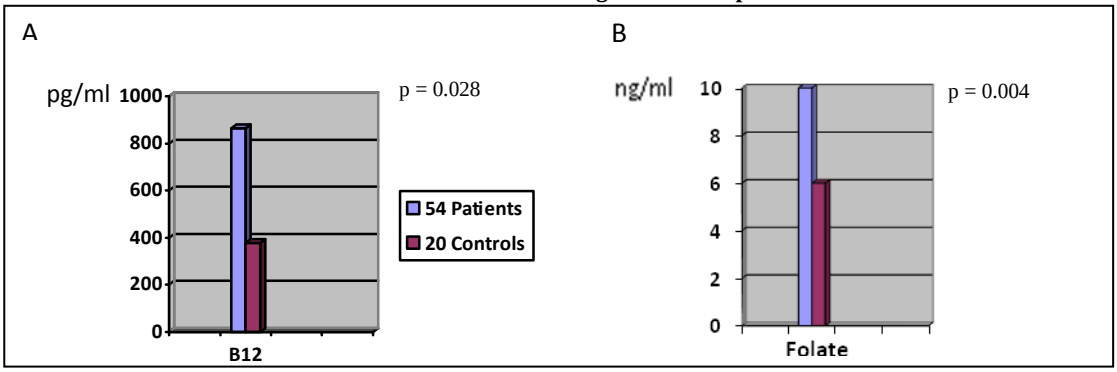
Statistical analysis was done using SPSS version 21. Non-parameteric Mann–Whitney U-test was used to compare continuous data. Spearman's correlation was used to prove correlations between continuous data. Exact test was used in the statistical analysis. P-value <0.05 was considered significant.

RESULTS:

The current study included 54 patients with the diagnosis of MPN (32 males and 22 females) categorized as 36 patients with PV, 6 patients with ET and 12 patients with IMF. Patients with PV and IMF were further divided according to their *JAK2*^{V617F} state into 2 groups (patients positive for *JAK2*^{V617F} and others negative for the mutation).

The mean serum B12 (pg/ml) among all MPN patients was 863 ± 790, while for control group was 377 ± 94. The mean serum folate (ng/ml) for patients was 10 ± 6, whereas it was 6 ± 3 for control group. When non-parametric Mann Whitney U test was done to compare B12 and folate levels between patients and control groups, a statistically significant difference was found with p-value of 0.028 for B12 and 0.004 for folate (Figure 1).

Figure 1: MPN patients and controls mean values



Distribution of serum B12 and folate levels with JAK2^{V617F} status in MPNs patients:

Of the 36 patients with PV; 33 were positive for *JAK2*^{V617F} and 3 were negative. High serum B12 level was only encountered in patients with positive mutation. Eight out of 36 had low serum B12, and in 9/36 the level was higher than the upper normal range while the rest had a normal level (Table 1).

In patients with positive mutation, serum folate was low in 4 patients, and high in only 2 patients while the rest had normal level.

Among 12 patients diagnosed with IMF, 9 of them were positive for *JAK2*^{V617F} mutation and 3 were negative. Only 2 patients had a high serum B12 level and both were among the *JAK2*^{V617F} positive group, one patient had a low B12 who was negative for *JAK2*^{V617F} mutation.

Serum folate was low in 2 patients one was positive and the other was negative for *JAK2*^{V617F}, whereas it was high in 3 patients all of them were positive for *JAK2*^{V617F}.

Six patients, diagnosed as ET, were enrolled in the study four of them were positive for *JAK2*^{V617F} mutation and two were negative. Serum B12 was low in one patient who was negative for *JAK2*^{V617F} and it was high in one patient who was positive for *JAK2*^{V617F}.

Regarding serum folate; low level was found in 5 patients four of them were positive for *JAK2*^{V617F} and only one patient had a high serum folate and was negative for *JAK2*^{V617F}.

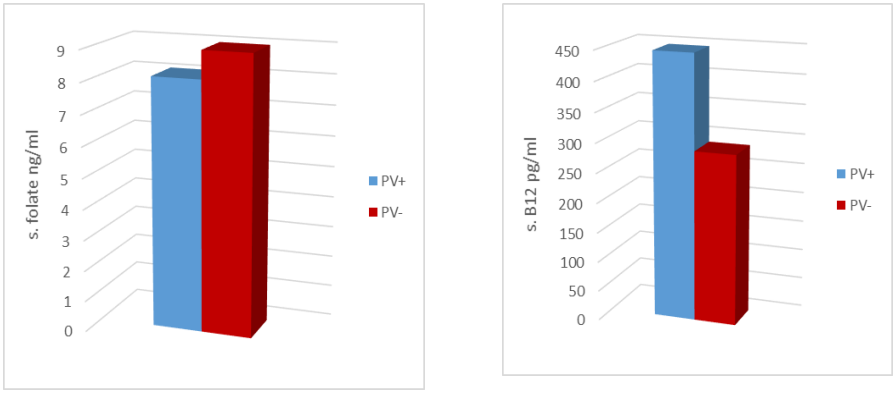
Table 1: Distribution of serum B12 and folate levels with *JAK2*^{V617F} status in 54 MPNs patients.

Serum folate		JAK2^{V617F} State		Total
		Negative	Positive	
PV (n= 36)	Low level	0	4	4
	Normal level	3	27	30
	High level	0	2	2
IMF (n= 12)	Low level	1	1	2
	Normal level	2	5	7
	High level	0	3	3
ET (n= 6)	Low level	1	4	5
	High level	1	0	1
Total		8	46	54

Serum B12		JAK2^{V617F} State		Total
		Negative	Positive	
PV (n= 36)	Low level	1	7	8
	Normal level	2	17	19
	High level	0	9	9
IMF (n= 12)	Low level	1	0	1
	Normal level	2	7	9
	High level	0	2	2
ET (n= 6)	Low level	1	0	1
	Normal level	1	3	4
	High level	0	1	1
Total		8	46	54

Correlation of serum folate and B12 with JAK2^{V617F} status in patients with PV:

When Mann Whitney U test was done to compare the median serum levels of B12 and folate between the 2 groups, significant difference was found between *JAK2*^{V617F} positive and negative patients with PV for serum B12 with a p-value of 0.040 while for serum folate the difference was insignificant with p-value of 0.630 (Figure 2).



p = 0.630

Figure 2:Median levels of serum folate and B12 in patients with PV categorized according to their *JAK2*^{V617F} mutation status.

Correlation of serum folate and B12 with hematological parameters in PV patients:
When Spearman's correlation was used to test for correlations between serum B12 and folate, and various hematological parameters; significant correlations were found between B12 median level and total white blood cell (WBC) count, absolute neutrophil count (ANC) and platelet count (PLT) with p-values of 0.009, 0.012, and 0.002 respectively (Table 2). Hematocrit (Hct), mean cell volume (MCV) and mean cell hemoglobin (MCH) didn't show any significant association. Serum folate displayed no significant correlation with any of the tested hematological parameters.

Table 2:Correlations between B12 and folate, and hematological parameters.

		S. B12	S. Folate	Hct	WBC	ANC	PLT	MCV	MCH
S. B12	Spearman's correlation*	1	-.147	.106	.427	.415	.501	-.088	-.122
	Sig.(2-tailed)		.392	.540	**.009**	**.012**	**.002**	.611	.507
	N	36	36	36	36	36	36	36	36
S. Folate	Spearman's correlation*	-.147	1	.154	.226	.226	.009	-.177	-.062
	Sig.(2-tailed)	.392		.369	.186	.186	.069	. 207	.734
	N	36	36	36	36	36	36	36	36

***** Spearman's correlation coefficient

Correlation of serum B12 and folate with JAK2^{V617F} mutation and hematological parameters in patients with IMF:
When Mann Whitney U test was used to compare the median serum levels of B12 and folate between the 2 groups; insignificant differences were found in patients with IMF positive and negative for *JAK2*^{V617F} mutation with p-values of 0.140 and 0.098 respectively (Figure 3).
When Spearman's correlation was used to test for correlations between serum folate and B12, and the different hematological variables (Hct, WBC, ANC, PLT, MCV, and MCH) no significant correlation was found with any of these parameters.

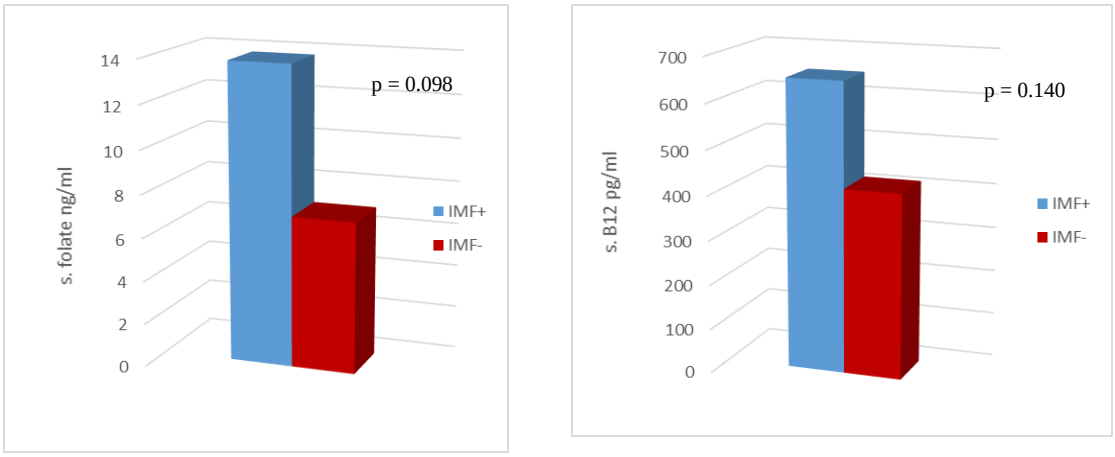


Figure 3: Median levels of serum folate and B12 in patients with IMF categorized according to their *JAK2*^{V617F} mutation status.

Correlation of serum B12 and folate with JAK2^{V617F} mutation and hematological parameters in patients with ET:

For ET patients; Mann Whitney U test showed insignificant difference regarding median level of serum B12 (p= 0.133) and median level of serum folate (p= 0.800) between patients positive and negative for *JAK2*^{V617F} mutation (Figure 4).

Also no significant correlations were found between the serum levels of B12 and folate, and various hematological parameters in ET patients.

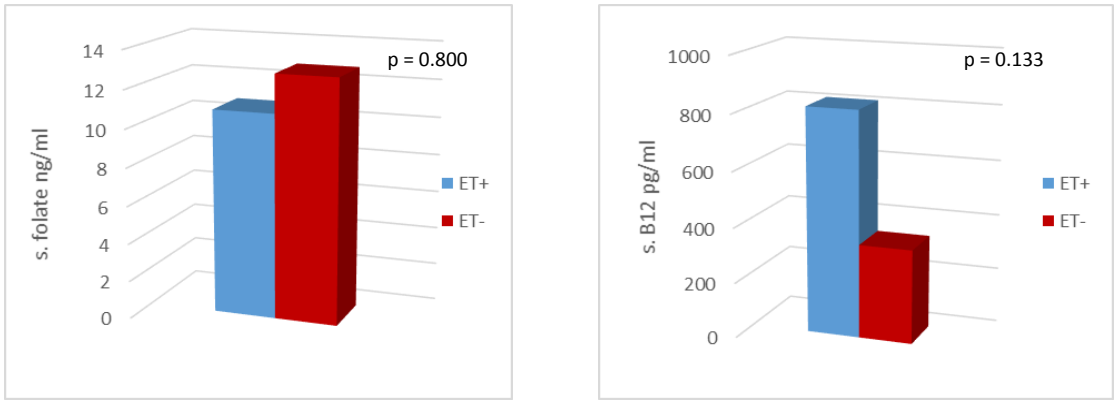


Figure 4: Median levels of serum folate and B12 in patients with ET categorized according to their *JAK2*^{V617F} mutation status.

DISCUSSION:

Derangement in the levels of serum B12 and folate have long been detected in patients with MPNs. Serum B12 had been included in the original PVSG work as a minor criterion for the diagnosis of PV.[10] In this study, the levels of both serum B12 and folate showed statistically significant difference when comparing MPN patients with the control group with p-values of 0.028 and 0.004 respectively. An elevated serum cobalamin (vitamin B12) level in myeloid neoplasms is primarily linked to the release of haptocorrins by the granulocytes and their precursors in these disorders.[11] In the current study; a significant association was found between the median serum level of B12 and *JAK2*^{V617F} mutational status with p-value of 0.04, all the patients with high level of B12 were positive for *JAK2*^{V617F}. Only three of our PV patients did not have a positive *JAK2*^{V617F} and none of them had an elevated serum B12 indicating that, possibly, patients with *JAK2*^{V617F} positive mutation may actually develop a more rapid proliferation of cells as compared to negative ones, as the unique valine to

phenylalanine substitution at position 617 of *JAK2* results in proliferative advantages for hematopoietic precursors.[12] The significant positive correlation of raised serum B12 level, in PV patients in this study, with increased WBC count, ANC and PLT (p-values of 0.009, 0.012, and 0.002 respectively) supports this assumption.

For patients with IMF or ET; no significant statistical association was found between serum B12 or folate levels and *JAK2*^{V617F} mutational status or with the other hematological parameters although the majority of patients with IMF and ET had a normal or elevated serum B12. The small number of patients in both these disorders could have influenced the results or a genuine difference in respect to the pathogenesis of these two disorders might be the reason for this negative finding.

Many of the previous studies have demonstrated marginal depletion of B12 or of folate that can possibly be masked by normal serum levels[13,14] and many of them advise for the use of serum homocysteine or methylmalonic acid (MMA) for accurate assessment of cobalamin content in the body.[15] In the present study; low level of folate was found in 4/36 patients with PV and in 2/12 patients with IMF, and 5/6 patients with ET, while serum B12 was low in 8/36 patients with PV, 1/12 with IMF and 1/6 patient with ET. The present study design does not support the detection of masked deficiencies, therefore no one can assume that some of the MPN patients with normal or even high serum levels of B12 or folate could not possibly harbor a state of deficiency reflected as raised serum homocysteine or methylmalonic acid (MMA) levels, the latter tests are recommended for the early diagnosis of B12 deficiency sufficient to cause neuropathy, even in the absence of subnormal levels of serum B12 or folate or before the appearance of anemia.

Some of the MPN patients with normal or even high serum levels of B12 or folate may have masked deficiency reflected as raised serum homocysteine or methylmalonic acid (MMA) levels which are necessary for the diagnosis of early deficiencies.

The current study demonstrate a significant association between serum B12, and *JAK2*^{V617F} mutational state but it does not allow to conclude that high levels of serum B12 can be used as a reflection of *JAK2*^{V617F} mutational state. Future studies including larger number of patients and measuring other parameters of assessing B12 such as MMA and homocysteine are needed to clarify this issue.

CONCLUSION:

There is derangement in the levels of serum B12 in patients with PV. *JAK2*^{V617F} mutation may have a possible impact on PV patients, in particular, reflected by higher level of serum B12 through the associated increase in WBC count and ANC.

REFERENCES:

1. Campbell PJ, Green AR. The Myeloproliferative Disorders. *N Engl J Med* 2006; 355: 2452-66.
2. Spivak JL and Silver RT. The revised World Health Organization diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis: an alternative proposal. *Blood* 2008; 112: 231-39.
3. McLornan D, Percy M, McMullin MF. JAK2 V617F: A Single Mutation in the Myeloproliferative Group of Disorders. *Ulster Med J* 2006; 75:112-19.
4. Tefferi A. Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: JAK2, MPL, TET2, ASXL1, CBL, IDH and IKZF1. *Leukemia* 2010;24:1128-38.
5. Serraj K, Mecili M, Housni I, and Andr`es E. "Hypervitaminemia B12 (high level of cobalamin): physiopathology, role and interest in clinical practice." *Presse Medicale* 2011;40:1120–27.
6. Gauchan D, Joshi N, Gill AS et al. "Does an elevated serum vitamin B12 level mask actual vitamin B12 deficiency in myeloproliferative disorders?" *Clinical Lymphoma Myeloma and Leukemia* 2012;12: 269–73.
7. Gisslinger H, Rodeghiero H, Ruggeri M et al. Homocysteine levels in polycythaemia vera and essential thrombocythaemia. *British Journal of Haematology* 1999;105:551–55.
8. McMullin MF. The classification and diagnosis of erythrocytosis. *International Journal of Laboratory Hematology* 2008;30:447-59.
9. Murphy S, Peterson P, Iland H, Laszlo J. Experience of the Polycythemia Vera Study with essential thrombocythemia: a final report on diagnostic criteria, survival, and leukemic transition by treatment. *Semin Hematol* 1997;34:29-39.

10. Pearson TC. Diagnosis and classification of erythrocytoses and thrombocytoses. *Baillières Clin Haematol* 1998;11: 695.

11. Andre`s E, Serraj K, ZHU J and Vermorken AJM. The pathophysiology of elevated vitamin B12 in clinical practice. *Q J Med* 2013. doi:10.1093/qjmed/hct 051, downloaded from http://qjmed.oxfordjournals.org/ (accessed 20 November 2013).

12. Kralovics R, Passamonti F, Buser AS et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med* 2005;352:1779-90.

13. Chanarin I. Folate deficiency in myeloproliferative disorders. *American journal of clinical nutrition* 1970;23:855-60.

14. Faurschou M, Nielsen OJ, Jensen MK and Hasselbalch HC. "High prevalence of hyperhomocysteinemia due to marginal deficiency of cobalamin or folate in chronic myeloproliferative disorders." *American Journal of Hematology* 2000;65:136–40.

15. Savage DG, Lindenbaum J, Stabler SP, Allen R. Sensitivity of serum methylmalonic acid and total homocysteine determinations for diagnosing cobalamin and folate deficiencies. *Am J Med* 1994;96:239–46.