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Evaluation of the pattern and prognostic implications of anti-p53 in hepatocellular carcinoma

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ABSTRACT

Introduction: The p53 antigen is onco-protective and when damaged, leads to production of anti-p53. It also predisposes to various cancers , including hepatocellular carcinoma (HCC). Serum anti-p53 has been proven to have prognostic and other values in patients with HCC. The objectives of this study were to determine the serum pattern, prevalence, diagnostic and prognostic utility of serum anti-p53 in Nigerians with HCC.

Methods: 41 subjects with HCC and 45 apparently-healthy controls were matched for age and sex. Serum anti-p53 was determined using p53-autoantibody ELISA kit.

Results: The mean age of the patients was 48.9 (+/- 13.8) years, and that of controls was 49.4 (+/- 13.7) years. There was male predominance among the patients, 31 men (75.6 percent) versus ten women (24.4 percent), with a male-to-female ratio of 3.1:1. Similar values among controls were 33 men (73.3 percent) versus 12 women (26.7 percent), with a male-to-female ratio of 2.75:1. Anti-p53 was detectable in the sera of five (12.2 percent) patients and four (8.9 percent) of controls (p-value is greater than 0.05). All the patients with positive sera were males, while one of the controls was a female. Three (60 percent) of the positive patients were in the age range 40–49 years, while in the control group, they were in the age range 50–59 years.

Conclusion: There is a low prevalence of serum anti-p53 in our study population, and this is commoner in men. It is also present in the control group and therefore may not be useful as a diagnostic tool in this study population.

Keywords: anti-p53, hepatocellular carcinoma, serum anti-p53

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INTRODUCTION

The p53 protein is involved in DNA repair, genomic stability, chromosomal segregation, and senescence. The p53 antigen is thus an oncoprotective antigen and when damaged, leads to production of anti-p53 and also predisposes to various cancers, including hepatocellular carcinoma (HCC), as well as autoimmune disorders[1]. Mutations and allelic deletions of the p53 gene are the most common genetic alterations found in human tumours[2], and are considered to be predictive of poor prognosis in HCC with induction of humoral response[3]. In HCC, allele losses have been frequently documented on chromosomes 1, 4, 5, 8, 10, 11, 13, 16, 17 and 22, irrespective of the hepatitis B virus (HBV) status[4]. Deletion, suppression or destruction of the p53 gene with production of antibody (anti-p53) against it has been found to predispose to HCC[5].

Serum anti-p53 has been proven to have prognostic and other values in patients with HCC and has been reputed to be a reliable expression of the p53 status in cancers.[6,7] Anti-p53 have been reported in the sera of 25%–50% of patients with HCC and there is a statistical correlation between its presence and chronic hepatic diseases[8]. Serum anti-p53 could therefore represent a new and sensitive method to identify preneoplastic lesions and a useful prognostic factor for HCC patients[8]. Previous studies in Nigeria have looked into the aetiology and risk factors such as aflatoxins, HBV and antibody to hepatitis C virus (anti-HCV), but none has focused on the usefulness of anti-p53 diagnosis or prognosis in HCC. Reid and Liang have suggested that a search for the ultimate screening test for HCC should be continued[9]. The objectives of this study were to determine the serum pattern, prevalence, diagnostic and prognostic utility of serum anti-p53 in Nigerians with HCC.

METHODS

Patients for this case-control prospective study were drawn from referrals to the Liver Unit at the University

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College Hospital, Ibadan after obtaining informed consent. 41 consecutive consenting adults with untreated HCC diagnosed by clinical features of HCC and confirmation by histology, ultrasonography and/or serum alpha-foetoprotein were enrolled between October 2003 and September 2004. Patients with cancers other than HCC, metastatic liver cancer or negative histology were excluded. 45 age- and sex-matched apparently healthy individuals, from hospital staff, medical students, relations of patients and patients with non-hepatic benign illnesses, such as hypertension, were recruited as controls.

Questionnaires were administered to the patients to collect demographical data, past medical history relating to risk factors for viral agents as well as alcohol and mouldy grain ingestion. Ten millilitres of venous blood was collected from all subjects, spun and sera stored at –20 °C, until they were analysed. Serum antibodies to p53 gene were assayed using the commercially-available p53-autoantibody enzyme-linked immunosorbent assay (ELISA) kit (Immunobiological Laboratories, Hamburg, Germany). Cut-off absorbance value was calculated according to the manufacturer's instructions (cut-off=0.748), and all sera with absorbance values greater than the cut-off were regarded as positive, while all sera with an absorption less than or equal to the cut-off value were considered negative. Liver function tests, including prothrombin time, were done using conventional methods.

Results of continuous variables were expressed in mean ± standard deviation (SD). The student's t-test and chi-square test were used to compare means and proportions, respectively. Where appropriate, other statistical instruments were employed using EPI-info version six. Statistically significant p-value was specified at <0.05.

RESULTS

A total of 86 adults, which comprised 41 patients with HCC and 45 apparently-normal control subjects, were studied. The mean age of the patients was 48.9 (±13.8) years, while that of the controls was 49.4 (± 13.7) years (Table I). There was no significant difference in the mean ages of patients and controls ($p > 0.86$). The peak age in both groups was 40-49 years. There was male predominance among the patients, 31 men (75.6%) versus 10 women (24.4%), with a male-to-female ratio of 3.1:1. Similar values were seen among the controls, with 33 men (73.3%) versus 12 women (26.7%), with a male-to-female ratio of 2.75:1. There was, however, no significant difference in the sex ratios of patients and controls ($p = 0.809$).

Alcohol ingestion was observed in 21 (51.2%) patients, out of whom only four (9.7%) had a significant (≥80g/day for ≥five years or ≥40g/day for ≥ten years) history of consumption. Only one (2.4%) patient had a positive history of mouldy grain ingestion. The mean duration of illness was 3.6 (± 2.0) months, with a range of one to seven months. Only two (4.9%) patients had evidence of distant metastasis, one to the chest and the other to the spine. Anti-p53 was detectable in the sera of five (12.2%) patients and four (8.9%) controls ($p > 0.05$). All the patients with positive sera were males, while one

Table I. Serum anti-p53 and demographical characteristics of patients with HCC and controls.

	Anti-p53 status	Patients	Controls	p- value
	Negative	36 (87.8%)	41 (91.1%)	
	Positive	5 (12.2%)	4 (8.9%)	0.617
	Total	41 (100%)	45 (100%)	
Gender				
Male	Positive	5 (16.1%)	3 (9.1%)	
	Negative	26 (83.9%)	30 (90.1%)	0.395
Female	Positive	0 (0%)	1 (8.3%)	
	Negative	10 (100%)	11 (91.7%)	0.350
Age group (years)				
< 40	Positive	1 (11.0%)	1 (10.0%)	
	Negative	8 (88.9%)	9 (90.0%)	0.937
40–49	Positive	3 (21.4%)	–	
	Negative	11 (78.6%)	14 (100%)	0.067
50–59	Positive	–	2 (18.2%)	
	Negative	10 (100%)	9 (81.8%)	0.156
≥ 60	Positive	1 (12.5%)	1 (10.0%)	
	Negative	7 (87.5%)	9 (90.0%)	0.867

of the controls was a female. Three (60%) of the positive patients were in the age range of 40-49 years, while in the control group, they were in the age range 50-59 years.

The prothrombin time was deranged in 65.2% of patients, while other liver function parameters were deranged in about 70% of the patients. Three (60.0%) of the anti-p53 positive patients had a history of alcohol ingestion, but only one (20%) had significant consumption, while three (8.3%) of the negative patients had significant alcohol consumption. The only patient with a history of mouldy grain consumption was negative for serum anti-p53. The presenting symptom in three (60%) of the anti-p53 positive patients was abdominal swelling. None of the positive patients presented with jaundice. The duration of illness in four (80%) of the positive patients was from one to six months, whereas in 24 (66.7%) of the negative patients, it was less than three months.

Three (60%) of the positive patients had ascites, which was haemorrhagic in one (20%). However, 24 (66.7%) of the negative patients had ascites out of which seven (19.4%) was haemorrhagic. Two (40%) of the positive patients had hepatic encephalopathy, which was observed in 16 (44.4%) of the negative patients. Metastasis to the chest was observed in one of the positive patients. All the five (100%) positive patients for serum anti-p53 were in Okuda stage two. Of the 36 patients with negative serum anti-p53, only 26 were staged, and of these, 17 (65.4%) and 9 (34.6%) were in stages three and two, respectively.

DISCUSSION

The prevalence of anti-p53 in this study is low and is consistent with studies conducted in other parts of the world. A study of 130 European patients with PLCC showed a prevalence of 7%[3]. Similarly, a low prevalence was reported by Soini et al[10], Sitruk et al[11], Tangkijvanich et al[12] and De Benedetti et al[13] among patients with HCC. Even though the sample sizes varied in these studies, the same ELISA method was used for qualitative analysis of serum anti-p53. There is however no other study in Africa and Nigeria on serum anti-p53 to compare our study with. A study conducted by Ndububa et al in Ile-Ife, Nigeria, showed mutation at codon 249 of the p53 gene in one (5.5%) of the 18 HCC patients studied[5]. However, they used amplification of exon seven of p53 gene from DNA samples of HCC tissue by nested polymerase chain reaction followed by restriction enzyme analysis, and not serum anti-p53.

In contrast to the results obtained in our study, Ryder et al at the Institute of Liver Studies, Kings College Hospital, London, found a prevalence of 50% of serum anti-p53 among HCC patients; again the sample size was lower though the same method was used. Their study concluded that serum anti-p53 could be of value in the diagnosis and characterisation of patients with HCC[14]. A high prevalence of serum anti-p53 was also observed by Charuruks et al (18.4%)[15], Raedle et al (22.7%)[16] and Shiota et al (32%)[17] in their separate studies. The reasons for the differences though not yet fully understood, may be related to some yet unidentified bio-geographical differences in the study populations.

The finding of anti-p53 in the sera of four (8.9%) of our controls is higher than that observed by Raedle et al in their study where only 25 (3.5%) subjects without underlying malignancy were p53 autoantibody-positive[16]. Nigeria, in contrast to Western Europe, has a high prevalence of HCC as a result of endemicity of hepatitis B infection, and this may account for the high prevalence of anti-p53 among controls. It would be advisable to follow-up the positve control subjects for markers of HCC. Measurement of serum aflatoxins and hepatitis B viral markers, known to contribute to genetic mutations of the p53 gene, may shed some light on the differences, as these factors are known to be more prevalent among Nigerian population.

This study revealed a higher prevalence of serum anti-p53 in patients who were in the intermediate stage of the Okuda staging system for HCC, compared to those in the advanced stage. Further studies need to be carried out to elucidate the absence of anti-p53 in advanced HCC, and larger studies are required to substantiate these findings. Qin and Tang, in a prospective study of Chinese patients with HCC, found that the three- and five-year overall survival rates of patients with positive p53 nuclear accumulation were much lower than those with negative p53 expression, although in this particular study, serum anti-p53 was not studied[7]. There is however no previous study comparing Okuda stage with serum anti-p53.

We conclude that there is a low prevalence of serum anti-p53 among patients with HCC and controls in our study population. Serum anti-p53 is commoner in men than in women. Anti-p53 may not be useful as a diagnostic tool in the study population.

We suggest that anti-p53, serum aflatoxins, HBV and HCV be studied in different populations for their interactive role in hepatic carcinogenesis.

REFERENCES

1. Di Cesare E, Previti M, Lombardo F, et al. Serum anti-p53 antibodies in patients with type 1 diabetes. Ann Clin Lab Sci 2001; 31:253-8.
2. Harris CC, Hollstein M. Clinical implications of the p53 tumor-suppressor gene. N Engl J Med 1993; 329:1318-27. Comment in: N Engl J Med 1994; 330:864-5..
3. Saffroy R, Lelong JC, Azoulay D, et al. Clinical significance of circulating anti-p53 antibodies in European patients with hepatocellular carcinoma. Br J Cancer 1999; 79:604-10.
4. Ding SF, Habib NA. Loss of heterozygosity in liver tumours. J Hepatol 1995; 22:230-8.
5. Ndububa DA, Yakicier CM, Ojo OS, et al. p53 codon 249 mutation in hepatocellular carcinomas from Nigeria. Afr J Med Sci 2001; 30:125-7.

6. Soussi T. The humoral response to the tumor-suppressor gene-product p53 in human cancer: implications for diagnosis and therapy. Immunol Today 1996; 17:354-6.
7. Qin LX, Tang ZY. The prognostic molecular markers in hepatocellular carcinoma. World J Gastroenterol 2002; 8:385-92.
8. Shiota G, Kishimoto Y, Suyama A, et al. Prognostic significance of serum anti-p53 antibody in patients with hepatocellular carcinoma. J Hepatol 1997; 27:661-8.
9. Reid AE, Liang TJ. The search for the ultimate screening test for hepatocellular carcinoma continues. Hepatology 1994; 19:255-7.
10. Soini Y, Chia SC, Bennett WP, et al. An aflatoxin-associated mutational hotspot at codon 249 in the p53 tumor suppressor gene occurs in hepatocellular carcinoma from Mexico. Carcinogenesis 1996; 17:1007-12.
11. Sitruk V, Vaysse J, Chevret S, et al. [Prevalence and prognostic value of serum anti-p53 in hepatocellular carcinoma. A study of 159 patients]. Gastroenterol Clin Biol 2000; 24:1159-63. French.
12. Tangkijvanich P, Janchai A, Charuruks N, et al. Clinical associations and prognostic significance of serum anti-p53 antibodies in Thai patients with hepatocellular carcinoma. Asian Pac J Allergy Immunol 2000; 18:237-43.
13. De Benedetti VM, Welsh JA, Trivers GE, et al. p53 is not mutated in hepatocellular carcinomas from Alaska natives. Cancer Epidemiol Biomarkers Prev 1995; 4:79-82.
14. Ryder SD, Rizzi PM, Volkmann M, et al. Use of specific ELISA for the detection of antibodies directed against p53 protein in patients with hepatocellular carcinoma. J Clin Pathol 1996; 49:295-9.
15. Charuruks N, Tangkijvanich P, Voravud N, et al. Clinical significance of p53 antigen and anti-p53 antibodies in the sera of hepatocellular carcinoma patients. J Gastroenterol 2001; 36:830-6. Comment in: J Gastroenterol 2001; 36:865-6.
16. Raedle J, Oremek G, Truschnowitsch M, et al. Clinical evaluation of autoantibodies to p53 protein in patients with chronic liver disease and hepatocellular carcinoma. Eur J Cancer 1998; 34:1198-203.
17. Shiota G, Kishimoto Y, Suyama A, et al. Prognostic significance of serum anti-p53 in patients with hepatocellular carcinoma. J Hepatol 1997; 27:661-8.