The Effects of Pegylated Interferon Alpha-2a and Alpha-2b Therapy on Chromosomal Aberrations and Mitotic Index in Patients with Chronic Hepatitis B

Kronik Hepatit B'li Hastalarda Pegile-İnterferon Alfa-2a ve Alfa-2b'nin Kromozomal Düzensizlikler ve Mitotik İndeks Üzerine Etkileri

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ABSTRACT Objective: We aimed to prospectively evaluate the effects of pegylated interferon alpha-2a and alpha-2b therapy on chromosomal aberrations and mitotic index in patients with chronic hepatitis B. **Material and Methods:** Fifty patients with chronic hepatitis B who underwent pegylated interferon alpha-2a or 2b therapy were evaluated for chromosomal aberrations and mitotic index before the treatment and at the end of 6 months of therapy. **Results:** Cytogenetic examinations revealed out that there was no significant difference between pre- and post-treatment frequencies of mitotic index and chromosomal aberrations. **Conclusion:** Interferon alpha-2a and alpha-2b therapy which is associated with common hematologic adverse effects has no significant cytogenetic effect.

Key Words: Hepatitis B; interferon alfa-2a; interferon alfa-2b; chromosome aberrations; mitotic index

ÖZET Amaç: Bu çalışmada kronik hepatit B'li hastalara uygulanan pegile interferon alfa-2a veya alfa-2b tedavisinin kromozomal düzensizlikler ve mitotik indeks üzerinde etkisinin prospektif olarak araştırılması amaçlanmıştır. **Gereç ve Yöntemler:** Pegile interferon alfa-2a ve alfa-2b tedavisi uygulanan elli kronik hepatit B hastası, tedavi öncesi ve altı aylık tedavi sonrası kromozomal düzensizlik ve mitotik indeks yönünden değerlendirilmiştir. **Bulgular:** Yapılan sitogenetik inceleme sonucunda tedavi öncesi ve tedavi sonrası kromozomal düzensizlik ve mitotik indeks sıklığında istatiksel olarak anlamlı bir farklılık saptanmadı. **Sonuç:** Yaygın hematolojik yan etkilerle ilişkilendirilen interferon alfa-2a ve alfa-2b tedavisinin, hepatit B'li hastalarda olumsuz sitogenetik etkiye yol açmadığı sonucuna varılmıştır.

Anahtar Kelimeler: Hepatit B; interferon alfa-2a; interferon alfa-2b; kromozom aberasyonları; mitotik endeks

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Hepatitis B virus (HBV) is the 10th leading cause of death and related to one million deaths/year due to chronic hepatitis, cirrhosis or hepatocellular carcinoma.[1,2] Interferon therapy; one of the main therapeutic agents for chronic hepatitis B infection, is associated with adverse effects such as flu-like syndrome, lack of appetite, alopecia, visual symptoms, leukopenia and thrombocytopenia that response to dose reduction or discontinuation of therapy.[3-5] Hematologic adverse effects are common and severe and use of growth factors may be required.[6] Recent reports mentioned interferon-related chromosomal aberrations and secondary malig-

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nancies.[7-9] Conversely, it has been claimed that interferon may have anticlastogenic and antitumor activity.[10-14] Various studies established the protective effect of interferons against multiple mutagens such as gamma irradiation, ultraviolet light or biological agents.[15-17] Additionally, induction of repair of DNA damage by human interferon-alpha was observed in peripheral lymphocytes of patients with hepatitis B.[18] However, to our knowledge, there are no other reports mentioning the effects of interferon therapy on chromosomal aberrations and altered mitotic index in patients with chronic hepatitis B.

There are several mutation detection protocols for determining the genotoxic effects of physicial and chemical agents on DNA.[19] Cytogenetic analysis of metaphase chromosome spreads in point of chromosomal aberrations and analysis of mitotic index in cultured cells can be used for detecting the genotoxic effect of an agent.[20,21] Chromosomal aberration is a term that used to define deletion, duplication, translocation and breakage of chromosomes. Mitotic index (MI) is a measure for the proliferation status of a cell population. It is defined as the ratio between the number of cells in mitosis and the total number of cells.[22,23]

We aimed to evaluate the effects of pegylated interferon (peg-interferon) alpha-2a and alpha-2b therapy on the development of chromosomal aberrations and mitotic index in patients with chronic hepatitis B.

MATERIAL AND METHODS

Fifty eight patients with chronic hepatitis B that were planned to undergointerferon therapy for six months in Gastroenterology Department of Dicle University, School of Medicine were enrolled in the study. The demographic features such as age, gender, alcohol and smoking habits were recorded. Patients aged between 16 and 57 years were divided into two equal groups. The patients in group A (n=29; 19 male19 male19 males) underwent to peg-interferon alpha-2a (Pegasys®, Roche, Basel, Switzerland) subcutaneous injections 180 mcg/week, and the patients in group B (n=29; 18 males and 11 females) were treated with peg-interferon alpha-2b (Pegintron®, Schering, Las Piedras Puerto Rico) subcutaneously 1.5 mcg/kg/week. Blood samples were collected at the beginning and at the sixth month of therapy. Informed consent was obtained from the subjects who were enrolled in the study. The Ethics Committee of Dicle University approved the study (Approval No: B.30.2 DİC.0.01.00.00/49).

For each subject, two parallel whole blood cultures were performed, and the blood samples were incubated for 72 h at 37°C, in RPMI 1640 medium, supplemented with 20% fetal bovine serum, 100 IU/mL penicillin G and 100 μg/mL streptomycin. Lymphocyte growth was stimulated by 1% phytohemoagglutinin.[24] For detecting chromosomal aberrations, 2 h before harvesting, colchicine (0.2 μg/ml) was added. After a total of 72 h culture, the cells were collected by centrifugation, resuspended in a pre-warmed hypotonic solution (0.075 M KCl) for 20 min and fixed in acetic acid/methanol (1:3v/v).[25] The centrifuged cells were placed on dry slides. One day later the slides were banded by Giemsa-trypsin banding (G-banding) technique.[26] Forty metaphases for each subject scored to determine the percentages of chromosome breakages. Chromatid breakage, chromosome breakage, fragments and deletions were accepted as 1; translocation, dicentric and rings were accepted as 2 breakage.[26-28] Representative chromosomal aberrations in metaphase spreads are shown in Figure 1.

To assess MI, the slides were stained with solid staining method. Two slides were examined for each sample and at least 1000 cells were counted under x10 magnification. For the MI values, the percentage of metaphases were assessed on 1000 blast nuclei.[22,23]

All analyses were conducted using SPSS 11.5 statistical program (SPSS for Windows 11.5, Chicago, IL, USA). The normality of distributions were evaluated with the Kolmogorov-Smirnov test. The results were expressed as means±standard deviation. The mean age of patients were measured by t test and Chi-square test was used to compare the distribution of gender and smoking habits.

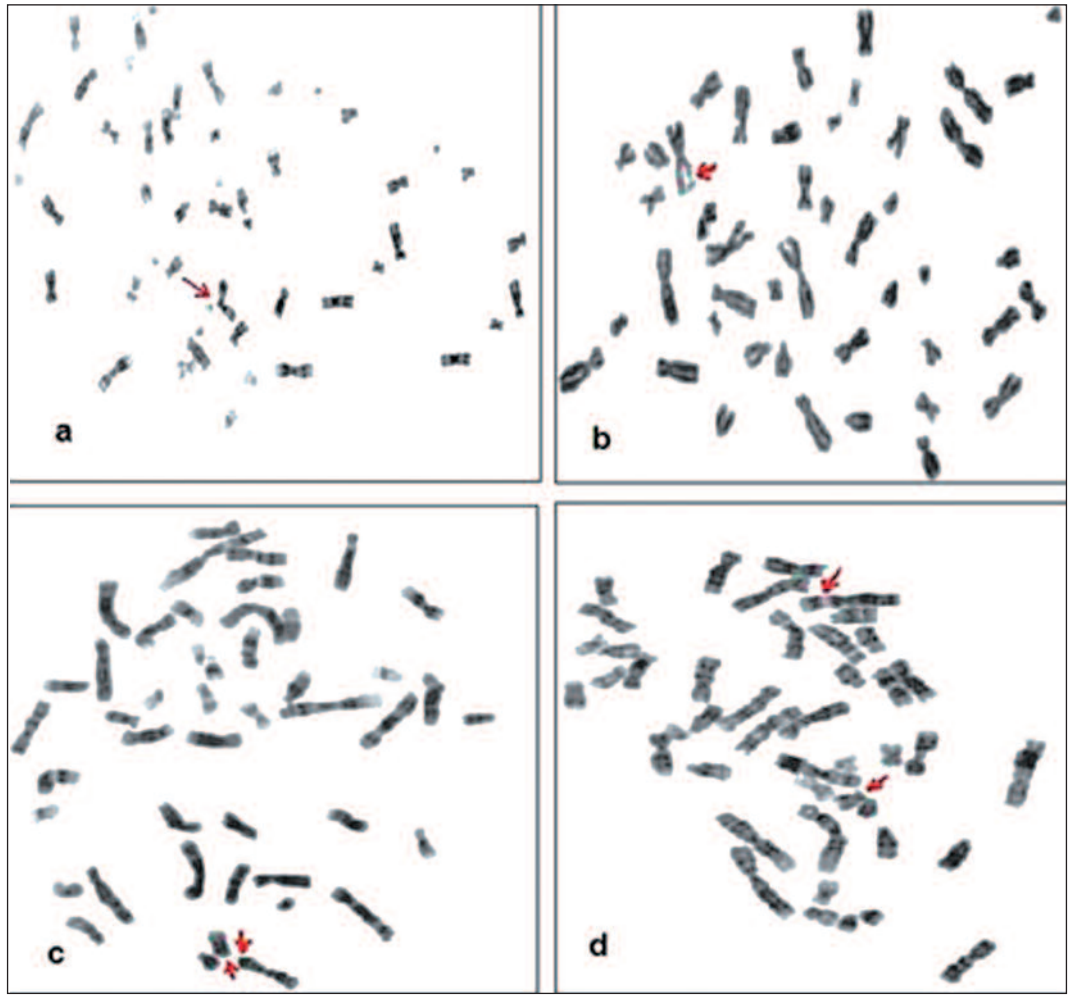

FIGURE 1: Representative chromosome aberrations in metaphase spreads in this study. **a:** Chromatid break, **b:** Chromatid gap, **c:** Deletion, **d:** Translocation. (See for colored form http://tipbilimleri.turkiyeklinikleri.com/)

Paired Samples t test and Wilcoxon test were conducted to assess the variables of the pre- and post-treatment period. Two independent groups were compared by Independent sample t test and Mann Whitney-U test. All statistical tests were two-sided, and a p value ≤0.05 was considered significant.

RESULTS

Mean age of patients in group A (16 males and 9 females) and group B (16 males and 9 females) were 33.17±18 and 36.89±13.6 years old; respectively (p=0.302). Eight patients (4 patients from group A and 4 patients from group B) were excluded from the study due to irregular follow-up and therapy. Chromosomal aberrations and MI are affected by demographic features of patients such as age and alcohol or smoking habits. No statistical significance was observed between groups in terms of smoking habits (p=0.765). None of our patients had a history of alcohol intake. Demographic features of patients are shown in Table 1.

TABLE 1: The demographic features of the study group.

	Group A (n=25)	Group B (n=25)	p
Age (mean±SD years)	33.17±18	36.89±13.6	0.302
Sex (male/female)	16/9	16/9	1.00
Smoking	16/9	17/8	0.765

Patients in the study groups were evaluated for MI before the treatment and at the 6th month of

therapy. There was no significant difference between pre- and post-treatment frequencies of MI (p=0.862 and 0.504, respectively). Table 2 indicates the MI parameters of patients with chronic hepatitis B before therapy and at the 6th month of therapy.

The difference of the frequencies of chromosomal aberrations between the initial evaluation and the 6th month of therapy were not significant (p=0.611 and 0.659, respectively). Table 3 indicates the frequencies of chromosomal aberrations before therapy and at the 6th month of therapy.

As it is shown in Table 4, the difference between groups A and B for the pre- and post-treatment comparisons of MI and chromosomal aberrations were not significant.

TABLE 2: The comparison of pre- and post-treatment frequencies of mitotic index.

Variable	Pre-treatment (Mean±Std. Deviation)	Post-treatment (Mean± Std. Deviation)	Significance (p)
Group A	1.96±0.98	2.01±0.91	0.862
Group B	2.14±0.94	2.00±0.63	0.504

TABLE 3: The comparison of pre- and post-treatment frequencies of chromosomal aberrations.

Variable	Pre-treatment Median (Min-Max.)	Post-treatment Median (Min-Max.)	Significance (p)
Group A	0 (0-10)	0 (0-25)	0.611
Group B	0 (0-25)	0 (0-30)	0.659

Min: Minimum; Max: Maximum.

DISCUSSION

Some studies carried out in patients with hepatitis B virus infection determined that hepatitis B virus was associated with significant cytogenetic changes, chromosomal aberrations and low mitotic index.[29,30] However, as we reviewed the literature, we could not find any other reports mentioning the relationship between interferon therapy and cytogenetic abnormalities or reporting that low MI was observed in patients with hepatitis B.

The relationship of interferon therapy with chromosomal aberrations or DNA damage is still being debated. Several experimental studies supported the protective effect of interferon therapy against several mutagens.[14] Zasukhina et al. analyzed the repair activity of interferon in eucaryotic cells and stated that interferon decreased the chromosomal aberrations induced by gamma and ultraviolet irradiation.[31] Bolzan et al. determined that recombinant interferon alpha-2a has an inhibitor activity against chromosomal aberrations that was induced by bleomycin therapy in hamsters.[32] Mertens et al. carried out an in vitro study in amniotic fluid cells and showed that recombinant interferon alpha and beta reduced dose-related sister chromatid exchange.[33] Higano et al. achieved permanent cytogenetic remission by interferon alpha-2a therapy without life-threatening toxicity in patients with chronic myeloid leukemia who relapsed subsequent to bone marrow transplantation.[13]

On the other hand, some studies that were performed on different study groups emphasize that interferon therapy may lead to chromosomal aberrations and secondary malignancies.[7-9] Jasny and Tamm established that interferon therapy increased chromosomal gap and breaks depending on

TABLE 4: The difference between group A and B for the pre- and post-treatment comparison of mitotic index and chromosomal aberrations.

	Group A (1st-6th month) Median (Min-Max)	Group B (1st-6th month) Median (Min-Max)	Significance (p)
Mitotic index	0.12 (-3.84-2.16)	-0.06 (-1.93-2.10)	0.377
Chromosomal aberrations	0 (0-25)	0 (0-30)	0.983

Min: Minimum; Max: Maximum.

the dose of therapy in Indian muntjac skin fibroblast cells.[34] Georgian et al. examined the role of human alpha interferon on the mitotic activity of normal and tumoral cells and observed that interferon therapy had a dose related inhibitor effect on the MI.[35] Ozturk et al. examined the in vitro effect of interferon alpha-2a on the MI, development of cytogenetic abnormalities and sister chromatid exchange of peripheral blood lymphocytes.[7] They observed that higher doses of interferon alpha-2a reduced the MI, however had no significant effect on the development of cytogenetic abnormalities and sister chromatid exchange.

We have determined that pegylated interferon alpha-2a and alpha-2b therapy for 6 months has no significant effect on the frequencies of chromosomal aberrations and MI of lymphocytes of patients with hepatitis B. The differences between the groups for frequencies of MI and chromosomal aberrations were statistically nonsignificant. When we compared our studies with the other studies that investigated the effect of interferon therapy in terms of chromosomal aberrations and MI, our results were similar to those of Ozturk et al. and Bolzan et al. while they were not similar to those of Jasny and Tamm in terms of frequency of chromosomal aberrations.[7,32,34] For the MI, our results were not similar to the results of Ozturk et al. and Georgian et al.[7,35]

These results represent critical and meaningful data. Although hepatitis B virus has a genotoxic effect, interferon therapy for 6 months does not lead to increased cytogenetic aberrations or decreased MI. Further studies with large number of individuals are required to clarify these data.

CONCLUSION

In conclusion, our results have pointed out that pegylated interferon alpha-2a and alpha-2b therapy for 6 months are well tolerated and have no significant cytotoxic effects.

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