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TP53 mutations in circulating free DNA from Egyptian patients with non-Hodgkin's lymphoma

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ABSTRACT

Background: P53 protein plays important role in the maintenance of genome stability in mammalian cells; it acts in many processes including cell-cycle checkpoint, DNA repair, apoptosis, and angiogenesis. Mutations of P53 have been reported as common mutations in solid tumours, including non-Hodgkin lymphoma, NHL, and have been implicated in drug resistance, aggression and poor prognosis. Chronic infection with hepatitis C, HCV, has been associated in some studies with increased risk of NHL. HCV is a widespread infection in the Egyptian population. Circulating free DNA (CFDNA) has been shown to be a good source of liver tissue-derived DNA in African and Asian patients with chronic liver disease or hepatocellular carcinoma, HCC. Our previous results have shown TP53 mutations in 5% of CFDNA and 10% of tumours of HCC, with underlying HCV.
Objective: Since previous studies have shown p53 mutations in the DNAs extracted from the NH lymphoid tumours, we have assessed the presence of p53 mutations from exons 5 to 9 in CFDNA in patients with NHL, from Alexandria, Egypt, where HCV is highly prevalent, in a first attempt case-control preliminary study.
Methods: CFDNA was extracted from sera of 20 cases with NHL and 20 negative control individuals. The retrieved serum DNAs were screened for TP53 mutations from exons 5 to 9 using direct sequencing and a PCR-restriction digestion analysis (RFLP). Concentrations of CFDNA were measured using Fluorometric assay.
Results: Concentrations of CFDNA were significantly higher among NHL patients compared to the negative control individuals indicating a very high release or turn-over of DNA from the tumour into the blood stream among NHL patients. Mutations of p53 determined in NHL cases (30%) were of Arg-176 (1/20: 5%), Phe-238 (1/20: 5%), Ser-249 (2/20; 10%), Lys-249 (1/20: 5%) and Phe-250 (1/20: 5%). No mutations were detected among controls. However, Arg-213 polymorphism was found in 2 cases of NHL (10%) and in 1 case of controls (5%).
Conclusion: Our findings of higher DNA concentrations with some p53 mutations in CFDNA from patients with NHL that match the previous reported p53 mutations from tumour DNA may hold promises that CFDNA may serve as a convenient source of tumour-derived DNA to serve as a promising tool of a non-invasive, low-cost new strategy for earlier detection, diagnosis and follow up of the disease. A large-scale prospective study utilizing CFDNA and DNA from tumours of NHL patients will be required to validate this first trial of utilizing CFDNA from NHL patients.

1. Introduction

Non-Hodgkin lymphomas, NHLs, are estimated at 287,000 new cases in the world annually and accounts

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for 6% of all incident cancers worldwide [1]. Age-standardized incidence rates of NHL in USA have been reported to be among the highest in the world. Rates have been reported to be low in East Asia, intermediate in Africa and the Middle East, and high in Western Europe, Australia and Canada. The international variations reflect differences in exposure to risk factors [2]. Among the risk factors highlighted are lifestyle and occupational exposures [2], immunological status [3,4], and viral infections, including human immunodeficiency virus (HIV), Epstein Barr virus (EBV) and hepatitis C virus [5–9]. HCV infection represents a major health problem worldwide and specifically in Egypt [8–10]. It is the principle causative agent of chronic hepatitis, cirrhosis and HCC [10]. Association between infection with HCV and increased risk of NHL has been reported in studies performed on Egyptian patients [8,9].

Low levels of circulating cell-free DNA (CFDNA) can be isolated from the plasma or serum of healthy individuals at average of 30 ng/ml. These levels are often increased in patients with cancer or autoimmune diseases [11–13]. Our earlier results have shown p53 mutations in CFDNA as well as in tumours from Egyptian patients with HCC, indicated that CFDNA may serve as a convenient tool in monitoring the process of tumourigenesis [14].

p53 is essential to keep the genomic stability and suppresses the development of malignancy. Mutations of p53 have been associated with various types of cancers. They were detected in tumours of colon, lung, breast, esophageal, liver, brain and other organs [15–17]. The genetic alterations of p53 in lymphoid malignancies have been reported by many investigators and were correlated to drug resistance, aggression and poor prognosis [18–25]. The incidence of p53 mutations among NHL was reported to reach up to 40% [19–22,25,26] and correlated with poor survival.

In this study, we have assessed the presence of genetic alterations in p53 from exons 5 to 9, in CFDNA isolated from the sera of 20 subjects with NHL and 20 negative control individuals. We show that levels of CFDNA vary with pathological status, and that TP53 mutations are detectable in patients, suggesting that they occur as a turn-over from cancer development.

2. Materials and methods

2.1. Patients and specimens

Specimens evaluated in the current case-control study included forty serum samples, obtained through routine clinical care and evaluated at the Department of Hematology, University Hospital, Faculty of Medicine, University of Alexandria, Egypt. The specimens included 20 NHL and 20 control individuals. The NHL cases were *de novo* diagnosed by pathological review, and did not receive any chemotherapeutic treatment yet. Disease dissemination was evaluated by physical examination, bone marrow biopsy and computed tomography (CT) scan of the chest and abdomen. Blood specimen were collected at the time of routine clinical examination, anti-coagulated with EDTA, processed immediately after collection and stored at −70 °C, until shipping in dry ice to IARC, France. Ethical approval for performing this joint Egyptian–French study was gotten in order to carry out this study.

2.2. DNA extractions

Circulating free DNA was extracted from human sera using QIAmp® DNA Blood Mini kit (Qiagen, Hilden, Germany). Simultaneous DNA extraction was performed from 300 μl serum sample according to the manufacture's blood and body fluid spin protocol. Purified DNA was eluted from the QiAmp Silica column with two volumes (2× 100 μl) of water (PCR-grade, Sigma Chemical Co., St. Louis, MO).

2.3. DNA quantification

Concentrations of the blood-extracted DNA were measured using Quant-iT™ PicoGreen dsDNA Assay Kit (Invitrogen/Molecular Probes, Eugene, OR; Invitrogen Detection Technologies) according to the manufacture's instructions. The fluorescence was read on Fluo/luminometer; Fluoroskan Ascent FL Fluoremeter (Labsystems, Helsinki, Finland) attached to a computer system. DNA concentrations were calculated using Microsoft Excel 2003 utilizing a calibration curve with different concentrations of DNA standards.

2.4. PCR, restriction digestion and direct sequencing (RFLP, TP53 mutation detection)

Mutations in exons 5 to 9 were detected by performing PCR/direct sequencing. Mutations in codon 249 of p53 (in exon 7; a reflection of environmental exposure to Aflatoxins [27]) were detected by performing PCR/RFLP in exon 7 of TP53 gene followed by direct sequencing. DNA (5–10 μl of resuspended, purified material) was used for amplification of different exons of the TP53 gene with 0.2 μM primers (final concentration) using HotStarTaq polymerase (Qiagen) or GoTaq (Promrga). Primers flanking exon 7 were: sense-333-: 5′-CTTGCCACAGGTCTCCCCAA-3′, antisense-313-: 5′-AGG GGTCAGCGGCAAGCAGA-3′; exons 5&6 were: sense-236-: 5′-TGTTCACTTGTGCCCTGACT-3′, antisense-240-: 5′-TTAA CCCCTCCTCCCAGAGA-3′; and exons 8&9 were: sense-314-: 5′-TTGGGAGTAGATGGAGCCT-3′, antisense-315-: 5′-AGT GTTAGACTGGAAACTTT-3′. In parallel with all reactions of PCR, a series of controls without DNA template (blank samples) were run to monitor for possible contamination. Negative (wild-type genomic DNA) and positive (DNA of PLC/PRF/5 cells, homozygous for Ser-249 mutation in exon 7 amplification) controls were included in PCR and restriction analyses. PCR reaction for exon 7 involved a 15-min HotStarTaq polymerase activation at 95 °C, 50 cycles of denaturation (94 °C, 30 s), primer annealing (60 °C, 30 s), and extraction (72 °C, 30 s), followed by a final 5-min extension at 72 °C. The size of the final PCR fragment was 177 bp. PCR reaction for exons 5&6 and 8&9 involved a 2-min GoTaq polymerase activation at 94 °C, 20 cycles of denaturation (94 °C, 30 s), primer annealing (63 °C, 45 s), and extraction (72 °C, 1 min), followed by another 30 cycles of denaturation (94 °C, 30 s), primer annealing (60 °C, 45 s), and extraction (72 °C, 1 min), then followed by a final 10-min extension