Screening of Arterial Atherosclerosis in JAK2 Positive Philadelphia Negative Myeloproliferative Neoplasms Among Egyptian Patients Below 60 Years Old

Original Article

Ahmed Mohamed Saeed[1], Noha M. El Husseiny[2], Marwa Salah Mohamed[3], Hany Hafez[4], Mohamed Khalaf[5], George B. Soryal[6], Sara Abd El-Ghani[7]

Departments of [1]Internal Medicine,[2,3]Hematology, Armed Forces College of Medicine, [4]Radiology, [5,6]Hematology, Military Medical Academy, [7]Internal Medicine, Faculty of Medicine, Cairo University, Cairo, Egypt.

ABSTRACT

Background: It has been discovered that patients with myloproliferative neoplasms (MPNs) have an increased risk of thrombosis in presence of JAk2V617F mutation that causes a thrombophilic phenotype. Ejection fraction (EF) and lipid profile assessment are accepted as markers of subclinical atherosclerosis and can help the clinician to reclassify the cardiovascular risk of patients into a lower or higher risk category.

Objective: The aim of the study was to identify whether the risk of arterial atherosclerosis increases in MPNs patients below 60 years in comparison to normal population regardless the known risk factor of atherosclerosis as diabetes, Hypertension and smoking.

Patients and Methods: The study included 48 patients of MPNs and 48 control samples were selected from the same places .the control were chosen as one by one to match the case in age, sex, smoking, HTN and DM. All the patients were subjected to: history taking and physical examination. Laboratory investigations include CBC and lipid profile. Radiological investigations in form of Trans thoracic Echo for EF assessment.

Results: The MPNs patients Showed statistically significant difference (*P value 0.035*) in the EF assessment when compared to controls and significant increase in lipid profile (LDL, HDL, TGs) despite similar risk in DM and HTN, With no significant difference between PV and ET cases regarding measurement of EF and lipid profile assessment (*p value 0.352*). This may point to the main drive for impaired EF and lipid profile is related mainly to JAK2 gene mutation.

Conclusion: JAK2 gene mutation is a risk factor for atherosclerosis.

Key Words: Ejection fraction, essential thrombocythemia, lipid profile, polycythemia vera.

Received: 10 April 2023, **Accepted:** 10 November 2023

Corresponding Author: Ahmed Mohamed Saeed, MSc, Departments of Internal Medicine, Armed Forces College of Medicine (AFCM), Cairo, Egypt. **Tel.:** 01005048778, **E-mail**: ahmedsa3eed47@gmail.com

ISSN: 2812-5509, 2023, Vol. 1, No. 1

INTRODUCTION

Myeloproliferative neoplasms are a diverse category of disorders caused by aberrant growth of any of the terminal myeloid cell types in the peripheral circulation. Myeloproliferative neoplasms include Chronic myeloid leukemia (CML), Polycythemia Vera (PV), Primary myelofibrosis (PMF), and Essential thrombocythemia (ET) Chronic eosinophilic leukemia (CEL), Chronic neutrophilic leukemia (CNL), and MPN, not otherwise specified[1].

The fourth edition of the World Health Organization (WHO) classification for acute and myeloid leukemia was revised in 2016 as a result of recent advances in hematology, including the recognition of prognostic markers and molecular markers, which has resulted in a much better understanding of the genetics of hematological malignancies and molecular pathogenesis[2].

An estimated 11% to 22% of patients with myeloproliferative neoplasms (MPNs) median age 68 years who should receive medication for the management of comorbidities associated with thrombotic risk were not prescribed adequate care, according to data presented at the 2021 European Hematology Association (EHA) Virtual Congress[3,4].

Ejection fraction and lipid profile are accepted as a markers of subclinical atherosclerosis and can help the clinician to reclassify a substantial proportion of intermediate cardiovascular risk patients into a lower or higher risk[5,6].

DOI: 10.21608/ARCMED.2023.205336.1025

PATIENTS AND METHODS:

Study design:

This case control study was conducted during the period from September 2021 to June 2022 and included 48 patients of myloproliferative disorders (Mainly PV and ET) attending clinical haematology outpatient clinic in Maadi hospital, and internal medicine department at kobry koba hospital and 48 control samples were selected from same places .the control were chosen as one by one to match the case in age, sex, smoking, HTN and DM.

Inclusion criteria:

Adult Egyptian cases with myeloproliferative neoplasms Philadelphia negative diagnosed based on WHO criteria 2016 and with positive JAK 2 mutation and age between (18-60 years).

Exclusion criteria:

Cases of MPNs above 60 years. Cases under lipid lowering agents treatment or under antiplatelet therapy due to previous thrombotic event.

Data collection:

Clinical data were obtained including patient demographics (age, gender, comorbid conditions (diabetes, hypertension, smoking).Full history taking and physical examination are applied to all patients. Clinical assessment of symptoms (MPNs score to all patients).Laboratory investigations included complete blood count (CBC), lipid profile in the form of (serum cholesterol, triglycerides (TGs) low-density lipoprotein cholesterol (LDL) and high-density lipoprotein cholesterol (HDL).

Transthoracic echocardiography are performed to all cases and control. All measurements were obtained according to the American society of echocardiography guidelines. Left Ventricle Ejection fraction will be calculated and expressed as percentage with patient in supine position.

Ethical consideration:

The study proposal was revised and approved by the Armed Forces College of Medicine Ethical Review Committee. Policy of data confidentiality was strictly followed. The aim and nature of the study was explained for each patient before inclusion. An informed written consent was obtained from all participants before enrollment. The study design conformed to the requirements of Revised Helsinki Declaration of biomedical ethics.

Statistical analysis:

Analysis of data will be done by using SPSS (statistical program for social science version 16) as follows: Description of quantitative variables as mean, SE and range. Description of qualitative variables as number and percentage. Chi-squared test will be used to compare variables, in parametric data (SD < 50% mean)

Mann Whitney test will be used instead of unpaired t-test in non-parametric data. One-way ANOVA test will be used to compare more than two variables as regard quantitative variable. Spearman correlation test will be used to rank variables versus each other positively or inversely (*p-value<0.05* significant). Linear regression and logic regression to assess relation between ejection fraction and significant correlating independent factors.

RESULTS:

Demographic data of the studied groups:

Regarding the age of the studied groups: the mean age of cases and control were 47.02 ranged from (18 -60) years and mean age of controls was 46.9 ranged from (18 – 60) years. Twenty three 23(47.9%) cases were males and 25 (52.1%) of the cases were female. And regarding the comorbidities of the cases 23 (47.9%)of them were hypertensive, 19(39.5%) were diabetic, and 21(43.7%) of them were smokers, the controls were chosen as one by one to match the case in age, sex, smoking, HTN and DM. The study included 23 patients (47.9%) had polycythaemia rubra Vera, 24 patient (50%) had essential thrombocytosis and 1 (2.1%) patient had Primary myelofibrosis as demonstrated in (Table 1).

Clinical presentations of the myloproliferative patients:

Regarding the clinical presentations of the patients (MPN10 score): 28 cases (58.3%) presented with generalized fatigue while 26 cases (54.1%) presented with lack of concentration, 23 cases (47.9%) of patients presented with bony pains and 20 (41.6%) of them presented with increased satiety Data shown in (Table 1). There was significant difference found between PV cases and ET cases regarding sex with predominance male sex in PV rather than ET. (*P value < 0.05*).

No significant difference was detected between PV cases and ET cases regarding comorbidities or presenting symptoms. (*P value > 0.05*).

Laboratory data of the cases and control:

Regarding the results of routine work up of our patients and controls the mean value of LDL for cases was 147.9

mg/dl and for controls was 105.1 mg/dl , the mean value of HDL for cases was 54.2mg/dl while for controls was 60 mg/dl Regarding mean value of haemoglobin in cases it was 14.47g/dl while in controls was 12.18 g/dL, the mean value of TLC in cases was 7.11cells/mm^3 while in controls was 6.706 cell/mm^3 and the mean value of PLT in cases was 459.65 109 /ml while in controls was 257.49 109 /ml as shown in (Table 2). The results showed significant difference between cases and controls in HDL, LDL, TGs, Hb and platelets. (*P value < 0.05*) While there was no difference between cases and controls regarding Cholesterol and TLC. (*P value > 0.05*).

The results showed significant difference between cases and controls regarding ejection fraction assessment. (*P value < 0.05*) as demonstrated in (Table 2).

Laboratory values, Radiological assessment and Treatment between PV patients and ET patients:

The results showed significant difference between PV cases and ET cases regarding values of Hb, platelets and duration of illness. (*P value < 0.05*). The results showed that there is no significant difference between PV and ET patients regarding echo assessment (*P value > 0.05*) as demonstrated in (Table 3).

Correlation between Ejection fraction and the different parameters:

Results show correlation analysis between EF statistically negative with total cholesterol, fatigue, age, bony pains, sweating, LDL and Diabetes.

A linear regression including, Total Cholesterol, LDL. and age statistically significantly predict EF of ECHO, $F(3,44) = 7.7$, ($p = .0001$) and it accounted for 34.5.% of the explained variability in ECHO. Age and LDL significantly associated with the decreased EF ECHO ($p = 0.008$, 0.04 respectively) as shown in following (Table 4) and (Figure 1).

Table 1: Demographic and clinical presentations data of the studied groups

Item	Cases	percent
Males	23	47.9%
Females	25	51.2%
DM	19	39.5%
HTN	23	47.9%
Smoking	21	43.7%
PV	23	47.9%
ET	24	50%
Primary myelofibrosis	1	2.1%
Generalized fatigue	28	58.3%
Lack of concentration	26	54.1%
Bony pains	23	47.9%
Early Satiety	20	41.6%
Abdominal pain	19	39.5%
Itching	19	39.5%
Fever	18	37.5%
Weight loss	16	33.3%
Sweating	14	29.1%
Thrombosis	12	25%
Hydroxyurea	25	52%
Thrombonorm	3	6.2%
No Treatment	20	41.6%

Table 2: Laboratory and radiological data among the myloproliferative patients and controls

Item	Mean for cases ±SE	Mean for controls ±SE	T	*P*
Cholesterol mg/dl	205.96± 2.925	202.243.525±	0.815	*0.417*
HDL mg/dl	54.251.25±	60.0632.2±	2.304	*0.023**
LDL mg/dl	147.983.84±	105.134.9±	6.891	*0.00**
TGs mg/dl	167.193.71±	153.474.43±	2.375	*0.02**
Hb g/dl	14.4770.41±	12.180.25±	4.685	*0.00**
PLT 10^9 /ml	459.6535.26±	257.4912.00±	5.381	*0.00**
TLC Cell/mm^3	7.11± 0.36	6.7060.319±	0.836	*0.405*
Ejection fraction	57.8330.799±	60.4680.94±	2.138	*0.035**

Table 3: Laboratory values, Radiological assessment and Treatment between PV patients and ET patients

Item	Mean of PV ±SE	Mean of ET ±SE	T	*P*
Cholesterol mg/dl	204.39 ±4.411	206.88 ±4.079	0.414	*0.681*
HDL mg/dl	55.478 ±1.812	53.371.78±	0.826	*0.413*
LDL mg/dl	148.785.965±	145.96 ±5.101	0.361	*0.720*
TGs mg/dl	171.17 ±6.07	161.75 ±4.23	1.282	*0.206*
Hb g/dl	17.16 ±0.197	12.170.357±	15.274	*0.00**
PLT 10^9 /ml	239.22 ±16.7	684.21 ±30.62	16.685	*0.00**
TLC Cell/mm^3	6.913 ±0.477	7.391 ±0.557	0.650	*0.519*
Duration of diagnosis(in month)	44.39 ±26.75	70.12 ±36.91	-2.75	*0.009**
Ejection fraction(EF)	57.081.29±	58.621±	0.941	*0.352*

Table 4: showing the correlation between Ejection fraction and the different parameters

item correlated to EF	R	*P*
Age	-0.49	*0.000*
Total chol.	0.29	*0.04*
HDL	0.19	*0.19*
LDL	-0.42	*0.003* *
TG	-0.25	*0.07*
PLT	0.003	*0.95*
Duration of disease	-0.23	*0.1*
Bone pains	-0.38	*0.007*
Sex	0.03	*0.8*
HTN	0.21	*0.13*
Smoking	-0.17	*0.23*
DM	-0.31	*0.03*
HB	0.284	*0.070*
TLC	0.13	*0.35*
CIT	-0.246	*0.092*
Thrombosis	-0.04	*0.7*
Satiety	0.14	*0.34*
Abdominal pain	-0.04	*0.7*
fever	0.08	*0.57*
Fatigue	-0.3	*0.03*
Lack conc.	0.010	*0.947*
Night sweat	-0.34	*0.015*
Itching	0.06	*0.68*
Weight loss	-0.23	*0.1*

Partial regression plot Dependent variable: EF

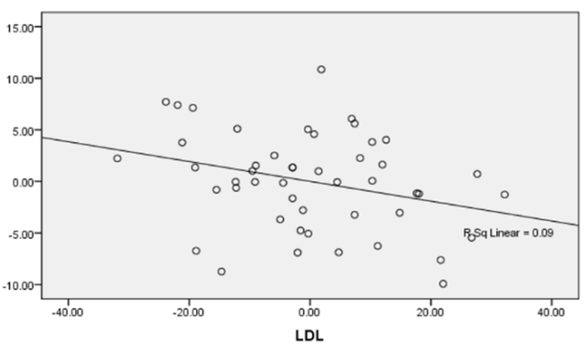

Fig. 1: shows the linear regression analysis of EF with LDL, *P value = 0.043*

DISCUSSION

Our study was conducted on 48 patients with MPN JAK 2 positive age from 18 years to 60 years which is to the best of our knowledge the first study to be done in young age below age of 60, Twenty three (47.9%) cases were males and 25 (52.1%) of the cases were females who underwent echo heart for ejection fraction and lipid profile as markers for atherosclerosis Who were compared to 48 sex, age and comorbidities matched control group.

Leiva *et al* stated that the risk of thrombotic consequences is raised in myeloproliferative neoplasms. The Janus-associated kinase signaling pathway is constitutively activated by somatic mutations in the genes encoding Janus kinase 2, as the JAK 2 mutated macrophage have impaired ability to engulf dead cells leading to enlargement in necrotic cores[3].

Another theory explained that Macrophages with the JAK2V617F mutation have faulty efferocytosis, which causes the necrotic cores to expand. As opposed to wild-type JAK2 macrophages, JAK2V617F macrophages exhibit enhanced erythrophagocytosis. Hemoglobin degradation releases iron, which through the Fenton reaction can increase local oxidative stress[7].

Matter *et al* studied the incidence of silent thrombosis in patients younger than 60 years and recommended routine screening for venous thrombosis in any patient with MPNs when diagnosed even if asymptomatic and if patients with MPNs present with increasing pruritus or abdominal pain, they should be also screened for venous thrombosis[8].

In the Philadelphia-negative myeloproliferative neoplasms, there is mounting evidence that persistent inflammation may play a significant role in clonal development and disease progression (MPNs), a variety of proinflammatory cytokines with abnormal expression and activity are linked to MPNs, particularly MF, where immunological dysregulation is prominent as shown by dysregulation of multiple immune and inflammatory genes. In addition, chronic inflammation has been suggested to share in the development of premature atherosclerosis and may also lead to the development of other cancers in MPNs, both non-hematologic and hematologic[9].

Our results showed significant increase in lipid profile (LDL, HDL, and TGs) despite similar risk in DM, HTN and smoking.

Mattar *et al* found that there is a positive statistically significant correlation between the JAK2 allele's burden and the hemoglobin level and LDH, and negative statistically significant correlation with the platelet count. While there is no significant correlation between JAK2 allele's burden and white cell count[10].

Results in our study show correlation analysis between ejection fraction(a parameter of left sided cardiac function) was statistically negative with total cholesterol, fatigue, age, bony pains, sweating, LDL, Diabetes

Moreover, we found a linear regression between EF and, Total Cholesterol, LDL. And age and it accounted for 34.5% of the explained variability in EF, while age and LDL significantly associated with the decreased EF.

Weber *et al* stated that EF showed a decrease in indices of diastolic function in Type 1 diabetic patients when compared to the control group, which may be the early cardiac lesion in diabetes[11].

Jørgensen *et al* found that in patients with type 2 diabetes, a subtle decrease in left ventricular function is present with increasing levels of remnant cholesterol and triglyceride levels indicating an effect of these on cardiac function that is not detectable by conventional echocardiography[12].

Mattar *et al* studied the correlation between JAK2 allele burden and pulmonary arterial hypertension in MPN patients but they don't found any correlation between JAK2 allele's burden and pulmonary artery hypertension[10].

Our study included 23 patients (47.9%) with Polycythemia rubra Vera, 24 patient (50%) having Essential thrombocytosis and 1 (2.1%) patient with Primary mylofibrosis.

Regarding the clinical presentations of the patients (MPN10 score): 28 cases (58.3%) presented with generalized fatigue while 26 cases (54.1%) presented with lack of concentration, 23 cases (47.9%) of patients presented with bony pains and 20 (41.6%) of them presented with increased satiety.

Harrison *et al* found that fatigue is the most common prevalent symptom in MPN cases[13], this results comes with agreement to our results as fatigue is the most common symptoms.

Regarding the treatment 25 patients (52%) were on Hydroxyurea, 3 patient (6.2%) on Thrombonorm and 20 patients (41.6%) on no treatment (follow up).

Our study found no correlation between ET and PV with presenting symptoms, comorbidities and treatment.

Our results showed significant difference between PV and ET cases regarding hemoglobin level and platelet count which go with Hemoglobin >16.5 g/dL in men or > 16 g/dL in women is a major criteria in PV diagnosis and Platelets ≥450 × 109/L is a major criteria in ET diagnosis[14].

This is also explained that 40% the patients were not under medical treatment.

Our study showed no significant difference between PV and ET cases regarding measuring EF and lipid profile assessment this may point to that the main drive for impaired EF is related mainly to JAk2 mutation.

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, *et al*. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022 Jun 22;36(7):1703–19.

2. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, *et al*. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood [Internet]. 2016;127(20):2391–405. Available from: https://www.ncbi.nlm.nih.gov/pubmed/27069254/

3. Leiva O, Hobbs G, Ravid K, Libby P. Cardiovascular Disease in Myeloproliferative Neoplasms. JACC: CardioOncology [Internet]. 2022 Jun [cited 2022 Aug 1];4(2):166–82. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9270630/

4. Baumeister J, Chatain N, Sofias AM, Lammers T, Koschmieder S. Progression of Myeloproliferative Neoplasms (MPN): Diagnostic and Therapeutic Perspectives. Cells [Internet]. 2021 Dec 1 [cited 2022 Sep 5];10(12):3551. Available from: https://www.mdpi.com/2073-4409/10/12/3551/htm

5. Bloomfield GS, DeLong AK, Akwanalo CO, Hogan JW, Carter EJ, Aswa DF, *et al*. Markers of Atherosclerosis, Clinical Characteristics, and Treatment Patterns in Heart Failure: A Case-Control Study of Middle-Aged Adult Heart Failure Patients in Rural Kenya. Global Heart [Internet]. 2016 Mar 1 [cited 2021 Oct 22];11(1):97–107. Available from: https://www.sciencedirect.com/science/article/abs/pii/S2211816015003191

6. Bordeianu G, Mitu I, Stanescu RS, Ciobanu CP, Petrescu-Danila E, Marculescu AD, *et al*. Circulating Biomarkers for Laboratory Diagnostics of Atherosclerosis—Literature Review. Diagnostics. 2022 Dec 13;12(12):3141.

7. Michel JB, Libby P, Franck G. Internal Bleeding. JACC: Basic to Translational Science. 2018 Aug;3(4):481–4.

8. Mattar MM, Nassef S, El Husseiny NM, El Masry MR, Salah M, Morad MA, *et al*. Incidence of Silent Thrombosis in Patients Younger Than 60 Years With Myeloproliferative Neoplasms: Single-Center Egyptian Study. Clinical Lymphoma Myeloma and Leukemia [Internet]. 2019 Aug 1 [cited 2022 Sep 19];19(8):e425–9. Available from: https://www.sciencedirect.com/science/article/abs/pii/S215226501930254X

9. Hasselbalch HC, Bjørn ME. MPNs as Inflammatory Diseases: The Evidence, Consequences, and Perspectives. Mediators of Inflammation. 2015;2015:1–16.

10. Mattar MM, Morad MAK, El Husseiny NM, Ali NH, El Demerdash DM. Correlation between JAK2 allele burden and pulmonary arterial hypertension and hematological parameters in Philadelphia negative JAK2 positive myeloproliferative neoplasms. An Egyptian experience. Annals of Hematology. 2016 Jul 29;95(10):1611–6.

11. Weber TR, Silva RL da, Cossul S, Lofrano Alves MS, Lee SV der S, Brum Marques JL. Echocardiographic evaluation in type 1 diabetes mellitus. Revista Portuguesa de Cardiologia (English edition) [Internet]. 2021 Oct 1 [cited 2022 Aug 2];40(10):757–65. Available from: https://www.revportcardiol.org/en-echocardiographic-evaluation-in-type-1-articulo-S217420492100249X

12. Jørgensen PG, Jensen MT, Biering-Sørensen T, Mogelvang R, Galatius S, Fritz-Hansen T, *et al*. Cholesterol remnants and triglycerides are associated with decreased myocardial function in patients with type 2 diabetes. Cardiovascular Diabetology. 2016 Sep 22;15(1).

13. Harrison CN, Koschmieder S, Foltz L, Guglielmelli P, Flindt T, Koehler M, *et al*. The impact of myeloproliferative neoplasms (MPNs) on patient quality of life and productivity: results from the international MPN Landmark survey. Annals of Hematology. 2017 Aug 5;96(10):1653–65.

14. Tefferi A, Vannucchi AM, Barbui T. Polycythemia vera treatment algorithm 2018. Blood Cancer Journal [Internet]. 2018 Jan;8(1). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5802495/