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Thesis Title	**Detection of *JAK2* V617F Mutation and Estimation of Serum Erythropoietin among Blood Donors with High Hematocrit**			
Year	**2014**			
Abstract	**It is not uncommon to observe in blood donors a hematocrit or hemoglobin value above or close to the upper limit of normal, the clinical evaluation and diagnosis may be complicated by regular blood donations that can mask an underlying disease. Such values have been investigated to exclude or confirm the presence of secondary causes of polycythemia as well as a primary myeloproliferative neoplasm such as polycythemia vera.** **True polycythemia is a condition that results in an increased level of circulating red blood cells in the blood stream, which usually manifests itself as raised hemoglobin concentration and\or hematocrit, while relative polycythemia is due to a reduction in plasma volume without an increase in total red cell mass.** **True polycythemia can be further subdivided into two categories; primary and secondary. Patients who cannot be assigned to either primary or secondary polycythemia are grouped together under the category of idiopathic erythrocytosis.** **Erythropoietin is a hemopoietic growth factor that is essential in terminal maturation of erythrocyte precursor to mature erythrocytes. Estimation of serum erythropoietin level could be potentially helpful in distinguishing between the different types of polycythemia. Its production is increased in (anemia) or in (hypoxia).** **Polycythemia vera is a myeloproliferative neoplasm characterized by excessive proliferation of erythroid, myeloid and megakaryocytic elements in the marrow, increased cell count in peripheral blood and increased erythroid mass.** **The cause of Polycythemia vera is not fully understood, almost, all patients with Polycythemia vera have an acquired mutation of the Janus kinase 2 (JAK2) gene on chromosome 9 and is found in the majority of patients with myeloproliferative neoplasm and has become a valuable marker for diagnosis of myeloproliferative neoplasm.**			

However, it has also been found in many other hematological diseases, and some studies even detected the presence of *JAK2* V617F in normal blood samples.

There are various techniques used to detect the genetic mutation underlying *JAK2*V617F, one of them is polymerase chain reaction. Using it; specific sequences within a DNA can be amplified. Quantification of amplified product is obtained using fluorescent probes or fluorescent DNA binding dyes and real-time polymerase chain reaction instruments that measure fluorescence while performing temperature changes needed for the polymerase chain reaction cycles.

Aim of study:

1. **Screening of *JAK2* V617F mutation in blood donors and the impact of the counted mutant ratio on clinical and laboratory parameters.**
2. **To determine the actual need to defer blood donors with high hematocrit.**

Patients and methods:

This prospective case control study was done over a period of 7 months, started on 16th of December 2012 and completed on 8th of July 2013. Venous blood samples from (116) blood donors who attended the National Blood Transfusion Center were collected. All of them were males and their age ranged between 20 and 62 years. Their hematocrit was ≥ 48%. Of the blood donors (22) were excluded; (14) of them had failure in DNA extraction, (8) of them had PCR failure. Also the study included a control group of 20 patients known to have JAK2 V617F positive PV. Those patients were diagnosed according to the BCSH criteria. Fifteen healthy Iraqi individuals run as a control group, to set the highest mutant ratio cutoff value for this Iraqi study, which was 1.3%. The following investigations were done for both the study and the control groups: complete blood count, blood film, serum erythropoietin, and real time PCR.

Results:

1) **The study revealed significant high counted mutant ratio of the *JAK2* V617F in the control group of PV patients versus donors with positive *JAK2* V617F mutation with a mean (%) value of 58 ± 26.9 versus 11.10 ± 12.6, respectively.**
2) **Out of (94) blood donors, (20) had positive *JAK2* V617F mutation, 18/20 (90%) were smokers.**
3) **Regarding the effect of smoking and its amount on the mutation of *JAK2* V617F; as 22.8% (18/79) of smokers were positive for *JAK2* V617F, while 13.3% (2/15) of non smokers showed positivity for the mutation.**
4) **There was insignificant difference for erythropoietin level between positive and negative *JAK2* V617F mutation blood donors groups and between the control group of PV patients**

and the positive *JAK2* V617F donors.

Conclusion:

1. **The frequency of *JAK2* V617F mutation was much higher than that anticipated in blood donors.**
2. **The donated blood from those with upper normal or even slightly elevated Hct does not necessarily denote to complete safety of blood as being devoid of *JAK2* V617F mutation.**
3. ***JAK2* V617F mutation as an exclusive test to diagnose patients with MPN can not be dependable as a surrogate for the known classification for the diagnosis of PV.**
4. **The significance of detecting *JAK2* V617F mutation in completely healthy donors is doubtful.**
5. **Including subnormal EPO among minor diagnostic criteria for PV was not found fruitful in this study.**
6. **No significant clinical or laboratory differences were found between donors who show the *JAK2* V617F mutation and those who are negative for the mutation.**
7. **Pruritis was found in blood donors with high normal or elevated Hct.**
8. **There is 1.7 and 2.24 folds increment in mutation in association with cigarette smoking and when the smoking index being $\geq$10 pack year, respectively.**