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RESEARCHPAPER

Mutational analysis of the Janus kinase II (V617F) gene in patients with β-Thalassemia major

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ABSTRACT:

β-Thalassemia is a group of congenital hemolytic anemia that characterized by the underproduction of the indispensable hemoglobin molecule, the oxygen and carbon dioxide carrying protein inside the red cells. With our current study, we've screened if some extent mutation at Valine 617 Phenylalanine of Janus kinase II genes detectable in β-thalassemia major in our region.

The present study for the screening of JAK II V617F mutation was conducted on (50) patients with β-thalassemia major, restriction fragment length polymorphism with restriction endonuclease enzyme AflIII was used to identify a flaking region of 617 for JAK II gene.

In the existing study, no mutation has been detected within the patients suffering from β-thalassemia major. Our results indicate that *JAK II* ^{V617F} mutation appears to not be associated with thromboembolic complications related to β-Thalassemia and therefore the incidence of β-Thalassemia. To the best of our knowledge, this study is the first evidence to determine the status of *JAK II* ^{V617F} mutation in patients with Thalassemia major in our region and expands the international published literature on it.

KEYWORDS:β-Thalassemia, *JAK II, V617F* mutation, Thromboembolic complications.
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1. INTRODUCTION

Thalassemia is a congenital nonspherocytic hemolytic disorder that arises from β-globin chain abnormality or mutation, requiring regular blood transfusions to sustain life. However, excess body iron overload develops and may accumulate in heart, liver and endocrine glands, leading to progressive failure of those organs (Melchiori, 2010, Sipahi, *et al.*, 2009, Nihad, A. 2016)

It has long been known that chronic hemolytic anemia particularly β-thalassemia, is characterized by a hypercoagulable state (Ali, T *et al*., 2008. patients with hemolytic anemia are implicated in the existence of a prothrombotic hemostatic anomalies, which is found almost in half patients with β-thalassemia (Eldor, *et al.*, 2002, Ataga, 2009, Ahmed, *et al.* 2019).

Furthermore, the manifestation of thromboembolic complications, including recurrent arterial occlusion, venous thromboembolism which resulting in deep venous thrombosis, pulmonary embolism and stroke (Ataga, 2009, Cappellini, 2007).

Several other risk factors are also contributing to the hypercoagulable state, like abnormal RBCs formation and high expression of negatively charged phosphatidylserine at the outer surface, results from specific changes within the lipid membrane, which can cause post-splenectomy, liver, and cardiac dysfunction, subsequently

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resulting in protein C and protein S reduction (Cappellini, 2007)

Janus kinase II (JAK II) is a cytoplasmic tyrosine kinase that has a vital role in signal transduction triggered by hemopoietic growth factors (Karaköse, *et al.,* 2005). The domain structure of Janus kinase is organized with 2 homologous kinase domains: One among these JAK homology JH1 is an activating domain while the other JH2 lacks kinase activity (Al-Thwani *et al.,* 2010)

Previous studies revealed that the JAK II mutation in healthy individuals is extremely rare and is found on chromosome 9p. During this mutation, guanine is substituted to thymidine (G to T mutation), causes an amino acid substitution of valine with phenylalanine at codon 617 in JH2 pseudokinase domain of the JAK II gene (Al-Thwani *et al.,* 2010). As a result of point mutation leading to growth factor hypersensitivity and dependence which results in up-regulated constitutive kinase activity causing enhanced hematopoiesis (Amarapurkar, *et al.,* 2011).

Abnormalities in *Janus kinase II* $^{(V617F)}$ mutation has been implicated during a several disease disorders including essential thrombocythemia, polycythemia Vera and idiopathic Myelofibrosis, while it's contribution to the β-thalassemic patients complications is still unknown and it remains unclear if the V617F mutation is related to blood clots and embolus (Alabdulaali, 2009, Steensma,2006, Steensma, *et al*., 2005) .

In this prospective cohort study, we evaluated the prevalence and the incidence of the *Janus kinase II* $^{(V617F)}$ mutation and its clinical correlation in patients with β-Thalassemia.

2. MATERIALS AND METHODS

2.1.Patient specimens

The present screening study for non-receptor protein tyrosine kinases (Janus kinase II) mutation was carried out on (50) patients with β-Thalassemic major with the mean age (24) years, ranging from (5-45) years of age, all examined samples were collected from previously diagnosed patients with β-thalassemia by a clinician using electrophoresis and the WHO 2008 criteria.

For genomic DNA extraction, the patient specimens were obtained using venous blood (5 ml), the blood samples were collected into EDTA blood collection tubes. After separation, the samples were either used directly for the study or kept frozen (-20 °C) until further analysis.

2.2.Molecular detection assay protocol for JAK2 V617F mutation

DNA extraction

The human genomic DNA was isolated from whole blood samples using a efficient genomic DNA Mini kit (Geneaid Biotech Ltd) according to manufacturer recommendations. The purity and the concentration of the extracted DNA samples were assessed by thermo scientific Nano drop 1000 spectrophotometer and the results were checked by 1% Agarose gel electrophoresis.

PCR/RFLP analysis

The V617F mutation analysis of JAK2 gene was assessed by restriction site generating polymerase chain reaction (RG-PCR). The full coding sequence of Janus kinase II gene at exon 14 was amplified by using forward primer (5′-TTT GGT TTT AAA TTA TGG AGT ACG-3′) and Reverse primer (5′- CTA TTG TTT GGG CAT TGT AAC C-3′).

RG-PCR was then performed in a total reaction volume of 50 µl, containing 15 µl extracted genomic DNA (30 ng/µl), 2.5µl reveres primer (42.8pmol/µl), 2.5µl forward primer (59 pmol/µl), 1µl buffer (10X(200 mM Tris-HCl (pH 8.4), 500 mM KCl.)), 2µl $MgCl_2$ (50mM), 0.2 µl dNTPs (10mM), 1.25 µl Taq polymerase (5U/µl) and 25.55 µl dH_2O .

The samples were amplified on the thermal cycler starting with an initial denaturing phase for 5 min at 94°C . The run consisted of 30 cycles, containing a step at 94 °C for 30 sec., Tm (Melting temperature) 59°C for 35 sec. and 72°C for 1 minute The samples were held at 72°C for 10 min. afterward. The PCR product was stored at -70 °C until it had been loaded and analyzed on an 1% W/V Agarose gel.

Restriction fragment length polymorphism (RFLP) was used to assess the V617F mutation in a total volume of 20µl, as the following: 5 µl amplicon products were digested overnight with 20 units/ 2µl *AflIII* restriction endonuclease enzyme in appropriate buffer (Acetylated BSA 0.4 µl and 2 µl buffer) at 37°C, Then the digested products were analyzed by visualization of

digested amplicon after separation by 2% (w/v) agarose gel electrophoresis at 100V current for 30 min and then staining with ethidium bromide.

When only wild type DNA is present, two fragments of 242 bp and 22 bp are generated, whereas mutated DNA (V617F-positive) JAK II allele) remains undigested (264 bp) fragment (Vlackaki, et al., 2012) .

2.3.Ethical consideration

Ethics approval was granted by the professional research committee of the College of Medicine -HMU. The patient's venous blood was used for our prospective study, Informed consent and permission were obtained from the patients before phlebotomy and the sample taking.

3. RESULTS

A total of fifty β-thalassemia patients with the mean age (24) years, ranging from (5-45) years of age were enclosed into the study

A 264 bp fragment from exon 14 of the Janus kinase II gene among patients with β–thalassemia major were successfully amplified with the precise primers containing *AflIII* endonuclease restriction sites as shown in Fig (1).

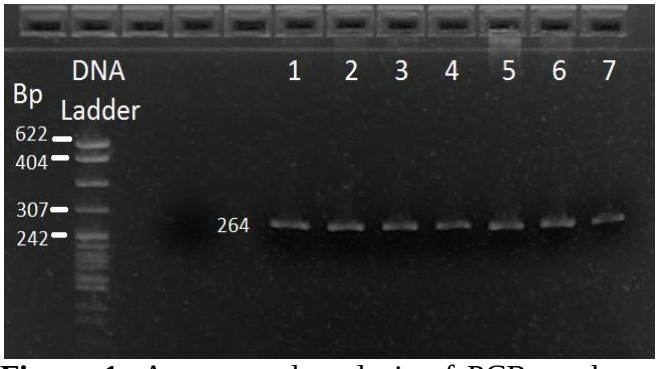

Figure 1: Agarose gel analysis of PCR products corresponding to 264 (bp) fragment of the Janus kinase II gene. DNA markers are shown on the right (Bp). Whereas lines from 1 to 7 correspond to PCR products of the amplified DNA from 7 different samples of patients with β–thalassemia major.

In our study for mutational region screening, we demonstrate that none of the β -thalassemic patients was positive and carried any of the described *kinase II* $^{(V617F)}$ mutation, as shown in Fig 2.

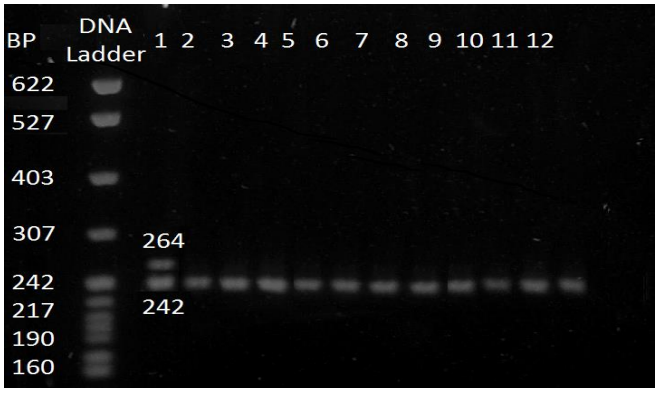

Figure 2: Agarose gel electrophoresis pattern of some RFLP products of the Janus kinase II gene. DNA markers are shown on the right (Bp), Line no. 1 control positive for *JAK2* ^{V617F} mutation, The other lanes are negative and did not have *JAK2* ^{V617F} mutation

4. DISCUSSION

It is well established that homozygous carriers of β-globin gene mutation suffer from severe hematological, thromboembolic complications, biochemical, and other serious complications from an early child (Eldor, *et al.*, 2002, Faris, *et al.*,2019). Although, the life expectancy of patients has markedly improved over the previous couple of decades but patients are still suffer from many complications of this hereditary disease (Syed, *et al.*, 2018). Of something, additional complications are now being recognized. Especially, an increased risk of hypercoagulability and thrombosis (Hadeer, *et al.*, 2017)

Despite longstanding evidence the pathophysiology of blood clots and embolus remain unclear (Ataga, 2019, Gregory, *et al.*, 2017).

So, in Beta-Thalassimia, The mechanisms responsible for the increased thrombotic risk are probable multifactorial. Several factors are identified. While the likelihood of a genetic basis is still not very clear (Amiram, *et al.*, 2002, Maria-Domenica *et al.*, 2011).

In our prospective study, we focused on the *JAK II* ^{V617F} mutation in patients with β-Thalassemic major because a better rate of thrombotic complications has been reported in some non thalassemic patients with the *JAK II* ^{V617F} mutation; however, It remains unclear if the V617F mutation of Janus kinase is related to an increased risk of blood clots and embolus in thalassemic patients

To the best of our knowledge, ours is that the first molecular study in this region. no other group has done any work about the V617F mutation of the *JAK II* gene and β-Thalassemia major.

In our analysis for β-Thalassemic major individuals, we observed that the *JAK II* ^{V617F} mutation was completely absent among the study subjects and *JAK II* ^{V617F} point mutation doesn't related to thrombotic complications in thalassemic patients, Our results are in agreement with the results obtained by Vlackaki *et al*, which they found that there is no association between *JAK II* ^{V617F} mutation and β- Thalassemia major.

5. CONCLUSIONS

In our study, we found no evidence for an association between *JAK II* ^{V617F} mutation and β-Thalassemia major, and that's means in patients with β- Thalassemia mutation at Valine 617 Phenylalanine of *Janus kinase II* genes does not seem to play a role in the blood clotting, hematological and thrombotic complications.

Conflict of Interest

The author declares that he has no conflicts of interest.

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