RESEARCH ARTICLE

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Myeloproliferative Neoplasms and Aspirin: Does Increased Platelet Turnover Matter?

Myeloproliferatif Neoplaziler ve Aspirin: Artmış Platelet Döngüsü Önemli mi?

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Abstract

Objective: Platelet aggregation tests and the analysis of thromboxane A2 metabolites [serum thromboxane B2 (TXB2) and urine 11-dehydro TXB2] are used to evaluate the efficacy of aspirin. In myeloproliferative neoplasms (MPNs), the immature platelet fraction (IPF) rises due to enhanced platelet turnover, and this has been thought to reduce the efficacy of aspirin. This phenomenon is overcome by the recommendation of aspirin intake in divided doses. We aimed to evaluate aspirin efficacy in patients who were receiving aspirin treatment of 100 mg/day.

Materials and Methods: Thirty-eight MPN patients and 30 control patients (non-MPN patients who received a single daily dose of aspirin at 100 mg for nonhematological conditions) were enrolled. IPF, serum TXB2, and urine 11-dehydro TXB2 levels were measured and aggregation tests with arachidonic acid and adenosine diphosphate were performed by light transmission aggregometry (LTA).

Results: Mean IPF and TXB2 levels were higher in the MPN group (p=0.008 and p=0.003, respectively). IPF levels were lower in patients on cytoreductive therapy in the MPN group (p=0.001), but these values were similar between patients on hydroxyurea and the non-MPN group (p=0.72). TXB2 levels did not differ according to hydroxyurea treatment status but were higher in the MPN group compared to non-MPN patients (23.63 ng/mL and 19.78 ng/mL, respectively; p=0.04). TXB2 values were higher in patients with essential thrombocythemia and a history of thrombotic events (p=0.031). No difference was observed in LTA between the MPN and non-MPN patient groups (p=0.513).

Conclusion: Higher levels of IPF and TXB2 in the MPN patient group indicated platelets that could not be inhibited by aspirin. It was

Öz

Amaç: Aspirin etkinliğini değerlendirmek için, trombosit agregasyon testleri ile tromboksan A2 metabolitlerinin analizi [serum tromboksan B2 (TXB2) ve idrar 11-dehidro TXB2] kullanılmaktadır. Myeloproliferatif neoplazilerdeki (MPN) artmış trombosit döngüsü, immatür trombosit fraksiyonunun (İTF) artışına sebep olmakta, bunun da aspirin etkinliğini azalttığı düşünülmektedir. Bu durumu aşmak için, aspirinin bölünmüş dozlarda verilmesi önerilmektedir. Çalışmamızda, günde tek doz 100 mg aspirin tedavisi alan hastalardaki aspirin etkinliğini değerlendirmeyi amaçladık.

Gereç ve Yöntemler: Otuz sekiz MPN tanılı hasta ile 30 kontrol hastası (hematolojik olmayan durumlar sebebiyle günde tek doz 100 mg aspirin kullanan, MPN tanısı olmayan hastalar) çalışmaya alındı. İTF, serum TXB2 ve idrar 11-dehidro TXB2 düzeylerine bakıldı. Işık transmisyon agregometride (ITA) agregasyon testleri yapıldı.

Bulgular: Hasta grubunun TXB2 ve İTF mutlak değer ölçümleri, kontrol grubuna göre anlamlı düzeyde yüksek görüldü (sırasıyla p=0,008 ve p=0,003). Hidroksiüre alan hastaların İTF değerleri hidroksiüre almayan hastalardan daha düşük (p=0,001), ancak kontrol grubu ile benzerdi (p=0,72). TXB2 seviyeleri hidroksiüre alan ve almayan hastalarda değişiklik göstermedi, ancak MPN grubunda kontrol grubuna göre daha yüksek saptandı (sırasıyla 23,63 ng/mL ve 19,78 ng/mL, p=0,04). Tromboz öyküsü olan esansiyel trombositemi hastalarında TXB2 seviyeleri daha yüksek görüldü (p=0,031). Gruplar arasında ITA ile bakılan trombosit agregasyon testlerinde anlamlı bir farklılık izlenmedi (p=0,513).

Sonuç: MPN hasta grubundaki daha yüksek İTF ve TXB2 seviyeleri, aspirin tarafından inhibe edilememiş trombositler olduğunu gösterdi. Sitoredüktif tedavi altındaki hastaların İTF değerlerinin daha düşük

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Abstract

observed that patients under cytoreductive therapy had lower IPF values, but the expected decrease in TXB2 levels was not observed. These findings suggest that a lack of response to aspirin may be due to additional intrinsic factors rather than increased platelet turnover.

Keywords: Thromboxane B2, Platelet aggregation, Platelets, Arachidonic acid, Aspirin

Öz

olduğu, ancak TXB2 seviyelerinde beklenen düşüşün olmadığı görüldü. Bu bulgular aspirin yanıtsızlığının, artmış trombosit döngüsünden ziyade intrinsik ek faktörlere bağlı olabileceği kanaatini oluşturmaktadır.

Anahtar Sözcükler: Tromboksan B2, Trombosit agregasyonu, Trombositler, Araşidonik asit, Aspirin

Introduction

Myeloproliferative neoplasms (MPNs) are a group of disorders characterized by leukemic transformation, myelofibrosis, and thrombosis. It has been shown that 41% of all deaths in cases of polycythemia vera (PV), which is a subgroup of MPNs, are caused by thrombotic events [1,2]. To reduce the risk of thrombosis, aspirin is recommended as a prophylactic agent in line with studies performed among PV patients [3,4]. The benefits of thrombosis prophylaxis in essential thrombocythemia (ET), however, are unclear. Recommendations for aspirin treatment in patients diagnosed with ET are based on studies involving PV patients. Thrombotic events may occur in ET patients even under aspirin treatment [5,6], which may be due to aspirin non-responsiveness.

Aspirin efficacy can be tested in many ways. Previous measurements of thromboxane A2 (TXA2) metabolites showed that low-dose aspirin twice a day provided better cyclooxygenase-1 (COX-1) inhibition than once-a-day low-dose aspirin [5]. Currently, platelet functions can be evaluated by aggregation tests or by testing the levels of serum thromboxane B2 (TXB2) and urine 11-dehydro TXB2 levels, which are stable metabolites of TXA2 [7]. Aspirin non-responsiveness in MPN patients is caused by increased platelet production, leading to increases in the immature platelet fraction (IPF) and naive platelet that are free from the effect of aspirin. Dividing the daily aspirin dose is recommended as a solution to overcome this problem [5,8]. This study aimed to determine the efficacy of a single dose of aspirin (100 mg/day) in patients diagnosed with MPN and compare it with that in a non-MPN patient group.

Materials and Methods

Patients with PV and ET who fulfilled the relevant diagnostic criteria of the World Health Organization, were aged between 18 and 80 years, and were prescribed treatment with a single daily dose of enteric-coated aspirin at 100 mg were included in the MPN patient group. Patients aged between 18 and 80 years who received a single dose of daily enteric-coated aspirin at 100 mg for nonhematological conditions were selected for the control group (non-MPN patient group). Patients in both groups were on aspirin treatment for at least 2 weeks at the time of the research. Patients who did not consent to participate, pregnant patients, and patients with glomerular filtration rates of <30 mL/min, platelet levels lower than 150,000/mm^3 or higher than 1,000,000/mm^3, and hemoglobin levels of <10 g/dL were excluded. Patients who had used non-steroidal anti-inflammatory drugs (NSAIDs), antidepressants or anticonvulsants, or antiaggregant/anticoagulant agents in the last 7 days and patients receiving aspirin treatment at 81 mg, 150 mg, or 300 mg were also excluded from the study.

Data on demographics, comorbidities, medications, alcohol and smoking habits, and biochemical, hematological, and pathological findings were collected from patient files retrospectively. Patients with MPN had already been tested for *JAK-2* mutations before our study. Ethical permission for this study was granted by the İstanbul Medeniyet University Göztepe Research and Training Hospital Ethics Committee on May 22, 2019 (decision number: 2019/0230). The study was conducted in line with the Declaration of Helsinki and good clinical practices.

Thirty-eight patients were included in the MPN group (27 patients diagnosed with ET and 11 patients diagnosed with PV), whereas 30 patients were included in the non-MPN group. Fasting blood samples were collected from all individuals. All blood samples were drawn in the morning immediately before the patients took their daily aspirin doses. Complete blood count analyses were performed with a Mindray BC-6800 Auto Hematology Analyzer to determine the IPF values of the patients. Blood and urine samples were stored at -80 °C and tested within 6 months for serum TXB2 and urine 11-dehydro TXB2 levels. MyBioSource Human 11-Dehydro-thromboxane B2 ELISA Kits (MBS161319) and MyBioSource Human TXB2 ELISA Kits (MBS766152) were used to test urine 11-dehydro TXB2 and serum TXB2 levels, respectively (MyBioSource, Vancouver, Canada). Platelet aggregation curves were interpreted based on the platelet agonists arachidonic acid (AA; 1 mM final concentration) and adenosine diphosphate (ADP; 2 µM final concentration) by light transmission aggregometry (LTA). All tests were run within 3 h after collecting blood samples. As in previous studies, patients with residual platelet aggregation of >20% by LTA using AA and patients with residual platelet

aggregation of >50% by LTA using ADP were accepted as non-responsive to aspirin [9,10,11,12,13].

Statistical Analysis

NCSS 2007 (NCSS Statistical Software, Kaysville, UT, USA) was used for statistical analysis. The Shapiro-Wilk test was used to determine whether quantitative variables complied with normal distribution. For comparisons of quantitative variables between two groups, the Student t-test was used for variables that showed normal distribution and the Mann-Whitney U test was used for variables that did not show normal distribution. The Pearson chi-square test and Fisher exact test were used to compare qualitative data. Values of $p<0.05$ were considered statistically significant. The Cohen effect size for serum TXB2 was found to be 0.63. With that effect size, the power of the study in post hoc analysis was 81% for an error rate of 0.05 with 38 and 30 patients in the two groups according to G*Power 3.1.9.4 (Dusseldorf University, Dusseldorf, Germany).

Results

The mean ages of the patients in the MPN group and the non-MPN group were 54.4 and 54 years, respectively. Nineteen patients (50%) were on hydroxyurea treatment and 2 patients (5%) were on anagrelide or interferon treatment. The characteristics of the patients according to the groups are summarized in Table 1.

Aggregation percentages were tested by measuring ADP values in LTA. The percentages varied between 4.14% and 156.38%, and the mean value was 25.3% [standard deviation (SD): 8.5]. Aggregation values as tested by AA in LTA varied between 1.22% and 84.80%, and the mean value was 9.7% (SD: 17.7). There were no significant differences in aggregation test results by LTA between the groups (p=0.513). LTA testing showed that 4 patients in the MPN group were non-responsive to aspirin treatment (ADP of >50% and/or AA of >20%), whereas no aspirin non-responsiveness was detected in the non-MPN group by the LTA method.

TXB2 levels were significantly higher in the MPN group [25.3 ng/mL (SD: 8.5)] than in the non-MPN group [19.7 ng/mL (SD: 6.9)] (p=0.003). No significant difference was found in urine 11-dehydro TXB2 levels between these two groups (p=0.45), but the mean IPF value was higher in the MPN group (p=0.008) (Table 1). Platelet levels and IPF values of the MPN patients who were on hydroxyurea treatment were lower than those of the patients who were not taking hydroxyurea, but serum TXB2 and urine 11-dehydro TXB2 levels did not differ between these patients (Table 2).

The mean platelet count was significantly higher among hydroxyurea-treated MPN patients than non-MPN patients (460,000/mm^3 vs. 238,333/mm^3, $p<0.0001$) (Table 2). There was no difference in absolute IPF values between hydroxyurea-treated MPN patients and non-MPN patients (1301/mm^3 vs. 1208/mm^3, respectively, p=0.72) (Table 2).

Compared to the non-MPN group, serum TXB2 levels were higher in MPN patients taking hydroxyurea [23.6 ng/mL (SD: 5.5) vs. 19.7 ng/mL (SD: 6.9), p=0.047]. Urine 11-dehydro TXB2 levels were also higher in MPN patients taking hydroxyurea than non-MPN patients [108.9 pg/mg creatinine (SD: 21.9) vs. 102.3 pg/mg creatinine (SD: 23.1), p=0.321] (Table 2).

The TXB2 levels of ET patients with a history of a thrombotic event were significantly higher than those of ET patients without any thrombotic events [28.7 (SD: 5.6) vs. 24.8 (SD: 10.3), (p=0.031) (Table 3). Only one patient had a thrombotic event among the PV patients. Among all MPN patients, 27 patients (71%) had

Table 1. Characteristics of the patients according to the groups.

	MPN group	Non-MPN group	p
Age	54.45 (SD=11.38)	54 (SD=10.8)	0.879
Gender (female)	18 (47%)	16 (53%)	0.625
Body mass index	28.43 (SD=5.27)	29.31 (SD=5.05)	0.493
Smoker	8 (21%)	7 (23%)	0.822
Hypertension	19 (50%)	21 (70%)	0.096
Diabetes	10 (26.3%)	18 (60%)	0.005
Hyperlipidemia	5 (13.2%)	21 (70%)	0.001
IPF (%)	4.3 (SD=3.3)	5.59 (SD=4.9)	0.21
Absolute IPF value (/mm^3)	1967 (SD=1208)	1208 (SD=984)	0.008
TXB2 (ng/mL)	25.3 (SD=8.5)	19.7 (SD=6.9)	0.003
11-dehydro TB2 (pg/mg creatinine)	166.5 (SD=107)	145.7 (SD=142)	0.45
ADP (%)	25.3 (SD=8.5)	34.8 (SD=12.4)	0.513
Arachidonic acid (%)	9.7 (SD=17.7)	8 (SD=3.5)	0.024

ADP: Adenosine diphosphate; IPF: immature platelet fraction; TXB2: thromboxane B2; MPN: myeloproliferative neoplasm.

Table 2. IPF and TXB2 values according to cytoreductive therapy.

	MPN patients on hydroxyurea (n=19)	MPN patients, hydroxyurea-naive (n=19)	Non-MPN group (n=30)	p
Platelets (/mm^3)	460,000 (SD=165,000)	539,000* (SD=249,000)	238,333** (SD=38,300)	0.052* <0.0001**
IPF (%)	2.6 (SD=1)	5.9 (SD=4.1)*	5.59 (SD=4.9)**	0.008* 0.008**
Absolute IPF value (/mm^3)	1,301 (SD=685)	2,672 (SD=1255)*	1,208 (SD=984)**	0.001* 0.72**
TXB2 (ng/mL)	23.63 (SD=5.5)	27 (SD=10.6)*	19.78 (SD=6.9)**	0.22* 0.047**
11-dehydro TB2 (pg/mg creatinine)	108.9 (SD=21.9)	104.1 (SD=23.9)*	102.3 (SD=23.1)**	0.532* 0.321**

*: MPN patients on hydroxyurea vs. hydroxyurea-naive MPS patients.
**: MPN patients on hydroxyurea vs. non-MN patients.
IPF: Immature platelet fraction; TXB2: thromboxane B2; MPN: myeloproliferative neoplasm.

Table 3. Results of ET patients according to thrombotic event status.

	History of a thrombotic event (n=20)	No thrombotic event (n=7)	p
ADP (%)	35.40 (SD=16.61)	32.86 (SD=25.08)	0.439
Arachidonic acid (%)	5.76 (SD=2.64)	15.22 (SD=30.75)	0.268
TXB2 (pg/mL)	28.74 (SD=5.63)	24.80 (SD=10.35)	0.031
11-dehydro TB2 (pg/mg creatinine)	175.66 (SD=23.8)	139.4 (SD=39.7)	0.445

ADP: Adenosine diphosphate; ET: essential thrombocythemia, TXB2: thromboxane B2.

JAK-2 V617F mutations. No significant differences in serum TXB2 levels and urine 11-dehydro TXB2 levels were found between patients carrying the *JAK-2* V617F mutation and patients who did not carry the *JAK-2* V617F mutation [22.9 ng/mL vs. 25.5 ng/mL, respectively, for serum TXB2, p=0.57, and 1038.5 pg/mg creatinine vs. 1074.6 ng/mg creatinine, respectively, for urine 11-dehydro TXB2, p=0.41).

Discussion

Thrombosis is a major complication in MPN, and despite current treatment strategies, it may not be completely prevented. The cause of thrombosis in these patients may be related to a failure to respond to aspirin due to naive platelets that are unaffected by aspirin. In our study, serum TXB2 and IPF levels were higher in MPN patients than in the non-MPN group. MPN patients taking hydroxyurea had lower IPF levels than patients who were not taking hydroxyurea. The term "aspirin response" is still under debate and a topic of interest in many studies. Cardiovascular events are more frequent among patients who are considered non-responsive to aspirin [13]. LTA was previously accepted as an important method for testing "aspirin response," but, in fact, LTA was not specifically designed to test for this issue. In our study, aspirin response was tested with LTA and no significant difference was found between the MPN group and the non-MPN group. Previously, LTA was accepted as the gold standard for detecting aspirin non-responsiveness, but the value of this method is now controversial. Other methods are currently being explored and are more widely researched, some of which were evaluated in an earlier study. TXB2 tests were able to show aspirin non-responsiveness, but LTA tests failed to show it [14]. In another study, it was suggested that LTA can be used to test aspirin responsiveness, but that study did not have a control group, which makes it difficult to evaluate the outcomes [15]. Other novel studies assessed aspirin response using the VerifyNow system (Werfen, Bedford, MA, USA), serum TXB2, and urine 11-dehydro TXB2 levels [5,14]. In our study, the serum TXB2 levels of MPN patients were higher than those of non-MPN patients, as expected, but urine 11-dehydro TXB2 levels did not differ. Although urine 11-dehydro TXB2 levels are sometimes tested to evaluate aspirin response, this testing is not standardized. In a recent randomized controlled trial, urine 11-dehydro TXB2 levels were tested in two similar study groups to evaluate aspirin response, but the researchers did not obtain consistent results between the groups [5]. Changes in urine 11-dehydro TXB2 levels were reported in that study, but the test had limited value in evaluating aspirin response.

Previous studies have also shown weak correlations between serum TXB2 and platelet counts [16]. Many previous studies showed that high IPF levels were related to increased platelet turnover rate [14,16]. In one recent study in which IPF was

not measured qualitatively, IPF was calculated as 10% of all platelets and aspirin non-responsiveness was subsequently attributed to an increased platelet turnover rate [17]. The IPF levels in our MPN group were higher than those in the non-MPN group, and our findings suggested that higher IPF values and higher serum TXB2 levels were correlated in the MPN group. However, the IPF levels of MPN patients taking hydroxyurea were lower. These levels were even lower than the IPF levels of the non-MPN group [2.8% (1301/mm^3 vs. 5.59% (1208/mm^3) for patients using hydroxyurea in the MPN group and non-MPN group, respectively]. The decrease in IPF levels among hydroxyurea-treated MPN patients did not correlate with serum TXB2 levels as expected. This finding suggests that increased platelet turnover is a confounding factor in evaluations of aspirin response. A randomized clinical trial also found conflicting aspirin response results between patients taking 100 mg of aspirin twice a day and patients taking 200 mg of aspirin once a day [14]. In that study, IPF levels were found to be related to TXB2 levels; however, patients receiving cytoreductive therapy were not evaluated in that study. In our study, patients receiving cytoreductive therapy had lower IPF levels, but their TXB2 levels did not decrease. These findings suggest that aspirin non-responsiveness may not be caused by platelet renewal rates but rather by other mechanisms. It was observed that the levels of TXB2 were elevated in individuals undergoing treatment with hydroxyurea. These patients exhibited higher platelet counts as well. Although it is generally thought that low IPF values do not affect TXB2 levels and do not change the effect of aspirin, this correlation may be attributed to elevated platelet counts and may suggest that the effect of aspirin is mitigated in individuals with high platelet counts. This factor should be taken into consideration in future studies. However, *JAK-2* is also a key feature of MPN physiopathology, as suggested by Perrier-Cornet et al. [17], and although there has been much research on the subject, genetic status had no effect on aspirin response in our study.

Study Limitations

One limitation of this study is that the control group with non-MPN patients did not include healthy controls. These patients received aspirin therapy with a diagnosis of diabetes, cardiovascular disease, or hyperlipidemia, and these diseases have been suggested to impair the antiplatelet effect of aspirin [18,19,20]. Nevertheless, there was a difference in responses to aspirin between the patient groups. Another limitation of our study is that we did not use other methods that can be applied to evaluate aspirin efficacy. Furthermore, we did not assess the clinical significance of aspirin non-responsiveness; rather, the in vitro results of aspirin non-responsiveness were evaluated in our study. The long-term clinical significance of these in vitro results was not assessed, which can also be considered a limitation.

Conclusion

The findings of this study confirm that aspirin non-responsiveness can be seen in MPN patients, which might not be caused by platelet renewal rates. Responses to aspirin at different dosing schedules should be evaluated in light of these results. New anticoagulation treatment protocols other than aspirin should also be investigated.

Ethics

Ethics Committee Approval: This was a prospective study conducted in accordance with the Declaration of Helsinki, good clinical practices, and the guidelines of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use. An independent ethics committee or institutional review board reviewed the ethical, scientific, and medical appropriateness of the study at each point.

Informed Consent: Signed informed consent forms were obtained from patients before any study-related procedures were performed.

Authorship Contributions

Surgical and Medical Practices: I.K.G., I.E.Ö., A.H.K., G.Y., M.O.A., E.Ö.; Concept: I.K.G., E.Ö.; Design: I.K.G., I.E.Ö., E.Ö.; Data Collection or Processing: I.K.G., I.E.Ö., E.Ö.; Analysis or Interpretation: I.K.G., A.G., E.Ö.; Literature Search: I.K.G., A.G., E.Ö.; Writing: I.K.G., A.G., E.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

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