Efficacy and Safety of Azathioprine Therapy in Turkish Patients with Inflammatory Bowel Disease: A Retrospective Long Term Follow Up Study

İnflamatuar Bağırsak Hastalığı Olan Türk Hastalarda Azatioprinin Etkinliği ve Güvenliliği: Uzun Dönem Takipli Retrospektif Çalışma

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ABSTRACT Objective: Effectivity and safety of azathioprine in the treatment of inflammatory bowel disease have been proved and cited in many articles. Objective of this study is to evaluate the efficacy and side effects of azathioprine particularly in the long term treatment and postsurgical prophylaxis of Crohn's disease in Turkish patients with inflammatory bowel disease. **Material and Methods:** Patients treated with azathioprine were enrolled in this study and the efficacy of azathioprine was assessed with Truelove-Witts and Crohn's Disease Activity Indexes, retrospectively. **Results:** A total of 122 patients treated with azathioprine with a median duration of 24 months, including 109 patients with Crohn's disease and 13 with ulcerative colitis, were evaluated. Response (complete or partial remission) was observed in 106 (98+8, 87%) of 122 patients. The median duration of remission during the treatment was 21 months. Adverse effects were observed in 16% (19/122) of our patients and myelotoxicity was the most frequent adverse effect seen in 6.6% (8/122) of the patients. Drug discontinuation rate due to side effects was 9.8%. Overall response (complete and partial remission)was seen in 38 (36+2, 90.5%) out of 42 patients that had been taking azathioprin for more than four years with a median period of 60 months. The median of remission duration in those patients that had similar side effects was 53 months. Clinical recurrence was seen in six of 31 patients treated with azathioprine for post-surgical prophylaxis in a median follow-up of 42 months. **Conclusion:** Our study re-affirmed the safety and the effectiveness of azathioprine in the treatment of inflammatory bowel disease, especially for post-surgical prophylaxis and the long term treatment.

Key Words: Inflammatory bowel diseases; azathioprine; adverse effects

ÖZET Amaç: Azatioprinin inflamatuar bağırsak hastalığı (İBH) nın tedavisindeki etkinliği ve güvenilirliliği hakkında derlenmiş kanıtlar bulunmaktadır. Çalışmamızın amacı, İBH olan Türk hastalarda özellikle de Crohn's hastalığının uzun dönem tedavisi ve cerrahi sonrası profilaksisi üzerine vurgu yapmak üzere ilacın etkinliği ve yan etki durumunu ortaya koymaktır. **Gereç ve Yöntemler:** Azatioprin tedavisi almış toplam 122 hasta geriye dönük değerlendirilmek üzere çalışmaya alınmıştır. İlacın etkinliğini belirlemede Truelove-Witts ve Crohn Hastalığı Aktivite İndeksleri kullanılmıştır. **Bulgular:** Toplam 109 Crohn ve 13 ülseratif kolit hastası, medyan değeri 24 ay olmak üzere azatioprin tedavisi almıştır. Tedavi alan 122 hastanın 106'sında (98+8, %87) etkinlik genel olarak (tüm ve kısmi) gözlemlenmiştir. Remisyon süresinin medyanı 21 bulunmuştur. Hastaların %6'sında tedaviye bağlı yan etki gözlenirken, en sık gözlenen yan etki myelotoksisite (8/122, %6.6) olmuştur. Yan etkiye bağlı ilaç kesilme oranı %9.8 olmuştur. Azatioprin kullanımı 4 yıldan fazla olan 42 hastada, genel olarak yanıt gözlenen (remisyon ve parsiyel remisyon) 38 hastada (36+2, %90.5) tedavi süresinin medyanı 60 ay olmuştur ve medyan remisyon süresi benzer yan etki profili ile 53 ay olmuştur. Cerrahi sonrası profilaksi için verilen azatioprin tedavisi sonrası, takip medyan süresi 42 ay olan 31 hastanın altısında klinik olarak rekürrens görülmüştür. **Sonuç:** Çalışmamız İBH'nin cerrahi sonrası profilaksisi ve uzun dönem tedavisi için kullanılan azatioprinin kabul edilebilir güvenilirlik profilini ve etkinliğini doğrulamaktadır.

Anahtar Kelimeler: İnflamatuar bağırsak hastalıkları; azatiyoprin; istenmeyen etkiler;

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Azathioprine (AZA) is the prodrug of 6-mercaptopurine which is a purine analogue. It inhibits cell growth by directly interfering with nucleic acid synthesis. However the mechanism of immunomodulation is by inducing T cell apoptosis through modulating cell (Rac1) signalling.[1] Since the first report in the late 1960s,[2] several studies evaluated the role of thiopurines in inflammatory bowel disease (IBD). The results implicated that AZA was effective both in inducing and maintaining remissions in Crohn's disease (CD) and ulcerative colitis (UC), the two major forms of IBD.[3-6] In clinical practice, the onset of full activity may take more than three months, and their use is complicated by several side effects. Allergic, non-dose related side effects include pancreatitis, fever, rash, malaise, nausea, diarrhea, and hepatitis. Secondly, there are non-allergic and presumably dose- related side effects such as leukopenia and some forms of hepatitis.[7] Practical advice for patients with either CD or UC who are on AZA is to continue treatment for 3-4 years and then stop, except in those with evidence of continuing disease activity. [7]

In the present study, we retrospectively investigated the efficacy and safety of azathioprine therapy in a group of Turkish patients with active IBD. The study group included patients on AZA therapy for more than 4-year duration so that the results may be important regarding the long term therapeutic and safety profile of the drug.

MATERIAL AND METHODS

Turkey Yuksek Ihtisas Education and Training Hospital is a tertiary reference center. The gastroenterology department of the hospital has a clinic specialized in inflammatory bowel disease. Among 1800 IBD patients who were followed between the years 1993 and 2009, AZA was prescribed to 140 of them (7.8%). The analysis was performed on 122 patients who had used AZA for at least six months (109 CD, 13 UC). Complete blood count was performed every 7-14 days for the first three months and then every three months. Blood chemistry was performed at the beginning, and then at every screening. Indications were steroid dependence, steroid resistance, prophylaxis after surgery, fistulizing disease and extraintestinal involvement. In a group of patients AZA was preferred as the initial treatment without those indications. Steroid-dependence was defined as a partial or complete clinical response to treatment with prednisone or equivalent, and relapse with a dose reduction of prednisone at doses ≤15-25 mg/day for at least six months or relapse within 30 days of stopping prednisone treatment. Steroid-resistance was defined as failing to respond within 30 days to prednisone treatment at doses of 40-60 mg/day.[8]

The activity of the disease and the response to AZA was evaluated by means of Truelove-Witts Index in UC patients, or by means of 'Crohn's Disease Activity Index' (CDAI) in CD patients.[9-11] Remission was defined as a reduction of CDAI to a score below 150 points in CD patients and a mild Truelove-Witts score in UC patients. Partial remission was defined as reduction of Truelove-Witts score (but without achieving a mild disease index) in UC patients, and a reduction >70 points of CDAI index (but without achieving a score <150) in CD patients. The response was regarded absent when no clinical effect was observed over six months and/or additional treatment (steroids) or surgery became necessary within one year from the beginning. Side effects, long-lasting treatment, patient's decision, neoplasm, inefficacy and infection were the reasons for discontinuation. Rachmilewitz endoscopic index was used to evaluate endoscopic activity in UC patients.[12]

The main side effects were myelotoxicity, liver toxicity, systemic toxicity, pancreatitis and infection. Myelotoxicity was defined as white blood cells count <3000 mm^3 and/or platelets <70.000 mm^3 and/or hemoglobin <10 g/dl. Liver toxicity was defined as transaminases and/or gamma glutamyl transpeptidase (GGT) and/or alkaline phosphatase (ALP) > 1.5 times of the upper limit of normal in at least two repeated measurements. Pancreatitis was diagnosed by abdominal symptoms accompanied by amylase and/or lipase increase. Systemic toxicity was noted when one of the following signs or symptoms occured: Fever, skin rash, arthralgias, asthenia, myalgia, diarrhea, nau-

sea and abdominal pain. Infection was included among the side effects only when it required specific treatment and/or hospitalization and/or drug discontinuation. The study was approved by the local ethics committee (reference no: B.10.4.ism. 4.06.00.15).

All quantitative data, including clinical and laboratory variables were presented as mean ± standard deviation (SD). Paired sample t test was used to compare parametric values before and after treatment. Mc-Nemar test was used for analyzing the proportion of patients with elevated CRP before and after treatment. Sign test was used for the analysis of Truelove-Witts class before and after treatment. The statistical analyses were performed using SPSS version 11.0 (SPSS, Chicago, IL). When actaul type I error probalities (P) was found less than 0.05, it was considered to be statisticaly significant.

RESULTS

Clinical and demographic characteristics of patients are summarized in Table 1. Because of the small sample size of UC patients, no comparative analysis was aimed between UC and CD patients. Indications for treatment with AZA and the efficacy of the treatment in CD and UC patients are shown in Table 2. We observed an overall response (complete and partial remission) in 106 (98 + 8) out of 122 (87%) IBD patients. Nine out of 98 (9.2%) patients relapsed after remission. The median duration of treatment was 24 months (6-120 months). The median time to get remission was 4 months (1-12 months). The median duration of remission under AZA treatment was 21 months (0-110 months). The effect of AZA treatment on clinical and laboratory indices of CD and UC patients is demonstrated in Tables 3 and 4. AZA was temporarily withdrawn in seven (5.7%) and discontinued in 12 (9.8%) out of 122 patients. In 12 patients whom AZA was discontinued, the median duration of remission after the end of treatment was 29 months (range, 6-50 months). Four out of 12 (30%) patients relapsed after two years of follow up. Reasons for temporary withdrawal were as follows: Myelotoxicity in four (3.2%), systemic to-

TABLE 1: Demographic and clinical features of IBD patients undergoing azathioprine treatment.

	Ulcerative colitis n=13	Crohn's disease n=109
Men/women	11/2	62/47
Mean age at diagnosis (years)[a]	39.4 ± 16.5	33.4 ± 11.7
Time to the beginning of AZA (months)[a]	33.8 ± 41.8	37.3 ± 50.2
Smoking (%)	5 (38.5)	61 (56.5)
Appendectomy (%)	0 (0)	15 (13.9)
History of surgery related to IBD	1 (7.7)	45 (41.7)
Family history of IBD	2 (15.4)	7 (6.5)
Location/extension, n (%)		
Ileum	-	34 (31.8)
Ileum and colon	-	54 (50.5)
Colon	-	17 (15.9)
Upper GI	-	2 (1.8)
Pancolitis	7 (53.8)	-
Extensive colitis	6 (46.2)	-
Behaviour, n (%)		
Perforating-Peritonitis	-	3 (2.8)
Perforating-Fistulizing	-	7 (15.6)
Perforating-Abscess	-	17 (6.4)
Stricturing	-	33 (30.3)
Non-stricturing, non-penetrating	-	49 (45)

[a] mean ± standard deviation.

TABLE 2: The indications for azathioprine and the dose, duration and efficacy of treatment.

	Ulcerative colitis n= 13	Crohn's disease n= 109
AZA treatment duration, months[a]	24 (6-36)	30 (6-120)
AZA dosage, mg/kg/day[b]	2.33 ± 0.23	2.21 ± 0.33
Indications for AZA treatment (%)		
Preferential treatment[c]		36 (33.1)
Prophylaxis after surgery		31 (28.4)
Steroid dependence	9 (69.2)	27 (24.8)
Fistulizing disease		9 (8.3)
Steroid resistance	4 (30.8)	6 (5.5)
Clinical response (%)		
Remission	11 (84.6)	87 (80.5)
Relapse after remission		9 (10.4)[d]
Partial response		8 (7.4)
No response	2 (15.4)	14 (12)

[a] median and range in parenthesis.

[b] mean ± standard deviation. AZA: Azathioprine.

[c] The cases in which AZA was preferred as the initial treatment without the indications below.

[d] 9 out of 87 patients who were under treatment.

TABLE 3: The clinical and laboratory parameters of the patients with Crohn's disease before and after azathioprine treatment.

	Before AZA treatment	After AZA treatment	P value[b]
CDAI group (%)			
<150	62 (56.9)	95 (88)	
150-220	24 (22	12 (11.1)	< 0.001
220-450	22 (20.2)	1 (0.9)	
>450	1 (0.9)	0 (0)	
CDAI[a]	150 ± 104	64.93 ± 54.43	< 0.001
Hemoglobin (g/dl)	12.79 ± 4.62	13.68 ± 1.76	< 0.001
Hematocrit	37.32 ± 7.13	41.44 ± 4.67	< 0.001
White blood cell (per µl)	9523 ± 5228	8744 ± 13451	0.957
Platelet (per µl)	443045 ± 356178	316449 ± 115592	0.028
ESR (mm/h)	30.44 ± 23.08	18.67 ± 16.40	< 0.001
CRP	10.90 ± 24.40	2.95 ± 6.3	0.113
Elevated CRP (% of patients)	50 (51.5)	20 (19.6)	< 0.001

CDAI: Crohn disease activity index, AZA: Azathioprine, ESR: Erythrocyte sedimantation rate, CRP: C reactive protein.
[a]Except for the CDAI group and the ratio of the patients with elevated CRP, all values were presented as mean ± standard deviation.
[b]Statistically significant if p< 0.05.

TABLE 4: The clinical and laboratory parameters of the patients with ulcerative colitis before and after azathioprine treatment.

	Before AZA treatment	After AZA treatment	P value[a]
Truelove-Witts criteria (%)			
Mild	1 (1)10	(83.3)	
Moderate	2 (16.7)	2 (16.7)	0.002
Severe	10 (83.3)	1 (1)	
Rachmilewitz score	10.33 ± 0.78	4.72 ± 3.37	< 0.001
Hemoglobin	11.10 ± 3.06	13.71 ± 13	0.05
Hematocrit	33.69 ± 10.37	41.43 ± 4.80	<0.11
White blood cell (per µl)	9123 ± 2044	6550 ± 2125	<0.004
Platelet (per µl)	407769 ± 122815	283692 ± 92049	0.001
ESR (mm/h)	27.30 ± 15.07	13.23 ± 11.21	0.006
CRP	2.53 ± 3.15	1.49 ± 2.23	0.335
Elevated CRP (% of patients)	3 (27.3)	2 (16.7)	0.5

AZA: Azathioprine, ESR: Erythrocyte sedimantation rate, CRP: C reactive protein.
[a]Statistically significant if p< 0.05.

xicity in two (1.6%) and liver toxicity in one (0.8 %). Reasons for discontinuation were as follows: Myelotoxicity in four (3.2%), systemic toxicity in two (1.6%), pancreatitis in two (1.6%), long lasting treatment in two (1.6%), patient's decision in one (0.8%) and neoplasm in one (0.8%). Four cases of myelotoxicity and one case of liver toxicity could be managed by dose reduction. The neoplasm case (0.8%) was a lung cancer which was diagnosed 11 months after the beginning of AZA treatment.

In 42 patients who took AZA for more than four years, the median duration of treatment was 60 months (range, 48-120 months). In this group of patients we observed an overall response (complete and partial remission) in 38 (36 + 2) (90.5%) and relapses were observed in five (13%) of them. The median duration of remission under AZA treatment was 53 months (range, 14-110 months). The median duration of remission after AZA was stopped was 32 months (range 6-50 months). Side effects observed in six (14%) patients were as follows: systemic toxicity in three (7.1%), myelotoxicity in two (4.7%), liver toxicity in one (2.3%). The response, the relapse rate, the frequency of side effects and the duration of remission without AZA were not significantly different from the patients having AZA treatment for less than four years (p> 0.05).

In the CD patients who took AZA as postsurgical prophylaxis, the overall efficacy (optimal + partial) was observed in 29 (23+ 6) (93.5%) out of 31 patients, and relapse was later observed in four (16%) of them. The median duration of remission under AZA treatment was 41 months (3-110 months) in this group of patients.

The the location/extension of disease did not influence the efficacy of treatment (p> 0.05). Efficacy was not different regarding the various indications for AZA (p> 0.05).

DISCUSSION

In this study, we assessed the use as well as the therapeutic and safety profiles of AZA in Turkish patients affected by IBD. It confirmed the safety and efficacy of AZA for the treatment of IBD in the largest group of patients reported from Turkey so far.

We found that 87% of the patients with IBD responded to AZA treatment (92% achieved remission and 8% had partial remission). The rates of remission were 84.6% for UC and 80.5% for CD patients (Table 2). AZA significantly improved cli-

nical and various laboratory parameters in our patients (Table 3 and 4). The overall efficacy of thiopurines was observed in two out of three IBD patients in the previous reports which were performed in clinical practice.[7,13-15] In one series, 85% response rate was observed but complete remission was less than 50%.[16] Our data is in agreement with the results of other studies reporting AZA as an efficient drug in the treatment of CD and UC. Higher remission rates observed in our series may be due to the retrospective nature of our study and the relatively small size of our patient population compared to other series.[7,13-15]

Adverse effects were observed in 16% (19/122) of our patients and the most frequent ones were myelotoxicity in 6.6% (8/122) and systemic toxicity reported in 4% (4/122). Drug discontinuation rate due to side effects was 9.8%. In previous studies, thiopurine discontinuation rate due to side effects varied from 5–6% to 30%.[7,17-19] The prevalence of the single side effects varies among the different studies possibly due to non-univocal definitions. The rates of myelotoxicity (10%), systemic toxicity (4%), liver toxicity (1.6%) and pancreatitis (1.6%) observed in our study do not significantly differ from literature data.[15,17,18] No drug-related mortality was experienced in our series. It was previously shown that risk of lymphoproliferative disorder was five times higher in patients exposed to thiopurines than in those never exposed to these drugs, and old age, male sex, and longer duration of inflammatory bowel disease were also associated with increased risk.[20] However absolute cumulative risk of lymphoproliferative disorder in young patients receiving a 10-year course of thiopurines was low (<1%).[20] We did not observe any lymphoma in our patients. The relative young age of our patients and the small size of our series may explain this finding. Another reason may be that the possible lymphoma cases might have admitted to hematology or oncology clinics which are not present in our hospital. However the risk of malignancy does not undermine the positive risk-benefit ratio of these drugs.[20]

Except for the classical indications for AZA which are steroid dependence and refractoriness, fistulating course and extraintestinal involvement, we started AZA in one third of our patients as an initial treatment. D'Haens et al. showed that combined immunosuppression (infliximab and AZA) was more effective than conventional management for induction of remission and reduction of corticosteroid use in patients with CD.[21] During the last years we have had more of a tendency to prefer AZA more frequently as initial treatment because three out of four CD patients have a poor outcome and complications in their lifetime, and almost all patients are complication-free at diagnosis.[22] As D'Haens et al. showed, initiation of more intensive treatment early in the course of the disease could result in better outcomes.[21] It was shown that mucosal healing, as a relevant clinical outcome, was achieved with AZA treatment in CD patients.[23,24] Maintaining an endoscopically normal mucosa over time might become the target of IBD therapy in the near future. We could evaluate mucosal healing only in our UC patients and we observed a significant decrease in the Rachmilewitz score after AZA treatment (Table 4).

The long-term effectiveness of AZA in the maintenance of both CD and UC was demonstrated in several clinical trials.[6,7,16,25,26] The appropriate duration of AZA treatment remains controversial once remission of IBD has been achieved with this drug. However, a number of studies suggest that discontinuing AZA would accelerate relapse.[6,7,27,28] Therefore, at present, many authors recommend not to withdraw AZA, even after several years of treatment maintaining remission, except in the presence significant adverse effects.[29-31] In our 42 patients who took AZA for more than four years the median duration of remission under AZA treatment was 53 months (range 14-110 months). The efficacy and the relapse rate were not significantly different from the patients having AZA treatment for less than four years, but lack of increase in side effects and prolonged remission period under treatment does not support the idea that AZA should be continued indefinitely in patients with remission.

The role of AZA as a prophylactic agent in the postsurgical setting is controversial. Previous studies argued the effectiveness AZA in preventing endoscopic progression (with a reduction of 20-30%

in the progression rates over 1 year) after surgery.[32] In another series, the occurrence of mucosal lesions was not avoided but could merely be delayed.[33] In this study, endoscopic progression was 69% whereas four out of 56 patients showed clinical recurrence at the end of five years. In our series the number of clinical recurrence was six out of 31 during a median follow up period of 41 months (range 3-110 months) but we could not accurately documentate endoscopic progression because of the absence of an objective scoring system (e.g. Ruutgerts score).[34] Our results support the previous studies in that AZA seems to be the best treatment at the present to prevent postoperative recurrence in CD patients although it is not an ideal long-term prophylactic strategy.

There are limitations of our study especially because of its retrospective nature. Another disadvantage is the relatively small size of the our patient population compared to the other similar series in the literature.[7,13-16] Most of our patients have CD and they were evaluated by means of CDAI. However, numerous problems exist with the CDAI. Many variables on the score are subjective and a large proportion of the score depends on the patients' perception of the disease. Substantial interobserver variability exists when different observers review the same case notes to calculate the CDAI. It is well known that the CDAI underestimates CD activity when compared to excretion of autologous indium labelled granulocytes in feces or with endoscopic evaluation. Finally CDAI may not accurately reflect the disease activity in postoperative patients.[35,36] These may explain why an important proportion of our patients had a CDAI <150 before AZA treatment (Table 3). (In these patients we performed endoscopic follow up to monitor response to treatment). However, bearing these limitations in mind, we believe that the present study may contribute to the issue of AZA utilization in IBD because it represents a picture of what may happen in clinical practice regarding AZA in the different settings, such as postoperative prophylaxis and aggressive initial (top-down) therapy.[37] The experience with relatively prolonged duration of AZA treatment is another important point of our study.

CONCLUSIONS

In summary, our study confirms the efficacy and the acceptable safety profile of AZA in Turkish patients with IBD in the short and long term. The drug also seems to be effective in the postsurgical prophylaxis of relapse in CD. Our results argue in favor of more widespread and earlier use of AZA in these patients.

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