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Evaluation of Philadelphia-negative myeloproliferative neoplasms in Erbil Iraq; analysis of JAK 2, CALR, and MPL mutations

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Abstract

Introduction: The prevalence of Janus kinase 2 V617F, Jak 2-Exon12, calreticulin (CALR) gene mutations and myeloproliferative leukemia mutations in myeloproliferative neoplasms (MPNs) without BCR-ABL1 gene fusion rearrangement in Iraq remains unknown due to inadequate data.

Objectives: The aim of this study was to ascertain the prevalence of these mutations and assess the clinical characteristics of polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) in Erbil, Iraq.

Patients and Methods: In this cross-sectional study, 80 patients were evaluated for the presence of JAK 2 V617, CALR, and MPL mutations. Specific primers were utilized to amplify MPNs-associated mutations (JAK 2 V617F, Jak 2 -Exon12, CALR, and MPL) using DNA extracted from whole blood.

Results: The presence of the JAK 2 V617F mutation was identified in 96% of patients diagnosed with PV and 35% of patients diagnosed with ET. Additionally, JAK 2 exon 12 was detected in 4% of PV patients. CALR mutation was identified in 65% of ET patients. No detection of MPL mutation was observed. A higher prevalence of the CALR mutation was observed in younger age groups, whereas the JAK 2 V617F mutation was predominantly found in older age groups.

Conclusion: The incorporation of genetic tests for JAK 2 V617F, JAK 2 exon 12, MPL exon 10, and CALR exon 9 significantly improves the diagnostic effectiveness for BCR ABL1-gene fusion negative MPNs.

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Introduction

Excessive production of specific myeloid cells is the result of abnormal stem cells in myeloproliferative neoplasms (MPNs), which represent a collection of disorders. The classification of polycythemia vera (PV) and essential thrombocythemia (ET) with primary myelofibrosis (PMF) and chronic myelogenous leukemia as neoplasms was first proposed by the American hematologist William Dameshek in 1951 (1). PV is one of the primary classic MPNs characterized by excessive production of predominantly red blood cells. The main focus of concern in ET lies in the excessive generation of platelets. PMF is characterized by an excess of megakaryocytes, resulting in fibrosis within the bone marrow. The significance of morphology and clinical laboratory analysis in delineating these conditions remains crucial. The World Health Organization (WHO) highlights the importance of molecular testing in MPN, specifically by incorporating JAK 2, MPL, and calreticulin (CALR) mutations as one of the diagnostic criteria in the 2016 update on the classification of myeloid neoplasms (2). The advancements in genetic research have significantly enhanced our comprehension of the molecular foundation of MPN.

Key point

In a study on 80 patients with myeloproliferative neoplasms, we found that the presence of the JAK 2 V617F mutation was identified in 96% of patients diagnosed with PV and 35% of patients diagnosed with ET.

Starting from 2005, clonal abnormalities have been observed in the classic MPNs, indicating the presence of the acquired clone. The presence of the Janus kinase 2 (JAK2) V617F mutation was identified in 95% of patients diagnosed with PV and around 50% of individuals with ET and PMF (3). CALR mutations have been observed in individuals who do not possess a JAK 2 mutation (4,5).

The primary clinical presentation of MPN, known as splenomegaly, is widely recognized for causing distressing symptoms such as early satiety, weakness, abdominal pain, and

potential progression to severe complications such as cytopenias resulting from splenic sequestration.

Spleen enlargement, present in 10%-20% of ET patients at diagnosis (6). Splenomegaly is widely regarded as a distinguishing characteristic of PV; however, its reported occurrence in the literature varies between 20% and 75%. (7).

Objectives

The primary aim of this study is to assess the prevalence of JAK 2 V617F, JAK 2 Exon 12, MPL, and CALR exon 9 mutations in patients with MPNs in the city of Erbil. Additionally, the study aims to describe the demographic data, clinical manifestations, and laboratory findings of these patients.

Patients and Methods

The inclusion criteria in this cross-sectional study encompass cases diagnosed with PV, ET, and PMF, along with being BCR-ABL1 negative. Any cases that do not fulfill the criteria established by WHO 2016 and are positive for BCR-ABL1 will be excluded from the study.

Following the diagnosis of MPN in several patients, polymerase chain reaction (PCR) testing was conducted. Concurrently, additional cases were identified and included in this study. Around 80 patients of MPN had their blood samples collected in ethylenediaminetetraacetic acid (EDTA) tubes. In collaboration with the bio-laboratory and the Nanakaly teaching hospital for blood diseases and oncology in Erbil, Iraq, our ongoing research was conducted. The period from September 2022 to December 2023. The participants were classified into three groups, including PV, ET, and idiopathic PMF, according to the diagnostic criteria for MPN published by the WHO in 2016 (8). The research has obtained ethics approval from Gaziantep university's ethics committee, ensuring its ethical and responsible implementation in accordance with the principles of the declaration of Helsinki. The patient enrolled in the study provided consent.

Blood samples in EDTA tubes were collected from patients who satisfied the inclusion criteria for MPN diagnosis. Meanwhile, DNA was the extracted from peripheral blood samples using the GeneAll: Exgene Blood SV mini kit (GeneAll, Korea, cat. no 105-152 and Lot. No 10523C09021). The DNA samples were analyzed using a micro-volume spectrophotometer, specifically the OneDrop TOUCH Pro/Lite micro-volume UV-Vis spectrophotometer.

Furthermore, serum samples were obtained from the participants to assess C-reactive protein and erythropoietin (EPO) levels through serology testing. The objective was to investigate whether there is a notable correlation between these levels and the MPNs patients.

In our study, DNA extracted from whole blood was utilized to amplify specific primers targeting mutations associated with MPN (JAK2, CALR and MPL). These primers enable the amplification of specific genetic regions for mutation identification and analysis. The amplified 5 µl DNA fragments will undergo sequencing and analysis to ascertain the molecular profile of the patients belonging to the investigated MPN subtype. The amplification of polymerase chain reaction was conducted in a total volume of 20 µL utilizing the SNP Biotechnology MPN (Turkey, cat. No: 23R-20-10), screening kit's master mix. The steps were as follows; initial denaturation for one minute at 96 °C followed by 32 cycles of denaturing at 96 °C for two seconds, annealing at 60 °C for 30 seconds.

Statistical analysis

GraphPad 9 Prism (GraphPad Software, USA) was used to apply the normality tests (Shapiro-Wilk, Kolmogorov-Smirnov, D'agostino) and determine if the data were parametric or not. In light of the data's non-normal distribution, we employed non-parametric tests across the board. The data was presented in the form of median and interquartile range (IQR). The Kruskal-Wallis and one-way analysis of variance (ANOVA) tests were conducted to compare over two groups. We also employed the Tukey test. Meanwhile, the Dunn's test was employed as a post hoc test for conducting multiple comparisons. To assess the association between categorical variables, specifically genotype and allele frequencies, the chi-square ($\chi 2$) test was utilized. Statistical significance was established when the p-value fell below 0.05. A single asterisk (*) was conducted to indicate a *P* value of less than 0.05; two asterisks (**) if the *P* value was beneath 0.01; three asterisks (***) if the p-value was less than 0.001; and four asterisks (****) when the *P* value was smaller than 0.0001.

Results

The most prevalent MPNs were PV 53 (66.25%), followed by ET 23 (28.75%) and PMF 4 (5%) (Figure 1).

Among patients with MPN, the JAK 2V617F mutation was detected in 77.5% of cases, the CALR mutation in 18.75% of cases, and the JAK 2 exon 12 mutation in 3.75% of cases. The occurrence of JAK2 V617F mutation was observed in 96% of patients diagnosed with PV and in 35% of patients diagnosed with ET. The prevalence of JAK 2 exon 12 mutation in patients with PV was found to be 4%, and it was observed to be mutually exclusive with the JAK2 V617F mutation. The presence of CALR mutation was detected in 65% of patients with ET, and no MPL were detected as illustrated in Figure 2.

The occurrence of mutations varies significantly among the different subtypes of MPN, with PV exhibiting a high prevalence of JAK 2 V617F, while ET and MF display a comparatively lower prevalence. On the other hand, the occurrence of CALR gene mutation is more pronounced in ET compared to PV and MF. The occurrence of JAK 2 Exon 12 mutation is relatively rare across all subtypes.

This study examined the hematological and clinical characteristics of patients with MPN, as indicated in

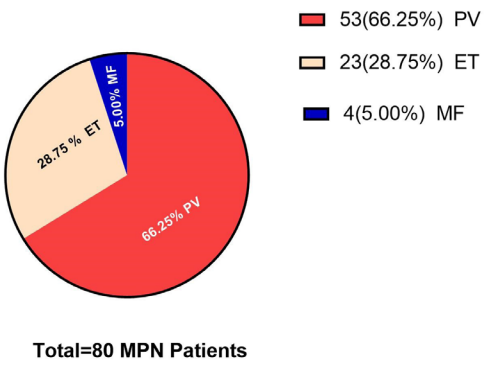

Figure 1. Frequency of polycythemia vera, essential thrombocythemia and myelofibrosis patient in myeloproliferative neoplasms patients. Abbreviations: PV, Polycythemia vera; ET, Essential thrombocythemia; MF, Myelofibrosis; MPN, myeloproliferative neoplasm.

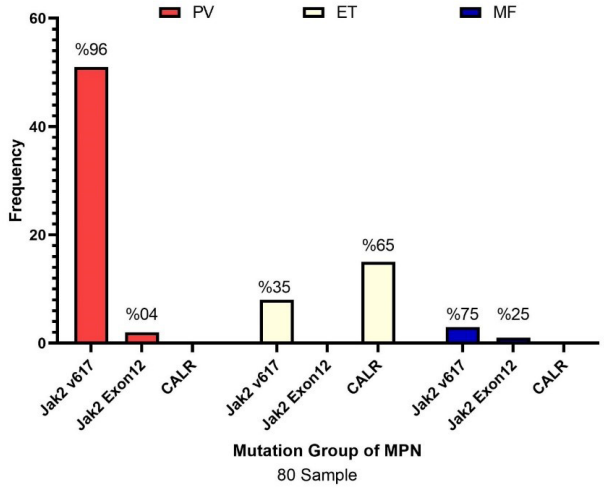

Figure 2. Frequency mutation rate of JAK2 v617f, JAK2 exon 12, and CALR gene mutation in polycythemia vera, essential thrombocythemia, and myelofibrosis patients. Abbreviations: PV, Polycythemia vera; ET, Essential thrombocythemia; MF, Myelofibrosis; MPN, myeloproliferative neoplasm.

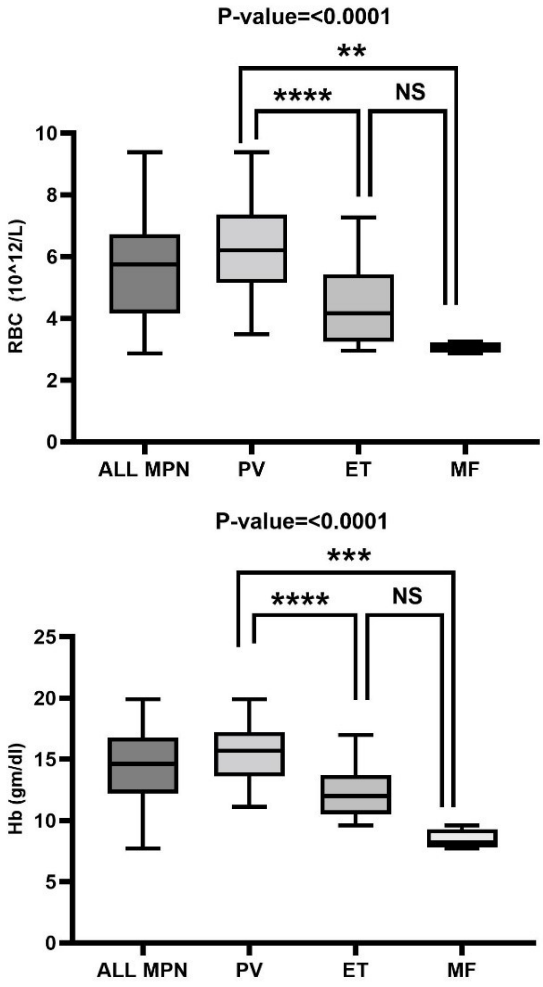

Figure 3. Evolution of hemoglobin (Hb) and red blood cell (RBC) in patients with myeloproliferative neoplasms (MPNs). One-way ANOVA was conducted for the comparison of (a) Hb; (b) RBC. $^{**}P<0.01$, $^{***}P<0.001$ and $^{****}P<0.0001$. Abbreviations: PV, Polycythemia vera; ET, Essential thrombocythemia; MF, Myelofibrosis.

(Table 1). As expected, the levels of hemoglobin and red blood cells were significantly higher in patients with PV compared to those with ET ($P<0.0001$; Figure 3).

Conversely, there were no notable distinctions observed among leukocyte patients diagnosed with PV and ET. Patients with ET exhibited significantly higher platelet levels compared to those with PV, with a median of 1121×10^9/L versus 661.0×10^9/L respectively ($P<0.0001$). We found that the median (IQR) for age was 56 (years) in total MPNs patients, which were statistically not observed any significant differences in comparison between the median (IQR) for these parameter levels in total MPNs patients ($P>0.05$; Table 1).

Serum parameters including EPO and CRP in MPNs patients and the control group are depicted in Table 1. The results obtained showed that the median (IQR) for EPO level was 7.195 (mlU/mL) and serum CRP level was 3.690 (mg/L) in total MPNs patients, which were statistically not observed any significant differences in comparison between the median (IQR) for these parameter levels in total MPNs patients ($P>0.05$; Figure 4).

Discussion

This study examined the frequency of JAK 2 (V617F), JAK2 Exon 12, CALR, and MPL mutations within the context of this research. Additionally, it investigated their clinical significance, along with clinical and laboratory parameters, among patients with MPNs. Notably, the utilization of enzyme-linked immunosorbent assay (ELISA) technology allowed for the assessment of EPO levels in our region for the first time.

This study aimed to analyze the prevalence of JAK2 V617F, CALR, MPL, and JAK2 exon 12 mutations among patients in Erbil, Iraq. The findings presented here substantiate the significance of incorporating JAK2 V617F, CALR, and JAK2 exon 12 mutations as important indicators in the diagnostic criteria for BCR-ABL rearrangement negative MPN (7).

Within this study, PV was detected to be the most

Table 1. A comparison of demographic factors and laboratory biomarkers among PV, ET, and MF groups

Parameters	All MPNs Median (IQR)	PV Median (IQR)	ET Median (IQR)	MF Median (IQR)	*P* value
N	80	53	23	4	-
Gender (M/F)	50/30	38/17	8/13	4/0	0.040
Age (y)	56.00 (46.75-65.00)	56.00 (48.00-65.00)	56.00 (43.00-63.00)	67.00 (51.25-68.50)	0.679
BMI (kg/m^2)	26.52 (23.64-29.64)	27.06 (24.62-30.73)	24.37 (21.41-27.79)	25.98 (23.51-27.27)	0.064
CRP (mg/L)	3.690 (2.423-6.668)	3.680 (2.390-6.260)	3.920 (2.275-7.060)	3.760 (3.530-6.953)	0.936
WBC (10^9/L)	11.31 (8.585 -14.80)	11.50 (9.165-14.60)	11.20 (8.205-15.03)	11.15 (5.575-19.65)	0.991
EPO (mIU/mL)	7.195 (3.768-10.24)	7.580 (4.540-10.47)	5.880 (3.595-11.39)	6.920 (3.710-9.305)	0.925
RBC (10^{12}/L)	5.745 (4.160-6.725)	6.210 (5.155-7.370)	4.170 (3.235-5.425)	3.075 (2.893-3.228)	<0.0001
Hb (g/dL)	14.63 (12.21-16.80)	15.70 (13.60-17.21)	12.00 (10.50-13.73)	8.250 (7.825-9.275)	<0.0001
Platelets (10^9/L)	577.0 (397.8-744.3)	488.0 (377.0-658.0)	879.0 (661.0-1121)	293.0 (179.0-437.0)	<0.0001

BMI: body mass index, PV: Polycythemia vera, ET: Essential thrombocythemia, MF: Myelofibrosis, EPO: Erythropoietin, CRP: C-reactive protein, RBC: Red Blood Cell, WBC: White Blood Cell, Hb: Hemoglobin.
Comparison of variables among groups was conducted by one-way ANOVA, post comparison was done by Tukey test. A statistical significance was assumed if the *P* value was less than 0.05.

prevalent MPN in this study, comprising 53 (66.25%), followed by ET 23 (28.75%) and PMF 4 (5%). Among patients with MPN, the JAK 2 (V617F) mutation was showed in 77.5% of cases, while the CALR mutation was present in 18.75% of cases. Furthermore, the presence of the JAK2 exon 12 mutation was identified in 3.75% of the subjects. In the study population, the absence of MPL mutation was observed. In patients diagnosed with PV, the JAK2 V617F mutation was observed in 96% of cases, while in patients with ET, the mutation was present in 35% of cases. In PV patients, the occurrence of JAK 2 exon 12 mutation was observed to be 4%, and it was identified as being mutually exclusive with the JAK 2 V617F mutation. The presence of a CALR mutation was observed in 65% of individuals diagnosed with ET. The findings align with prior publications, where the JAK 2 V617F mutation was detected in approximately 80-95% of PV patients and 20-70% of ET patients (9-11) .

The JAK 2 V617F mutation is the primary molecular abnormality found in MPNs, occurring in 95% of PV cases and 50%-60% of ET and PMF cases (12). According to studies, the occurrence of the JAK2 V617F mutation varies across different MPN subtypes. The prevalence is observed to be lower than anticipated in PV cases, possibly due to over-diagnosis, while higher rates are found in ET and PMF patients (13). Moreover, the JAK2 V617F mutation has been associated with distinct clinical and hematological features in patients with MPN. For instance, JAK2 V617F-mutated ET cases exhibit increased hemoglobin and erythrocyte volume fraction, while CALR-mutated ET cases show elevated platelet levels (14). A study conducted in the city of Sulaimaniyah, Iraq (15) revealed the presence of JAK 2 V617F mutation in patients with PV, as well as CALR and MPL mutations in patients with ET and PMF. The proportions of JAK2 V617F, CALR, and MPL W515L mutations were recorded as 50.7%, 22%, and 16.4%, respectively, highlighting the importance of accurately identifying these mutations through genotyping

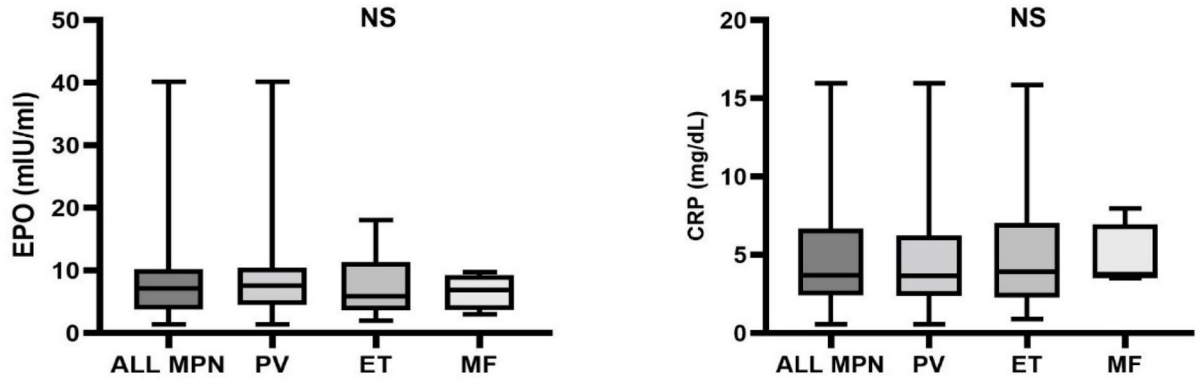

Figure 4. Kruskal-Wallis test for EPO and CRP correlation in MPN patients. The median (IQR) for serum EPO level was 7.195 (mlU/mL) in total MPNs patients, but the median (IQR) for serum CRP level was 3.690 (mg/L) in total MPNs patients. Abbreviations: PV, Polycythemia vera; ET, Essential thrombocythemia; MF, Myelofibrosis.

in the diagnostic process. This emphasizes the significance of these mutations in the identification and prediction of the respective diseases.

Another study conducted in Pakistan demonstrated a significant recurrence rate of JAK2 V617F in patients with MPN, with 96% of cases exhibiting positivity for this mutation (16).

In a parallel, an investigation carried out in Baghdad city, Iraq, findings mirrored our own. An observation was made indicating that the JAK2V617F mutation was present in 97.9% of PV patients and 61.7% of ET patients (11). In addition, authors have observed a decreased occurrence of the JAK2 V617F mutation, with a prevalence of 31% (17) and 35.7% (18). Moreover, it was found that JAK2 exon 12 was present in 3.1% of patients diagnosed with PV. In patients diagnosed with ET, the presence of CALR mutation was identified in 38.3% (11).

The studied population showed a notable absence of MPL mutations. The results of this study are in line with a recently published systematic review and meta-analysis, which demonstrated a complete absence of MPL mutation in PV patients (11,19). Moreover, associations between specific mutations and hematologic parameters were observed, such as higher platelet counts in CALR-mutated patients and higher age and hemoglobin and RBC counts in JAK2 V617F-mutated individuals. The significance of genetic testing is underscored by these findings, as it enables accurate diagnosis and prognostic evaluation of BCR-ABL1 negative MPNs in the Middle Eastern population (20).

Our study revealed a significant increase in platelet count among patients with ET, with a median value of 879 (10^9/L). Our results are similar to other findings by Ojeda et al (20) and Saaed et al (21). The researchers observed that patients with CALR mutation in the ET study demonstrated a lower age and elevated platelet counts in contrast to individuals with JAK2 V617F and triple negative. Additionally, a recent study has revealed that patients harboring the JAK2 V617F mutation exhibited elevated levels of leukocyte and hemoglobin in contrast to CALR-mutated patients. This observation aligns with previous reports.

Nevertheless, our current study found, no patients with positive MPN have elevated serum EPO levels. Our study also investigates the levels of serum EPO in untreated ET patients, revealing a potential correlation between subnormal EPO values and disease characteristics. Likewise, the study conducted by Szuber et al (22) highlights that a substantial number of individuals diagnosed with ET exhibited lower-than-normal serum EPO values, suggesting a potential connection between EPO levels and variations in the disease.

The current study has reaffirmed the distinct laboratory and clinical characteristics of PV and ET patients, as previously reported in prior publications. Additionally, our findings demonstrate a similarity in the occurrence of JAK 2 V617F, JAK2 exon 12, and CALR mutations to the data reported in prior scientific publications.

Conclusion

To achieve an accurate diagnosis of BCR-ABL1-negative MPN, the inclusion of molecular tests such as JAK2 V617F, CALR, and MPL mutations, along with other hematological analysis like complete blood count, can provide substantial diagnostic value.

Limitations of the study

The study included 80 patients, which, although significant, may not represent the entire population of MPN patients in Erbil Iraq regions. The MF group had a significantly lower number of participants compared to the PV and ET groups. A larger sample size would yield more reliable results.

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Authors' contribution

Conceptualization: Isik Didem Karagoz and Mohammed Hashm.
Data curation: Mohammed Hashm.
Formal analysis: Isik Didem Karagoz and Mohammed Hashm.
Funding acquisition: Mohammed Hashm.
Investigation: Mohammed Hashm.
Methodology: Mohammed Hashm.
Project administration: Isik Didem Karagoz.
Resources: Mohammed Hashm.
Software: Mohammed Hashm.
Supervision: Isik Didem Karagoz.
Validation: Isik Didem Karagoz.
Visualization: Isik Didem Karagoz and Mohammed Hashm.
Writing–original draft: Isik Didem Karagoz and Mohammed Hashm.
Writing–review & editing: Isik Didem Karagoz and Mohammed Hashm.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The research conducted in this study adhered to the principles outlined in the Declaration of Helsinki and was approved by the ethics committee of Gaziantep Islamic Science and Technology University (Ethical code #2024-2ÖNP-0177). This study was conducted in collaboration with the bio-laboratory and the Nanakaly Teaching Hospital for blood diseases and oncology in Erbil, Iraq. Before the study, and at the time of admission, patients routinely provided the informed consent for any intervention. Additionally, this study is derived from the MSc thesis of Mohammed Jarjees Hashm at the Gaziantep Islamic Science and Technology University, Faculty of Arts and Sciences, Department of Biology, Gaziantep, Turkey. Accordingly, ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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