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Impact of the Hepatitis B Virus pRE-S2 Mutant on p53 and PLK1 Biomarkers Expression in Patients with Hepatocellular Carcinoma in Brazzaville

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Abstract

Hepatocellular carcinoma (HCC) is the most common primary liver tumor and a major public health problem, particularly in sub-Saharan Africa. Hepatitis B virus (HBV) is an important etiological factor, and its mutants, particularly pRE-S2, are associated with an increased risk of HCC. To evaluate the impact of the HBV pRE-S2 mutant on the expression of p53 and PLK1 biomarkers in patients with HCC in Brazzaville. A case-control analytical study was conducted between July and December 2024, *i.e.*, 6 months, including 82 participants divided into two groups: 22 patients with HBV-related HCC (cases) and 60 controls, including 30 controls infected with HBV without HCC (sick controls) and 30 healthy control subjects. Serum expression of pRE-S2, p53, and PLK1 was analyzed by ELISA. Data were processed by GraphPad Prism with a significance threshold of $p < 0.05$. Mean age was 56 ± 11 years in cases, 40 ± 15 years in diseased controls, and 38 ± 13 years in healthy controls. Mean serum levels were 2955 ± 69 ng/mL for pRE-S2 in cases versus 185.7 ± 26 ng/mL and 64.5 ± 14 ng/mL in diseased and healthy controls, respectively. p53 expression was significantly higher in cases (6.92 ± 4.8 ng/mL) compared to controls (2.73 ± 1.7 and 1.45 ± 0.8 ng/mL). PLK1 levels were also increased

(181.5 ± 31 ng/mL in cases). After adjustment for age, pRE-S2 and p53 were strongly associated with HCC, while PLK1 was less significant. The high expression of pRE-S2 and p53 on the one hand and of PLK18 to a lesser extent suggests their potential role as biomarkers of HBV-related HCC. However, PLK1 seems rather a biomarker of chronic infection than specific for HCC.

Keywords

Hepatocellular Carcinoma, Hepatitis B Virus, pRE-S2 Mutant, p53 and PLK1 Biomarkers, Congo

1. Introduction

Hepatocellular carcinoma (HCC), or primary liver cancer, is one of the leading causes of cancer-related death worldwide. According to GLOBOCAN 2022 data, it is the sixth leading cause of cancer in terms of incidence and the third leading cause of mortality, with more than 866,000 new cases and 758,000 deaths annually [1]. This disease particularly affects regions with a high viral endemic, notably sub-Saharan Africa, where it remains a major public health problem [2] [3].

In the Republic of Congo, HCC is one of the deadliest cancers, with an estimated adjusted mortality rate of 36.3 deaths per 100,000 population [4]. Chronic infection with hepatitis B virus (HBV) constitutes the main etiological factor in these regions, significantly increasing the risk of hepatic carcinogenesis, especially in patients with cirrhosis [5] [6].

HBV-induced liver oncogenesis is based on several biological mechanisms: chronic inflammation, repeated liver regeneration, integration of the viral genome into that of the host, and expression of mutated viral proteins [7]. Among the latter, a common and particularly oncogenic mutation concerns the deletion in the pRE-S2 region of the surface antigen (HBsAg), leading to the production of the mutant protein pRE-S2. This accumulates in the endoplasmic reticulum (ER), causes oxidative stress, and activates signaling pathways such as mTOR, promoting the proliferation and malignant transformation of hepatocytes [8] [9].

Recent work has reinforced these observations. Zhang *et al.* (2022) demonstrated that expression of the pRE-S2 mutant induces increased cell proliferation via activation of endoplasmic reticulum stress [10]. Similarly, Liu *et al.* (2023) highlighted direct interactions between the pRE-S2 mutant and the PI3K/AKT/mTOR pathway, both *in vitro* and *in vivo* models, confirming its central role in liver carcinogenesis [11]. Furthermore, the pRE-S2 mutant could also influence the expression of key cell cycle biomarkers, such as p53 and PLK1. p53 is a tumor suppressor protein, involved in genomic stability and regulation of apoptosis [12] [13], while PLK1 (Polo-like kinase 1) plays a crucial role in the control of mitosis and cell proliferation [14]. Their deregulation is frequently observed in many can-

cers, including HCC. In particular, overexpression of PLK1 is often correlated with poor therapeutic response and unfavorable prognosis [15]. In the Congolese context, marked by a high prevalence of HBV and a high incidence of HCC, it appears essential to further study the molecular mechanisms involved in tumor progression [16]. Analyzing the impact of the pRE-S2 mutant on the expression of p53 and PLK1 could thus contribute to a better understanding of liver carcinogenesis and open up perspectives in terms of prognostic biomarkers and therapeutic monitoring strategies.

Thus, the present study aimed to evaluate the impact of the pRE-S2 mutant of HBV on the expression of p53 and PLK1 in patients with HCC in Brazzaville.

2. Materials and Methods

A case-control analytical study was conducted from July to December 2024.

2.1. Study Population

Consisted of 82 participants divided into two groups: cases and controls (sick and healthy controls).

- Cases: 22 patients with HCC and HBV-positive confirmed after anatomopathological and virological analysis.
- Controls: 30 patients with HBV without HCC (patient controls) and 30 healthy subjects without HBV or HCC (healthy controls). The sample size is limited by the availability of cases over the study period (6 months), but remains comparable to other similar studies on pRE-S2 and CHC.

2.2. Selection Criteria

Informed consent and age ≥ 18 years were the selection criteria.

- Inclusion criteria: were defined based on the EASL 2018 international guidelines. Cases had a histological diagnosis of HCC and were HBsAg positive; patient controls had HBsAg positive results without malignant liver lesions, and healthy controls were seronegative for HBV or HCC.
- Patients with co-infections (HIV, HCV, HDV), alcoholic cirrhosis, or other cancers were not included.

2.3. Assay

The procedure consisted of collecting a blood sample (4 - 5 mL) from the elbow crease into a dry tube. After centrifugation at 5000 rpm for 5 minutes, the samples were stored at −20˚C before analysis. The samples were analyzed by ELISA technique (Nanjing Pars Biochem kits, China) to measure pRE-S2, p53, and PLK1.

2.4. Statistical Analysis

Data were entered into Excel and processed using GraphPad Prism. Comparisons were performed using the chi-square test and Student's t-test. The significance threshold was $p < 0.05$.

2.5. Ethical Approval

This study was approved by the Ethics Committee of the Faculty of Health Sciences at Marien Ngouabi University (Ref: 2024/CE-FSS/UNM). Written informed consent was obtained from all participants.

3. Results

3.1. Socio-Demographic Characteristics

The mean age was 56 ± 11 years for the case group, 40 ± 15 years for the control group without HCC, and 38 ± 13 years for the healthy control group. Females were predominant in the case group (54.5%), while the control group was predominantly male (Table 1).

Table 1. Distribution of patients by sociodemographic characteristics.

Variables	Control group		Case group
	Healthy (n = 30)	HBV (n = 30)	HBV + HCC (n = 22)
Age (years), X ± SD	**38 ± 13**	**40 ± 15**	**56 ± 11**
≤30	10 (33.33%)	11 (36.67%)	0
30 - 45	12 (40%)	9 (30%)	4 (18.18%)
>45	8 (26.67%)	10 (33.33%)	18 (81.82%)
Sex			
Feminine	14 (46.67%)	14 (46.67%)	12 (54.55%)
Masculine	16 (53.33%)	16 (53.33%)	10 (45.45%)
Occupational status			
Student	5 (16.66%)	3 (10%)	0
Employee	14 (46.67%)	20 (66.66%)	10 (45.46%)
Retired	3 (10%)	2 (6.67%)	4 (18.18%)
Unemployed	8 (26.67%)	5 (16.67%)	8 (36.36%)
Marital status			
Unmarried	21 (70%)	17 (56.67%)	6 (27.28%)
Married	9 (30%)	13 (43.33%)	16 (72.72%)

3.2. Expression of Biomarkers

The mean values of pRE-S2 expression were 2955 ± 69 ng/mL for the case group compared to 185.7 ± 26 ng/mL and 64.5 ± 14 ng/mL for the without HCC and healthy control groups, respectively.

The mean value of p53 was 6.92 ± 4.8 ng/mL for the case group versus 2.73 ± 1.7 ng/mL for the control group without HCC, and 1.45 ± 0.8 ng/mL for the healthy control group.

For PLK1, the mean values were 181.5 ± 31 ng/mL for the case group versus

199.2 ± 20 ng/mL for the control group without HCC, and 35.3 ± 12 ng/mL for the healthy control group.

A significant difference in pRE-S2 concentration was observed between the case group versus the healthy control group (p = 0.001), while no significant difference was observed between the control group without HCC and the case group (p = 0.77).

The analysis of our data from p53 showed a significant difference between the case group versus the healthy control group on the one hand (p < 0.0001) and that of control group without HCC on the other hand (p < 0.0001). This trend was also observed with the concentration of PLK1 between case and healthy control groups only (p = 0.0035). All results are reported in Figure 1.

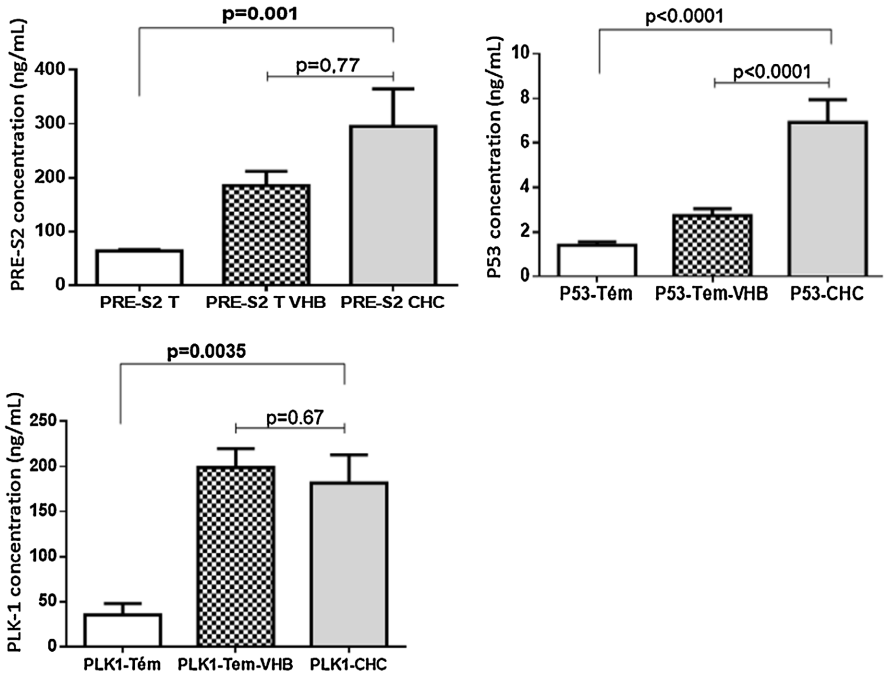

Figure 1. Analysis of the mean concentration of the different biomarkers with the Pre_S2 mutation.

3.3. Multivariate Analysis

After adjusting for age, multivariate analysis of the data showed a significant difference observed between biomarkers in the case and control groups for pRE-S2 and p53 (Table 2).

4. Discussion

Hepatocellular carcinoma is among the most common liver tumors, accounting for about 50% of cases associated with hepatitis B virus (HBV) infection [3] [5]. In the Republic of Congo, the incidence of HCC is steadily increasing, partly due to the high prevalence of HBV [4] [6]. Previous studies demonstrated that mutations in the large surface HBV antigen, particularly deletions in the pRE-S2 region,

Table 2. Crude and age-adjusted ORs (Odds Ratio) for pRE-S2, p53, and PLK1.

Variable	Raw OR (95% CI)	Age-adjusted OR (95% CI)	Adjusted p-value	Interpretation
pRE-S2	25.3 (10 - 60)	21.5 (8 - 55)	<0.001	Strong age-independent association, pRE-S2 remains a robust biomarker of HBV-related HCC
p53	5.1 (2.0 - 12.0)	4.3 (1.6 - 11.2)	0.002	Significant association independent of age, p53 reflects oncogenic stress related to HCC
PLK1	2.1 (0.9 - 4.8)	1.8 (0.7 - 4.5)	0.12	Association weakened after adjustment, not significant; PLK1 appears less specific for HCC

are associated with increased resistance to antiviral therapy and elevated risk of HCC in patients with chronic infection [7]-[9]. Assessing the impact of these mutants on cell cycle biomarkers, such as p53 and PLK1, is therefore essential to elucidate HCC pathogenesis and optimize therapeutic strategies.

In our study, 82 patients were included and divided into two groups: 22 cases of HCC-HBV and 60 controls, including 30 patients with hepatitis B and 30 healthy individuals. Among HCC-HBV cases, women predominated (sex ratio M/F = 0.83), with a mean age of 56 ± 11 years. Our findings contrast with observations by Séhonou *et al.* in Benin, who reported a male predominance [15], which may reflect epidemiological, methodological, or contextual differences across populations. In the control group without HCC, men predominated (53.53%, sex ratio M/F = 1.14) with a mean age of 40 ± 15 years, which is consistent with the study of Kaberladjouz *et al.* [16].

The mean serum pRE-S2 levels were 64.5 ± 14 ng/mL in the healthy control group, 185.7 ± 26 ng/mL in the control group without HCC, and 2955 ± 69 ng/mL in HCC-HBV cases. Our results showed a significant increase in pRE-S2 expression in HCC-HBV cases compared to controls, aligning with Ding *et al.* and Huang *et al.* [8] [9]. Previous studies also showed positive expression of the pRE-S2 mutant in the majority of patients with chronic hepatitis B [9]. Accumulation of the pRE-S2 mutant within the endoplasmic reticulum of infected hepatocytes triggers chronic cellular stress, activating oncogenic pathways such as mTOR, MAPK, and NF-κB, promoting malignant transformation and progression to HCC [7]. Moreover, recent work (Zhang *et al.*, 2022; Liu *et al.*, 2023) confirms the oncogenic role of the pRE-S2 mutant through activation of the mTOR and PI3K/AKT pathways [17] [18].

p53, a tumor suppressor regulating cell cycle, and apoptosis, exhibited mean serum levels of 1.45 ± 0.8 ng/mL in the healthy control group, 2.73 ± 1.7 ng/mL in the control group without HCC, and 6.92 ± 4.8 ng/mL in HCC-HBV cases. Our findings indicate a significant overexpression of p53 in HCC-HBV cases, probably in response to cell damage and oncogenic stress induced by chronic HBV infection. Our findings align with those of Salima *et al.* and Attallah *et al.*, who reported a gradual

increase in p53 with severity of liver disease [10] [11]. PLK1, a key cell cycle kinase and potential therapeutic target in HCC, showed mean levels of 35.3 ± 12 ng/mL in healthy control groups, 199.2 ± 20 ng/mL in control group without HCC, and 181.5 ± 31 ng/mL in HCC-HBV cases. The overexpression of PLK1 in the HCC-HBV and diseased HBV groups suggests early activation in tumor progression, aligning with studies of Mok *et al.*, Yousef *et al.*, and Wei *et al.* [12]-[14]. These observations highlight the role of PLK1 in abnormal cell proliferation and tumor development.

After adjusting for age, multivariate analysis revealed a highly significant association between HBV pRE-S2 mutant and hepatocellular carcinoma (HCC) (aOR = 21.5; 95% CI: 8 - 55; $p < 0.001$). This strong association suggests that pRE-S2 is an independent and robust biomarker of HBV-associated HCC. This result corroborates previous data showing that pre-S region mutants, including pRE-S2, promote abnormal viral replication, endoplasmic reticulum stress, and activation of oncogenic pathways such as MAPK and PI3K/AKT [19] [20]. These mechanisms contribute to genomic instability and malignant transformation of chronically infected hepatocytes.

Furthermore, the p53 biomarker also showed a significant and independent association (ORa = 4.3; 95% CI: 1.6 - 11.2; $p = 0.002$). This observation supports the hypothesis that the pRE-S2 mutant interferes with the p53 pathway, leading either to functional inactivation of this tumor suppressor protein or to compensatory overexpression linked to oncogenic stress [21]. Deregulation of p53 constitutes a key step in the progression of HCC, promoting resistance to apoptosis and uncontrolled proliferation of infected liver cells.

In contrast, PLK1 protein, although involved in cell cycle regulation and mitosis, did not show a statistically significant association after adjustment for age (ORa = 1.8; 95% CI: 0.7 - 4.5; $p = 0.12$). This lack of a strong association could indicate that PLK1 acts more as a non-specific tumor proliferation marker, rather than as a biomarker directly induced by the pRE-S2 mutant [22]. Nevertheless, its slight overexpression observed in some cases could reflect an indirect activation by pRE-S2-stimulated oncogenic pathways.

Overall, these results suggest that the HBV pRE-S2 mutant exerts a direct oncogenic effect, altering p53 regulation and contributing to HCC progression. Thus, pRE-S2 and p53 appear as complementary molecular biomarkers, the joint study of which could improve the early diagnosis and prognosis of HBV-related HCC in Brazzaville.

The primary limitations of this study remain the small sample size and the moderate sensitivity of the ELISA method, compared to more precise techniques such as RT-PCR, immunohistochemistry, or Western Blotting. Further studies, with larger cohorts and more sensitive molecular tools, are needed to clarify the molecular mechanisms involved [10] [11] [14].

5. Conclusions

The HBV pRE-S2 mutant shows a strong and independent association with hepa-

tocellular carcinoma (HCC), confirming its major oncogenic role. The observed p53 overexpression suggests deregulation of the genomic surveillance pathway and oncogenic stress induced by pRE-S2. In contrast, PLK1 did not show a significant association after adjustment, indicating a less specific role in carcinogenesis.

These results highlight that pRE-S2 and p53 constitute complementary molecular biomarkers of HBV-associated HCC. They could improve early diagnosis and prognosis of infected patients.

Ultimately, their integration into molecular screening tools would strengthen HCC surveillance in Congo. Expanded and translational studies are needed to confirm these observations and explore their therapeutic applications.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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