Correlation Between Serum Level of Alpha-Fetoprotein and Histological Differentiation Grade of Hepatocellular Carcinoma

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Abstract. In HCC, the tumor marker alpha-fetoprotein (AFP) is increased, but it might be normal in 40% of cases. Increased AFP implies worse tumor differentiation. This study aimed to clarify and determine the correlation between the histological differentiation grade of HCC and serum AFP level in patients managed at the RSCM (Dr. Cipto Mangunkusumo Hospital). In total, 32 of HCC cases of patients who underwent hepatectomy at RSCM in 2010–2016 were enrolled in a retrospective study. The subject characteristics, AFP levels, and histological differentiation grade were the variables analyzed in this study. A P value of <0.05 was considered to be significant. Among the subjects, 59.4% were HBsAg-positive and 12.5% were anti-HCV-positive. The tumor size was more than 5–10 cm in diameter in 50% subjects; 50% subjects were cirrhotic and 68.8% subjects had microvascular invasion. Overall, the AFP levels ranged from 0.5 to 400000 ng/mL (mean, 20183 ± SD 75580.08). The histological grade was well differentiated in 12.5%, moderately differentiated in 28.1%, and poorly differentiated in 59.4%. In cases with poorly differentiated HCC, the AFP level was <20 ng/mL in 42.1% and >20 ng/mL in 57.9%. Correlation analysis revealed an r value of 0.203 (P > 0.05). There was no correlation between the serum AFP level and histological differentiation grade of HCC.

1. Introduction

Alpha-fetoprotein (AFP) is one of the tumor markers that is important and has been used to diagnose hepatocellular carcinoma (HCC) since 1970 [1]. In most patients with HCC, the serum AFP level is increased but the normal value of AFP can be less than 40% of early cases of HCC and in 15% to 20% of advanced cases of HCC [1,2]. Small tumors are more likely to express low AFP levels; however, the correlation between AFP expression and tumor size still remains unclear because the capacity to synthesize AFP can differ among tumors. For example, a large tumor may contain many necrotic cells and few healthy cells. Moreover, a high number of HCC cells that produce AFP was reported to indicate poor tumor differentiation, high tumor load, and high risk for a recurrence after tumor resection and a poor outcome [3-5].

Several scientists found that an increase in serum AFP was consistent with an increasing number of tumor cells. However, others reported that there was no clear correlation between tumor size and AFP level. Moreover, some highly differentiated small tumors have been shown to express undetected
levels of AFP in serum; however, some undifferentiated tumor cells have shown AFP levels higher than 400 ng/mL. Another important factor that was reported to correlate with increased serum AFP level was the presence of blood vessel invasion [6]. This study aimed to find the correlation between serum AFP level and histological differentiation of HCC.

2. Methods
This cross-sectional study design included HCC patients who underwent hepatectomy at the Digestive Surgery Division of RSCM from January 2010 to October 2016. The serum AFP level was measured before the operation was conducted and before the AFP level was examined by the Anatomical Pathology department. Research data were collected from the Anatomical Pathology department.

The patient variables included age, gender, total number and size of the tumors, presence and absence of cirrhosis, anti-HCV, HBsAg, blood vessel invasion, and the Child-Pugh stage of the patients. The correlation between the serum AFP level and histological differentiation was analyzed using the Spearman analysis. Statistical analysis was performed with SPSS version 22. A $P$ value less than 0.05 was considered to be significant.

3. Results
In total, 38 cases of HCC were eligible for study inclusion; after exclusion, 32 patients were included for analysis. We determined the inclusion and exclusion criteria for the patients. Anatomical pathology data showed that 12.5% of the patients had good differentiation, 28.1% had moderate differentiation, 59.4% had poor differentiation, and none had undifferentiated result.

Table 1. Demographic Characteristic of the Patients (n = 32).

| Variable          | Total (%) |
|-------------------|-----------|
| **AFP (ng/mL)**   |           |
| Mean ± SD         | 20183.60 ± 75580.08 |
| Age (years)       |           |
| Mean ± SD         | 51.75 ± 12.34   |
| Gender            |           |
| Male              | 27 (81.3)   |
| Female            | 6 (18.8)    |
| HBsAg             |           |
| Positive          | 19 (59.4)   |
| Negative          | 13 (40.6)   |
| Anti-HCV          |           |
| Positive          | 4 (12.5)    |
| Negative          | 18 (87.5)   |
| Tumor size        |           |
| $\leq 5$          | 3 (9.4)     |
| >5–10             | 16 (50)     |
| >10               | 13 (40.6)   |
| Tumor number      |           |
| Single            | 28 (87.5)   |
| Multiple          | 4 (12.5)    |
| Child-Pugh        |           |
| A                 | 32 (100)    |
| B                 | 0           |
| C                 | 0           |
Table 1. Continue

| Variable            | Total (%) |
|---------------------|-----------|
| Differentiation     |           |
| Good                | 4 (12.5)  |
| Moderate            | 9 (28.1)  |
| Poor                | 19 (59.4) |
| Not differentiated  | 0         |
| Cirrhosis           |           |
| Yes                 | 16 (50)   |
| No                  | 16 (50)   |
| Vascular invasion   |           |
| Yes                 | 22 (68.8) |
| No                  | 10 (31.3) |

Table 2. Pathological characteristics of the HCC patients.

|                     | Good differentiation | Moderate differentiation | Poor differentiation | Not differentiated |
|---------------------|----------------------|--------------------------|----------------------|--------------------|
| Gender              |                       |                          |                      |                    |
| Male                | 3 (11.5%)             | 12 (46.2%)               | 11 (42.3%)           | 0                  |
| Female              | 1 (16.7%)             | 3 (50%)                  | 2 (33.3%)            | 0                  |
| HBsAg               |                       |                          |                      |                    |
| Positive            | 0                     | 10 (52.6%)               | 9 (47.4%)            | 0                  |
| Negative            | 4 (30.8%)             | 5 (38.5%)                | 4 (30.8%)            | 0                  |
| Anti-HCV            |                       |                          |                      |                    |
| Positive            | 0                     | 2 (50%)                  | 2 (50%)              | 0                  |
| Negative            | 4 (14.3%)             | 13 (46.13%)              | 11 (39.3%)           | 0                  |
| Size                |                       |                          |                      |                    |
| ≤5                  | 1 (33.3%)             | 1 (33.3%)                | 1 (33.3%)            | 0                  |
| >5–10               | 2 (12.5%)             | 5 (31.2%)                | 9 (56.2%)            | 0                  |
| >10                 | 1 (7.7%)              | 3 (23.1%)                | 8 (69.2%)            | 0                  |
| Number              |                       |                          |                      |                    |
| Single              | 3 (10.7%)             | 13 (46.4%)               | 12 (42.9%)           | 0                  |
| Multiple            | 1 (25%)               | 2 (50%)                  | 1 (25%)              | 0                  |
| Cirrhosis           |                       |                          |                      |                    |
| Positive            | 1 (6.25%)             | 10 (62.5%)               | 5 (31.2%)            | 0                  |
| Negative            | 3 (18.8%)             | 5 (31.2%)                | 8 (50%)              | 0                  |
| Blood vessel invasion |                    |                          |                      |                    |
| Positive            | 0                     | 13 (59.1%)               | 9 (40.9%)            | 0                  |
| Negative            | 4 (40%)               | 2 (20%)                  | 4 (40%)              | 0                  |
| Child-Pugh          |                       |                          |                      |                    |
| A                   | 4 (12.5%)             | 15 (46.9%)               | 13 (40.6%)           | 0                  |
| B                   | 0                     | 0                        | 0                    | 0                  |
| C                   | 0                     | 0                        | 0                    | 0                  |

Patients with positive HBsAg or anti-HCV showed moderate differentiation. The AFP levels were higher in patients with positive HBsAg than in those with anti-HCV-positive. Tumors that measured 5–10 cm and >10 cm had poor differentiation; among these, tumors that measured >10 cm had serum AFP levels higher than 20 ng/mL. Patients with cirrhosis had higher AFP levels compared with the
patients with no cirrhosis. This fact is in line with lymphovascular invasion in which is moderate-poor differentiation with AFP < 20 ng/mL or >20 ng/mL.

Table 3. Characteristics of the HCC patients based on the AFP levels.

|                         | AFP < 20 ng/mL | AFP > 20 ng/mL |
|-------------------------|----------------|----------------|
| Gender                  |                |                |
| Male                    | 17 (65.4%)     | 9 (34.6%)      |
| Female                  | 2 (33.3%)      | 4 (66.7%)      |
| HBsAg                   |                |                |
| Positive                | 11 (57.9%)     | 8 (42.1%)      |
| Negative                | 8 (61.5%)      | 5 (38.5%)      |
| Anti-HCV                |                |                |
| Positive                | 2 (50%)        | 2 (50%)        |
| Negative                | 17 (60.7%)     | 11 (39.3%)     |
| Size                    |                |                |
| ≤5                      | 2 (66.7%)      | 1 (33.3%)      |
| >5−10                   | 10 (62.5%)     | 6 (37.5%)      |
| >10                     | 7 (53.8%)      | 6 (46.2%)      |
| Total number            |                |                |
| Single                  | 15 (53.6%)     | 8 (46.4%)      |
| Multiple                | 4 (100%)       | 0 (0%)         |
| Cirrhosis               |                |                |
| Positive                | 9 (56.2%)      | 7 (43.8%)      |
| Negative                | 10 (62.5%)     | 6 (37.5%)      |
| Blood vessel invasion   |                |                |
| Positive                | 12 (54.5%)     | 10 (45.5%)     |
| Negative                | 7 (70%)        | 3 (30%)        |
| Child-Pugh              |                |                |
| A                       | 19 (59.4%)     | 13 (40.6%)     |
| B                       | 0              | 0              |
| C                       | 0              | 0              |
| Differentiation         |                |                |
| Good                    | 4 (100%)       | 0              |
| Moderate                | 4 (44.4%)      | 5 (55.6%)      |
| Poor                    | 11 (57.9%)     | 8 (42.1%)      |
| Not differentiated      | 0 (0%)         | 0 (0%)         |

Table 4. Correlation between the characteristics and pathological features of HCC.

| Characteristics          | Pathological features |
|--------------------------|-----------------------|
| HBsAg                    | r = −0.307            |
|                          | p = 0.088             |
| Anti-HCV                 | r = −0.112            |
|                          | p = 0.540             |
| Cirrhosis                | r = 0.086             |
|                          | p = 0.642             |
| Blood vessel invasion    | r = −0.217            |
|                          | p = 0.234             |
Correlation analysis among the number of patients with positive HBsAg, anti-HCV-positive, and blood vessel invasion was quite far from 1 and may have a negative value. Consequently, HBsAg, anti-HCV, and blood vessel invasion had weak negative correlations with the histological differentiation. Referring to four variables with a $P$ value of $>0.05$ indicated that the HCC variables did not significantly correlate with the pathological characteristics. Conversely, both AFP levels had weak correlations with the HCC histological differentiation is approaching +1.

**Table 5.** Correlation between the pathological features and AFP levels in HCC.

| Pathological features | $r$ | $p$ |
|-----------------------|-----|-----|
| AFP                   | 0.203 | 0.265 |

Regression test was done to determine the coefficient of determination ($r^2$). In this study, we obtained an $r^2$ value of 0.041, which meant that the pathological features of HCC varied by 4.1%; this result was attained from the AFP levels and other factors. Furthermore, there was not enough evidence that the presence of a variation of the HCC pathological features was caused by a number of $p > 0.05$.

4. Discussion

AFP is a tumor marker that is mostly found in patients with HCC. To date, some related studies have been conducted to determine the correlation between the AFP level and the clinicopathological features of HCC. In this study, the average age of the HCC patients was 51.75 years. More than 50% of the male patients had moderate to poorly differentiated HCC; this is probably accounted for by the high levels of androgen in men [7,8]. Cirrhosis was found in 50% of the patients. Regardless of the presence of cirrhosis, the histopathologic differentiation did not affect the prognosis of HCC. However, well-differentiated HCC was more common in non-cirrhotic HCC, whereas third-fourth degree differentiation and blood vessel invasion were more common in cirrhotic HCC. Blood vessel invasion has not always been parallel to the differentiation features of HCC. This finding was in line with the results of other studies that found a good correlation between the differentiation features and vascular invasion [1,5,11,12,13-16].

In this study, the mean serum AFP level was 20183.6 and ranged from 0.5 to 400000; the highest AFP level was found in only one patient. In the extreme value of AFP (>800 ng/mL). Most patients had poor to moderate differentiation, and no patient had a good differentiation. Most of the male patients who were HBsAg-positive had cirrhosis and blood vessel invasion.

The results of this study were contrary to the previously reported result that AFP value was related to large tumor size, bilateral lobe involvement, vein thrombosis, and poor differentiation. An increased AFP correlated with the $p53$ mutation, which may increase tumor cell growth, whereas a decreased AFP expression with activation of $p53$ may stop the growth and apoptosis of HCC cells in humans. The AFP receptors may be expressed in AFP-positive cells but are not usually expressed in AFP-negative cells. Therefore, cells that express the AFP receptor are more sensitive to AFP and may extend proliferation. AFP is not only a diagnostic marker but also a growth factor that may be important in tumor progression and cell differentiation and as a poor prognosis marker [17,18].

In a study in China, the histopathologic differentiation was better in AFP-negative patients than in AFP-positive patients; moreover, the value of AFP was higher in HCC patients with cirrhosis, incomplete capsule, and poor differentiation [1]. In this research, poor differentiation was more common in AFP-negative patients (<20 ng/mL) than in AFP-positive patients. These results contradict with other previously reported results and raise the question on whether other factors aside from AFP influence histopathologic differentiation or whether these HCC cells are severely damaged to produce AFP. Based on our results, preoperative AFP levels did not correlate with HCC differentiation.
5. Conclusion
There was no correlation between serum AFP level and histological differentiation grade of HCC.

Abbreviations:
HCC    : Hepatocellular carcinoma
AFP    : Alpha-fetoprotein

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