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Full Length Research Paper

The p53 status in patients with common variable immunodeficiency

Sara S. Kilic[1]*, Berrin Tunca[2], Gulsah Cecener[2] and Ünal Egeli[2]

[1]Department of Pediatrics, Division of Pediatric Immunology, Uludag University Medical Faculty, Bursa, Turkey
[2]Department of Medical Biology, Uludag University Medical Faculty, Bursa, Turkey

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Common variable immunodeficiency (CVID) comprises a heterogeneous group of primary antibody deficiencies with complex clinical and immunological phenotypes. A high risk for cancer has been described for some types of cancer among patients with CVID. Mutations in p53, a critical tumor suppressor gene, are one of the most common genetic alterations in human cancers, therefore contributes to the complex network of molecular events leading to tumor formation. This prompted us to investigate the incidence of p53 gene mutations in patients with CVID and evaluated the predictive risk for tumor development. We investigated the presence of p53 mutations in patients with CVID, tumor samples and in the surgical margins of 34 patients with head and neck cancer using single strand conformational polymorphism and sequencing analysis. We investigated the presence of p53 mutations in genomic DNA samples of 20 patients with CVID and 10 healthy controls using polymerase chain reaction and heteroduplex analysis. None of the patients were found to have p53 gene mutations. Only one patient developed non-Hodgkin lymphoma (NHL) during nine years follow-up. P53 mutations was not also detected in tumor biopsy sample. We found no statistically significant association between the presences of p53 mutations in patients with CVID.

Key words: Common variable immunodeficiency, p53 gene, tumor development, apoptosis

INTRODUCTION

Common variable immunodeficiency (CVID) includes a heterogeneous group of conditions characterized by reduced levels of serum immunoglobulins and primary antibody failure (Chapel et al., 2008). Genetic defects in transmembrane activator and calcium modulator and cyclophilin ligand interactor -TACI (*TNFRST13B*), inducible costimulator (ICOS), B cell-activating factor receptor (BAFF-R) and CD19 account only for a minority (15 to 20%) of cases of CVID; the molecular pathophysiology of the remaining cases remains undefined (Castigli and Geha, 2006). Chronic infections and chronic inflammatory conditions seen in CVID result in a prolonged oxidative and nitrosative stress and an increased cancer risk.

Patients with CVID have an increased risk of malignancy, particularly lymphoma and gastric cancer (Cunningham-Rundles and Bodian, 1999). CVID involves T-lymphocyte abnormalities, which may in part explain the increased incidence of lymphoproliferative and autoimmune diseases seen in patients with CVID. Patients require administration of intravenous immunoglobulins monthly or every three weekly (Chapel and Cunningham-Rundles, 2009).

Apoptosis is a programmed cell death process that plays role in regulation of cell count, organ size and tissue hemostasis throughout the development of the organism. The defects in apoptosis mechanism lead to autoimmunity, immunodeficient state and tumor development. The p53 tumor suppressor gene plays an important role in the regulation of the apoptotic response of cells following exposure to genotoxic stress. The dual role played by p53 in hematopoesis, inducing proper

*Corresponding author. E-mail: sebnemkl@uludag.edu.tr, sarasebnemklc@gmail.com. Tel: +90 224 295 04 18. Fax: +90 224 442 81 43

cellular maturation as well as maintaining the quiescence of the stem cell population contributes to the homeostasis of the hematopoietic system, assuring the prevention of malignant transformation. p53 has many mechanisms of anticancer function, and plays a role in genomic stability, and inhibition of angiogenesis. It can initiate apoptosis, the programmed cell death, if DNA damage proves to be irreparable. P53 can activate DNA repair proteins when DNA has sustained damage (Levine et al., 1991). Inactivating mutations at the p53 gene represent the most common genetic lesion of human primary tumors and have etiologically been associated with the onset of neoplasia. Mutant alleles with single missense base substitutions, predominantly within exons 5 to 8, encode for p53 proteins (Molchadsky et al., 2010).

Several alteration in tumour suppressor genes have been reported not only in cancers but also in precancerous processes, suggesting that mutation of the p53 gene is an early event in cancer development. Because malignancy is seen frequent in CVID patients, we planned to study p53 gene mutations in CVID and evaluated the predictive risk for tumor development.

MATERIALS AND METHODS

We analyzed 20 patients with CVID (12 males and 8 females; mean age 7.8 years, range 3.8 to 25 years) and 10 healthy children as a control group (9 males and 1 female, mean age 6.5, range 2.5 to 15 years). In the CVID group there was one patient who also had non-Hodgkin lymphoma (NHL).

Genomic DNA was isolated using standard methods from peripheral blood lymphocytes. (Proteinase K incubation and phenol-chloroform extraction). Exons 5 to 8 of p53 gene were amplified by polymerase chain reaction (PCR) and heteroduplex analysis (HDA) with a sensitivity of 80-90% in small DNA fragments was used to investigate the point mutations in the central hydrophobic core of the molecule coded in exons 5 to 8 where most mutations seemed to be clustered (Kiaris et al., 2005). The primary sequences of p53 genes were as below:

E5F 5′-TCA ACT CTG TCT CCT TCC TCT TCC-3′
E5R 5′-CTG GGC AAC CAG CCC TGT CGT-3′
E6F 5′-TTG CTC TTA GGT CTG GCC CC-3′
E6R 5′-CAG ACC TCA GGC GGC TCA TA-3′
E7F 5′-TAG GTT GGC TCT GAC TGT ACC-3′
E7R 5′-TGA CCT GGA AAT CTA CTG GGA CGG-3′
E8F 5′-AGT GGT AAT CTA CTG GGA CGG-3′
E8R 5′-ACC TCG CTT AGT GCT CCC TG-3′

Heteroduplex analysis (HDA) was carried out from the high-quality PCR products (Figure 1). Samples were HDA positive by visual inspection if two bands migrated apart from the wild-type bands.

RESULTS

None of the patients were found to have p53 gene mutations by HDA (Figure 2). No mutation was detected in exons 5 to 8 of p53 gene in any of our patients or control group. The analysis of p53 mutation on tumor tissue of CVID patient who developed NHL was negative.

During 9 years follow up, only one patient in 20 developed malignity and chemotherapy does not cure metastatic NHL. The patient died in a short time of the first chemotherapy regimen.

DISCUSSION

The incidence of malignancy appears overall increased in CVID, occurring in up to 15% of subjects. About 2 to 8% of subjects with CVID are diagnosed with NHL (Chua et al., 2008). Kinlen et al. (1985) reported a 30-fold increase in the risk of lymphoma and a 47-fold increase in the risk for stomach cancers. For 176 subjects in a European study, the observed to expected ratio for lymphoma in CVID was 12.1 and for stomach cancer was 10.3 (Mellemkjaer et al., 2002). In our study group, only one patient developed NHL during 9 years follow-up period.

There is no sufficient number of studies that disclose the predictive risk factors for cancer development among patients with CVID. To our knowledge, p53 mutations on blood sample have not been searched in CVID patients. In this study, we analysed these patients for mutations in p53 which is one of the tumor suppressor genes frequently involved in neoplasia and found no mutation both in patients and controls. There is only one study showed that Bcl-6 mutations, proto-oncogene, have been proposed as a genetic marker for defining the histogenesis of B-cell lymphoproliferation in patients with CVID (Ariatti et al., 2000). Rearrangements of *BCL-6* were detected in two thirds of patients with CVID and NHL.

Nearly 50% of gastric cancers show p53 overexpression, and some studies report p53 gene mutations in precancerous lesions, suggesting a role in the early stages of gastric carcinogenesis. The mutated protein has a longer half-life than native p53 (Starzynska et al., 1994; Shiao et al., 1994). Zullo et al. (1999) assessed both histological alterations and p53 over expression in the gastric mucosa of patients with CVID, and to correlate these findings with *Helicobacter pylori* infection. In the present study, p53 overexpression was found in 18% of patients, including one with normal gastric mucosa. They hypothesised that both *H. pylori* and p53 alterations play a role in the gastric carcinogenesis of patients with CVID. We have studied p53 mutations in the tumor tissue obtained from the patient with CVID and NHL. Unfortunately, no mutation was detected.

We conclude that although the number of our patients is not sufficient to make strict comments, p53 gene mutations appears to play no role in the higher incidence of neoplasia in these patients. This study could be done with more extensive patient populations and furthermore investigations of the other apoptose regulator factors, such as bcl-2, bax and bcl-x, besides p53 mutations are necessary in order to identify the underlying aetiology of malignancy in CVID patients.

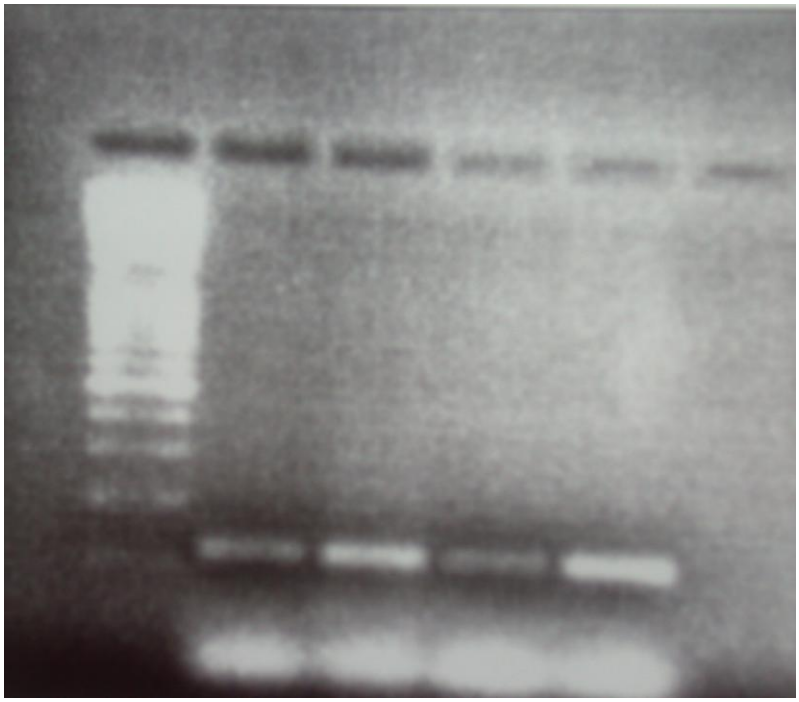

Figure 1. The PCR products were controlled on 2% agarose gel stained with ethidium bromide and photographed under UV light with imaging software system (Vilber Laurmat /France) after electrophoresis.

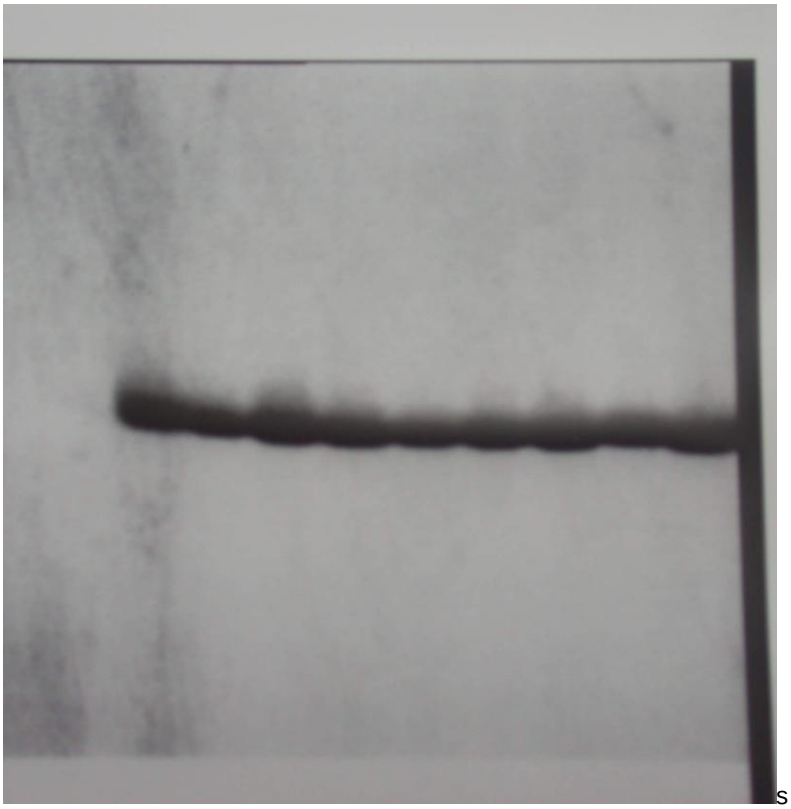

Figure 2. Heteroduplex analysis of exon 7 of p53 gene in lymphocytes from 9 patients.

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