Alpha-Fetoprotein and the Early Diagnosis of Hepatocellular Carcinoma

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1. Abstract

Hepatocellular carcinoma is the most common primary malignant tumor of the liver. Cirrhosisis associated with its carcinogenesis, so periodic surveillance is necessary. Ultrasonography is currently the most appropriate test for screening hepatocellular carcinoma, and alpha-fetoprotein is the most used biomarker despite its low sensitivity. Given the discussion and lack of consolidated information on the topic, the importance of alpha-fetoprotein in the early diagnosis of hepatocellular carcinoma in a reference service in hepatology was assessed. An observational, cross-sectional, retrospective study was carried out, in which the population consisted of patients treated at a referral center for liver disease in north eastern Brazil, where 13,500 medical records were analyzed. After applying the inclusion and exclusion criteria, 42 medical record seligible for this study were selected. Of these patients, 54.8% had normal alpha-fetoprotein and 45.2% altered values. From ultrasounds, 66.7% had neoplastic characteristics,

while 33.3% were healthy. After a statistical analysis of the relationship between the levels of the biomarker and the

early diagnosis of hepatocellular carcinoma, a value of p=0.079 was found. It was concluded, therefore, that the alpha-fetoprotein dosage did not make a significant difference for the diagnosis of hepatocarcinoma in the analyzed sample. Regarding the sensitivity found for this biomarker and ultrasound, the findings were similar to those found in the literature, with alpha-fetoprotein below the predicted. Thus, the dosage of this biomarker alone is not indicated for screening hepatocellular carcinoma. The diagnosis must contain a serial ultrasound with or without the measurement of alpha-fetoprotein.

2. Keywords: Alpha-fetoprotein; Ultrasonography; Diagnostic imaging; Diagnosis; Hepatoma; Hepatocellular carcinoma.

3. Introduction

Hepatocellular Carcinoma (HCC) or hepatocarcinoma is the most common primary malignant tumor of the liver. It has its origin in the principal cells of this organ, hepatocytes, being the 5th most common cancer in the world, and the 2nd leading cause of death associated with the disease, with about 854,000 new cases and 810,000 deaths annually [1,2]. It has a wide world wide distribution, with about 85% of cases in developing and under developed countries, especially those with high

rates of hepatitis B virus (HBV) infection, regions in Asia, and sub-Saharan Africa [2]. In Brazil, the incidence of HCC is considered low. However, in recent years there has been an increasing focus on the hepatitis C virus (HCV), which is found in most Brazil in regions, one of the main risk factors [3,4]. Brazilian data demonstrate that this neoplasm is not among the most incident in the country, but in 2017 it was the sixth leading cause of death among men and the eighth among women [4-6]. The most important conditions for its carcinogenesis are chronic infection by hepatotropic viruses, hepatitis B and C, alcohol, and exposure to a flat oxin B [5]. Most of these risk factors lead to a standard route, liver cirrhosis, which is found in most patients with HCC [2]. Therefore, periodic surveillance and screening of liver disease patients, especially cirrhotic patients, is necessary, since the liver disease is the underlying cause of most cases of this neoplasm. The Ministry of Health (MS)/Brazil, the American Association for the Study of Liver Diseases (AASLD), and the European Association for the Study of the Liver (EASL) currently indicate ultrasound (USG) as a method of choice for screening hepatocarcinoma. It is widely available, non-invasive, does not emitioniz in gradiation, has a low risk, and an excellent cost-benefitratio, which must be carried out every sixmonths [4,6]. However, only the MS and AASLD recommend its performance associated or not with the measurement of alpha-fetoprotein (AFP) [6-8]. AFP is the most used biomarker for the early detection of HCC. There are several questions about the use of AFP due to its low sensitivity, with a result below the ideal, as this can lead to false-negative results [1, 9]. However, a randomized and an observational population-based study showed precisely the opposite, showing that AFP measurement can be useful mainly in some instances and in places where USG is not accessible [1]. When used for diagnosis, at 20ng/mL levels, its dosage has good sensitivity with low specificity. For levels greater than 200ng/mL, the situation is reversed, with low sensitivity, but high specificity [1-3]. Due to these factors and information conflicts, the isolated dosage is not recommended AFP [7-8]. Further more, the removal of this biomarker is still doubtful, due to the deficiencies and failures of the USG observed in the clinical routine, a fact also explained in other national studies that mention its limitation in Brazil [6,9]. Such imaging examination is a method that depends on the operator, knowledge clinical and technical, of the patient and the type and quality of the equipment used [10-12]. Besides, the diagnosis of HCC in cirrhotics is technically tricky, which makes it difficult to identify certain tumors. Thus, the performance in the early diagnosis of HCC depends on the relationship between experienced operator and equipment quality, demonstrating the need for qualified training of radiologists [6,7]. Besides, it was shown in a recent meta-analysis that the addition of AFP to USG improved the sensitivity in detecting hepatoma, thus demonstrating the usefulness of that marker [13]. It is recognized, therefore, that USG is the most appropriate test currently for screening HCC and that it can be associated or not with AFP measurement [1, 7-8]. However, given the world wide trend to withdraw the dosage of this marker, in addition to the frequent discussion on the subject and the lack of consolidated in formation on the topic, the present study evaluated the importance of AFP for the early diagnosis of HCC in a reference service in hepatology in north eastern Brazil. Besides, other relevant epidemiological in formation about these patients was found, such as mean age, presence of cirrhosis, and its causes.

4. Methods

An observational, cross-sectional and retrospective study was carried out, approved by the Ethics and Research Committee of Hospital Universitario Onofre Lopes (CEP-HUOL) on December 14, 2018, under CAAE 96620618.4.0000.5292/Plataforma Brazil/ Ministerio da Saude. The study consisted of patients seen at the Liver Study Center (LSC) of HUOL, located in Natal, capital of the State of Rio Grande do Norte - Brazil, which is a reference in Hepatology of the Unified Health System (SUS) in theState. A total of 13,500 medical records were analyzed, the first of which was dated October 1995 and the last of December 2018. It was a non-probabilistic sample collected for convenience. The included patients had HCC with a confirmed diagnosis. Patients who had illegible in accurate information recorded in medical records or lack of fundamental data for the study, such as performing USG and AFP dosing, were excluded. Only the AFP values and the USG results obtained during the investigation of HCC or at the first moment of diagnosis were considered to assess there levance of these tests for the early diagnosis of neoplasia. After analyzing the medical records and applying the inclusion criteria, 93 cases were initially selected, then, using the exclusion criteria, 42 remained. Information was collected from 4/4/2019 to 7/31/2019 and was carried out by two medical students, with a third in case of divergence between the inclusion or not of doubtful cases. Recommendations from the MS and EASL to consider patients with HCC were used. In which the diagnosis must be confirmed by radiological methods such as Computed Tomography (CT), Magnetic Resonance Imaging (MRI), ultrasound with contrastor biopsy, the latter being more used in patients without cirrhosis, or in case of diagnostic doubt. For imaging methods, those patients who had no dules with characteristic ssuggestive of HCC were taken into account, including arterial hyper vascularization and bleaching in the portal phase.The cutoff points that separated normality, both for AFP and USG, were also defined according to data from the MS and EASL. In there sults records, average AFP values upto 20ng/mL were con-

sidered. As AFP has a higher specificity for HCC in values above 200ng/mL, patients were divided between those with border line values of 20-200ng/mL and those above 200ng/mL. Regarding the USG information, the exams that presented liver nodules larger than 1cm with high pre-test valueor liver masses with a neoplastic aspect suggestive of HCC were considered altered. Data analysis was performed using descriptive statistics using Microsoft Excel (2016), with the calculation of percentage, average, median, and standard deviation. For the statistical analysis, the IBM /SPSS Statistics version 20 program was used, where the p-value was calculated using Pear son's chi-square test, $p \leq 0.05$ statistically significant, significance level of 95%.

5. Results

(Figure 1) shows the division of the sample according to AFP levels. Among the 42 patients analyzed, 23(54.8%) had normal levels, 8 had values between 20-200ng/mL, and 11 degrees above 200ng/mL, so 19(45.2%) patients had AFP values changed. The sensitivity found for the examination in these patients was approximately 45%. (Figure 2) shows the relationship between the USG and AFP levels. Of the total analyzed, 28(66.7%) cases had USG with characteristics suggestive of HCC, and 14(33.3%) were standard or had no changes that indicated malignant neoplasia. The sensitivity found for such imaging exams among the cases was approximately 66%. Among the USG suggestive of neoplasia, 18 had normal AFP; two were between 20-200ng/mL and eight values above 200ng/mL. Therefore, 10(37.7%) patients out of 28 had values above normal. Among the cases with USG without alterations suggestive of neoplasia, 5 had normal AFP, six between 20-200ng/mL, and three levels above 200ng/mL. Thus, 9(64.3%) ofthe 14 cases had AFP altered.

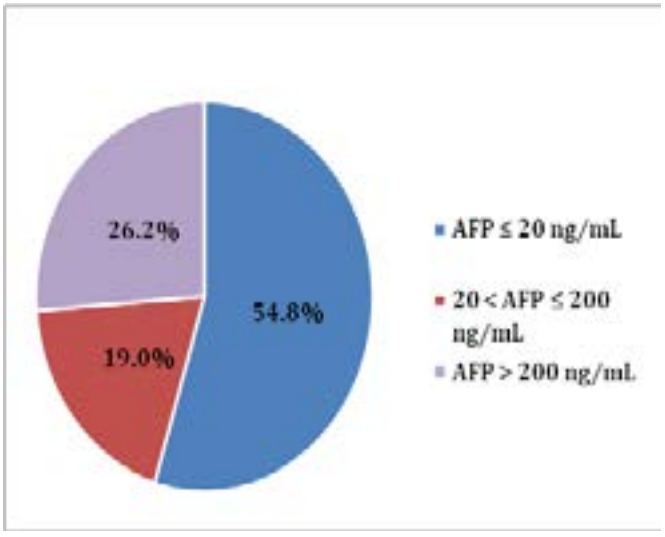

Figure 1: Alpha-fetoprotein levels among the patients analyzed. Source: Authors.

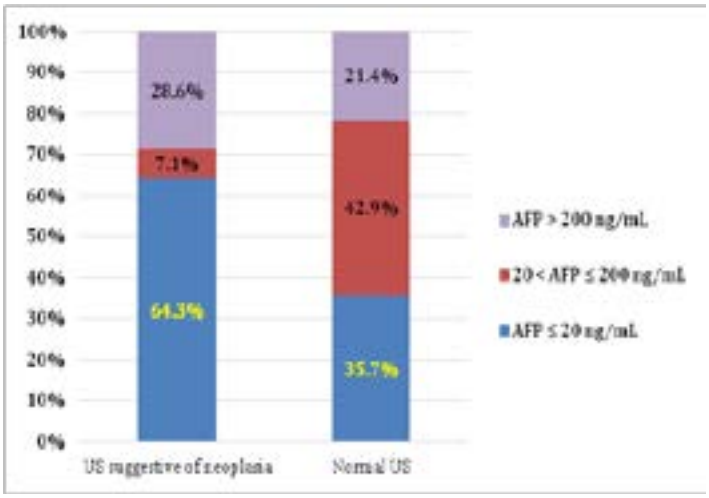

Figure 2: Relationship between alpha-fetoprotein levels and ultrasound results. Source: Authors.

According to the data related to AFP dosage and USG results, a statistical analysis was carried out to verify whether the dosage of this biomarker was relevant or not for the early diagnosis of HCC, with a p=0.079. (Table 1) shows the number of cases per age group between men and women, as well as the calculation of the mean and median age at the time of diagnosis. Of the total, 66.7% were men, and 33.3% were women. The average age for the general population was 60.8 years. Most diagnoses occurred in patients over 40 and had a peak incidence among the total number of cases between 60-70 years, with 38.1% of cases, as well as among men, with 46.4% of cases. HCC was two times more common in males than in females, with a male/female ratio of 2:1. It is worth mentioning that among females, the distribution between age groups was equal, while between the general population and males, the distribution was classically presented as a Gaussian curve tending the normal distribution to the average found.

Table 1. Patients diagnosed.

Variables	Groups		
	Total (n=42)	Men (n=28)	Women (n=14)
Average age + standard deviation	60,8 ± 11,7	60,3 ± 10,8	61,6 ± 13,6
Median age	62	62	61
Age Ranges			
Age ≤ 40 (%)	3 (7,1)	2 (7,1)	1 (7,1)
40 <Age ≤ 50 (%)	6 (14,3)	3 (10,7)	3 (21,4)
50 <Age ≤ 60 (%)	8 (19,0)	6 (21,4)	2 (14,3)
60 <Age ≤ 70 (%)	16 (38,1)	13 (46,4)	3 (21,4)
70 <Age ≤ 80 (%)	7 (16,7)	3 (10,7)	4 (28,6)
Age> 80 (%)	2 (4,8)	1 (3,6)	1 (7,1)

Of the 42 cases, 8(19.0%) did not have cirrhosis, and of these, only 1 of them had a known underlying disease that would be associated with the etiology of HCC, being HBV. The remaining 7 had no known cause for carcinogenesis. The other 34(81.0%) had cirrhosis. (Figure 3) shows the distribution of patients with cirrhosis and its leading associations. In 11 patients the only cause of cirrhosis was alcohol, 7 had an association between HCV and alcohol, two between HBV and alcohol, 6 had only HCV, one only HBV and 1 carried HCV and HBV, 3 were cases of non-alcoholic fatty liverdisease (NAFLD), 1 of auto immune hepatitis and 2 of cryptogenic cirrhosis, so 10 (29.4%) patients were associated with more than one cause. Thus, 32 individuals had a well-defined etiology of cirrhosis, and 2 of them had no apparentor known reason.

6. Discussion

The role of AFP dosing in early diagnosis and screening for HCC is a hotly debated and questioned world wide since this marker has a sensitivity considered low, around 60% [14-15]. In the present study, it was found that 54.8% of the patients had normal AFP levels, and only 45.2% had levels considered high. The sensitivity of the exam in this situation was approximately 45%. Findings were lower than expected for their subtlety, because their levels may be distorted due to cirrhosis, out breaks of HBV and HCV, exacerbation of liver disease, germ cell cancers, and gastric neoplasms [15,16]. Further more, only a fractionof 10-20% of tumors in the early stages have changes AFP [1]. However, in other studies, it has been shown that about 50-70% of the total HCCs have altered levels of the marker, this way, the series in question is close to the cases of this neoplasia that present alterations in AFP, but even so it remains below expectations [8,14]. Based on this, it was observed that the dosage of this markers

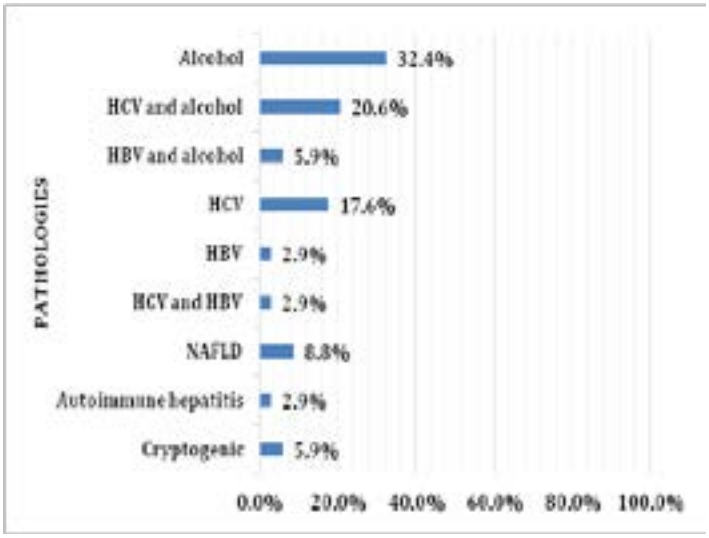

Figure 3: Patients with cirrhosis. NAFLD: Non-alcoholic fatty liver disease. HCV: hepatitis C virus. HBV: hepatitis B virus. Source: Authors.

howed an insufficient result for the diagnosis of the patients analyzed. In addition to presenting low sensitivity and suffering influences on their levels naturally, in the present study, a conclusion was still found below the predicted [17-19]. Regarding USG, it is the most wide spread and used test for the early diagnosis of HCC, with sensitivity ranging from 58-89% and specificity of more than 90% [20-22]. A meta-analysis demonstrated a sensitivity of 63% for early detection 15, while another showed a sensitivity of 84% for any stage of HCC and 47% for its early detection 10. In the present trial, 66.7% of cases had USG findings suggestive of HCC, while 33.3% were healthy [23]. The sensitivity found was approximately 66%. These findings are, therefore, within the expected sensitivity of the diagnostic method in question [1,10,15]. Consequently, it became evident that USG was useful for these patients, despite its limitations, as it is perceived that it presented a reasonable rate of detection of HCC within the predicted error margin and its sensitivity was very similar to that wide spread in the world literature [24]. After the statistical analysis of the data correlating USG and AFP, it wasfound that the biomarker measurement was not statistically relevant for the early diagnosis of HCC among the patients analyzed ($p>0.05$) [25]. This finding supports the findings of international studies that demonstrated AFP's limitation for screening HCC [7, 26]. This is associated, in addition to the deficits in herent in AFP already described above, with the advancement of USG, which now adays can identify tumors smaller than were evidenced in the past and early [27]. Therefore, associated with still normal levels of AFP it is note worthy that its use in clinical practice is not ruled out, as it has already been demonstrated that the addition of AFP to USG has improved sensitivity in neoplastic detection [10,11,28]. Besides, benefits have been evidenced when the dosage has been dynamically evaluated [16]. HCC is rarely diagnosed before the age of 40 and has a peak approximately around the age of 70, with the prevalence rate being 2-4 times higher in men than in women [29,30]. Two Brazilian multi center studies carried out at different times showed an average age ranging from 55.9-59 years, with a predominance of males, ranging from 77-78% [2]. Similar findings have been found in other centers in the country more recently [4,6]. In our study, the average age of 60.8 years was found in the general population, with 66.7% men, with HCC being twice as common among men than in women. Other studies have shown that HCC has a peak incidence during old age, around 60 years old. Cirrhosis is the leading risk factor associated with the appearance of HCC, present in about 70-90% of cases [31]. In the present study, 81% of the patients were cirrhotic, which reinforces the strong correlation of this neoplasm with chronic liver disease and justifies the periodic monitoring of these patients. The leading causes of HCC in the world are HBC and HBV, which correspond to almost 80% of cases. 54% being

related to HBV, 31% to HCV, and 15% to other causes [32]. The associated risk factors, in addition to hepato tropic virus infection, are alcohol, exposure to a flatoxins, and non-alcoholic liver steatosis [1,9]. The global incidence varies according to the geographical area and the risk factors. In summary, it can be said that in under developed countries or with high frequency, the leading cause of HCC is HBV, while in more developed regions, with low incidence or better vaccination coverage for hepatitis B, HCV becomes the leading cause [2,5]. Two extensive Brazilian studies have shown the epidemiology of HCC in the country showed that chronic alcoholism was present in 36% of cases, HBV in 35% and HCV in 25%, while the study by [6] demonstratedthat HCV was present in 54% of cases, followed by HBV with 16% and alcohol with 14% [2-6]. Another study in Rio de Janeiro demonstratedthat HCV was associated with 65.6% of cases, alcohol at 9.2%, and NAFLD at 9.2% [2]. It was evident in the present study that the primary etiology found was alcohol, with 32.4% of cases, followed by HCV with 17.6% and NAFLD with 8.8%. In 29.4% of patients, there was more than one cause, of which 20.6% had an association between alcohol and HCV. Ethanol, as the leading cause of HCC, was also seen in the study by [17]. However, there sults found here differ from the findings by [10]. The findings of our study are consistent with what is expected among the leading causes of HCC, with alcoholism and HCV being the most prevalent. However, there is an essential correlation between alcohol and HCC in this population, which can be associated with the fact that ethanolisone of the leading causes of liver cirrhosis in the country [5, 21, 22]. The North east has the second-highest prevalence of alcoholism in Brazil, about 15.6%, above the national average of 13.7% [2,4,6]. However, this high rate of alcoholism found among the study patients cannot be attributed only to epidemiological factors of cirrhosis and data on alcohol use in the North east, and further data and studies are needed to understand this situation better [4]. Similar findings of the low incidence of HBV in this neoplasia, especially in areas with better vaccination coverage, have already been described in national and international studies [30-32]. This is associated, in Brazil, with the hepatitis B vaccine, present through out the national territory with a progressive increase in distribution since its implantation, even with the vaccine coverage still below the goale stablished by the Ministry of Health [2,4]. It is worth mentioning the vital synergism found between alcohol and HCV, which demonstrates that different liver-damaging agent scan act together and facilitate the appearance of HCC [25, 26].

7. Study Limitations

One of the main limitations of this study is associated with the fact that a large number of suspected cases of HCC lost follow-up and had no confirmed diagnosis, which made it impossible to be included in the study and, therefore, reduced the total number of the analyzed sample. Within this context, it was notice able that countless patients were referred and did not return to the service and not received a counter-reference, demonstrating a deficit in the follow-up. The vast majority of these patients had cirrhosis and still needed careat the hepatology out patient clinic LSC/HUOL. Another limitation was the collection of data through the consultation of medical records in physical form (onpaper), which led to the disappearance of files and the in adequate filling of clinical records, in which case loss of cases or non-inclusion of patients may have occurred due to non-compliance. Fulfill the criteria adopted in the present study due to the lack of information.

8. Conclusion

In conclusion, the AFP dosage didnot make a significant difference in the early diagnosis of HCC in the analyzed sample. There was a tendency to follow the data found in the world literature concerning the sensitivity of the USG, while the AFP indexes were even lower than expected. The results of this study corroborate the world wide guidelines regarding the performance of screening tests for HCC, demonstrating that USG is the besttest for early diagnosis of HCC. Therefore, it is necessary to follow there commendations of the MS, and international societies such as EASL and AASLD, in which AFP dosage should not be performed in isolation and screening for HCC should be carried out through serial USG with or without AFP. Therefore, the dosage of this marker is at the discretion of the service and protocols adopted in the management of these patients. The mean age found was similar toother studies, showing a peak incidence of the disease in old age, around 60 yearsold. Among the patients analyzed, most had cirrhosis, a fact that proves the strong association of HCC with chronic liver disease and reinforces the necessary vigilance in these patients. Finally, the data from this work converge to the main pathologies that cause HCC in Brazil, alcoholism, and HCV. However, a significant number of alcohol-related cases were evidenced, is this the primary etiology isolated in the study patients.

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