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Conventional and molecular cytogenetic analyses in Behçet's syndrome patients with gastrointestinal involvement

Gastrointestinal sistem tutulumlu Behçet sendromu hastalarında konvansiyonel ve moleküler sitogenetik analizler

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Abstract

Objective: We aimed to determine the frequency of trisomy 8 and other potential chromosomal abnormalities among Behçet's syndrome (BS) patients with gastrointestinal system (GIBS) involvement and those without myelodysplastic syndrome.

Methods: Between September 2014 and April 2015, 29 GIBS patients and 23 healthy controls were enrolled. Peripheral blood samples were collected from these patients for conventional cytogenetic analysis and fluorescence in situ hybridization (FISH) analysis specifically targeting chromosomes 8 and 9.

Results: Conventional cytogenetic analysis of 29 GIBS patients revealed clonal chromosome losses in 14 patients. One patient had a clonal del(8)(q11q13), and one patient had a constitutional t(5;10)(q33;p13). Polyploid metaphases were observed in 27 of 29 patients. In 7 of 23 control cases clonal aneuploidies were observed, and in two cases structural abnormalities along with numerical ones were detected. No clonal anomaly was observed in 14 cases. Polyploidy was detected in 13 of 23 cases in the control group. While trisomy 8 and 9 were detected in one patient by FISH analysis, only trisomy 9 was detected in one patient. The patient with trisomy 8 was diagnosed with polycythemia vera.

Conclusion: The frequency of chromosomal abnormalities observed in GIBS patients was found to be consistent with the literature. Trisomy 8 does not seem to be a feature of GIBS unless there is a

Öz

Amaç: Bu çalışmada, miyelodisplastik sendromu eşlik etmeyen gastrointestinal sistem tutulumu olan Behçet sendromu (GIBS) hastalarında trizomi 8 ve diğer potansiyel kromozomal anomalilerinin sıklığını araştırmayı amaçladık.

Yöntem: Eylül 2014 ile Nisan 2015 tarihleri arasında, 29 GIBS hastası ve 23 sağlıklı birey kontrol grubu olarak çalışmaya dahil edildi. Bu hastalardan periferik kan örnekleri alınarak konvansiyonel sitogenetik analiz ve kromozom 8 ve 9'u hedefleyen floresan in situ hibridizasyon (FISH) analizi yapıldı.

Bulgular: Yirmi dokuz GIBS hastasında yapılan konvansiyonel sitogenetik analizler sonucunda 14 hastada klonal kromozom kayıpları gözlendi. Bir hastada klonal olarak del(8)(q11q13) gözlenirken, bir hastada konstitüsyonel olarak t(5;10)(q33;p13) saptandı. Yirmi dokuz hastanın 27'sinde poliploid metafazlar gözlendi. Kontrol grubunda yer alan 23 olgunun 7'sinde klonal kromozom kayıp ve artışları, 2'sinde sayı ve yapı anomalileri saptanırken, 14 olguda klonal anomali gözlenmedi. Kontrol grubunu oluşturan 23 olgunun 13'ünde poliploidi tespit edildi.

FISH analizi ile 1 hastada trizomi 8 ve 9 tespit edilirken bir hastada sadece trizomi 9 saptandı. Trizomi 8 olan hastaya polisitemia vera tanısı konuldu.

Sonuç: GIBS hastalarında gözlenen kromozomal anomali sıklığı literatürle uyumlu bulunmuştur. Trizomi 8, GIBS hastalarının bir özelliği gibi görünmemekle beraber varlığı eşlik eden bir hematolojik hastalığı kuvvetle düşündürmektedir.

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hematologic condition. However, its presence strongly suggests the possibility of such a condition.

Keywords: Behçet's syndrome, gastrointestinal, cytogenetics, FISH, chromosomal abnormalities

Anahtar Kelimeler: Behçet sendromu, gastrointestinal, sitogenetik, FISH, kromozom anomalileri

Introduction

Behçet's syndrome (BS) is a multisystemic and chronic vasculitis with unknown etiology and associated with chronic inflammation. Besides mucocutaneous symptoms such as oral and genital ulcers and papulopustular lesions, organ involvement can also be observed. Involvement of the ocular, vascular, nervous and gastrointestinal system is responsible for morbidity and mortality.[1]

The association between BS and myelodysplastic syndrome (MDS) is well-established, with several cases reported in the last two decades.[2,3] Previous reviews of these cases have indicated that gastrointestinal system involvement is a frequent characteristic of this association, which can be severe and unresponsive to treatment.[4-9] The frequency of gastrointestinal involvement in BS varies in different geographic regions. It is more commonly reported in BS patients from the Far East, with a prevalence of up to 30%, whereas the prevalence is lower in Europe and the Middle East, ranging from 3-8%.[10] However, gastrointestinal system involvement seems to be closely associated with MDS regardless of geographic location.[2] Possible theories that could explain the relationship between BS and MDS include the altered immune function due to BS or DNA damage caused by immunosuppressive agents that are used in the management of BS.

MDS is overall the most common hematological disorder in BS.[11] The frequency of trisomy 8 in BS is significantly higher than that seen in primary MDS (87% vs 6%).[9,12] Lee et al.[13] compared 61 MDS patients with autoimmune manifestations and 134 MDS patients without it and trisomy 8 was found to be associated with BS. Another study involving 46 MDS patients further demonstrated that intestinal ulcers were more common in patients with trisomy 8 than in those without it (3/8 vs 0/38).[14] Similarly, Ahn et al.[11] reported an association between gastrointestinal system involvement and trisomy 8 in BS associated with MDS. Fever has been identified as an inherent feature in BS patients with MDS and trisomy 8 since a higher frequency of fever observed in these individuals compared to those without trisomy 8 (79.5% vs. 33.3%).[2] Trisomy 8 has also been found to be associated with inflammatory fever in pediatric patients with fever of unknown origin.[15] In addition, BS patients with gastrointestinal system involvement have been observed to be more resistant to immunosuppressive therapies. Alternative treatment approaches directed at MDS were found to be more beneficial in managing gastrointestinal manifestations, even if MDS itself does not require treatment.[3] Lastly, constitutional trisomy 8 has been linked to an increased risk of developing features of BS.[16,17] Taken together, these findings suggest that trisomy 8 may trigger inflammation in BS.

Several retrospective series have suggested the presence of trisomy 8 as a risk factor for gastrointestinal system involvement in BS.[4-6,8,18,19] Trisomy 9 is another chromosomal anomaly that is commonly seen in BS associated with MDS with gastrointestinal system involvement.[8,9,20] In this study, we aimed to determine the presence of trisomy 8 and trisomy 9 and possible chromosomal changes that may play a role in the pathogenesis of gastrointestinal system involvement of BS.

Materials and Methods

Patients

Between September 2014 and April 2015, 29 BS patients with gastrointestinal system involvement were included in the study. Gastrointestinal system involvement had been confirmed with colonoscopy. We did not include our 2 patients with MDS who were already known to have trisomy 8. For control group, 23 healthy individuals were enrolled. After obtaining signed informed consent forms, 5 cc of heparinized peripheral blood was taken from all participants for conventional cytogenetics and fluorescence *in situ* hybridization (FISH) analyses. Patient charts were reviewed for demographic data, BS manifestations, and medications. The study was conducted in accordance with the Declaration of Helsinki. Approval was obtained from the ethics committee (2014/83045809-876). The study was funded by Scientific Research Projects Coordination Unit (project no: 40937).

Cytogenetics and FISH Analysis

Conventional cytogenetic analyses were performed using standard 72 h peripheral lymphocyte culture technique. A mitogen mix [phorbol 12-myristate 13-acetate (PMA) + pokeweed mitogen (PWM) +Phytohemagglutinin (PHA)] was used to induce both T and B lymphocytes. Fifty (31-65) GTL-banded metaphases were analyzed whenever possible.

Slides were examined using a Nikon Eclipse E600 W light microscope. Metaphases were evaluated according to International System for Human Cytogenetic Nomenclature 2016[21] and photographed using an automated imaging system (Applied Imaging-Powergene). FISH was performed using liquid alpha satellite probes specific to centromere regions of chromosomes 8 and 9. At least 200 cells were counted under a Nikon Eclipse E600 W fluorescence microscope using a triple filter (DAPI-FITCH-Texas red).

Statistical Analyses

Statistical analyses were performed using SPSS 20.0. We used descriptive statistics to describe variables. Continuous variables were represented as mean and standard deviation. Chi-square test was used for comparison of categorical variables.

Results

We studied 29 BS patients with gastrointestinal system involvement (13 women and mean age: 40.1±9.7 years). All patients fulfilled International Study Group Criteria for BS.[22] The mean age at diagnosis was 27±11 years for BS and 32±11 years for gastrointestinal system involvement. BS preceded the diagnosis of gastrointestinal system involvement of BS in 19 patients. The mean duration from BS diagnosis to the diagnosis of gastrointestinal involvement of BS was 7.3±5 years. Among the remaining 10 patients, BS and gastrointestinal involvement of BS were diagnosed concomitantly in 7 and 3 patients were diagnosed with BS after 2, 3, and 5 years of gastrointestinal system involvement. Apart from gastrointestinal system involvement, 9 patients also had involvement of another major organ/s (eye involvement in 5, vascular involvement in 5, and central nervous system involvement in 2). At the time of sampling, 22 patients were on azathioprine therapy, 6 were on 5-ASA compounds, and the remaining 1 had never used any medication for BS previously (Table 1).

Conventional cytogenetic analysis of 29 BS patients with gastrointestinal system involvement revealed clonal chromosome losses in 14 patients. The most common monosomies were monosomy 20, 19, 21, and 18, respectively. One patient had a clonal del(8)(q11q13) and, one patient had a constitutional t(5;10)(q33;p13). In 13 patients, no clonal chromosomal abnormality was found. Polyploid metaphases were observed in 27 of 29 patients. Trisomy 8 was not detected in any of the patients (Table 2). Among the 23 control cases, 17 had clonal aneuploidies, and 2 had both structural and numerical abnormalities. No clonal anomaly was observed in 14 cases. Polyploidy was detected in 13 of 23 cases in the control group (Table 3). Polyploidy was significantly more frequent in BS patients with gastrointestinal system involvement compared to the control group (p=0.002). However, there was no significant difference in the frequency of structural or numerical abnormalities between the two groups (p=0.25).

The cut-off values for FISH analyses were set as 5%. The FISH results for centromeres 8 and 9 of the patients are shown in Figure 1. FISH analysis of chromosome 8 revealed that only 1 patient had trisomy 8 in more than 20% of interphase cells while trisomy 8 was detected in fewer than 5% of interphase cells in the remaining 28 patients. In FISH analysis of chromosome 9, trisomy 9 was detected in fewer than 5% of interphase cells in 27 patients. One patient had trisomy 9 in 5-10% of interphase cells. In another patient, who also had trisomy 8 in more than 20% of interphase cells, trisomy 9 was observed in 10-20% of interphase cells. Subsequently, this patient was evaluated by hematology and diagnosed with polycythemia vera. During a 7-year follow-up, no hematological or solid malignancy developed in the remaining patients. In 8 patients, monosomy 8 was observed in 5-10% of the cells and, in 2 patients more than 20% of the cells. Monosomy 9 was shown in 5-10% of the cells in 11 patients and 10-20% in 4 patients. Two of the 11 patients with 5-10% monosomy 9 had that abnormality also in their

Table 1. Demographic and clinical characteristics of patients

Variable	Behçet's syndrome patients with gastrointestinal involvement (n=29)
Women, n (%)	13 (45)
Mean (SD) age at BS diagnosis, years	27±11
Mean (SD) age at GIBS diagnosis, years	32±11
BS manifestations, n (%)	
Oral ulcers	29 (100)
Genital ulcers	25 (86)
Papulopustular lesions	12 (41)
Erythema nodosum	8 (28)
Arthritis	5 (17)
Pathergy positivity	8 (28)
Eye involvement	5 (17)
Vascular involvement	5 (17)
Central nervous system involvement	2
Medications, n (%)	
Azathioprine	20 (69)
Azathioprine and TNFi	2
5-ASA compounds	6 (21)
No treatment	1

BS: Behçet's syndrome, GIBS: Gastrointestinal involvement of Behçet's syndrome, SD: Standard deviation, TNFi: Tumor necrosis factor alpha inhibitors

conventional cytogenetic analysis. None of the control group had aneuploidies of chromosomes 8 and 9 by FISH.

Discussion

In this study, we investigated the presence of trisomy 8, trisomy 9, and other potential chromosomal abnormalities in BS patients with gastrointestinal system involvement. Conventional cytogenetic analysis of 29 BS patients with gastrointestinal system involvement revealed clonal chromosome losses in 14 patients. The most common monosomies were monosomy 20, 19, 21, and 18, respectively. Only one patient exhibited structural abnormalities (del(8)(q11q13) and a marker chromosome). The patient who carried a constitutional balanced translocation (t(5;10)(q33;q13)) did not have any acquired chromosome abnormalities. Furthermore, FISH analysis detected trisomy 8 in one patient that was not observed by conventional cytogenetic analysis. This patient was further diagnosed with polycythemia vera.

Chromosomal abnormalities have been reported among BS patients with accompanying MDS or another hematologic disorder. The most common chromosomal abnormality observed in these patients was trisomy 8 (87%), followed by trisomy 9 (13%) and trisomy 15 (9%).[9] However, there are only a few studies investigating chromosomal abnormalities among BS patients without any hematologic disorder. In a study conducted by Denman et al.[23] in 1980, chromosomal abnormalities were found to be more common in BS patients (16 out of 38, 42%) compared to healthy controls (1 out of 17, 6%). However, the authors did not provide information on the type of chromosomal abnormalities, BS manifestations and medications of these 38 patients.[23] On the other hand, another study reported no numerical or structural abnormalities among 38 BS patients.[24] The authors did not report the BS manifestations, however, none of the patients had received any medications for BS prior to sampling. Interestingly, in our study, all except one patient showed chromosomal abnormalities, and the monosomies observed among the 14 BS patients have not been reported previously. However, we also observed rather more than expected, numerical and structural chromosomal abnormalities, as well as polyploidies in our control group.

Table 2. The types and frequencies of chromosomal abnormalities among the 29 BS patients with gastrointestinal involvement

Number of patients (%)	Numerical chromosomal abnormality	Structural chromosomal abnormality
27 (93)	Polyploidy	
8 (28)	-21	
7 (24)	-19	
6 (21)	-X	
4 (14)	-18, -20	
3 (10)	-16, -22	
2 (7)	-Y, -4, -6, -9, -10, -11, -12	
1 (3)	-3, -7, -8, -13, -14, -17	t(5;10))(q33;p13), del(8)(q11;q13)

**A variable number of polyploid cells ranging from 1 to 7 were detected. The ploidy levels ranged from 3n (69 chromosomes) to 6n (138 chromosomes)*

Table 3. The types and frequencies of chromosomal abnormalities in control group

Number of patients (%)	Numerical chromosomal abnormality	Structural chromosomal abnormality
13 (57)	Polyploidy	
4 (17)	-21	
3 (13)	-22	
2 (9)	-18	
1 (4)	-X, +1, -10, -12, -19, -20	del(1)(q32q42), del(6)(q14q16)

**A variable number of polyploid cells ranging from 1 to 5 were detected. The ploidy levels ranged from 3n (69 chromosomes) to 4n (92 chromosomes)*

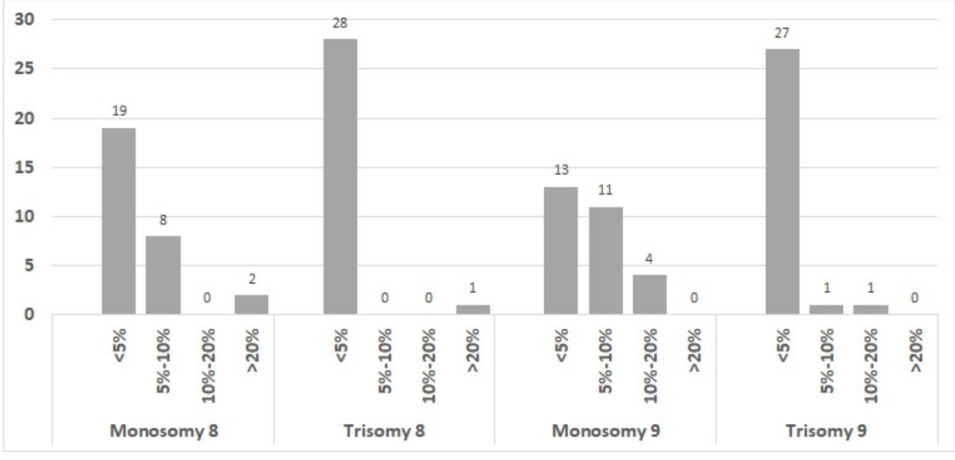

Figure 1. FISH results for centromeres 8 and 9 of 29 BS patients with gastrointestinal involvement
BS: Behçet's syndrome, FISH: Fluorescence in situ hybridization

This could be related to the fact that PMA which is included in our mitogen mix can induce mitotic dysfunction and cause poliploidisation.[25,26] There was no statistical difference between BS and control group regarding numerical and structural chromosome abnormalities. Therefore, in that respect our results may be considered in accordance with the literature. But polyploidy ratio was significantly higher in BS group than controls in our study.

Arimura et al.[27] conducted a study to investigate the presence of morphological myelodysplasia in BS patients. They observed significant trilineage myelodysplasia in bone marrow cells of 8 out of 15 BS patients. However, all these patients did not show any chromosomal abnormalities. The incidence of apoptotic bone marrow cells was lower in BS patients compared to patients with MDS, but higher when compared to normal controls. The authors suggested that this finding may explain the absence of peripheral cytopenia observed among BS patients. Unlike other rheumatologic disorders where lymphoproliferative disorders are the most common type of hematologic malignancy,[28] several studies have consistently reported MDS as the most common type of malignancy among BS patients.[11,29-31] In a meta-analysis, BS patients were found to have an increased risk of developing hematologic malignancies [pooled risk ratio (RR), 2.58; 95% confidence interval (CI): 1.61-3.55].[30] Another study specifically investigating BS patients with gastrointestinal system involvement reported that the risk of hematologic malignancy was significantly higher in both men and women.[32] The standardized incidence ratio (SIR) was 23.90 (95% CI: 2.89-86.32) for men and 34.47 (95% CI: 4.17-124.51) for women. The SIRs observed in BS patients with gastrointestinal system involvement were significantly higher compared to the SIRs observed in all BS patients, regardless of the type of involvement. Furthermore, another study reported a higher prevalence of gastrointestinal system involvement in BS patients with malignancy compared to those without malignancy.[31] Our findings, which show a high prevalence of chromosomal abnormalities among our patients, may provide an explanation for why BS patients with gastrointestinal involvement have a higher risk of developing hematologic malignancies.

We had previously reported that among our 198 BS patients treated with cyclophosphamide 15 (8%) patients developed malignancies, with bladder carcinoma being the most common.[33] Huang et al.[29] investigated the relationship between medications for BS and the risk of malignancy. They found that cyclophosphamide was associated with a 10-fold increase in the risk of developing malignancies.[29] In our patient population, none of them had been exposed to cyclophosphamide previously, but 76% was currently using azathioprine. Thiopurines have been implicated as a risk factor for the development of lymphoma among patients with inflammatory bowel disease,[34] which shares many similar clinical features with gastrointestinal system involvement of BS.[1] Furthermore, current use of thiopurines has also been associated with an increased risk of myeloid clonal disorders, including acute myeloid leukemia and MDS among patients with inflammatory bowel disease.[35] In our study, it should be noted that 22 of the 29 BS patients included in the analysis were taking azathioprine at the time of serum sampling, which could potentially provide an explanation for our findings.

Study Limitations

Our study has some limitations. First, we studied a small number of patients due to the low prevalence of gastrointestinal system involvement of BS in Turkey. Also, it would be preferable to perform conventional cytogenetic analysis on bone marrow samples. However, previous studies have demonstrated that conventional cytogenetic analysis performed on both peripheral blood and bone marrow samples yield similar results.[36] Second, it would be more preferable to study treatment-naive patients in order to minimize confounding factors related to drug-induced chromosomal changes.

Conclusion

In the future, further research with larger cohorts is warranted. Future studies should include appropriate control groups, such as healthy controls, adequate number of BS patients who have been exposed to immunosuppressive drugs and those who have never been exposed, as well as BS patients with different types of involvement. These additional studies will provide a more comprehensive understanding of the relationship between chromosomal abnormalities and BS. Finally, it should be noted that trisomy 8 is not typically considered a characteristic feature of BS with gastrointestinal system involvement, unless there is an associated hematologic condition. Considering that the patient with trisomy 8 in this study was also diagnosed with polycythemia vera, which is another myeloid clonal disorder, the presence of this chromosomal abnormality strongly suggests the possibility of a coexisting hematologic condition.

Ethics

Ethics Committee Approval: The study was conducted in accordance with the Declaration of Helsinki.

Approval was obtained from the ethics committee (2014/83045809-876).

Informed Consent: All participants gave their written informed consent.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Concept: S.N.E., G.H., A.F.Ç., Design: S.N.E., G.H., A.F.Ç., A.S., Data Collection or Processing: Ş.Y., A.Ç, R.H.K., İ.H, Y.Z.E., Y.T.A., A.D., S.H., A.S., Analysis or Interpretation: S.N.E., Ş.Y., A.Ç, R.H.K. Literature Search: S.N.E, Writing: S.N.E., G.H., A.F.Ç., Ş.Y., A.Ç, R.H.K.

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