Frequency of Janus Kinas 2V617F (JAK2V617F) Mutation among Children with Type 1 Diabetes Mellitus

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Authors’ contributions

This work was carried out in collaboration among all authors. Authors MHH, RT, HMS and OG designed the study and wrote the protocol. Authors MHH and NFZ wrote the first draft of the manuscript. Authors NFZ, MHH, HMS, RT and OG managed the analyses of the study. Authors MHH and NFZ managed the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Background and Objectives: Type 1 Diabetes mellitus (T1DM) is a disorder of glucose homeostasis results from the destruction of β- cells of the pancreas with subsequent insulin deficiency and hyperglycemia. Acquired mutation (V617F) affecting the JAK2 gene disrupts the auto-inhibition of JAK2 and results in constitutive activation of the Janus Kinase 2(JAK2)/signal transducer and activator of transcription (STAT) pathway. Once activated, the JAK/STAT pathway stimulates cell proliferation, differentiation, migration and apoptosis critically involved in growth control. Studies investigating the possible role of Janus kinas 2V617F (JAK2V617F) mutation and risk of T1DMor development of diabetic complications among such children are very few. So we

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aimed in this pilot study to estimate the relative frequency of Janus kinas 2V617F (JAK2V617F) mutation among a sample of children with T1DM.

**Patients and Methods:** This cross-sectional study included 25 Egyptian children with T1DM. Full clinical evaluation, routine laboratory investigations including kidney function, RBG, HbA1C and A/C ratio were done. Determination of JAK 2v617f gene mutation was performed using real time-PCR.

**Results:** The included diabetic children were 15 males and 10 females. Their mean random blood glucose was 272mg/dl±33 SD. Two cases exhibited mutation in the form of heterozygous type (Aa) representing 8% of the total included diabetic children and the remaining 23 (92%) diabetic children showed normal wild alleles (AA). There was a non-significant correlation between the glycemic control and the presence of JAK 2v617f gene mutation, p>0.05.

**Conclusions:** JAK 2v617f gene mutation present in a small percentage of non-complicated T1DM with lack of its correlation with the glycemic control.

**Keywords:** T1DM; JAK2; real-time PCR; gene.

**ABBREVIATIONS**

T1DM : Type 1 Diabetes mellitus  
JAK2 : Janus Kinase 2  
STAT : Signal transducer and activator of transcription  
RBG : Random blood glucose  
PCR : Polymerase chain reaction  
MPN : Myeloproliferative neoplasms  
PV : Polycythemia vera  
ET : Essential thrombocythemia  
PMF : Primary myelofibrosis

**1. INTRODUCTION**

Type 1 Diabetes mellitus (T1DM) is a disorder of glucose homeostasis characterized by autoimmune destruction of insulin-producing pancreatic b-cell that progressively leads to insulin deficiency and resultant hyperglycemia. If left untreated, insulin deficiency leads to progressive metabolic derangement, ketoacidosis, and death [1]. Acquired mutation (V617F) affecting the JAK2 gene disrupts the auto-inhibition of JAK2 and results in constitutive activation of the Janus Kinase 2(JAK2)/ signal transducer and activator of transcription (STAT) pathway. Once activated, the JAK/ STAT pathway stimulates cell proliferation, differentiation, migration, and apoptosis critically involved in growth control, and therefore, represents a crucial pathway implicated in promoting tumorigenesis [2]. A mutation in the JAK2 gene, V617F, has been identified in several BCR-ABL1 negative myeloproliferative neoplasms (MPN): polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) [3]. The signalling pathways mediated by JAK2 have been shown to stimulate replication and survival of β-cells [4], but no adequate researches could be traced in the literature regarding its association with T1DM, so the current pilot study aimed to assess the relative frequency of JAK2 gene mutation among diabetic children and its correlation with the glycemic control.

**2. PATIENTS AND METHODS**

A cross-sectional study conducted on 25 children with T1DM, recruited from outpatient pediatric clinics and inpatients pediatric department of Qena University Hospitals, Egypt, in collaboration with Medical Biochemistry and Medical Physiology Departments. The study period was 6 months from October 1st, 2018 to March 1st, 2019. Any children with chronic kidney or liver or haematological disease or any other associated co-morbidities, or with diabetic nephropathy, all were excluded from the study.

**2.1 Data Collections**

All index patients were subjected to:

1. Complete medical history will be taken including (Duration of disease, Type of insulin therapy).
2. Anthropometric measurement including weight, height, BMI according to the Egyptian growth chart.
3. General examination, systemic examination, cardiac, chest, and abdominal examination were performed to all included diabetic children.

**2.2 Biochemical and Molecular Assays**

A Routine investigations in the form of random blood glucose (RBG) level, glycated
haemoglobin (HbA1c %), serum urea, creatinine level and urinary albumin /creatinine ratio, all were measured to the included diabetic children.

B- Determination of JAK 2v617f gene mutation using 5 ml whole EDTA blood: Allele-specific real-time quantitative PCR was performed using the ABI Prism 7000 platform. JAK-2 Forward primer:5'-AAGCTTTTCTCACAAGCATTGGT TT-3'.Reverse primer:5'-AGAAAGGCATTAGAAAG CCTGTAGTT-3'. TaqMan probes JAK 2v617f were labelled with VIC and FAM fluorescent dyes, respectively, with the probe sequence as follows: JAK2 wild-type probe: 5'-TCTCCACAG ACACATAC (VIC)-3'. JAK2 mutant-type probe: 5'-TCCACAGAAACATAC (-FAM)-3'. The assay contains probes specific to the wild-type (G) and mutant (T) alleles labelled with VIC and 6-fluorescein (6-FAM), respectively. Wild type

2.3 Statistical Analysis

IBM SPSS Statistics for Windows version 20 and Medcalc version 15.8.0 was used for data analysis. Qualitative data were expressed as number and percentage. Quantitative data were expressed as mean±SD. Pearson's correlation was used. Level of significance was considered at p<0.05.

3. RESULTS

The included diabetic children were 15 males and 10 females. Their mean random blood glucose was 272 mg/dl±33 SD. Regarding the glycemic control status, 7 (28%) cases had poor glycemic control (HbA1c more than 8%) and the remaining 18 diabetic children (72%) had good glycemic control (HbA1c ≤8%). Two cases exhibited JAK 2v617f mutation in the form of heterozygous type (Aa) representing 8% of the total included diabetic children and the remaining 23 (92%) diabetic children showed normal wild alleles (AA), (Table 1 and Fig. 1). There was a non-significant correlation between the glycemic control and the presence of JAK 2v617f gene mutation, p>0.05.

4. DISCUSSION

Diabetes is a chronic disease that occurs when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin, it produces. There are two major types of diabetes: type 1 diabetes and type 2 diabetes [5]. Type 1 diabetes mellitus characterized by hyperglycemia secondary to inadequate production of insulin by the pancreas. This is due to T cell-mediated autoimmune destruction of the insulin-producing β cells in the islets of Langerhans [6]. The rate of T1D in children is rising by 3% annually with a projected 70% increase in prevalence between 2004 and 2020 [7]. The JAK2 V617F mutation is an acquired, somatic mutation present in the majority of patients with a myeloproliferative neoplasm (MPNs) i.e. nearly 100% of patients with polycythemia vera and in about 50% of patients with essential thrombocytosis and primary myelofibrosis [8].

An acquired mutation within the JAK2 gene, an amino acid substitution of valine-to-phenylalanine at position 617 (V617F), disrupts the auto-inhibition of JAK2 and results in constitutive activation of the JAK2/STAT signalling pathway. Once activated, the JAK/STAT pathway stimulates cell proliferation, differentiation, migration, and apoptosis critically involved in growth control, and therefore, represents a crucial pathway implicated in promoting tumorigenesis [2]. Recently, the JAK2 V617F mutation has been reported in patients with cerebral vein thrombosis with no previous diagnosis of myeloproliferative neoplasm (MPNs) [9]. The JAKs were found to be involved in signalling downstream of the insulin receptor, several receptor tyrosine kinases, and certain G-protein coupled receptors [10].

To the best of our knowledge, this study is the first to examine JAK2 V617F mutational status in T1DM children. In our study, 23(92%) of the index cases were negative for AA/JAK2 V617F mutation and 2(8%) were positive for Aa JAK2 V617F mutation. Additionally, we couldn't found any correlation between the presence of JAK2 V617F gene mutation and the glycemic control. Choi et al reported that β-cell-specific JAK2 knockout mice, using the RIPcre transgenic mice, did not present with any alterations in glucose homeostasis for up to 2 months of age [11]. Additionally, they reported that JAK2 does not appear to play a major role in regulating the compensatory increase in β-cell mass in response to a high-fat diet. However, it remains possible that in other conditions of β-cell expansion, JAK2 may still play an essential role.
Table 1. Frequency of JAK2 V617F mutation in children with non-complicated T1DM

| Variables                  | JAK2 V617F genotypes |
|----------------------------|----------------------|
|                            | AA   | %    | Aa  | %    |
| Children with T1DM (n=25)  | 23   | 92   | 2   | 8    |

Fig. 1. JAK2 V617F mutation among children with non-complicated T1DM

5. CONCLUSIONS

The relative frequency of JAK 2v617f mutation among T1DM is 8%. Larger scale studies including those with and without diabetic complications especially diabetic nephropathy are recommended to evaluate the possible role of JAK 2v617f gene mutation in glycemic control and development of diabetic complications.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

FUNDING

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CONSENT

Written informed consent has been obtained from the parents of every included child.

ETHICAL APPROVAL

The study has been done following the Declaration of Helsinki and has been approved by the Ethics Committee of Faculty of Medicine, South Valley University, Qena, Egypt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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