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Original Research Article

Assessment of alpha-fetoprotein clinical performances in the diagnosis of hepatocellular carcinoma at Sominé DOLO Hospital of Mopti

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ABSTRACT

Background: Serum AFP has a poor clinical performance values especially when it comes to dealing with the early and AFP-negative diagnostic of HCC. This study aimed to assess the contribution of AFP in the diagnosis of HCC.

Materials and Methods: A total of 95 subjects were in a prospective observational study by consecutive enrolment and divided into two groups. The first group was made up with subjects in whom the diagnosis of HCC had been retained the second was the control group which was free of HCC. AFP levels were performed by electrochemiluminescence immunoassay on the cobas e411®. Data were captured in Excel and analyzed by Ri386 version 4.1.2 binary for macOS 10.13.

Results: Log of AFP median of AFP in HCC subjects was significantly greater than in non HCC subjects 6.91 ng/mL versus 1.43 ng/mL, Wilcoxon *p-value* < 0.001. At the cut-off of 200 ng/mL, the clinical performances showed an acceptable sensitivity 97.1% CI 95% [93.7 – 100] but a poor specificity 73,8% CI 95% [64.9 – 82.6] and out of the 34 cases of HCC, one case (2.9%) was AFP-negative HCC.

Conclusion: Our data show an acceptable sensitivity but a weak specificity of AFP as a biomarker for HCC at a cut-off 200 ng/mL. This suggests that AFP should be used with other biomarkers, mainly for the early and AFP-negative HCC diagnosis.

1. Introduction

Cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020.[1] It is estimated that, by 2025, more than one million individuals will have liver cancer annually.[2] The incidence and mortality of liver cancer cases in Africa, according to the latest Global Cancer Statistics (GLOBOCAN 2020), represent 7.8 and 8.1% of the global cases, respectively.[3] In terms of the most common causes of cancer death in 2020, liver cancer ranks third with 830,000 deaths yearly. Hepatocellular carcinoma (HCC) is the most common liver cancer accounting for 75 to 85%.[4] In Mali, an old study reported 9.6% of HCC proportion among hospitalized patients.[5] The diagnosis of HCC is most often made very late, and the disease has a very high lethality in its terminal phase. Thus, early screening and monitoring are the significant policies allowing potentially curative therapies

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and a good prognosis.[6,7] The positive diagnosis of HCC is based on non-invasive criteria, including clinical, abdominal ultrasound, and biomarkers.[8] Alpha-fetoprotein (AFP), a glycoprotein of relative molar mass 67 kDa present during fetal life, which gradually decreases during the development process to reach a blood level < 9.8 IU/mL in adults.[9] In contrast, during the HCC pathogenesis process, AFP increases in 75% of the cases, reaching a serum level > 350 IU/mL, sometimes up to 7000 IU/mL.[10] Thus, the HCC is accurately diagnosed when we face a significant increase in serum AFP levels and subsequent ultrasound imaging features. However, the sensitivity and specificity of AFP for HCC diagnosis are only 68.8% and 87.6%, respectively.[11] Moreover, some HCCs are AFP-negative, and therefore difficult to diagnose, especially since most of them are small tumors which also offer little chance for imaging to be practical.[12] This study aimed to highlight the AFP profile in HCC patients and discuss the perspective markers of this disease.

2. Materials and Methods

This was a prospective observational study by consecutive enrollment from January to December 2020 at Sominé DOLO hospital of Mopti. The diagnosis of HCC was evoked in subjects using clinical symptoms plus liver ultrasound and histological examination of nodule fragments obtained by ultrasound-guided sampling.

2.1. Study design

Subjects who were examined in the Department of medicine, stiff, painful hepatomegaly, pain in the right hypochondria, and the presence of a hepatic nodule of more than two cm were enrolled by consecutive recruitment in this study and divided into two groups. The first group was composed of the subjects in whom the diagnosis of HCC had been retained while the second was the control group which was free of HCC. The differential diagnosis between HCC and the other hepatopathies were carried out by extensive assessments such as ultrasonography, biological, and anatomopathological assessments. The subjects suffering from other pathologies or hepatopathies, not HCC and who refused to give their informed consent were not included in this study. In each group of subjects, sociodemographic data, clinical data, and AFP serum levels were collected. AFP serum levels of 200 ng/mL and 400 ng/mL were used as the reference of the cut-off to assess the clinical performance of AFP testing.

2.2. Physical and clinical examination

The subjects underwent a complete clinical examination which aimed to search: hepatomegaly and its characteristics, pain in the right hypochondria, jaundice (current or old), the onset of the disease, duration, body mass index, the exposure to a risk factor: intravenous drug addiction, alcohol, tobacco, known viral hepatitis, a notion of viral hepatitis or jaundice in the family, and metabolic disorders.

2.3. Ultrasonography

Mindray® DC-70(Shenzhen, China) ultrasound machine was used for the abdominal ultrasound to check hepatic injuries and the associated symptoms of chronic liver disease like ascites, splenomegaly, jaundice, and collateral venous circulation.

2.3.1. Biological examinations

The cobas e 411® analyzer Roche Diagnostic (CH-4070 Basel, Switzerland). The cobas e 411 analyzer is a fully automated analyzer that uses patented ElectroChemiLuminescence (ECL) technology for immunoassay analysis. It is designed for both quantitative and qualitative in vitro assay determinations for a broad range of applications (including anemia; bone, cardiac and tumor markers; critical care; fertility/hormones; maternal care; and infectious diseases). The analyzer is available as a rack or disk sample handling system.

2.3.2. Sample collection and preparation

Whole blood was collected on standard serum collection tubes or containing a separating gel. The tube was gently inverted the tube 8-10 times, and sited in an upright position for at least 10 minutes at room-temperature to allow the blood to clot. Then, the tube was labeled with the appropriate study code. No identifying information of the subjects without ID numbers was included on the aliquot label. After the blood clotting, the tubes were spined at 1500 G using a standard room temperature centrifuge for 15 minutes. The primary tubes were used directly on the cobas e411® analyzer Roche Diagnostic (CH-4070 Basel, Switzerland) for the analysis. After the analysis, the remaining serum was recovered with serum transfer Eppendorf Research® pipette volume 100-1000 μL (22339 Hamburg, Germany) while placing two ml in a labeled cryovial tubes and then froze serum at -20°C until further analysis.

2.3.3. Precautions and warnings

The usual precautions were observed for handling the samples in the laboratory in terms of biosafety and biosecurity. Disposal of all waste was carried out per the legal provisions for managing biomedical waste.

2.3.4. Reagents preparation

The reagents in the kit Elecsys$^{\circ}$ AFP II Gen (100 tests) assay, # Ref. Code: 04481798190, Roche Diagnostic (CH-4070 Basel, Switzerland) were ready to use and cannot be used separately. All the information necessary for the proper

conduct of the test were stored on the bar code of the reagent bottles and have been entered by scanning the bar codes.

2.3.5. Principle of the Elecsys® AFP II Gen (100 tests) assay[13]

It is based on the "sandwich" method and uses the ElectroChemiLuminescence (ECL) technology for detection. The total duration of the analytical cycle: is 18 minutes and includes two incubation phases:

1^{st} incubation: 10 μL of the sample was placed in the presence of a specific biotinylated anti-AFP II Gen monoclonal antibody and a specific anti-AFP monoclonal antibody labeled with ruthenium (Ru(bpy): Tris(2,2'-bipyridyl) ruthenium(II)). A "sandwich" forms.

2^{nd} incubation: the microparticles coated with streptavidin are added to the reaction cuvette. The immune complex is attached to the solid phase by a streptavidin-biotin bond.

The reaction mixture is drawn into the measuring cell where a magnet holds the microparticles at the electrode's surface. The elimination of the free fraction is carried out by the passage of ProCell or ProCell M. A potential difference applied to the electrode triggers the production of luminescence which is measured by a photomultiplicator.

The results are obtained using a calibration curve. This is generated specifically for the analyzer used by a 2-point calibration and a reference curve stored in the bar code label or the reagent bar code.

Reagents - composition and concentrations. The reagent rackpack is labeled AFP.

-M: Streptavidin coated microparticles (transparent cap), one vial containing 6.5 mL: streptavidin coated microparticles $0.72.10^6$ ng/mL, preservative,

-R1: anti-AFP-biotin Ab (grey cap), one vial containing 10 mL: biotinylated anti-AFP (mouse monoclonal) antibody $4.5.10^3$ ng/mL; 100 mmol/L phosphate buffer, pH 6.0; conservative,

-R2: anti-AFP-Ru(bpy) antibody (black cap), one vial containing 10 mL: anti-AFP antibody (mouse monoclonal) labeled with ruthenium $12.0.10^3$ ng/mL; 100 mmol/L phosphate buffer, pH 6.0; conservative.

2.3.5.1. Calibration traceability. The method has been standardized against the first international preparation: 1^{st} IRP WHO reference 72/225. The Elecsys reagent bar code contains all the information needed to calibrate the batch. The reference curve is adapted to the analyzer using the respective CalSet. Calibration frequency: perform a batch calibration using a fresh reagent (that has been stored for a maximum of 24 hours on the analyser). Calibration frequency may be reduced after acceptable calibration verification by the laboratory.

The new calibration is recommended:

1. After one month (28 days) for the same batch of reagents,
2. After seven days for the same reagent box remaining on the analyzer, if necessary: e.g. if the quality control results fall outside the defined confidence limits.

2.3.5.2. Quality control. Use PreciControl Tumor Marker or PreciControl Universal.

Other appropriate controls may also be used.

It is recommended to perform a simple control assay at least once every 24 hours during a routine, for each new kit, and after each calibration.

The frequency of controls and the confidence limits must be adapted to the requirements of the laboratory. The results should fall within the defined confidence limits. Each laboratory should establish the procedure to follow if the results fall outside the defined limits.

If necessary, repeat an analysis of the samples concerned.

Comply with applicable local quality control regulations and guidelines.

2.3.5.3. Calculation of results. The analyzer automatically calculates the analyte concentration of each sample. The results are expressed optionally in ng/mL on the cobas e411 analysers.

2.3.6. HBsAg assay

Principle of HBsAg assay by chemiluminescence (sandwich method)

The total duration of the analytical cycle: is 18 minutes

1^{st} incubation: 50 μL of the sample are placed in the presence of two biotinylated anti-HBsAg monoclonal antibodies and a mixture containing an anti-HBsAg monoclonal antibody and anti-HBsAg polyclonal antibodies labeled with ruthenium (Ru(bpy): Tris(2, 2'-bipyridyl)ruthenium (II)) A "sandwich" is formed.

2^{nd} incubation: the microparticles coated with streptavidin are added to the reaction cuvette. The immune complex is attached to the solid phase by a streptavidin-biotin bond.

The reaction mixture is transferred into the measuring cell, and a magnet holds the microparticles at the level of the electrode. The elimination of the free fraction is carried out by the passage of ProCell or ProCell M. A potential difference applied to the electrode triggers the production of luminescence, which is measured by a photomultiplicator.

The software automatically determines the results by comparing the electro chemiluminescent signal generated by the reaction with the threshold value obtained during calibration.

2.3.7. Anti-HCV Ac assay

Principle of the assay of anti-HCV Ab by chemiluminescence (sandwich method)

The total duration of the analytical cycle: is18 minutes

1^{st} incubation: 30 μL of the sample, a reagent containing HCV-specific antigens labeled with biotin, and a reagent containing HCV-specific antigens labeled with ruthenium (Ru(bpy): Tris(2,2'-bipyridyl) ruthenium (II)) react to form a "sandwich".

2^{nd} incubation: the microparticles coated with streptavidin are added to the reaction cuvette. The immune complex is attached to the solid phase by a streptavidin-biotin bond.

The reaction mixture is drawn into the measurement cell where a magnet holds the microparticles at the electrode's surface. The elimination of the free fraction is carried out by the passage of ProCell II M. A potential difference applied to the electrode triggers the production of luminescence which is measured by a photomultiplicator.

The software automatically determines the results by comparing the electrochemiluminescent signal generated by the reaction with the threshold value obtained during calibration.

2.3.8. Data management and analysis

Data were captured in Excel and analyzed with the statistical software Ri386 version 4.1.2 binary for macOS 10.13. Pearson's chi-square or Fisher's exact test was used for the comparison of the proportions of qualitative data. Quantitative data normality were verified by using the Shapiro-Wilk test, the Wilcoxon Mann-Whitney test carried out comparison. We used AFP 200 ng/mL and 400 ng/mL as a cut-off for the positive diagnosis of HCC. The BioProbabillity R package and the cut-off of AFP II Gen 200 ng/mL and 400 ng/mL were used to access of the clinical performance estimation and their 95% confidence interval. The threshold for statistical significance was set up at $p \leq 0.05$.

2.3.9. Compliance with ethical standards

The study protocol was reviewed by Sominé DOLO hospital institutional ethical committee for its conformity to the declaration of Helsinki, and ethical clearance was obtained from the same institution. Each participant gave fully informed written consent prior to their enrollment. In particular, they were informed of the anonymous nature of the study and that the data concerning them, will only be used for the sole purpose of scientific production while guaranteeing their confidentiality by removing all subject's identifiers.

3. Results

A total of 95 subjects were enrolled in this study. Out of these, 34 cases were HCC 35.8%. HCC mainly affects men in our series, with a sex ratio 16 (Figure 1). The medians of age were 51.5 years IQR = [42.2-59.0] and 48.0 years IQR [39-60] for subjects with HCC and those without HCC, respectively. The age varied between 32 and 75 years for the HCC subjects and from 14 to 74 for the non-HCC subjects. The difference in median age between HCC subjects and non-HCC subjects was insignificant, with Wilcoxon p-value = 0.37. (Figure 2). Overall, the median AFP blood level was 592.1 ng/mL with the interquartile range (IQR) = [0.6 – 1210.0]. For the non-HCC subjects, the median AFP blood level was 5.0 ng/mL with the IQR = [0.6 – 592.0]. For HCC subjects, the median was 1210.0 ng/mL with IQR = [90.1 – 1210.0]. The Log of the median AFP in HCC subjects was significantly greater than in non-HCC subjects 6.91 ng/mL versus 1.43 ng/mL, with Wilcoxon p-value < 0.001. (Figure 3). At serum AFP levels > 200 ng/mL, the proportion of HCC was significantly greater than that of non-HCC (97.1% versus 26.2%; $p < 0.001$). In contrast, at serum AFP levels ≤ 200 ng/mL, the proportion of HCC was significantly less than that of non-HCC (2.9% versus 73.8%; $p < 0.001$).

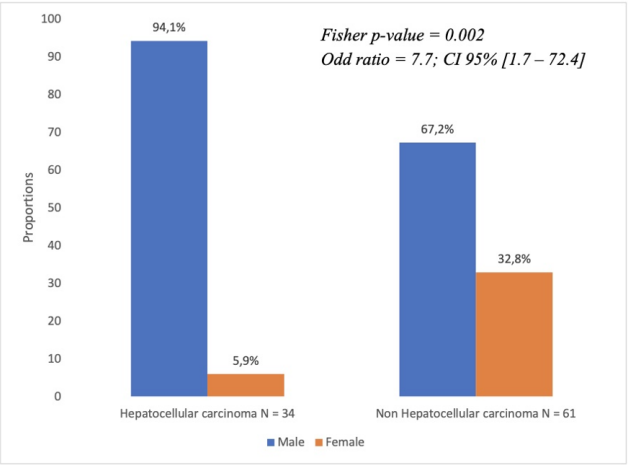

Fig. 1: Distribution of hepatocellular carcinoma in subjectsby sex. Theproportion of HCC was more significant in males compared to females, p-value =0.002, Odd ratio = 7.7 CI 95% [1.7- 72.4]

At a cut-off = 200 ng/mL, the clinical performance showed an acceptable sensitivity of 97.1% CI 95% [93.7 – 100] but poor specificity of 73.8% CI 95% [64.9 – 82.6]. (Table 2). The HCC proportion was significantly higher in the group with AFP serum levels > 400 ng/mL than AFP serum levels ≤ 400 ng/mL (94.1% versus 5.9%), Fisher p-value < 0.001; but this proportion has decreased compared to a cut-off of 200 ng/mL. In contrast, the proportion of non-HCC was significantly less in AFP serum levels > 200 ng/mL than in AFP serum levels ≤ 200 ng/mL (26.2% versus 73.8%). (Table 3). At the cut-off of 400 ng/mL, the clinical performances decreased in sensitivity: 94.1% CI 95% [89.4 – 98.8], but the specificity was constant at 73.8 CI [64.9 – 82.6]. (Table 1).

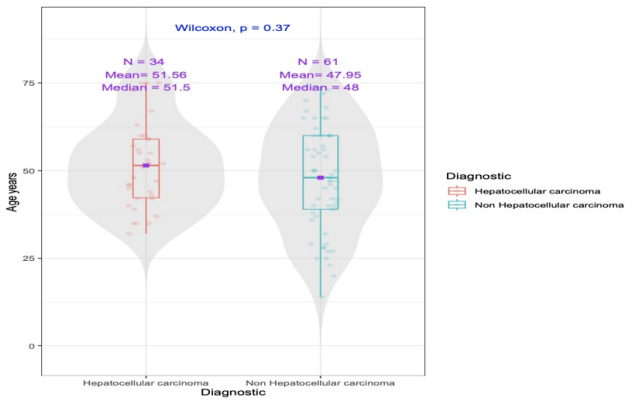

Fig. 2: Comparison of age between HCC subjects and non-HCC subjects

The median age were 51.5 years IQR [42.2-59.0] and 48.0 years IQR[39-60] for subjects with HCC and those without HCC, respectively. The age varied between 32 and 75 years for the HCC subjects and from 14 to 74 for the non-HCC subjects. The difference in mean age between HCC subjects and non-HCC subjects was not significant, Wilcoxon p-value =0.37

Table 1: Comparison of AFP profile between subjects with HCC and non-HCC when the cut of AFP was set at 200 ng/mL

Class AFP	HCC N(%)	Non HCC N(%)	Total N(%)
AFP >200ng/mL	33(97.1)	16(26.2)	49(51.6)
AFP < 200ng/mL	1 (2.9)	45 (73.8)	46(48.4)
Total	34 (100)	61 (100)	95(100)

The HCC proportion was significantly greater in AFP blood level > 200 ng/mL group than AFP ≤200 ng/mL (97.1% versus 2.9%), Fisher P-value < 0.001. In contrast, the proportion of non HCC was significantly less in AFP blood Level >200ng/mL than AFP blood level ≤ 200ng/mL (26.2% versus 73.8%), Fisher P- value < 0.001

Table 2: Diagnostic performance of AFP cut-off = 200 ng/mL

Clinical performance	E-Estimation	CI 95%
Sensitivity	97.1	93.7- 100
Specificity	73.8	64.9 - 82.6
Positive predictive Value	67.3	57.9 - 76.7
Negative predictive Value	97.8	94.8 - 100
Relative risk	31.0	6.0 - 158.9
Diagnostic O dd Ratio	92.8	11.7 - 735.2

At a cut- off = 200ng/mL, the clinical performances showed an acceptable sensitivity 97.1% CI 95% [93.7- 100] but a poor specificity 73,8% CI 95% [64.9 – 82.6]

Table 3: Comparison of AFP profile between subjects with HCC and non HCC when the cut of AFP was set at 400 ng/mL

Cut-off AFP II Gen	HCC N (%)	Non HCC N(%)	Total N(%)
AFP > 400 ng/mL	32 (94.1)	16 (26.2)	48 (50.5)
AFP ≤ 400 ng/mL	2 (5.9)	45 (73.8)	47 (49.5)
Total	34 (100)	61 (100)	95 (100)

The HCC proportion was significantly higher in AFP blood level > 400 ng/mL. group than AFP ≤ 400ng/mL. (94.1% versus 5.9%), fisher P- value < 0.001; but this proportion have decrease compared to a cut -off of 200 ng/mL. In contrast, the proportion of non HCC was significantly less in AFP blood level > 200 ng/mL than AFP blood level ≤ 200ng/mL. (26.2% versus 73.8%)

Table 4: Diagnostic performance of AFP cut – off = 400ng/mL

Clinical performance	Estimation	CI 95%
Sensitivity	94.1	89.4 – 98.9
Specificity	73.8	64.9 – 82.6
Positive predictive Value	66.7	57.2 – 76.2
Negative predictive Value	95.7	91.6 – 99.8
Relative risk	15.7	4.9 – 49.5
Diagnostic Odd Ratio	45.0	9.7 – 209.5

At the cut – off of 400ng/mL, the clinical performance decrease interm of sensitivity: 94.1% CI 95% [89.4 – 98.8], but the specificity was constant 73.8 CI [64.9 – 82.6]

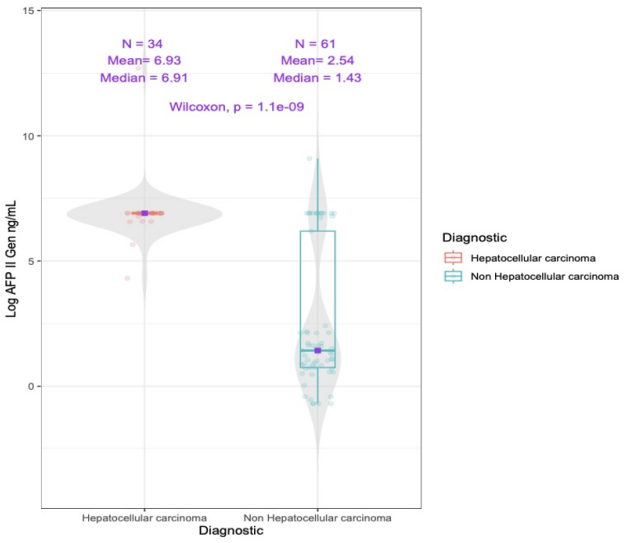

Fig. 3: Comparison of AFP II gen log of the median in serum

Overall, the median AFP blood level was 592.1 ng/mL with theinterquartile range (IQR) = [0.6 – 1210.0]. For the non-HCC subjects, the median AFP blood level was 5.0 ng/mL with the IQR = [0.6-592.0]. For HCC subjects, the median was 1210.0 ng/mL with IQR = [90.1-1210.0]. The Log median of AFP in HCC subjects was significantly greater than in non HCC subjects 6.91 ng/mL versus 1.43 ng/mL, Wilconxon p-value <0.001

4. Discussion

The contribution of alpha-fetoprotein (AFP) in the diagnosis of hepatocellular carcinoma (HCC) still feeds controversial debates, especially about its ability to highlight the diagnosis at the infra-clinical stage. We conducted a prospective consecutive recruitment studies in order to assess AFP's clinical performance in the diagnosis of hepatocellular carcinoma. Chronic infection with hepatitis B and C viruses are risk factors for HCC.[14] In our series, the hepatitis B virus surface antigen was found in 55.88% of HCC cases. The hepatitis C virus was found in 26.47% of cases. This virus also plays a role in liver carcinogenesis in our context. Dicko MY et al. found 63.8% and 33.8% of HCC cases carrying hepatitis B and hepatitis C viruses, respectively.[15] In our series, 26.47 of HCC patients had been transfused compared to 17.5% reported by Noah D et al.[16]

The infectious role of the hepatitis B virus has been demonstrated in liver carcinogenesis. In HCC cases co-infected with HBV and HCV, AFP positivity was 85.7%. In HBV-associated HCC alone, positivity was 62.9%, and 54.5% of AFB1-positive HCC cases showed AFP positivity. In HBV and HCV-negative HCC cases, the positivity was 20.5%, and in HCV-associated HCC cases, it was 17.6%. The HBV/HCV co-infected group and HCC cases positive for HBV alone had significantly elevated levels of AFP.[17] Multivariate analysis showed that positive hepatitis B surface antigen (HBsAg) status and bilobar tumor involvement were the independent factors in predicting elevated AFP values. However, it cannot be considered a sensitive tumor marker, especially during the early stages in HBsAg-negative patients.[18]

AFP is a glycoprotein present during fetal life, but will usually disappear in adults (usually < 17 ng/mL). The AFP serum level is linked to the regeneration of hepatocytes. It increases in 75% of liver cancer cases, reaching concentrations > 605 ng/mL, sometimes up to 12,100 ng/mL. AFP is the reference marker used in association with ultrasound. Although it lacks sensitivity and specificity, it is more beneficial for the monitoring of HCC treatments than in screening for primary tumors and these values are related to tumor growth.[10,19] The distribution of AFP level serum did not follow a normal distribution in our series, Shapiro-test, $p < 0.001$. The median and the interquartile range (IQR) were 592.1 ng/mL [3.5-1,210]. There was a significant difference between HCC subjects and non-HCC subjects in terms of the median of AFP level in the serum: 1,210 versus 5.1; $p < 0.001$. At serum AFP levels of > 200 ng/mL, the proportion of HCC was significantly greater than that of non-HCC (97.1% versus 26.2%; $p < 0.001$). In contrast, at serum AFP levels ≤ 200 ng/mL, the proportion of HCC was significantly less than that of non-HCC (2.9% versus 73.8%; $p < 0.001$). Ma Wj et al., reported a lower proportion of HCC (36.1%) at serum AFP levels > 339 ng/mL.[20] Police SMC et al., also reported a higher proportion of HCC (73%) at this interval of serum AFP levels than that of Ma Wj et al. The study from Tangkijvanich P et al., classified patients into group 1 considered as normal AFP (< 24 ng/mL), group 2 as moderately elevated AFP (24-483 ng/mL), and group 3 as markedly elevated AFP (≥ 484 ng/mL). Out of these groups, the authors reported 76 (24.6%), 78 (25.2%) and 155 (50.2%) patients in groups 1, 2, and 3, respectively.[18] Despite the controversial debate on AFP clinical performances in HCC diagnosis, it has been shown that the serum level of AFP was an independent risk factor for HCC grade (odds ratio (OR), 2.56; 95% confidence interval (CI), 2.07–3.16; $p < 0.001$), Tumor-Nodes-Metastasis-7 (TNM-7) stage (OR, 2.794; CI, 2.41–3.24; $p < 0.001$), and tumor size (OR, 1.748; 95% CI, 1.574–1.941; $p < 0.001$). Moreover, multivariate analysis with the Cox model showed that AFP serum level was an independent risk factor predicting the survival of HCC patients who underwent surgery (Hazard ratio (HR), 1.66; 95% CI, 1.53–1.80; $p < 0.001$), and those who had not undergone surgery (HR, 1.534; 95% CI, 1.348–1.745; $p < 0.001$).[21]

The sensitivity and specificity of AFP depend on the chosen cut-off value. Out of the 34 cases of HCC, at a cut-off of 200 ng/mL, in one case (2.94%) the AFP serum levels were lower and represented the case of AFP-negative HCC in our series. In contrast, out of the 61 non-HCC subjects, 16 (26,2%) had AFP serum levels > 200 ng/mL, which represented the false positive HCC proportion in our series. Globally at the cut-off of 200 ng/mL, the sensitivity and specificity of AFP were 97.1% and 73.8%, respectively and these clinical performances decreased when the cut-off was placed at 400 ng/mL. El-Husseini ME et al., have reported a lower sensitivity than ours, 68.2%.[22] In the study by Bruno D et al., using a threshold of 17 ng/mL in cirrhotic patients, reported a sensitivity of 60%, and the positive predictive value ranged from 9% to 50%, depending on HCC prevalence. However, the sensitivity and specificity were higher in hepatitis B carriers; 94.1% and 99.9%, respectively but the positive predictive value was only 5%.[8] The multitude of serum AFP cut-offs used for the diagnoses of HCC is due to its poor clinical performance. Thus, the research for other biological markers which can be used in association with the traditional marker or alone in the early diagnosis of HCC is necessary and it should occupy an essential place in ongoing research.

The mechanisms of carcinogenesis, which seem to depend on the etiologies and ethnicity of the subjects, could explain the absence of a sensitive and specific marker.[23] Currently, many studies have been conducted to find a reliable biomarker in the diagnosis of AFP-negative HCC.[24] In this vein, Dave L et al., have used a combined tool of AFP and the AFP-L3 fraction for the

diagnosis of HCC and have got a sensitivity and specificity of 56% and > 95%, respectively.[25] A logistic regression combining several hematological parameters (mean platelet volume, the volume of distribution of red blood cells, mean platelet volume-PC ratio, neutrophil-lymphocyte ratio, and platelet-lymphocyte count ratio) showed better diagnostic efficiency with an area under the ROC curve of 0.92, a sensitivity of 83.0% and a specificity of 93.1% in HCC-AFP-negative patients.[26] Moreover, nuclear ß-catenin positivity was frequently found more frequently among AFP-negative liver tumors.[27] Still to find more sensitive and specific biomarkers, other HCC biological markers have been used in clinical biology, these were the cases of des-gamma-carboxyprothrombin (DCP).[28,29] and alpha-L -fucosidase.[19] DCP is the incompletely carboxylated form of prothrombin. An immunoenzymatic measures it method, and its usual value is < 2 g/L. Its plasma concentration is increased in 2/3 of HCCs but is not correlated with that of AFP. Thus, it will be interesting the simultaneous determination of AFP and DCP in the diagnosis of HCC.[30] DCP pooled sensitivity, specificity, PLR, NLR, and DOR were 0.71 (95% CI: 0.59, 0.80), 0.93 (95% CI: 0.87, 0.96), 9.5 (95% CI: 5.2, 17.5), 0.32 (95% CI: 0.22, 0.46) and 30 (95% CI: 13, 72), respectively. The area under the ROC curve was 0.91 (95% CI: 0.88, 0.93). In the diagnoses of HCC in subjects with hepatitis B, DCP could be an ideal marker to take into account for surveillance purposes.[29] The area under ROC for AFP and Glypican-3 (GPC3) combined was 0.81 (95% CI 0.77-0.84) in the study by Xu C et al., They concluded that GPC3 is comparable to AFP as a serum marker for the diagnosis of HCC and that their combination may increase diagnostic sensitivity.[31] The pan-genomic, proteomic, and metabolomic studies have also showed importance in the research of new markers for HCC-AFP-negative. Beyond analysis by mass spectrometry coupled with liquid chromatography (MS-LC) or mass spectrometry assisted by laser ionization, desorption time of flight (MALDI-TOF), functional and molecular imaging represents an additional technology to identify potential biomarkers for HCC.[9] Indeed, the development of new radiotracers beyond 18F-Fluorodexoyglucose (FDG) for positron emission tomographic (PET) imaging could link the results of proteomic and metabolomic analyses to imaging and thus add useful information and be important for the diagnosis of HCC.[32]

Studies have shown that many circulating aberrantly expressed microRNAs can be used as a diagnostic tool for HCC. In this vein, a meta-analysis was undertaken by Peng C et al., to summarize the diagnostic accuracy of circulating miRNAs and have reported that the combination of miRNAs and AFP has excellent potential as a new strategy for diagnosing HCC.[33] From a clinical development point of view, among the biomarkers, some are in phase II clinical trials including DCP,[28] and others in phase III, including osteopontin,[34] Midkine[24] and GALAD.[35]

In terms of molecule perspective, AFP does not only act as a tumor marker, it is now known that AFP is not only a fetal form of carrier protein and a tumor marker. It is also involved critically in regulating several essential cellular functions, such as cell growth, differentiation, apoptosis, angiogenesis, and immune regulation and in this vein, it still keeps all attention for the research of biomarkers of certain diseases.[10]

Our study has limitations, such as the absence of computational tomodensitometriy scan (CT-Scan) and magnetic resonance imaging (MRI) examination, which was not available at the time of this study. CT-scan and MRI could improve the diagnosis quality of HCC mainly the early diagnostic at the infra-clinical stage and AFP-negative HCC.

5. Conclusion

Our data suggest a better sensitivity of serum AFP at a cut-off of 200 ng/ml than at 400 ng/mL as a diagnostic marker for HCC. However, the low specificity reported makes it necessary to combine the use of AFP with other biological markers, especially in cases of early diagnosis where the size of the tumor is not sensitive to ultrasonography. Also, the search for new markers retains all its importance in cases of AFP-negative HCC.

6. Source of Funding

None

7. Disclosure of Conflict of Interest

Authors certify that there is no actual or potential conflict of interest with this article.

8. Statement of Informed Consent

Each participant gave fully informed written consent prior to enrollment. The protocol was reviewed and approved by the Mopti Hospital ethical committee.

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