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cfDNA and associated risk factors in the early diagnosis of cancers in Côte d'Ivoire: The case of primary liver cancer related to viral hepatitis B

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Abstract

Introduction: In Côte d'Ivoire (CI), according to Globocan 2020 primary liver cancer (PLC) had an incidence of 10.1/1.0E+05 inhabitants in men (2nd rank) and 5.5/1.0E+05 inhabitants among women (4th rank). This situation has so far been favored by late diagnosis and ignorance of the risk factors responsible for the tumor in people at risk.

Objective: To detect free circulating DNA (cfDNA) and evaluate its possible diagnostic role in order to contribute to the prevention of PLC in CI.

Methodology: Prospective experimental study carried out at the Institut Pasteur in CI in patients coming for alfa-fetoprotein assay. Sociodemographic, clinical, paraclinical and epidemiological data were collected. A blood sample was taken for biological analysis. Data collected in Excel and processed with the GraphPad Prism 5.

Results: 142 predominantly male patients with an average age of 45 were included. More than 50% had the same dietary habits including the consumption of tap water, rice, cassava, plantain, millet, maize and yam and 18% regularly consumed alcohol. 66.66% were carriers of chronic viral hepatitis B (HVB) including 2.81% at the cirrhosis stage and 0.70% at the PLC stage. 20% had a history of familial hepatitis and 9.6% a notion of familial cancer. The cfDNA concentration ranged from 38ng/ml to 10763ng/ml for an average of 571ng/ml. The elevation of AFP and transaminase levels was related to the presence of HBsAg (P<0.001; P=0.02). In addition, the concentration of cDNA was strongly linked to HBsAg carriage as well as to other factors including the notion of familial cancer (P=0.03), and consumption of alcohol (p =0.04) and/or tobacco (p=0.02). Conclusion: HVB remains the predominant risk factor that can lead to PLC. The dosage of cfDNA and the monitoring of food and life hygiene in a chronic carrier of HVB could contribute to the prevention of PLC in the latter.

Keywords: HBsAg; cfDNA; Risk factors; Liver cancer

1. Introduction

Cancer is a major public health concern in both developed and developing countries [1]. It is the second most common cause of death in Western countries after cardiovascular disease and is becoming an increasingly important threat for the health of low- and middle-income countries [2]. According to the latest WHO estimates, the global annual incidence of human tumours could reach 15 million cases by 2030, with 70% of new cancer cases occurring in developing

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countries if no action is taken. Among these cancers, primary liver cancer (PLC) remains the 5th most common cancer in the world in terms of the number of cases (5.4% of new cancer cases) and the 2nd in terms of mortality. Hence, the epidemiology of liver cancer is characterized by major geo-anthropological (or bio-geographical) variations due to widespread differences in the distribution of major risk factors and potentially to variations of patient genome architecture ie susceptibility. This complex situation implies that this tumour must be analyzed and understood through the specificities of local contexts and that it is difficult to consider *a priori* that a single clinical-biological model of liver cancer is valid for all populations on the planet [3].

In French-speaking sub-Saharan Africa, liver cancer ranks 3rd in terms of frequency after cervical and breast cancer, with a rate of 10.3% [4].

In Ivory Coast, according to the last version of Globocan [5], liver cancer had an incidence of 10.1/1.0E+05 inhabitants in men and 5.5/1.0E+05 inhabitants in women with a mortality rate of 9.6/1.0E+05 inhabitants and 5.3/1.0E+05 inhabitants respectively. The proximity of these figures (mortality represents 95-96% of incidence) indicates that the prognosis of PLC remains particularly dismal in CI. This situation is partly due to the fact that its diagnosis in routine practice is hampered by a lack of sensitive biomarkers and a lack of knowledge of the associated risk factors by the populations concerned. In CI, PLC is therefore generally diagnosed at a very late stage when palliative medicine is the only reasonable treatment [6].

The aim of the current study is to explore the potential of cfDNA as a novel biomarker (*and associated risk factors*) that could be useful in the management of patients at risk to develop a PLC in Côte d'Ivoire.

2. Material and methods

This is a prospective, experimental study that began on 30 January 2020 at the Institut Pasteur of Ivory Coast (IPCI) and involved patients who came for biological investigations at IPCI in whom alpha fetoprotein (AFP) assay was requested. Patients were informed of the advantages and disadvantages of the study before signing an informed consent form. Sociodemographic, clinical, para-clinical and epidemiological data were collected in a document coded to ensure confidentiality. A venous blood sample was taken from the elbow for biological analysis. Centrifugation was performed at 5,000 rpm for 10 minutes. 2 aliquots of 500 µL of plasma and 500 µL of serum were made for each patient included. Sera were stored at -20 °C for serological and biochemical analyses. Plasma was stored in liquid nitrogen or in a freezer at -80°C for molecular analyses. Serological analyses were carried out using ARCHITECT Plus i1000 SR (ABBOTT) and COBAS 6000 (ROCHE) for HBsAg.

Biochemical analyses involved the determination of AFP using the COBAS 6000 and other parameters (ASAT, ALAT, PAL, BILT, GGT, U, C, ALB) using the COBAS C311 (ROCHE). The molecular analysis was carried out in three stages: extraction, quantification and qualification of the cfDNA. For the cfDNA extraction, the method of Zhi *et al*. [7] modified by Marchio *et al*.[8], which is commonly used and adapted to different contexts was used. This took place over three days. On the third day, the resulting cfDNA pellet was dried in a Thermo mixer, then resuspended with elution buffer and left at 4°C for one minute.

The quantity and quality of the cfDNA was assessed using the "NANODROP Thermoscientific" automated system based on a complete absorbance spectrum for micro-volume measurement and analysis. Any cfDNA extract with a 260/280 yield ratio of between 1.8 and 2 and a 260/230 yield ratio of between 1.8 and 2.2 is considered pure. Outside these margins the cfDNA is considered impure. These samples were re-extracted once by a phenol-chloroform, precipitated, rinsed with ice-cold 70% ethanol and quantified again.

Data were collected in Excel and analyzed using Graph Pad Prism 5 software. Variables were compared using Chi2 or Fisher exact tests at a significance level of α=5%.

3. Results

3.1. Socio-demographic and Clinical data (Table1)

A total of 142 patients with an average age of 45 were included in the study. The sex ratio was 1.58. The study population was dominated by self-employed workers (64.78%) and salaried workers (35.22%). They lived mainly in Abidjan (41.54%). The Akan people predominated with 34.23%, followed by the Krou (25.35%). More than 50% of this population had the same eating habits, namely drinking tap water, rice, cassava, plantain, millet, maize and yam. 18%

of our population drank alcohol regularly, while 60% drank occasionally. A large majority (70.62%) was referred by gastroenterologists proceeding to the routine surveillance of patients with chronic liver disease (55.24%). Clinically, the main causes of consultation were asthenia (38%) and pain in the right upper quadrant of the abdomen (27%). Another significant subset reported to be either currently (12%) or regularly in the recent past affected by fever (20%).

A subset of 23.9% had a history of familial hepatitis and 15.4% reported cases of cancer in close family members (father, mother, brother, sister, aunt, uncle, cousins). A small proportion reported to have been previously vaccinated for hepatitis B (16.1%, n=23). Thirteen (56%) of these previously immunized patients were nevertheless carriers of HBsAg.

85.2% of our patients were referred for liver disease, compared with 14.8% suffering from extra-hepatic conditions. Concerning nosocomial infectious risk factors, 47% received dental, 28% had surgical antecedents, and 21% received blood transfusion. Among esthetic and traditional risk factors of infection, 38% of the patients were either circumcised for the men or excised for women and 16% had a piercing.

Exposition to toxic substances known to be truly or potentially deleterious for liver health (alcohol, tobacco, herbal remedies, pesticides) was mild in this population, ranging from 7 % (alcohol) to 19% (herbal remedies).

Table 1 Distribution and frequency of appearance among the 142 patients of socio-demographic, cultural and clinical variables

Parameters studied		**N**	**Frequency (%)**
Male		87	61.26
Female		55	38.74
Occupation	Private employee	92	64.78
	Employee	50	35.22
Place of residence	Abidjan	59	41.54
	Outside Abidjan	83	58.46
Ethnic group	Akan	49	34.23
	Krou	36	25.35
	Gours	30	21.12
	Mandé	10	7.04
	Etranger	17	11.97
Tobacco consumption		33	23.23
Alcohol consumption	regular	25	18
	occasional	85	60
Source	Gastroenterology	100	70.62
	External	42	29.4
Reason for consultation	Asthenia	54	38
	Abdominal pain	38	27
	Fever	28	20
	Other	17	12
Family notion	hepatitis	34	23.9
	cancer	22	15.4
Hepatopathy		121	85.2
Extra-hepatic pathology		21	14.8

Medical history	Dental treatment	66	47
	Surgery	40	28
	Blood transfusion	30	21
Circumcision/Excision		54	38
Tattoo		23	16
Traditional treatment		27	19

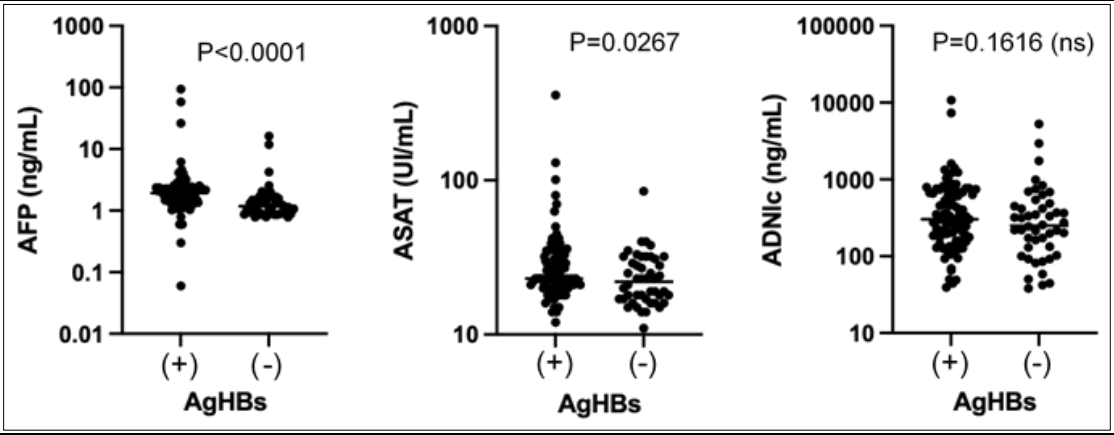
3.2. Biological data

Biologically, 66.66% of the study population were carriers of chronic viral hepatitis B. There were 4 cases of cirrhosis (2.81%) and 1 case of cancer (0.70%). Liver markers were slightly elevated in the cirrhotic patients and much more severe in the cancer patients.

A total of 284 extractions were performed with 2 extractions per plasma. The concentration of cfDNA ranged from 38.14 ng/ml to 10763.12 ng/ml with a mean of 571.02 ng/ml. 47.88% (68/142) of our cfDNA extracts were pure; 15.49% (22/142) contained phenol as an impurity and 36.61% (52/142) contained protein as an impurity after the first extraction.

3.3. Correlation between sociodemographic and biological data.

Increased concentrations of liver markers such as AFP and transaminases were linked to the presence of HBsAg (P<0.001; P=0.02 respectively) (fig.1A; 1B). On the other hand, the increase in cfDNA concentration did not depend solely on the presence of HBsAg (P=0.16) (fig.1C), but rather was strongly linked to the presence of HBsAg and other associated factors such as the notion of a family cancer (P=0.03) (fig.2), alcohol consumption (p=0.04) and smoking (p=0.02). The concentration of cfDNA did not depend on the value of AFP (p=0.45), indicating that cfDNA and AFP are 2 independent markers in the diagnosis of liver cancer. HbsAg carriage was related to occupation, depending on whether the individual worked in a professional sector or was self-employed (p=0.03), and to place and type of residence (p=0.03) (slum dwellers were more exposed than those in well-off neighborhoods). The study showed that the quality of cfDNA was linked to the presence of HBsAg (p=0.006) but did not depend on the value of AFP (p=0.45).



1A : [AFP]/ HbsAg	1B : [ASAT]/ HbsAg	1C : [ADNLc]/ HbsAg

Figure 1 Concentration of AFP, ASAT and ctDNA as a function of HBsAg

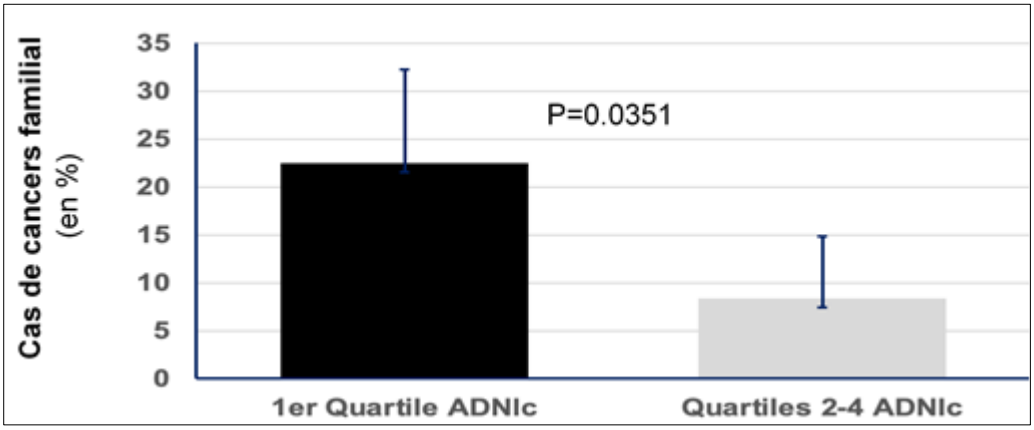


Figure 2 Concentration of ctDNA in relation to the notion of familial cancer

3.4. Correlation between clinical and biological data

In terms of clinical data, HBsAg carriage was linked to medical history such as blood transfusion (p=0.06), surgery (0.03) and dental treatment (0.02). On the other hand, these various factors were not linked to cfDNA concentration (p=0.58, p=0.22, p=0.20 respectively).

4. Discussion

4.1. Socio-demographic data

Our study showed a male predominance, as did that of Bekraoui [9] at the Hassan II University Hospital in Fez, Egypt, with a sex ratio of 1.5. Alcohol consumption and lifestyle are important risk factors in the development of PLC in our study, as in the studies by Wan-Shui Yang et al [10] in China and Mc Glyn [11]. In our study, the carriage of HBsAg was linked to occupation, with greater exposure among people working in the private sector. This finding was consistent with that of Mebarki et al [2]. This could be explained by the fact that employees have health cover (health insurance, mutual insurance company, etc.) which provides them with better medical monitoring.

4.2. Clinical data

The results obtained in our study from the clinical data indicate that blood transfusion, surgical operations and dental care could be risk factors in the contraction of the hepatitis B virus and the main risk factor for hepatocellular carcinoma. This assertion is in line with that of Noah et al in Cameroon [12], who also proved in their study that the blood route, in particular through the use of needles for personal and collective use, and uncontrolled transfusions would be risk factors associated with cancer.

4.3. Biological data

In our study, HBsAg positivity is linked to AFP levels, as indicated in the study by Chunfeng Qu [13], who went further and indicated that AFP could be recommended in the early detection of PLC in chronic carriers of viral hepatitis B. Chronic infection with the hepatitis B virus would contribute to a high risk of developing hepatocellular cancer in our study, as stated in the study by Fanny Lebossé and Fabien Zoulim [14].

As for cfDNA, although it has been identified as a predictive marker in several cancers [15], its analysis comes up against several obstacles. From the pre-analysis phase onwards, the volume of plasma, the storage temperature, the interval between blood sampling and plasma isolation, the centrifugation protocol and the purification methods are all parameters that influence the results [16].

The lack of comparison and standardization of the different methods is also an obstacle. This could explain the fact that its concentration was not linked to liver status in our study, unlike in many other studies. However, the correlation of cfDNA concentration with familial cancer and alcohol consumption has been confirmed by several studies, such as that by Wan-Shui Yang et al. [10]

5. Conclusion

In conclusion, we would say that viral hepatitis B associated with the notion of familial cancer remains the most predominant risk factor that could lead to primary liver cancer. Testing for cfDNA and monitoring the dietary and lifestyle habits of chronic carriers of viral hepatitis B could be one of the keys to preventing liver cancer in these patients. However, given the difficulties involved in obtaining quality cfDNA, insistence on the cfDNA extraction method and mutation testing could prove vital in the fight against cancer through prevention.

Compliance with ethical standards

Disclosure of conflict of interest

All authors of the manuscript have no conflict of interests to declare.

Statement of ethical approval

The study was conducted in accordance with international ethical regulations for biomedical research involving human subjects. Information and consent forms were read, approved, and signed by each study participant. It received approval from the National Ethics Committee for Health and Life Sciences (Ref n°173-21/MSHP/CNESVS-kp)

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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