

## Screening of V617F mutation in *JAK2* gene with acute myeloid leukemia in the Saudi population

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Progress in pathogenesis and therapy of acute myeloid leukemia (AML) is presently accelerating. The Janus kinase 2 gene (*JAK2*) mutations are rare in de novo AML. The gene codes for the tyrosine kinase that has a significant role in the signal transduction in hematopoietic cells. The aim of this study was to induce V617F mutation in the *JAK2* gene in the AML patients diagnosed in the Saudi population. In this case-control study, 100 AML patients and 100 healthy controls were recruited. Genotyping was performed with polymerase chain reaction followed with restriction fragment length polymorphism analysis. The mean age of the AML patients and healthy controls was found to be almost similar ( $p=0.60$ ). In this study, 15% of VF mutation was documented in the AML cases and none of the mutations were documented either in FF mutation in AML cases or VF and FF mutations in the healthy control subjects. VF mutations [VF vs VV; OR-18.79; (95% CIs: 2.442–144.6) and  $p=0.0001$ ; F vs V; OR-87.76; (95% CIs: 11.76–654.7) and  $p<0.0001$ ] were found to be significantly associated when compared between AML cases and healthy controls. In conclusion, the V617F mutation showed the positive association in the AML patients diagnosed in the Saudi population.

**Keywords:** acute myeloid leukemia, V617F, *JAK2* and Saudi population

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**Abbreviations:** AML, acute myeloid leukemia; HWE, Hardy Weinberg Equilibrium; *JAK2*, Janus kinase 2 gene; H2, Jak homology domain 2; ET, essential thrombocythemia; MMM, myeloid myelofibrosis metaplasia; PV, polycythemia Vera; PCR, Polymerase Chain Reaction; SNP, Single nucleotide polymorphism; V617F, valine-617-phenylalanine

### INTRODUCTION

Acute myeloid leukemia (AML) is a type of cancer which involves bone marrow, blood, and other tissues by proliferative, highly undifferentiated, slightly abnormal and sometimes immature, and mesenchymal cells (Döhner *et al.*, 2015). AML is a highly heterogeneous and CD34<sup>+</sup>CD38<sup>-</sup> myeloid progenitor hematopoietic malignant disorder (da Cunha Vasconcelos *et al.*, 2021). The etiology of this disease is virtually unknown, but the relationship between environmental risk and genetic susceptibility is believed to be a potential trigger for the formation of AML (Botros *et al.*, 2021). The rate of occurrence rises considerably with the age at about 35/1000000 people. AML is therefore more frequent among people over 60 years of age (Sariani *et al.*, 2021). AML accounts

for 10–20% of infant leukemia and less than 30% of adults have a survival rate of AML for 5 years. In addition, older persons with AML are poorly tolerated and contagious (Yuan *et al.*, 2021). The acute promyelocytic leukemia variant should be treated as a distinct entity with distinct genetic defects, different treatment, and a better prognosis than patients with nonpromyelocytic variants, and the term AML applies to nonpromyelocytic variants (Hernandez-Valladares *et al.*, 2021). A combination of anthracyclines and cytosine arabinoside in the normal 3/7 regimen is the quality of care in community practice (Sasaki *et al.*, 2021). AML has been identified as the sixth leading cause of death among a variety of malignancies. During diagnosis, AML patients exhibit 50–60% in chromosomal anomalies, and karyotyping plays a significant role in disease-related prognostic factors for care. Despite major advancements in the diagnostic and therapeutic processes, patients with AML have a poor prognosis (Farasani, 2019).

For the majority of genetic variants, gene mutations are the most common ones. Gene polymorphisms are commonly a cause of genetic diseases, but not always. Single nucleotide polymorphisms (SNPs) is the most common form of nucleotide variations found in humans, representing the most common base pair variation (Khan *et al.*, 2016). Previous studies have identified many SNPs with leukemias and Janus kinase 2 (*JAK2*), was one of the SNPs documented with AML. The Janus Kinases (*JAK*) family of mammals includes four cytoplasmic tyrosine kinases (*JAK1*, *JAK2*, *JAK3* and *TYK2*) recruited and activated by particular cytokine receptors on the surface of certain cells and receptors which are critical in normal and disordered hematopoiesis (Steensma *et al.*, 2006). Previous studies showed that *JAK2* tyrosine kinase has recurring somatic activating mutations in polycythemia vera (PV), essential thrombocythemia (ET), and myeloid myelofibrosis metaplasia (MMM). In the Jak homology domain 2 (JH2) pseudokinase field of *JAK2*, V617F mutation leads to the valine-phenylalanine replacement for codon 617 (Levine *et al.*, 2005). Limited studies have been documented with V617F mutation in the *JAK2* gene with AML and no studies have been carried out in the Saudi population. So, the current study was carried out in the Saudi population between AML patients and V617F mutation in the *JAK2* gene.

### MATERIALS AND METHODS

In this case-control study, age and gender matching Saudi patients 100 AML patients and 100 healthy subjects were selected. All recruited samples were collected from Riyadh regional laboratory, Saudi Arabia. Both

**Table 1. Anthropometric information on the patients in this study**

Anthropometric parameters	AML cases (n=100)	Controls (n=100)	p-Value
Age (years)	38.9±15.1	39.9±12.06	0.60
Minimum and maximum ages (years)	19–82	18–63	–
Males (Gender)	61 (61%)	54 (54%)	–
Females (Gender)	39 (39%)	46 (46%)	–

AML cases and control subjects were recruited based on the inclusion and exclusion criteria, as described in the previous publication (Farasani, 2019). The inclusion criteria of AML subjects were recruited based on the diagnosis of the AML with histopathological and cytogenetic confirmation, and informed consent signed by Saudi adults. The exclusion criteria of AML cases were patients diagnosed with other cancers, and unsigned consent form with non-Saudi subjects. The AML cases were diagnosed with bone-marrow examination, complete blood count and flow cytometry. Additionally, cytogenetics and fluorescent in situ hybridization tests were implemented for reconfirmation. The inclusion criteria of the healthy controls were not diagnosed with any specific type of cancers or other diseases. The exclusion criteria were non-Saudi subjects. Ethical grant was obtained from Ministry of Health affairs. This study was performed as per the Declaration of Helsinki.

#### Sample selection

From each patient (n=200; 100 AML cases and 100 healthy controls) 2 ml of EDTA blood was collected and used for the DNA extraction and further use.

#### Molecular analysis

Genomic DNA extraction was carried out with specific kits used for separation of DNA and nanodrop was used to DNA quantification. Genotyping was performed towards V617F mutation in the *JAK2* gene using (F: TCCTCATCTATAGTCATGCTGAAA) and reverse (R:CTGACACCTAGCTGTGATCCTG) primers and polymerase chain reaction (PCR) was performed with the PCR master mix of 50 µl microliters consisting of 4 µl genomic DNA and 30 µl PCR mixes, which contained 10X, MgCl<sub>2</sub>, dNTPs and 10x Taq DNA polymerases. The Master mix was supplemented by 10 pmoles of 2 µl of forward and reverse primers followed by 12 µl of distilled water. For the final amount of 50 µl, the PCR reaction has been standardized. V617F primers from previous investigations have been adopted. The initial denaturation was carried out at 95°C – 5 min.,

denaturation at 95°C – 30 s, annealing at 60°C – 30 s, extension at 72°C – 45 s and final extension at 72°C – 5 min. with 35 cycles (Khan et al., 2015). The 305 bp PCR products were digested with *BsaXI* enzyme at 37°C for 18 hours, whereupon samples were loaded on 3% agarose gel stained with ethidium bromide.

#### Statistical analysis

SPSS software (version 20) was used to examine the clinical data. Hardy-Weinberg Equilibrium (HWE) was used for comparing the observed and anticipated genotype frequencies using control subjects. The odds ratios, upper, and lower ranges of 95% confidence intervals (95% CI) for V617T mutation in the *JAK2* gene were used in the genotype differences between AML cases and controls.  $p < 0.05$  was considered as indicating the statistical significance (Khan *et al.*, 2019).

#### RESULTS

This molecular analysis was carried in Riyadh, with 100 AML patients and 100 healthy control subjects. The mean age of AML cases was 38.9±15.1 years and 39.9±12.06 years was documented for the healthy controls. Both groups showed similar ages with non-significant association ( $p=0.60$ ). The minimum age of AML cases and healthy controls was documented between 19 and 18 years of age and 82 and 63 years of age was documented as maximum ages, respectively. The male gender in AML cases was documented for 61% and 54% in the control groups and 39% was documented for female subjects in AML cases and 46% in healthy controls. HWE was not in accordance with V617F mutation in (Table 1) the control subjects as none of the mutations was present. The genotype mutation frequencies between VV, VF and FF were found to be 85%, 15% and 0% in the AML cases, whereas in the control subjects no mutation was documented either in VF or FF group 15% of F allele and 85% of V allele mutations were documented between AML and, 100% of V allele mutation were present and 0% of F allele mutations were in the control

**Table 2. JAK2 mutation analysis in AML patients**

JAK2 (rs77375493)	AML Cases (n=100)	Controls (n=100)	Odds Ratio	(95% CI)	p value
	N (%)	N (%)			
VV	85 (85%)	100 (100%)	Reference	Reference	Reference
VF	15 (15%)	00 (00%)	18.79	2.442–144.6	0.0001
FF	00 (00%)	00 (00%)	1.17	0.07–19.05	0.90
V	170 (85%)	200 (100%)	Reference	Reference	Reference
F	30 (15%)	00 (00%)	87.76	11.76–654.7	<0.0001

subjects (Table 2). The genotypes present in this study were 100% VV [VF *vs* VV; OR-18.79; (95% CIs: 2.442–144.6) and  $p=0.0001$  and FF *vs* VV; OR-1.17; (95% CIs: 0.07–19.05) and  $p=0.90$ ; F *vs* V; OR-87.76; (95% CIs: 11.76–654.7) and  $p<0.0001$ ] was significantly associated.

## DISCUSSION

This study aimed to analyze rs77375493 (G>T) mutation in the *JAK2* gene, in the Saudi population diagnosed with AML. In this study, 15% of VF mutations were documented in AML cases. However, no FF mutations were observed in AML cases. None of the mutations were documented with VF and FF genotypes and 100% of VV genotypes were present in healthy controls. This study confirms that V617F mutation plays a major role in AML subjects. One of the strengths of this study was documented with 100 AML cases and 100 health subjects with age-matched subjects and one of the limitations of this study was not exhibiting the results of cytogenetic and FISH analysis. In this study, 60% of VF mutations were found in male AML participants and 40% in female AML subjects. In AML patients, the male and female frequencies were 69% and 31%, respectively.

AML is a deadly hematopoietic stem cell malignancy, resulting in a significant hematopoietic stem cell depletion, which further inhibits normal hematopoiesis. Early discovery and treatment for AML patients can help them survive, and a number of tests including cytogenetics and molecular analysis can help to estimate the likelihood of remission and survival. But because of many different molecular pathways of the disease, it is difficult to determine AML prognosis. Thus, a biomarker must be found that is more effective (Tong *et al.*, 2020). JAK2 is a cytoplasmic tyrosine kinase that plays a crucial role in signal transduction of various hematopoietic receptors. The *JAK2* gene has discovered a gain-of-function mutation resulting in position 617 of valine-to-phenylalanine substitution (G1894T) (Malhan *et al.*, 2014). JAK2 p.V617F is a missense mutation resulting in the activation of the JAK Signal Transducer and Transcription Pathway as a constituent of hematopoietic myeloid precursors in >90% of polycythemia vera patients and roughly half of essential thrombocythemia and myelofibrosis individuals (Alghasham *et al.*, 2016). Previous studies showed the positive and negative associations with V617F mutation and AML subjects in the global population (Aynardi *et al.*, 2018; de Noronha *et al.*, 2019; Illmer *et al.*, 2007; Lee *et al.*, 2006; Nahajevszky *et al.*, 2006; Steensma *et al.*, 2006; Vicente *et al.*, 2007). There are no meta-analysis studies have been documented with V617F mutation in the *JAK2* gene and AML diseases in the global population. Based on clinical trial results, Reddy and others (Reddy *et al.*, 2012) studies confirmed the role of *JAK2* mutation as it indicates the feasibility and efficacy of JAK2 targeted approaches. Furthermore, there is a limited number of examples using JAK2 inhibitors as drugs due to the dose-dependent toxicity and the requirement of combined treatment.

In this study, using *Bsa*XI restriction enzyme, PCR products were digested and randomly, 10% of PCR products were sequenced (Al-Otaiby *et al.*, 2021). Previous studies, however, validated the false-positive and false-negative results, and one of the simplest techniques is to conduct the experiment with allele specific ARMS, and quantitative PCR analysis (Ali, 2020; Guo *et al.*, 2020; McLornan *et al.*, 2006). Skipping of additional PCR techniques could be one of the limitations of this study.

## CONCLUSION

In conclusion, V617F mutation shows positive association with AML subjects in the Saudi population. Future studies should be performed in global population with a large sample size.

## Conflict of Interest

There is no conflict of Interest regarding this article.

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