INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com
ISSN 2230 – 8407

Research Article

INTERLEUKIN 1β LEVEL AND C- REACTIVE PROTEIN ROLES IN PRIMARY MYELOFIBROSIS PATIENTS TREATED WITH HYDROXYUREA AND RUXOLITINIB

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Article Received on: 25/05/17 Approved for publication: 18/06/17

DOI: 10.7897/2230-8407.08692

ABSTRACT

Primary Myelofibrosis (PMF) is one of development of the myeloproliferative neoplasms that occurs during change in the DNA of a single hematopoietic stem cell. About 50 % of people with MF have an alteration called V617F JAK2 that initiate in the Janus kinases (JAK2) gene. The gene alteration causes unusual signaling in the JAK pathway, which regulates the production of blood cell. There is many theories suggesting the deregulated irritation and immune genes have a role in patients with myeloproliferative neoplasm (MPN). Aim of this study is to characterize the serum levels of IL-1β and C-reactive protein in PMF patients, also to assess their relationship to the treatments and to the spleen size between different patients of PMF received hydroxyurea and ruxolitinib. The study was conducted between November 2014 up to September 2015, during this period 60 Iraqi patients of primary myelofibrosis receiving hydroxyurea for at least 6 months are taken and 30 samples were also taken from healthy persons as control group. Ultarsongraphy of abdomen to assess the spleen size and peripheral blood indices were taken from patient's records at time of sampling. Screening for interleukin 1β level and C-reactive protein were performed to all patients. Out of these 60 patients, 10 patients with high interleukin 1β level and high C-reactive protein switched to received ruxolitinib therapy to determine its effect in correlation with hydroxurea treated patients and control patients. There was significantly lower IL-1β and CRP in control normal group compared to both patients groups. Patients received ruxolitinib treatment had lower IL-1β and CRP compare to hydroxyurea group but it was not statistically significant. The relationship between CRP and IL 1β in all studied group was significant with non-linear relationship between CRP and IL-1β and only patients on hydroxyurea had significant relationship between CRP and IL-1β. In patients with PMF there ishigh level of IL 1βlevel and C reactive protein which may have a role in the pathogenesis of the disease and new treatment of PMF like ruxolotinib may have ability to reduce their levels through controlling the disease pathogensis, further follow-up and larger sample is needed to assess the cytokine levels on long term follow up.

Key words: Primary myelofibrosis, Ruxolitinib, Hydroxyl urea, C-reactive protein, Interleukin-1β.

INTRODUCTION

Primary Myelofibrosis (PMF) is a uncommon bone marrow disease in which the marrow is replaced by fibrous tissue; PMF primarily affects elderly patients [1]. The PMF develop when a change occur in the hematopoietic stem cell in DNA, In myeloproliferative neoplasm(MPN) there is progressive bone marrow fibrosis, extra medullary hematopoiesis, extreme creation of inflammatory cytokines, leading to reduced survival [2-4].

Primary myelofibrosis is characterized by stem cell derivative clonal myeloproliferation, bone marrow fibrosis, abnormal cytokine appearance, it's present with anemia, leukopenia or leukocytosis, and thrombocytopenia or thrombocytosis, characteristically with a leukoerythroblastic peripheral blood smear [5], splenomegaly, extramedullary hematopoiesis, cachexia, constitutional symptoms, leukemic development, and reduced survival [1,5].

A change in V617F JAK2 creates in the Janus kinases (JAK2) gene occurs in fifty percent of public with MF. The gene change resulted in abnormal signaling in the JAK pathway, which regulates the production of blood cell. Gene changes that result in myeloproliferative leukemia (MPL) occur from 5 to10 % of MF patients, which furthermore affects the pathway of JAK signaling. In addition, 5 -15 percent of patients suffering from myeloproliferative neoplasms have mutation in the TET2 gene [6].

Scientists are investigating the role of TET2 mutation, and other gene mutation in additional signaling pathways. The cause of this genetic change that occurs in MF is unknown [6]. A major detection of MF is improved signaling during the JAK-signal transducer and activator of transcription (STAT) pathway, the central mechanism of MF path biology is now recognized by dysregulated of JAK-STAT signaling [7].

Aim of the study

To assess the level and the relationship between serum levels IL-1β and C-reactive protein as inflammatory cascade markers in PMF patients using hydroxyurea and ruxolitinib.

MATERIALS AND METHODS

Patients

The study was conduct between November 2014 up to September 2015; during this period 60 Iraqi patients of primary

myelofibrosis, receiving hydroxyl urea 500-1500mgper day for at least 6 months and another 30 samples were taken from healthy persons as control group were evaluated at prospective multi-center study that permitted by the review ethical committee of haematology.

Spleen size of the PMF patients was recorded from ultrasonography of abdomen from patients records at time of blood sampling and on follow up records.
Peripheral blood sample used to assess interleukin 1β level and C-reactive protein performed to all PMF patients.

Out of these 60 patients we select 10 patients recorded with highly interleukin 1β level and high C-reactive protein switched them to received ruxolitinib to determine its effect in correlation with hydroxyurea treated patients and control patients.

Statistical analysis

Data analysis was approved by use the existing Statistical Packages for Social Sciences-version 20 "PASW" Statistics. The results were expressed as mean ± SD for quantitative variables and qualitative variables are expressed as percentages.
All measures performed in studies relating human participants were in agreement with the ethical standards of the institutional and/or national research committee.

RESULTS

Out of 90samples were included in the study, there was no significant difference in the mean age and gender, the mean age for PMF patients and control group was 59.1 ± 9.5,57.9 ± 0.1 and 53.4 ± 10.9 consequently as illustrated in table 1.

Male: female ratio among control group and PMF patients on hydroxyurea and ruxolitinib groups was 1.3:1, 1.3:1 and 1.5:1 in the 3 groups respectively as illustrated in table 2.

The control group had significantly lower IL-1β and CRP compared to both patients groups who received hydroxyurea and ruxolitinib. Patients on hydroxy urea with high level of IL-1β and CRP shifted to receive ruxolitinib had lower IL-1β and CRP compare to hydroxyurea group but it was not statistically significant as show in figure 1,2 respectively.

Regarding the relationship between CRP and IL 1βin all studied group was significant with non-linear relationship between CRP and IL-1β, this relationship follow 3 stages as illustrated in figure 3.

Only patients who received hydroxyurea had significant relationship between CRP and IL-1β, while in patients used ruxolitinib treatment there was strongest correlation but it was not significant (because very small sample) as illustrated in figure 4.
"Relationship follow 3 stages:1) 0 – 20 CRP there is direct relationship, 2) 20 – 60CRP the relationship inverse, and 3) 60 – 90 the relationship is direct".

In the present study, Ultrasound examination for the spleen size, revealed splenomegaly as principal result detected in all patients with the mean splenic size of 21.17±3.0 cm. Patients of PMF with ruxolitinib treatment had significantly lower spleen size compared to hydroxyurea treatment as illustrated in table 3.

Table 1: Mean age distribution

	All	Control	Hydroxyurea	Ruxolitinib	P- value
Age	57.5 ± 10.1	53.4 ± 10.9	59.1 ± 9.5	57.9 ± 0.1	0.871

One way ANOVA

Table 2: Gender distribution

		Control	Hydroxyurea	Ruxolitinib	Total	P value
Male	No	13	31	4	48	0.967
	%	43.3%	44.3%	40.0%	43.6%	
Female	No	17	39	6	62	
	%	56.7%	55.7%	60.0%	56.4%	

Chi square test

Table 3: spleen size (in cm) for each group

Hydroxyurea	Ruxolitinib	P value
21.59 ± 3.08	16.70 ± 3.23	<0.001

Independent -t- test

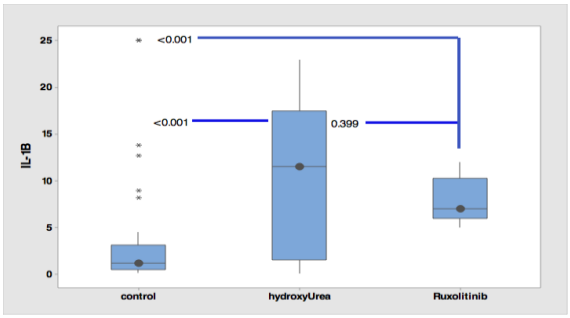

Figure 1: Boxplot of IL-1β for each group

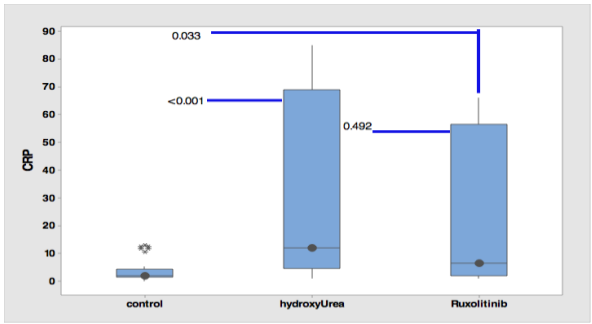

Figure 2: Boxplot of CRP for each group

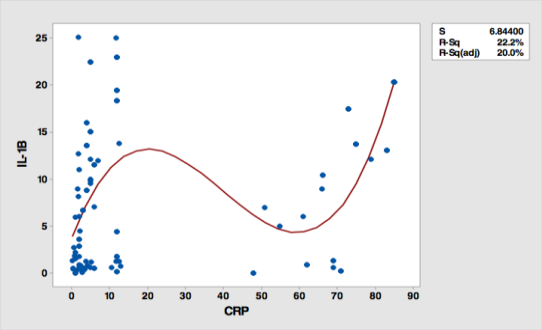

Figure 3: Scatter plot of the CRP and IL-1B for all patients

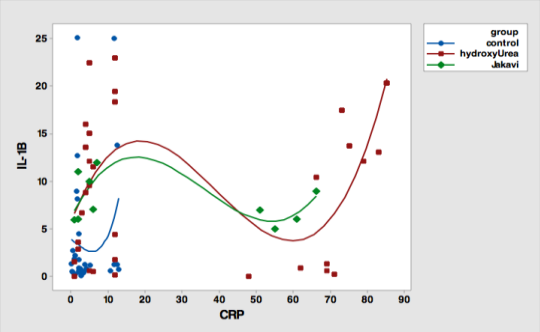

Figure 4: Scatter plot of the relationship between CRP and IL-1B for each group

DISCUSSION

Primary Myelofibrosis is a marrow fibrosis, in which the abnormal clone of hematopoietic stem cells undergoes proliferation. PMF is connected with a bad prognosis in assessment with the further classic BCR/ABL-negative myeloproliferative neoplasms, which may ultimately transform into acute leukemia [8].

This haematological disease is very unusual world broadly, usually develops gradually and was principally observed in people over the age of 50 years and these results are in agreement with studies available in Germany and Sweden, in which the median age were 57 and 55 years respectively [9,10].

The male gender domination was seen in the present study is in agreement with the prior worldwide data recommend equal gender distribution [11]. In conflict to this, the Indian and Thailand studies [12,13]. This dissimilarity could be qualified to genetic and ethnic variations but needs justification in larger population based studies from our county.

They are various clinical manifestation of PMF, patients are usually symptomatic, often with splenomegaly. Splenomegaly occurs in most percentage of patients. Subsequently one study in India reported 78% of their patients disclosed splenomegaly [12], in our study all patients were with splenomegaly.In conflict to Duangnapasatit, reported splenomegaly in 55% [13].

In healthy individuals, the inflammatory cascade is determined by interaction between cellular responses and neurohormonal stimulatory cytokines. Deregulation of this system is a characteristic feature of the PMF. All PMF disorders arise from genetic defects within stem cell populations that accumulate over the disease course. JAK2V617F, a signal transduction pathway in the Janus kinase member, was the recognized mutation in the PMF [14].

In this study, both patients groups who were taking hydroxyurea and ruxolitinib group had significantly higher IL 1βand CRP compared to control group, while those patients on hydroxyurea who had high level ofIL1β and CRP when their treatment shifted to ruxolitinib therapy, there was lower IL1β level and CRP when compare them to hydroxyurea group although it was not statistically significant beside significant decrease in spleen size. However, it was small sample and non significance statistical result but the ruxolitinib therapy may play a role as anti-inflammatory effect in reducing the cytokines release then leading to decreasing size of spleen.

Treatments such as hydroxyurea have been used mainly for the treatment of splenomegaly; on the other hand, their effectiveness was also modest and their use was connected with an increased adverse action [15]. Significantly, usual therapies have not established improvement of legal symptoms such as weakness and have not been exposed to cause in enhanced generally survival or disease adjustment [16]. Our study is comparable to other studies where the initial support of ruxolitinib was based on the consequences of two essential phase III clinical trials, restricted MF study with oral JAK inhibitor management (COMFORT)-I and –II [17,18]. Ruxolitinib reduced inflammatory cytokines circulating levels, eliminated neoplastic cells and prolonged myeloproliferative neoplasm survival [19,20]. The inflammatory cytokines gene might be probable to be up regulated in PMF as well.

C-reactive protein (CRP), which is an indicator of general inflammation formed in hepatocytes subsequent stimulus by cytokines, particularly IL-6, have been establish in MF[21,22]. The elevated levels of CRP could calculate the development to acute leukemia and mortality in patients with principal or post-ET/PV MF [23]. Other study suggests that the CRP level considered as a biomarker to observe treatment with JAK1/JAK2 inhibitors, which have been create to stimulate a rapid reduce of numerous inflammatory cytokines [20].

Findings of widely deregulated irritation and immune genes in patients with MPN are certainly helpful of this theory [24], the potency of counter regulation by anti-inflammatory reaction genes and immune striking genes. The remarkable effectiveness of JAK1-2 inhibitor management in dropping massive splenomegaly and enhance hyper metabolic symptoms in MF may mainly reproduce the extremely effective anti-inflammatory property of the agent [25,26].

However, the deficiency of inflammatory cytokines deregulation in our PMF patients may be explained by compromised immune purpose and immune deficiency resulting to extreme levels of circulating VEGF and TGF-beta–cytokines which in cooperation is greatly immunosuppressive by numerous mechanisms, as well as mutilation of NK-cell and dendritic cell purpose [27–28].

The production of the pro-inflammatory cytokines IL-6, TNF-α, and IL-1β fuel the acute phase rejoinder (APR), that – might be harmful if not quell down via counter regulatory mechanisms [29]. Chronic inflammation is well thought-out of the most important in the progress of cancer, including numerous hematological neoplasms[30], and the majority of newly chronic inflammation has been planned as the initiating occasion and a

driver of clonal development in patients with myeloproliferative neoplasms (MPN) [24].

CONCLUSION

The IL 1β level and C reactive protein may have a part in the pathogenesis of the PMF syndrome and new treatment era of PMF like ruxolotinib may have ability to reduce their levels and block one of the pathogenesis pathway, then controlling disease symptoms. Further follow-up and larger sample is needed to assess the cytokine levels on long term.

ACKNOWLEDGEMENTS

The authors like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq) Baghdad-Iraq for its support in the present work.

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Cite this article as:

Bassam Francis Matti *et al*. Interleukin 1β level and C- reactive protein roles in primary myelofibrosis patients treated with hydroxyurea and ruxolitinib. Int. Res. J. Pharm. 2017;8(6):30-34
http://dx.doi.org/10.7897/2230-8407.08692

Source of support: Nil, Conflict of interest: None Declared