Turk J Med Sci
30 (2000) 71–75

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Comparison of Sister Choromatid Exchange Frequency Between two Distinct Forms of Anterior Uveitis

Received: May 22, 1998

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Abstract: Acute anterior uveitis (AAU) is one of the most prevalent forms of intraocular inflammatory disorders. Although immunological and inflammatory factors are thought to play a part in the pathogenesis of the disease, its real etiology is unknown. AAU is mostly seen in two distinct types; the first one an idiopathic form, and the other frequently associated with a systemic disease such as ankylosing spondylitis (AS) and Behçet's disease (BD).

Sister choromatid exchange (SCE) analysis was performed on peripheral lymphocytes from the patients with either idiopathic AAU or AAU associated with a systemic disease, in order to explore the genomic instability and DNA damage related to AAU.

Twenty-four AAU patient with systemic diseases and 27 idiopathic AAU patients were compared with 20 healthy persons used as the control group. While the SCE frequencies of patients with AAU associated with AS and BD were statistically higher than those of the control group ($p<0.00001$), no significant difference was observed between those patients with the idiopathic type of AAU and the control group ($p>0.05$). Idiopathic AAU can be considered as a distinctive localized inflammation, and may have no more genetic basis than AAU associated with AS and BD.

Key Words: Sister choromatid exchange (SCE), idiopathic nonspecific anterior uveitis, Behçet's disease, ankylosing spondylitis.

Introduction

Acute anterior uveitis (AAU), the most prevalent form of intraocular inflammatory disease, has an incidence of 8.1/100.000 per year (1). AAU is predominantly seen in two distinct types: an idiopathic form, and another, frequently associated with certain HLA systemic diseases such as ankylosing spondylitis (AS) and Behçet's disease (BD) (2). Immunological and inflammatory factors are thought to play critical roles in the pathogenesis. Linsen et al. studied 103 AAU patients and showed that 49 (48%) were positive for HLA- B27 antigens. Of these HLA positive AAU patients, 27 (55%) had AS (3). Even though the clinical implications of HLA findings are known as diagnostic and prognostic parameters, the definite pathogenesis of AAU is still an enigma.

Sister chromatid exchange (SCE) is known as the reciprocal translocation of DNA between the homologous loci of sister chromatids in the replication process, and it occurs spontaneously in all cells of healthy populations at certain frequencies (5). It is believed that most chemical carcinogens and/or mutagens, and even cigarette smoking, have an influence on SCE frequency (6, 7). In addition, elevated SCE rates were found in some chronic diseases, viral and bacterial infections (8, 9)

Since SCE is believed to be a sensitive measurement of genomic instability and possible DNA damage, we aimed to measure the SCE frequency in the peripheral lymphocytes of the patients with AAU, either idiopathic or associated with AS and BD.

Patients and Methods

Twenty-seven idiopathic AAU patients (22 male, 5 female) and 20 healthy subjects as the control group (12 male, 8 female) were analyzed. The age of the patients and control groups ranged from 17 to 56. They were selected from among non-smoking, non-treated subjects. We excluded those patients who had formerly received

corticosteroid, cytotoxics and radiation therapy. Additionally, 24 AAU patients (21 male, 3 female) who had systemic disease were included in the study. These patients had either acute forms or attacks of AS or BD.

Heparinized peripheral blood samples were obtained following the diagnosis prior to the treatment. The lymphocyte cultures were performed according to the standard procedure for SCE analysis (10). Briefly, lymphocytes were cultured in darkness for 72 hours in culture tubes containing 5 ml RPMI- 1640 medium, 20% fetal calf serum, 2% phytohemagglutinin and 0.3 mg/ml BrdU. 0.1 mg/ml colcemid was added 1.5 hours before harvesting. Chromosome prepations were obtained following hypotonic treatment in 0.075 M KCL and fixation in a 1:3 dilution of acetic acid-methanol treatment. BrdU incorporated metaphase chromosomes were stained by the FPG (fluorescent plus Giemsa) method as described by Wolf and Perry (11). Twenty second division metaphases were evaluated for each subject. The Mann Whitney U test was used for statistical analysis of the results.

Results

The mean SCE rates per cell of patients with AAU associated with AS and BD and the SCE rates of the control group are shown in Table 1 and Table 2, respectively. The SCE results of idiopathic AAU patients are summarized in Table 3. A significantly higher SCE frequency was obtained in AAU patients with systemic disease than in the control group ($p<0.05$) (Table 4) (Figure 1). No significant difference was found between those patients with the idiopathic type of AAU and the control group ($p>0.05$) (Table 4).

Discussion

Despite complete medical evaluation including careful history, ophthalmic and general medical examination, and full laboratory testing, the real cause of AAU remains obscure in most cases. In addition to immunologic and inflammatory factors, the HLA system has been delineated in the pathogenesis of uveitis in recent years. Most studies on AAU patients agree on the importance of

Table 1. SCE data of the AAU patients with systemic diseases.

Case	Systemic Disease	Age	Sex	SCE counts (Mean Per metaphase)
1	BD	34	M	9.29
2	BD	27	M	10.24
3	BD	33	F	10.01
4	BD	17	M	10.22
5	BD	32	M	10.74
6	BD	44	M	10.55
7	BD	27	M	9.93
8	BD	23	M	11.01
9	BD	29	M	9.01
10	BD	22	M	10.15
11	BD	20	M	9.83
12	BD	34	F	10.57
13	BD	45	M	11.01
14	AS	38	M	11.17
15	AS	33	M	7.98
16	AS	46	M	6.76
17	AS	39	M	8.56
18	AS	44	M	5.87
19	AS	56	M	8.54
20	AS	31	M	9.24
21	AS	47	F	7.42
22	AS	36	M	7.49
23	AS	39	M	8.21
24	AS	40	M	8.76
Mean+SEM	9.27 -+ 0.29			

Table 2. SCE data of the control group

Case No	Age	Sex	SCE counts (Mean Per metaphase)
1	23	F	6.76
2	34	M	8.11
3	45	M	7.64
4	21	M	5.82
5	36	M	6.37
6	31	M	9.92
7	37	F	7.56
8	29	M	8.11
9	38	M	6.12
10	34	M	8.61
11	27	F	7.12
12	28	M	6.83
13	37	M	5.75
14	32	M	4.89
15	28	M	8.34
16	42	M	6.46
17	44	M	7.12
18	45	M	9.29
19	20	F	5.82
20	19	M	6.39
Mean+SEM			7.15 +- 0.29

a high association with HLA-B27 (12). However, the majority of the HLA-B27+ population will never develop AAU. The partial association of AAU and HLA-B27 subtypes are equally distributed among normal controls and AAU patients (12). HLA-Cw1 antigen was found in 64.3% of the patients with uveitis (13). HLA-BW51 has also been shown to be associated AAU with BD (14). According to the Breworton et al., the existence of antigen HLA-B27 increases the risk of AAU twentyfold when related to AS (15). However, these associations do not explain the etiopathogenesis of the disease. Therefore, factors other than HLA-B27 must contribute to the pathogenesis of HLA-associated immunologic diseases.

There is no adequate study in the literature on SCE frequency in the idiopathic form of AAU. However, there

Table 3. SCE data of the idiopathic AAU patients.

Case No	Age	Sex	SCE counts (Mean Per metaphase)
1	43	M	6.5
2	41	M	6.5
3	46	F	6
4	34	M	6.1
5	32	M	6.3
6	28	M	6.44
7	43	F	6.33
8	38	M	5.5
9	50	M	6.36
10	38	M	7.18
11	28	M	7.86
12	34	F	6.34
13	41	M	7.21
14	39	M	8.02
15	37	M	6.7
16	40	M	5.8
17	47	M	6.56
18	39	M	5.93
19	47	M	8.56
20	53	M	6.78
21	52	F	7.35
22	37	M	7.51
23	41	M	8.53
24	44	M	6.91
25	46	F	6.04
26	34	M	7.59
27	39	M	6.77
Mean+SEM			6.80 +- 0.16

are some articles on SCE frequency in AAU with certain connective tissue diseases such as scleroderma, systemic lupus erythematosus, juvenile chronic arthritis and periarthritis nodosa (16, 17). Furthermore, in some other HLA-associated autoimmune diseases such as ulcerative colitis, chron disease, multiple sclerosis, AS and recently in BD, elevated SCE frequencies have been demonstrated (18-20).

Groups	SCE's Per Metaphase (Mean ⁺SE)	Z value	P
Idiopathic AAU Patient-control (p=0.260669)	6.80 ⁺ 0.16-7.15 ⁺ 0.29	1.14	p>0.05
AAU patients with systemic Diseases - control	9.27 ⁺ 0.29 – 7.15 ⁺ 0.29	5.1	P<0.00001 P=0.00000769

Table 4. The comparison of the mean SCE frequencies in patients and control groups using Mann-Whitney U test

In the present study, we found no significant difference in SCE frequency between patients with idiopathic AAU and the control group ($p<0.05$). However, there was a significant alteration in the rates of SCE between the control group and the AAU patients having BD and AS ($P<0.05$). In light of our results, increased levels of SCE frequencies in AAU patients with BD and AS suggest a genetic predisposition to these diseases. Even though HLA B27 and HLA BW51 antigens themselves may be involved in the pathogenesis of these diseases, an important role may be played by some other genetic or perhaps environmental triggers causing DNA damage.

In conclusion, we believe that genetic factors other than HLA antigens, such as genomic instability and DNA damage, may play a more important part than previously realised in the etiology of AAU, associated with AS and BD. However, idiopathic AAU may be considered a distinctive localized inflammation and may have no more of a genetic basis than AAU with AS and BD.

Acknowledgement

We are cordially indebted to Sinan Sönmez.

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