The Influence of $C936T$ Gene in Wild of the Vascular Endothelial Growth Factor and Biochemical Variants Analysis for Alpha-Fetoprotein in Hepatocellular Carcinoma Patients in Egypt

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Abstract

Hepatocellular carcinoma (HCC) is one of the most frequent malignant tumors. It is important to detect disease and recurrence at its earlier period. The aim of this study was to evaluate the influence of the $C936T$ polymorphism of the VEGF and Alpha fetoprotein (AFP) on Hepatocellular carcinoma (HCC), Hepatitis C Virus (HCV) and control groups. Also, estimation of serum AFP and correlation with other parameters was studied. In this study was included 75 subjects, 25 patients with HCV, 25 patients with confirmed HCC and 25 healthy volunteers were subjected to abdominal ultrasonography, AFP and VEGF were assessed. The statistical analysis will include the Fisher exact test, T test and the multivariate regression. There was a nonsignificant increase in serum (AFP) level in the untreated HCC infected group compared to control group and also there was a nonsignificant increase in serum (AFP) activity in the HCC group compared to untreated HCV infected group with a (P value = 0.203), the influence of the $C936T$ polymorphism of the VEGF distribution of the studied groups showing that Positive gene have higher incidence of HCV and HCC infection than Negative gene, when compared to control group. Sensitivity and specificity of markers in diagnosis of HCC were 52% and 40% respectively for AFP using a cutoff value of 3.39 ng/mL. VEGF may be useful marker for detection of HCC in addition to traditional markers.

Keywords: Alpha Feto-Protein (AFP), Hepatocellular Carcinoma (HCC), Hepatitis C Virus (HCV), Vascular Endothelial Growth Factor (VEGF)

Introduction

The burden of Hepatocellular carcinoma (HCC) has been increasing in Egypt with a doubling in the incidence rate in the past 10 years [1]. Early diagnosis of HCC is of great importance in order to offer the possibility of curative treatment. Surveillance programs have been conducted in many countries to detect HCC at an early stage. Alpha fetoprotein (AFP) and ultrasoundography are usually used as diagnostic tools [2]. Most patients with HCV have two diseases, liver cirrhosis and HCC, and complex interactions between the two have major implications for prognosis and treatment choice [3]. However, the biological mechanism for the relationship between these diseases is not clear [4]. HCV is the commonest cause of chronic hepatitis, liver cirrhosis and liver cancer in Egypt, where 12% to 15% of the population have HCV antibodies (anti-HCV) [5]. The discovery of genetic factors associated with cancer risk may help lower the stage and improve survival [6,7].

Angiogenesis is an essential process in the development, growth and metastasis of malignant tumors. Vascular endothelial growth factor (VEGF) plays an important role in the angiogenesis. Increased VEGF expression is associated with tumor growth and metastasis [8]. The human VEGF gene is located on chromosome 6p21.3 and is organized into 8 exons separated by 7 introns. Several polymorphisms have been described in the VEGF gene. Three single-nucleotide polymorphisms (G+405C in the 5’-untranslated region, C+460T in the promoter region, and C+936T in the 3’-untranslated region) are common and related to VEGF protein production. Different factors designated VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placenta growth factor, and snake venom VEGF (VEGF-F). The receptors for VEGF are located on the surface of endothelial cells. VEGF, which has a mitogenic effect on endothelial cells and increases their migratory capacity [9]. Epigenetic mechanisms play an essential role in normal development and maintenance of physiological functions in humans. Emerging evidence indicates that aberrant epigenetic mechanisms are strongly involved in the genesis and progression of human disorders [10]. Take histone methyltransferase G9a for example, overexpression of this enzyme has been found to be associated with poor prognosis in different types of cancers [11]. Interestingly, previous study has shown that the expression of alpha-fetoprotein was significantly higher in patients with G9a-positive tumors [12]. In mammals, the main function of G9a is depositing of H3K9me1/2 at the euchromatin loci [13]. In addition, G9a also interacts with DNA methyltransferase and maintains DNA methylation at imprinted loci [14]. Therefore, understanding the patterns of H3K9me1/2 as well as DNA methylation at alpha-fetoprotein locus might reveal the potential epigenetic mechanisms that required for the regulation of this gene and shed light for the diagnosis of HCC in humans.

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Subjects and Methods

Study groups

This study was conducted on seventy five patients attending the Hepatology and Gastroenterology Department, Theodor Bilharz Research Institute (TBR) either as outpatients for evaluation and assessment or as inpatients to be investigated within the department, in addition to fifteen apparently healthy individuals served as a control group. The Samples of blood were collected from July 2014 up to October 2014. They were divided into three main groups (25 for each): group I: HCV, group II: HCC and group III: healthy controls. Full history and clinical evaluation to assess fitness for further surgical intervention as presence of cirrhosis, status of liver function using child's classification and presence of extra hepatic spread. A history of intraabdominal malignancy, breast or lung cancer or malignant skin caner particularly melanoma raises the possibility of liver metastases. This study was approved by the Ethics Committee of Faculty of Medicine, Fayum University.

Laboratory investigations

Complete blood count (CBC): This was performed using Sysmex XE-2100 is a haematology automated analyser according to manufacturer instructions [15].

Liver function tests: These were performed using Colorimetric method of serum (AST, ALT, ALP activities, Albumin, Total proteins, Total bilirubin levels) by using Human Gesellschaft für Biochemica und Diagnostica mbH (Germany) laboratories diagnostic kits [16,17].

Qualitative determination of serum (anti-HCV) antibodies: These are done by anti-HCV enzyme linked immunoassay kit (Diasorin S. A., Madrid, Spain) [18]. Alfa Fetoprotein (AFP) in human serum was performed using immunoenzymatic colorimetric method for qualitative determination of AFP concentration in serum or plasma by DiaMetra S.r.l. (Z.I Paciana- ITALY) laboratories diagnostic kits [19].

Evaluate the influence of the C936T Gene in wild of the VEGF on the studied groups was performed by three steps:

Step 1: DNA extraction from whole blood of human was performed using Bio Basic Canada Inc. (EZ-10 Spin Column Genomic DNA Minipreps Kit, Blood) [20].

Step 2: Polymerase chain reaction.

Genomic DNA was isolated from venous blood using a EZ-10 Spin Column Genomic DNA Minipreps Kit, Blood and stored at 4°C. The primer pair was designed to amplify full sequence of the VEGF gene, VEGF (C936T) primer 1 (5’-AAGGAAAGGAGACTCCTGCGC-3’), primer2 (3’TATGTGGGTGGGTGTCTACAG-5’). PCR cycling conditions were 2 min at 95°C followed by 35 cycles with 30 sec at 94°C, 30 sec at 60°C the annealing temperature and the Extension with 2 min at 72°C [21].

Step 3: DNA agarose gel electrophoresis was performed using the following:

*AGAROSE D1 LOW EEO (Employed in applications requiring electroendosmosis (EEO) of low value. Cat. No. 8010.11 (Laboratorios Conda S.A.).

* PCR marker: Ethidium bromide.

*50bp DNA ladder RTU (Ready-to-Use), Cat. No. DM012-R500. GeneDirex.

*50x TAE: (stock solution).

Statistical analysis

Data were collected and analyzed by computer program SPSS “version 17” (The Statistical Package for the Social Science Program), Chicago, USA. All data were expressed as mean ± SD and percentages. Unpaired t-test was used to compare a quantitative variable between two independent groups in parametric data. Mann Whitney test was used to compare quantitative variables between two independent groups when data were nonparametric (SD>25% of mean). Chi square test was used to compare qualitative variables between two independent groups. Correlation between studied parameters was performed by Spearman rank correlation coefficient.

P<0.05 was considered significant. Receiver operating characteristics curves (ROC) were constructed for studied variables, reporting area under the curve (AUC). The ROC analysis was used for the selection of the best diagnostic cut off values, and the related sensitivity and specificity were determined.

Results

Demographic data and routine investigations

Table 1 showed that a significant increase in the mean of serum AST, ALT, ALP activities and of creatinine, total bilirubin, direct bilirubin, HCV (ab) levels in HCC group when compared to the control group and there was significant decrease in the mean values of albumin, total protein, Hemoglobin, and Platelets in HCC groups when compared to the control group. Also, α-fetoprotein, the mean level was higher in the group of HCC when compared to either the control group or HCV group (P<0.05 for both). But the mean level of α-fetoprotein was decreased in HCC group when compared to the HCV group (P<0.203).

Influence of the C936T Gene in wild of the VEGF in the studied groups

Table 2 showed that the number of Positive gene within control, HCV group and the HCC groups were 0/25 (0%), 3/25 (12%) and 11/25 (44%) respectively, showing that Positive gene have higher incidence of HCV and HCC infection than Negative gene, when compared to control group (Figures 1-3) respectively.

6.3 The mean value ± S.D of the positive and negative gene C936T of the VEGF in all patients (untreated HCV and HCC patients ).

Table 3 showed that the mean value ± S.D of Alk. phos., GOT, GPT, and WBC parameters in Positive gene C936T in all patients (untreated HCV and HCC patients ). The mean value ± S.D of Alk. phos., GOT, GPT, and WBC parameters in Positive gene C936T in all patients were 754 ± 409 ng/5 µl, 42 ± 255 ng/5 µl, 190 ± 229 ng/5 µl and 12 ± 4.48 ng/5 µl respectively. There was a highly significant increase in Alk. phos. GOT, GPT and WBC activity in positive gene C936T polymorphism of the VEGF in all patients. P<0.05, P<0.001 respectively. Also there was a nonsignificant decrease in other parameters in the of positive and negative gene C936T polymorphism of the VEGF activity in all patients. Regarding sex distribution among discovered cases; the proportion of female 7 (36.8%) is slightly more than male proportion 6 (19.4%) this result in positive gene C936T polymorphism of the VEGF in all patients.

The mean value ± S.D of the positive and negative gene C936T in wild of the VEGF in HCC patients

Table 4 showed that the mean value ± S.D of WBC and PLT parameters in Positive gene C936T polymorphism of the VEGF HCC...
Table 1: Basic laboratory data of the study groups.

| Parameters     | Group I | Group II | Group III |
|----------------|---------|----------|-----------|
|                | Control (n=25) | HCV (n=25) | HCC (n=25) |
| Mean ± SD      |          |          |           |
| Age (years)    | 53.1 ± 7.75 | 55 ± 11.33 | 57.7 ± 5.99 |
| Gender         |           |          |           |
| Female         | 13       | 8        | 11        |
| Male           | 12       | 17       | 14        |
| ALT (U/L)      | 31 ± 15  |          |           |
| AST (U/L)      | 38 ± 29  |          |           |
| ALP (U/L)      | 202 ± 114|          |           |
| T.Bil (mg/dl)  | 1.3 ± 0.4|          |           |
| D.Bil (mg/dl)  | 0.4 ± 0.3|          |           |
| Albumin (g/dl) | 4.2 ± 0.5|          |           |
| T. protein (g/dl) | 8.1 ± 1.75 |          |           |
| Creatinine (g/dl) | 1.3 ± 0.24 |          |           |
| AFP (ng/ml)    | 2.12 ± 1.54|          |           |
| HCV (ab) (nm)  | 0.124 ± 0.004|          |           |
| Hgb (g/dl)     | 13 ± 2.03|          |           |
| WBC (10 × 3/µl) | 6 ± 1.7  |          |           |
| Plt (10 × 3/µl) | 242 ± 49 |          |           |

P value between group I versus group II  *P value between group I versus group III  **P value between group II versus group III
HCV=Hepatitis C Virus  HCC=Hepatocellular Carcinoma  P>0.05 is not significant (NS)  P ≤ 0.05 is significant

Table 2: Comparison of the VEGF Gene in the studied groups.

| Parameters | Group | Control (25) | HCV (25) | HCC (25) |
|------------|-------|--------------|----------|----------|
| VEGF       |       |              |          |          |
| positive (+ve) | %    | (0%)      | (12%)    | (44%)    |
| negative (-ve) | %    | (100%)     | (88%)    | (56%)    |
patients were 10.9 ± 2.99 ng/5 µl, 75 ± 37 ng/5 µl, respectively. There was a highly significant increase in WBC and PLT activity in Positive gene C936T polymorphism of the VEGF in HCC patients P<0.001,

Figure 1: Agarose gel electrophoresis of PCR genotyping in control group. M: DNA ladder (50 bp); Lane from (1 to 10) is negative gene (C936T gene polymorphism in VEGF) in control group.

Figure 2: Agarose gel electrophoresis of PCR genotyping in HCV group. M: DNA ladder (50 bp); Lane from (1, 3 and 4) is positive gene (200 bp) (C936T gene polymorphism in VEGF) in HCC group and other lane is negative in the same group.

Figure 3: Agarose gel electrophoresis of PCR genotyping in HCC group. M: DNA ladder (50 bp); Lane from (2, 3, 4, 8, 10, 11, 12, 15, 16 and 17) is positive gene (200 bp) in HCC group and other lane is negative in the same group.

Table 3: The mean value ± S.D of the positive and negative gene in all patients (untreated HCV and HCC).

| Parameter     | Positive gene (+ve) (ng/5µl) | Negative gene (-ve) (ng/5µl) | P-value |
|---------------|------------------------------|------------------------------|---------|
| Age           | 57 ± 5.7                     | 56 ± 10.7                    | 0.804   |
| T. Protein    | 5.5 ± 1.99                   | 5.36 ± 2.12                  | 0.822   |
| Alk. Phos.    | 754 ± 409                    | 409 ± 298                    | <0.05   |
| GOT           | 421.77 ± 255.21              | 199 ± 186                    | <0.05   |
| GPT           | 305.23 ± 229.2               | 95 ± 86                      | <0.05   |
| Albumin       | 2.4 ± 0.76                   | 3.47 ± 1.27                  | >0.05   |
| Creat         | 2.67 ± 1.7                   | 2 ± 1.34                     | 0.161   |
| T. Bil        | 1.86 ± 0.755                 | 1.58 ± 0.54                  | 0.170   |
| D. Bil        | 1.16 ± 0.64                  | 1.37 ± 1.34                  | 0.822   |
| HCV (ab)      | 1.65 ± 0.55                  | 1.63 ± 0.60                  | 0.932   |
| AFP           | 144 ± 49                     | 44.76 ± 26.45                | 0.312   |
| Hb            | 9.95 ± 1.84                  | 10 ± 2.2                     | 0.863   |
| WBC           | 12 ± 4.48                    | 5.49 ± 3.14                  | <0.001  |
| PLT           | 97 ± 76.8                    | 130 ± 71.7                   | 0.174   |
| Female (%)    | 6 (54.5%)                    | 5 (35.7%)                    | >0.05   |
| Male (%)      | 5 (45.5%)                    | 9 (64.3%)                    |         |

Table 4: The mean value ± S.D of the positive and negative gene in HCC patients.

| Parameter     | Positive gene (+ve) (ng/5µl) | Negative gene (-ve) (ng/5µl) | P value |
|---------------|------------------------------|------------------------------|---------|
| Age           | 57 ± 6.25                    | 58 ± 8.7                     | 0.744   |
| T. Protein    | 5.6 ± 2.17                   | 6.3 ± 1.94                   | 0.41    |
| Alk. Phos.    | 824 ± 397                    | 627 ± 343                    | 0.195   |
| GOT           | 394 ± 228                    | 277 ± 229                    | 0.219   |
| GPT           | 245 ± 220                    | 128 ± 127                    | 0.285   |
| Albumin       | 2.17 ± 0.446                 | 2.38 ± 0.437                 | 0.243   |
| Creatinine    | 2.61 ± 1.82                  | 2.7 ± 1.78                   | 0.897   |
| T. Bil        | 1.87 ± 0.8                   | 1.7 ± 0.83                   | 0.626   |
| D. Bil        | 1.28 ± 0.61                  | 1.05 ± 0.577                 | 0.327   |
| HCV (ab)      | 1.67 ± 0.59                  | 1.9 ± 0.37                   | 0.197   |
| AFP           | 149 ± 54                     | 67 ± 34                      | 0.605   |
| Hb            | 9.6 ± 1.9                    | 11.3 ± 2.0                   | 0.327   |
| WBC           | 11.4 ± 5.1                   | 8 ± 4.3                      | <0.001  |
| PLT           | 136 ± 71.8                   | 262 ± 67                     | <0.05   |
| Female (%)    | 6 (54.5%)                    | 5 (35.7%)                    | >0.05   |
| Male (%)      | 5 (45.5%)                    | 9 (64.3%)                    |         |
Figure 4: Correlation between serum AFP and HCV (ab) in HCC patients \( (r=-0.700, p<0.001) \) (significant correlation).

Figure 5: ROC curve of AFP between Control and HCC groups.

Figure 6: ROC curve of AFP between Control and HCV groups.

Figure 7: ROC curve of AFP between HCV and HCC groups.

Correlations study and ROC curve

There was a significant negative correlation between AFP and HCV (ab) in HCC group \( P<0.001 \). Also, there was a significant correlation between AFP and Age in HCV group \( P<0.05 \) but there were no significant correlation between serum AFP and other biochemical parameters ALT, AST, ALP, T. bilirubin, D. bilirubin, albumin, and T.-protein, creatinine, Hb, WBC, PLT. Cut off level, sensitivity, specificity, and the area under the receiver operating characteristic curve (AUROC) of different tests in the diagnosis of HCC were evaluated and the results tabulated (Figures 4-7).

Discussion

VEGF has a major effect in regulating angiogenesis, and its expression has been shown to correlate with carcinogenesis [22]. In the present study we found that the influence of the \( C936T \) polymorphism of the VEGF activity in all patients \( P<0.05, P<0.001 \) respectively. Also there was a nonsignificant decrease in other parameters in the of positive and negative gene \( C936T \) polymorphism of the VEGF in all patients \( P>0.05 \). Also there was no significant between positive and negative gene \( C936T \) polymorphism of the VEGF in HCC patients. Regarding sex distribution among discovered cases; the proportion of female \( 6 (54.5\%) \) is slightly more than male proportion \( 5 (45.5\%) \) this result in positive gene \( C93 \ 6T \) polymorphism of the VEGF in HCC patients.

There was a significant negative correlation between AFP and HCV (ab) in HCC group \( P<0.001 \). Also, there was a significant correlation between AFP and Age in HCV group \( P<0.05 \) but there were no significant correlation between serum AFP and other biochemical parameters ALT, AST, ALP, T. bilirubin, D. bilirubin, albumin, and T.-protein, creatinine, Hb, WBC, PLT. Cut off level, sensitivity, specificity, and the area under the receiver operating characteristic curve (AUROC) of different tests in the diagnosis of HCC were evaluated and the results tabulated (Figures 4-7).

Discussion

VEGF has a major effect in regulating angiogenesis, and its expression has been shown to correlate with carcinogenesis [22]. In the present study we found that the influence of the \( C936T \) polymorphism of the VEGF distribution of the studied groups that the number of Positive gene within control, HCV group and the HCC groups were \( 0/25 (0\%) \), \( 3/25 (12\%) \) and \( 11/25 (44\%) \) respectively, showing that Positive gene have higher incidence of HCV and HCC infection than Negative gene, when compared to control group. This study agreed with that reported by El-Sherif et al. [23] who found that VEGF was significantly higher among patients than controls and it was more significantly elevated in HCC cases than in those with other groups. The present study showed that in all groups There was a highly significant increase in Alk.phos., GOT, GPT and WBC activity in Positive gene \( C936T \) polymorphism of the VEGF in all patients \( P<0.05, P<0.001 \) respectively. Also there was a nonsignificant decrease in other parameters in the of positive and negative gene \( C936T \) polymorphism of the VEGF activity in all patients \( P>0.05 \).
In the present study we found that there was a highly significant increase in WBC and PLT activity in Positive gene C936T polymorphism of the VEGF in HCC patients \( P < 0.001 \), \( P < 0.05 \) respectively. Also there was a nonsignificant decrease in other parameters in the of positive and negative gene C936T polymorphism of the VEGF activity in HCC patients \( P > 0.05 \). Therefore, platelets may transport VEGF to its target cells, restricting the angiogenic activity of circulating VEGF to sites where coagulation takes place, such as a healing wound [24]. This view is supported by the fact that platelet aggregation with cancer cells is commonly observed during the process of angiogenesis and metastasis [25]. There was no significant change between positive and negative gene C936T polymorphism of the VEGF in all patients. Regarding sex distribution among discovered cases; the proportion of female 7 (36.8%) is slightly more than male proportion 6 (19.4%) this result in positive gene C936T polymorphism of the VEGF in all patients. but, there was no significant between positive and negative gene C936T polymorphism of the VEGF in HCC patients. Regarding sex distribution among discovered cases; the proportion of female 6 (54.5%) is slightly more than male proportion 5 (45.5%) this result in positive gene C936T polymorphism of the VEGF in HCC patients. In the present study we found that males have higher liver cancer rates than females ranges in HCV and HCC group about 64% and 56% for males in both groups respectively. That finding was in agreement with that reported by Hagymási and Tulassay, [26]. In addition, we found that the incidence of HCV and HCC is higher in the older patients in comparison to the control and this difference was significant when compared to control. That finding was in agreement with that reported by Velazquez et al. [27] who stated that a prospective Spanish analysis of risk factors for HCC found a 4-fold greater risk for developing HCC in patients older than 54 years, while El-Serag and Mason [28] found that the incidence of HCC increases in older age was associated with a higher risk, but the incidence among younger persons also rose progressively. This study also agrees with that reported by Mohamed et al. [29]. Arrieta et al. [30] found a greater proportion of men and a greater mean age for patients with HCC.

In the present study we observed significant increase in both serum ALT and AST in the HCC group compared to control group these results agree with the results obtained by Vincent [31] who reported that viral hepatitis was encountered with dramatic increase of liver enzymes and different degrees of hepatic inflammation as well as fibrosis. The present study showed that there was a highly significant increase in serum ALP activity in the HCC group when compared to control group and untreated HCV infected group \( P < 0.001 \), but there was a non significant increase in serum ALP activity in the untreated HCV infected group when compared to control group \( P = 0.249 \). This was in agreement with that reported by Sleisenger and Fordtran [32] and Merco et al. [1] who found that the conventional tests of hepatic function don’t distinguish hepatocellular carcinoma from other hepatic masses or from cirrhosis. In this study there is a significant increase in serum total bilirubin in the HCC groups compared to control group, but showing non significantly variations between HCC group and HCV group. This result agree with the results reported by Wahib et al. [33], while disagree with Tong et al. [34] who found that HCC patients had higher values of bilirubin, alkaline phosphatase, aspartate aminotransferase and, alanine aminotransferase than those of chronic carriers.

The present study suggested there was a highly significant decrease in serum albumin and T-Protein activity in the HCC group compared to control, but showing nonsignificant decrease in serum albumin activity in the untreated HCV infected group when compared to control group, this result agree with the result obtained by Shaker et al. [35] who reported that plasma Albumin level showed a significant decrease as compared to normal control group. In the present study there was a significant decrease in serum T. Protein level in the untreated HCV infected group when compared to control group, this result agree with that showed by Mahdy et al. [36].

In the present study there was a highly significant increase in serum creatinine activity in the HCC group compared to control and untreated HCV infected group \( P < 0.001, P = 0.004 \) respectively, this result disagree with Nguyen et al. [37] and Zakhary et al. [38] who reported that the level of creatinine non significantly decrease in HCC group compared to other studied groups.

In the present study there was a highly significant decrease in Hemoglobin and Platelets activity in the HCC group and untreated HCV infected group when compared to control group, and also, there was a non significant increase in white blood cells (WBC) activity in the HCC group and untreated HCV infected group when compared to control group. this result agree with El-Sherif et al. [23] and Noritake et al. [39] who reported that there was a highly significant increase in Hemoglobin and Platelets counts activity in the HCC group when compared to other studied groups \( P < 0.001 \).

In the present study there was a highly significant increase in serum (HCV) antibodies in the HCC group and untreated HCV infected group when compared to control group, and also, there was a highly significant increase in serum (HCV) antibodies activity in the HCC group when compared to untreated HCV infected group, this result agree with Baghdady et al. [2] who reported that In Egypt, HCV is the main risk factor for HCC,wherein 71% of the HCC cases are positive for anti-HCV antibodies.

In the present study a highly significant increase in serum (AFP) level in the HCC group compared to control group. This result agree with that stated by Baghdady et al. [2] who declared that significantly elevated serum levels of alpha fetoprotein (AFP) in patients with HCC compared to control participants as detected by enzyme-linked immunosorbsent assay, and also, There was a non-significant increase in serum (AFP) level in the untreated HCV infected group compared to control group.

In our study there was nonsignificant correlation between serum AFP and other biochemical parameters ALT, AST, ALP, T. bilirubin, D. bilirubin, albumin, and T. protein, creatinine, Hb, WBC, PLT in HCC group, This result agree with those supported by Nguyen et al. [37] Who found that no significant correlation was found between serum AFP levels and gender, various tumor factors. Also, there was a significant correlation between AFP and Age in HCV group \( P < 0.05 \), this result agree with Bruce et al. [40] stated that elevated AFP level was significantly correlation associated with increasing age in HCV group. In the present study we found that There was a significant negative correlation between AFP and HCV (ab) in HCC group \( P < 0.001 \), this result agree with those supported by Zainal et al. [41] who found that There is a growing number of evidence that AFP levels are significantly raised in anti-HCV- positive HCC group. In our study when using receiving operator characterizing curve (ROC curve) to reach the value of the best sensitivity and specificity for AFP, it was found that at a value of 3.39 ng/ml (the best cut off) yields, the sensitivity was 52% and the specificity was 40%. Zakhary et al. [38] reported a cutoff of as a diagnostic cutoff. According to our results, at a cut-off 28 ng/dl shows the sensitivity was (73.3%) and specificity was (75%). This result was in...
according to chen et al. [42] reported a cutoff of as a diagnostic cutoff. According to our results, at a cut-off 15.6 ug/L shows the sensitivity was (34.3%) and specificity was (83.6%). Concluding that reducing the cut-off means that more HCCs would be identified, but at the cost of a progressive increase in the false positive rate.

Conclusion
In conclusion, it has been found that the presence of gene C936T is strongly correlated with HCC cases, and Alpha fetoprotein (AFP) is serological marker specific for HCC group with HCC.

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