BioBacta

Journal of Bioscience and Applied Research
www.jbaar.org

# 8-hydroxy-2'-deoxyguanosine and TP53 in Egyptian Patients with Hepatitis C Viral Chronic Liver Diseases: Insight into the Pathogenesis and Predictive Force

**Hoda M. El-Emshaty[1,*], Somaia M. Osman [2], Fathy M. El-Taweel[2], Mohamed M. El-Hemaly[1], Hisham Ismail [3]**

[1]Gastrointestinal Surgery Center, Faculty of Medicine, Mansoura University, Mansoura, Egypt
[2]Chemistry Dept., Faculty of Science, Damietta University, New Damietta, Egypt.
[3]Biochemistry Division, Chemistry Dept., Faculty of Science, Minia University, Minia, Egypt.

**Running title:** Predictive force of serum 8-OHdG in HCV-chronic liver diseases

***Corresponding Author: Dr. Hoda Mohamed El-Emshaty**
*Professor of Biochemistry, Gastrointestinal Surgical Center, Faculty of Medicine, Mansoura University, Mansoura, Egypt*
**E-mail:** elemshaty_h@yahoo.com

DOI: 10.21608/jbaar.2022.223518

**Abstract**

Reactive oxygen species (ROS) is excessively generated during tumor development yielding the oxidatively modified products of proteins and DNA. These DNA alterations could contribute to the development of cancer through the activation of oncogenes and inactivating tumor suppressor genes (TSGs). Therefore, 8-OHdG DNA oxidative damage and TP53 protein expression were evaluated amongst HCV-Chronic liver disease patients to explore their possible role in hepatocarcinogenesis and to predict HCC development at early stages. A total of 141 patients with HCV-related liver diseases; 69 with hepatocellular carcinoma and 72 with liver cirrhosis were enrolled in this study in addition to 56 healthy subjects. Serum 8-OHdG and TP53 expression by ELISA were markedly elevated in HCC patients compared to LC and healthy individuals ($p<0.0001$). A significant correlation was noted for 8-OHdG and TP53 with disease progression and tumor differentiation but not with tumor site. 8-OHdG and TP53 were highly ($p<0.05$) predicting for HCC at early stages and the diagnostic performance for discriminating HCC from LC by ROC curve showed the best AUC was recorded for 8-OHdG (0.745) followed by TP53 (0.667) with accuracy (87.2% and 82% respectively). Therefore, HCV-induced oxidative DNA damage could increase the carcinogenic potential of HCC development through the activation of TP53.

***Keywords:*** 8-OHdG, TP53 Ag, ELISA, HCV, Hepatocellular carcinoma.

---

## Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death, primarily caused by viral infections. Only about 71 million chronic HCV-infected patients worldwide remain at high risk of HCC in the near future **(Zhao et al., 2021)**. Exposure to HCC accounts for 70.48% of all Egyptian liver tumors **(AbdelAtti, 2015)**. This remarkable increase may be due to the high incidence of HCV infection, a major risk factor for liver cirrhosis-induced HCC

Received: January 25, 2022. Accepted: February 27, 2022. Published: March 8, 2022

**(Abd-Elsalam et al., 2018)**. Though, active research aimed at elucidating the molecular pathogenesis of HCC and identifying new biomarkers will result in further advances in the prevention, diagnosis, and treatment of HCC **(Tinke and Haas-Kogan, 2012)**.

Reactive oxygen species (ROS) is excessively generated in tumor's create oxidative stress in the tumor microenvironment **(Ma-on et al., 2017)**. Accumulated ROS can result in structural cellular and/or genetic alterations modulating crucial triggers of tumorigenesis, especially at the initial steps of carcinogenesis. In nuclear and mitochondrial DNA, 8-hydroxy-2'-deoxyguanosine (8-OHdG) or 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) are the most universally observed single nucleotide-base lesions that might induce mutations in replicating DNA. These free radical-induced oxidative lesions are potential biomarkers of oxidative DNA damage **(Cadet et al., 2012)**, though continuously repaired by intracellular base excision repair (BER). These DNA alterations, that activate oncogenes and inactivate tumor suppressor genes (TSGs), could contribute to the development of cancer in oxidative stress-affected organs **(Nishida and Goel, 2011).**

Tumor suppressor P53 is a nuclear transcription factor that regulates several genes involved in apoptosis, cell cycle arrest, and growth. It is activated by genotoxic or cellular stresses such as oxidative stress, DNA damage, and malnutrition **(Lujambio et al., 2013)**. The p53 gene encompasses 20Kb of DNA with 11 exons and when transcribed, it produces 3.0Kb mRNA having a 1179bp open reading frame. On translation, this mRNA produces a 53KDa protein **(Foulkes, 2007)**. Either truncating mutations or point mutation of the P53 gene may lead to the absence or malfunctioning of the P53 **(Van Dyke, 2007)**, causing the loss of apoptosis control affecting senescence, cell cycle regulation, DNA repair damage, angiogenesis, and oxidative stress regulation; all are essential in carcinogenesis **(Vousden and Lane, 2007)**. Therefore, P53 is generally accepted as a component of the biochemical pathways central to human carcinogenesis, and detection of its abnormalities during tumor development may have diagnostic, prognostic, and therapeutic implications **(Inoue et al., 2012)**. Thus, it is interesting to study the correlative analysis of TP53 Oncoprotein expression with 8-OHdG DNA oxidative damage among HCV- induced chronic liver disease patients to explore their possible role in liver carcinogenesis and to predict the early development of HCC.

## Subjects and Methods

### Study population

A total of 141 Egyptian patients who suffered from HCV-chronic liver diseases admitted at Gastrointestinal Surgery Center (GISC), Mansoura University during the period from November 2019 to January 2021 were enrolled in this study. After clinical, radiological, and laboratory investigations, all selected patients had positive reactivity for HCV RNA with no serological evidence for other hepatotropic viruses or any other causes of liver diseases. All patients were histopathologically diagnosed and categorized into: 69 (54 men and 15 women; mean age 60.08 ± 7.43yrs) patients with HCC and 72 (54 men and 18 women; mean age 57.92 ± 8.86yrs) patients with LC. Tumor grading of HCC was categorized as grade I in 15 (21.7%) patients, grade II in 36 (52.2%), and grade III in 18 (26.1%) of HCC patients. HCC usually arose in the cirrhotic liver (69.6%) and as regards a tumor site, HCC was located mainly in the right lobe 78.3% (54/69) and only 15 (21.7%) were detected in the left lobe. Additionally, 56 (32 men and 24 women; mean age 43.93±6.48yrs) healthy subjects were selected without a clinical history of hepatitis and with no symptoms or signs of liver disease. This research was performed according to the ethical guidelines of the Helsinki Declaration **(World Medical Association, 2013)** and the study protocol was approved by the Ethics Committee of GSC, Mansoura University.

**Informed consent statement:** Informed written consent for all participants was provided before study enrolment.

**Biochemical and Virological analysis**

Blood samples of all individuals were centrifuged and the sera were stored at -70 °C until used or processed immediately for measuring the activity of the biochemical parameters; ALT, AST, total bilirubin, and albumin (Hitachi 750XRC Analyzer). HCV Abs was detected by third-generation ELISA (Biochem. Immunosystem Company) and HCV-RNA was quantitatively detected using RT-PCR according to the method described by **Pawlotsky (2003)**. AFP tumor marker was investigated using AFP kit (Abbott Laboratories, USA) and the results were automatically calculated using 1Mx Abbott equipment

**Assessment of Serum 8-OHdG**

Serum 8-OHdG concentrations were quantitatively measured by ELISA Kit using the competitive-ELISA principle (Elabscience Biotechnology Inc., USA) according to the manufacturer's instructions. 8-OHdG in the sample or standard competes with a fixed amount of 8-OHdG in a pre-coated microplate. Biotinylated detection antibody specific to 8-OHdG was applied next with avidin conjugated horseradish peroxidase (HRP) and TMB substrate solution. The developed color was measured spectrophotometrically at wavelength 450±2 nm (detection range 1.56 - 100 ng/mL).

**Assessment of Human TP53 (Tumor protein p53)**

Serum TP53 concentrations were quantitatively measured by ELISA kit using the Sandwich-ELISA principle (Elabscience Biotechnology Inc., USA). Samples and standards were added to the pre-coated micro ELISA plate with an antibody specific to human TP53. Biotinylated detection antibody specific to TP53 was applied subsequent with avidin-HRP conjugate and substrate. The developed color was measured spectrophotometrically at wavelength 450 ±2 nm (detection range 78.13 - 5000 pg/mL).

**Statistical Analysis**

Data were analyzed using SPSS (version 17, Sydney, NSW, Australia). Data for continuous variables were expressed as mean±SD or median (IQR) and for categorical variables as frequencies and percentages. The significance between categorical variables was analyzed by Chi-square test and for continuous variables; Kruskal Wallis and Mann-Whitney U tests were used. The receiver operating characteristic curve (AUROC) was utilized for the assessment of the diagnostic performance of investigated biomarkers.

**Results**

Clinical and laboratory investigations of study group patients and healthy individuals (HI) were listed in **table 1**. HCC patients showed higher expression of serum AST, ALT and lower expression of serum albumin, total bilirubin, platelet count (PLT) compared to LC ($p<0.05$). Liver cirrhotic patients compared to healthy subjects showed higher expression of AST, ALT, T.bilirubin with lower expression of albumin and PLT count ($p<0.0001$). Serum AFP was detected in HCC patients within normal limit (≤15 ng/mL) in 21/69 (30.4%), moderate elevation (15-400 ng/mL) in 27/69 (39.1%) and above the upper reference limit (>400 ng/mL) in 21/69 (30.4%). HCC patients showed significant differences as regard to Age, tumor site ($p<0.0001$), and cirrhotic liver ($p=0.002$).

Serum levels of 8-OHdG, TP53, and AFP expression with the clinicopathological characteristics of chronic HCV-liver disease patients compared to HI were listed in **table 2**. Serum 8-OHdG was significantly ($p<0.0001$) expressed in HCC patients compared to LC and HI and also, between LC and HI. 8-OHd8G gradually increased with tumor grading and the highest elevation at HCC grade III compared to grade I and II. According to the cut-off value (≥39.0 ng/ml), 8-OHdG was considered positive in 57/69 (82.6%) of HCC patients, 61.1% (44/72) of LC and 4/56 (7.1%) of HI ($p<0.0001$). Positive cases for 8-OHdG were frequently detected at HCC grade III compared to grades I and II (100% vs 40% and 91.7%, $p<0.0001$). TP53 Oncoprotein level showed progressive elevation from HCC to LC compared to

HI. Higher expression of TP53 was recorded in HCC grade III compared to grades I and II. TP53 positive cases (cut-off ≥0.85 ng/ml) was detected in 68.1% (47/69) of HCC patients; 6.7% (1/15) of HCC grade I, 77.8% (28/36) of grade II and 100% (18/18) of grade III ($p<0.0001$).

The serum level of AFP in HCC patients ranged from 1.53 to >2000ng/ml was highly elevated ($p<0.0001$) compared to LC and HI. Significant elevation was recorded in HCC grade III compared to grade I ($p=0.022$) and grade II ($p=0.004$) but no significant difference was recorded between grade I and II. Serum AFP was detected positive (> 15ng/ml) in 48/69 (69.6%) of HCC patients and within normal limit (≤15ng/ml) in 21/69 (30.4%) of already diagnosed HCC. As regard tumor sits, serum 8-OHdG and TP53 showed no significant difference but AFP was detected significant ($p<0.0001$). HCC patients showed higher expression of TP53 and 8-OHdG ($p<0.05$) evaluated by HCC with the cirrhotic liver.

The incidence of positive cases for OHdG and TP53 in HCC patients with positive AFP expression was illustrated in **figure 1**. Positive expression of 8-OHdG and TP53 was recorded in 70.4% and 55.6% of modestly elevated AFP (15-400 ng/mL), in 95.2% and 90.5% of AFP >400 ng/mL but AFP within the normal limit (≤15 ng/mL) showed 61.9% and 85.7% were positive for TP53 and 8-OHdG respectively suggesting its importance for the diagnostic sensitivity of HCC. The correlation of 8-OHdG, TP53, and AFP with the clinical characteristics of HCC patients was recorded in **table 3**. 8-OHdG was associated with TP53 and AFP ($p<0.0001$), and TP53 was associated with AFP ($p=0.047$). A significant correlation was recorded for 8-OHdG, TP53, and AFP with ALT, AST, tumor grading, and disease progression but no correlation was detected with tumor site. 8-OHdG, TP53, and AFP were significantly predicted for disease progression and HCC development at early stages (**table 4**). The diagnostic performance of 8-OHdG, TP53, and AFP for discriminating HCC from LC was listed in **table 5** and illustrated in **figure 2** using the ROC curve.

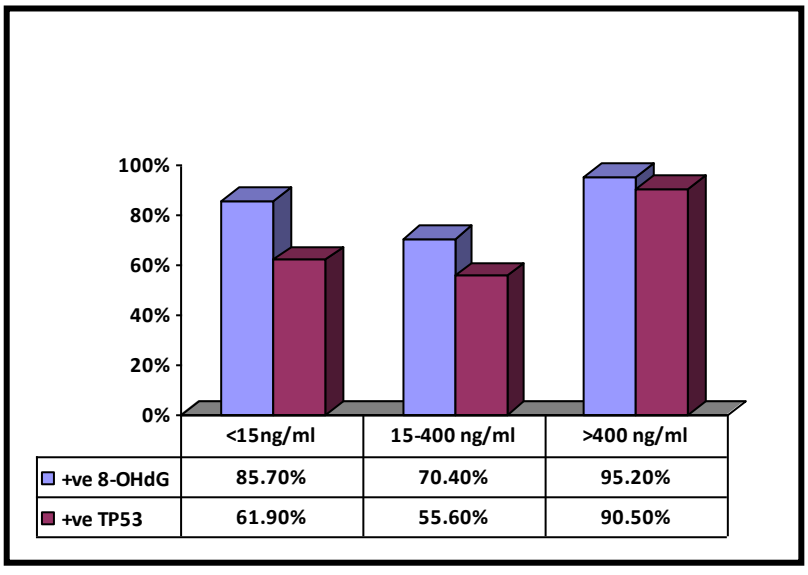


Figure 1 Incidence of positivity for serum OHdG and TP53 in HCC patients positive for AFP expression

Table 1. Clinical characteristics and laboratory investigations of the study groups

| | HCV-Chronic Liver Disease | | HI | |
|---|---|---|---|---|
| | HCC (n=69) | LC (n=72) | (n=56) | *P-value* |
| Gender (n%)<br>Female<br>Male | <br>15 (21.7%)<br>54 (78.3%) | <br>18 (25%)<br>54 (75%) | <br>24 (42.9%)<br>32 (57.1%) | |
| | *P <0.0001* | *P <0.0001* | *NS* | |
| Age (yrs) | 60.86±8.9 [a,b] | 54.01±6.9 [a,c] | 43.93±6.48 [b,c] | [a,b,c] ***P<0.0001*** |
| Albumin (g/dl) | 3.2 (0.3) [a, b] | 3.5 (0.77) [a, c] | 4.2 (0.8) [b,c] | [a,b,c] ***P<0.0001*** |
| T.bilirubin (mg/dl) | 2.0 (1.95) [a,b] | 2.7 (3.4) [a, c] | 0.7 (0.4) [b, c] | [a] ***P=0.019***<br>[b] ***P= 0.001***<br>[c] ***P< 0.0001*** |
| AST (U/ml) | 74.0 (54.0) [a, b] | 68.0 (33.0) [a, c] | 29.5 (7.0) [b,c] | [a] ***P=0.018***<br>[b,c] ***P <0.0001*** |
| ALT (U/ml) | 48.0 (41.5) [a, b] | 46.0 (40.0) [a, c] | 23.5 (6.75) [b,c] | [a] ***P=0.014***<br>[b,c] ***P <0.0001*** |
| Pt count ($x10^9$) | 65.0 (70.0) [b] | 84.5 (49.0) [c] | 254.0 (39.0) [b, c] | [b,c] ***P<0.0001*** |
| Creatinin (mg/dl) | 0.8 (0.6) | 0.75 (0.58) | 0.8 (0.1) | ***>0.05*** |
| AFP (ng/ml)<br><15 ng/ml<br>15-400 ng/ml<br>>400 ng/ml | <br>21/69 (30.4%)<br>27/69 (39.1%)<br>21/69 (30.4%) | <br>42/72 (80.8%)<br>10/72 (19.2%)<br>0/72 (0%) | <br>56/56 (100%)<br>0/56 (0%)<br>0/56 (0%) | ***P < 0.0001*** |
| Tumor site<br>Right lobe<br>Left lobe | <br>54 (78.3%)<br>15 (21.7% | | | |
| | ***P <0.0001*** | | | |
| Frequency of Cirrhosis<br>HCC associated with LC<br>HCC | <br>48 (69.6%)<br>21 (30.4%) | | | |
| | ***P= 0.002*** | | | |

Data expressed as mean±SD, median (interquartile range IQR), or frequency (%)

Significant difference at p<0.05 between HCC and LC (a), between HCC and HI (b), between LC and HI (c),

Abbreviations; Hepatocellular carcinoma (HCC), Liver cirrhosis (LC), Healthy individuals (HI)

Table 2. 8-OHdG, TP53 protein expression and AFP with the clinicopathological characteristics of patients with HCV-related liver diseases

| **Variable** | **8-OHdG (ng/ml)** | | **TP53 (ng/ml)** | | **AFP (ng/ml)** | |
|---|---|---|---|---|---|---|
| | **Median (IQR)** | **Positivity (%)** | **Median (IQR)** | **Positivity (%)** | **Median (IQR)** | **Positivity (%)** |
| **HCV-liver diseases** | | | | | | |
| **HCC (n= 69)** | 97.8 (92.0) [a, b] | 57/69 (82.6%) | 2.4 (2.05) [a, b] | 47/69 (68.1%) | 35.0 (1979.3) [a, b] | 48/69 (69.6%) |
| **LC (n= 72)** | 54.5 (36.0) [a, c] | 44/72 (61.1) | 1.17 (1.48) [a, c] | 31/72 (43.1%) | 3.45 (10.6) [a] | 8/52 (15.4%) |
| **HI (n= 56)** | 35.0 (8.0) [b, c] | 4/56 (7.1%) | 0.35 (0.6) [b, c] | 0/56 (0%) | 4.95 (2.43) [b] | 0/56 (0%) |
| ***P value*** | **[a, b, c] <0.0001** | **<0.0001** | **[a, b, c] <0.0001** | **<0.0001** | **[a, b] <0.0001** | **<0.0001** |
| **HCC Grading** | | | | | | |
| **Grade I (n=15)** | 50.0 (15.75) [a, b] | 6/15 (40%) | 0.85 (1.3) [a, b] | 1/15(6.7%) | 25.3 (1497.4) [b] | 12/15 (80%) |
| **Grade II (n=36)** | 97.4 (65.0) [a, c] | 33/36 (91.7%) | 2.45 (1.69) [a, c] | 28/36 (77.8%) | 34.3 (241.55) [c] | 21/36 (58.3%) |
| **Grade III (n=18)** | 165.0 (18.0) [b, c] | 18/18 (100%) | 3.25 ( 0.77) [b, c] | 18/18 (100%) | 2000 (1609.8) [b, c] | 15/18 (83.3%) |
| ***P-value*** | **[a, b, c] <0.0001** | **<0.0001** | **[a, b] <0.0001** **[c] 0.023** | **<0.0001** | **[b] P=0.005** **[c] P< 0.0001** | >0.05 |
| **Tumor site** | | | | | | |
| **Right lobe (n=54)** | 97.4 (85.0) | 43/54 (79.6%) | 2.4 (2.05) | 34/54 (63.0%) | 29.4 (468.9) | 33/54 (61.1%) |
| **Left lobe (n=15)** | 100.0 (70.0) | 14/15 (93.3%) | 2.8 (2.45) | 13/15 (86.7%) | 2000.0 (1709.0) | 15/15 (100%) |
| ***P* <0.0001** | >0.05 | >0.05 | >0.05 | >0.05 | **<0.0001** | **0.002** |
| **Cirrhosis** | | | | | | |
| **HCC on top of LC (n=48)** | 75.0 (80.95) | 30/48 (62.5%) | 1.8 (2.25) | 36/48 (75%) | 34.3 (1781.4) | 36/48 (75%) |
| **HCC (n=21)** | 140.0 (58.0) | 17/21 (81.0%) | 2.8 (1.3) | 21/21 (100%) | 35.0 (1988.9) | 12/21 (57.1%) |
| ***P*=0.002** | **<0.0001** | >0.05 | **0.003** | **0.013** | >0.05 | >0.05 |
| **Age (yrs)** | | | | | | |
| **<60 (55.0, 6.5)** | 60.0 (108.0) | 21/29 (72.4%) | 1.5 (1.94) | 19/29 (65.5%) | 26.6 (283.7) | 20/29 (69.0%) |
| **≥≥60 (66.0, 7.0)** | 100.0 (48.0) | 36/40 (90%) | 2.27 (2.36) | 28/40 (70.0%) | 35.75 (1988.9) | 28/40 (70.0%) |
| ***P<0.0001*** | >0.05 | >0.05 | >0.05 | >0.05 | **0.037** | >0.05 |

Data expressed as median (IQR) and the difference between quantitative variables was calculated by Kruskal-Wallis H and Mann-Whitney U. Qualitative data were expressed as frequencies (%) and the difference between variables was calculated by Pearson Chi-square

Positive TP53 was calculated at cut-off ≥0.85 ng/ml

Positive 8-OHdG was calculated at cut-off ≥39.0 ng/ml

P-value considered significant at <0.05 between HCC and LC (a), between HCC and HI (b), between LC and HI (c)

**Table 3** Spearman correlation (rho) of 8-OHdG, TP53, and AFP with the clinical characteristics and laboratory investigations of HCC patients

| | AST | ALT | Albumin | T.bilirubin | Pt count | 8-OHdG | TP53 | AFP |
|---|---|---|---|---|---|---|---|---|
| 8-OHdG | 0.711**<br>0.000 | 0.404**<br>0.001 | 0.316**<br>0.008 | -0.219<br>0.071 | 0.093<br>0.479 | 1 | 0.669**<br>0.000 | 0.440**<br>0.000 |
| TP53 | 0.633**<br>0.000 | 0.383**<br>0.001 | 0.120<br>0.325 | -0.027<br>0.827 | 0.187<br>0.153 | 0.669**<br>0.000 | 1 | 0.240*<br>0.047 |
| AFP | 0.520**<br>0.000 | 0.380**<br>0.001 | -0.09<br>0.46 | 0.048<br>0.69 | 0.423**<br>0.001 | 0.440**<br>0.000 | 0.240*<br>0.047 | 1 |

**Correlation is significant at the 0.01 level (2-tailed)
*correlation is significant at the 0.05 level (2-tailed)

**Table 4** Regression analysis for 8-OHdG, TP53, and AFP to predict disease progression from LC to HCC

| | B | SE | P | Exp (B) |
|---|---|---|---|---|
| 8-OHdG | -1.106 | 0.399 | 0.006 | 0.331 |
| TP53 | -1.039 | 0.351 | 0.003 | 0.354 |
| AFP | -2.531 | 0.465 | 0.000 | 0.08 |

**Table 5** ROC curve to discriminate HCC from LC by 8-OHdG, TP53, and AFP

| | AUC | SE | p | 95% CI | | Sensitivity | Specificity | Accuracy |
|---|---|---|---|---|---|---|---|---|
| | | | | Lower bound | Upper bound | | | |
| 8-OHdG | 0.745 | 0.041 | 0.000 | 0.664 | 0.826 | 75.8 | 59.9 | 87.2% |
| TP53 | 0.667 | 0.046 | 0.001 | 0.577 | 0.757 | 67.1 | 62.1 | 82% |

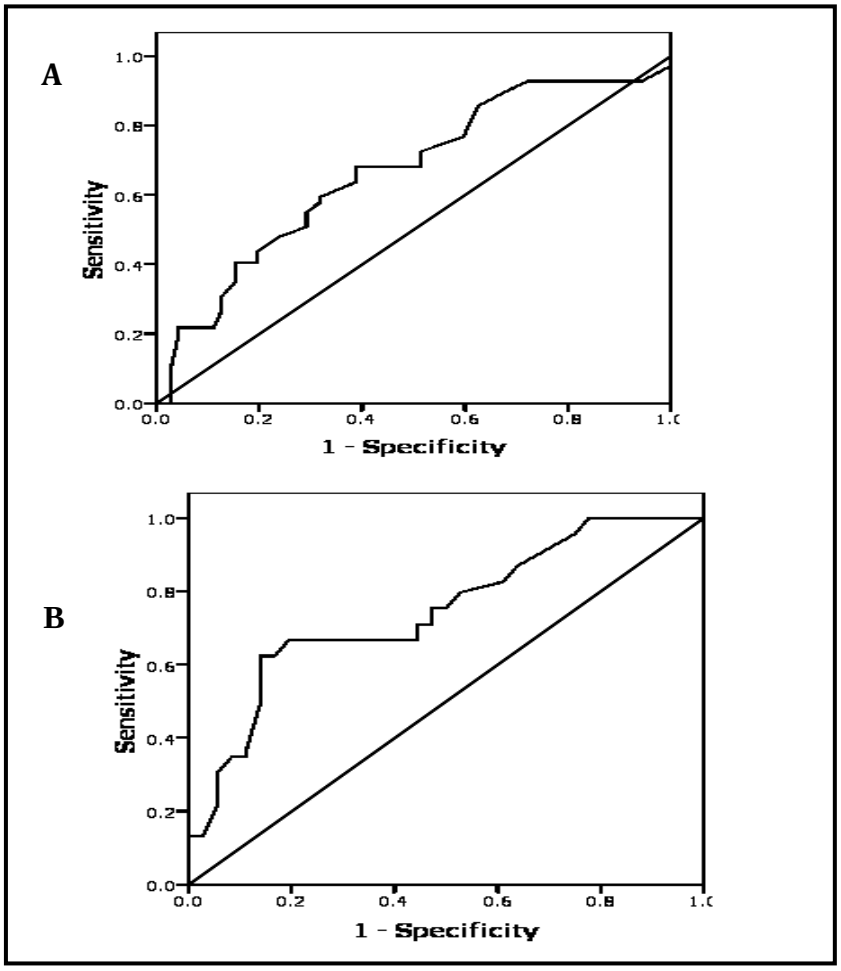


Figure **2** Receiver operator characteristic (ROC) curve of 8-OHdG (A) and TP53 (B) for discriminating HCC from LC patients

**Discussion**

Hepatocellular carcinoma (HCC) is the most primary liver cancer and usually arises in cirrhotic livers **(Rastogi, 2020)**. HCC patients in this study are on average age 60.09±7.43 yrs concur with others **(Zahran et al., 2017)** and HCC was developed in 69.6% of a cirrhotic liver.

The increased oxidative stress is associated with hepatitis viral infections and HCC progression **(Tanaka et al., 2008)**. 8-OHdG or 8-oxodG is generally accepted as a potential biomarker for measuring the effects of endogenous oxidative damage to DNA as an important factor in the initiation and promotion of carcinogenesis **(Korkmaz et al., 2018)**. 8-OHdG oxidative damage to DNA was elevated successively with disease progression in the current study and was significantly expressed in association with tumor differentiation ($p<0.0001$) but not to cirrhosis or tumor site. 8-OHdG was also associated with liver transaminases; AST and ALT, so, whether oxidative damage is the cause of these clinical features, thus upsetting tumor progression indirectly, or cooperates with these features to directly alter tumor progression requires further analysis **(Li et al., 2012)**. Yet, **Ma-on et al. (2017)** did not find a significant association of tumor differentiation ($p=0.152$), number of tumor mass ($p=0.538$), cirrhosis ($p=0.150$) and metastasis ($p=0.190$) with 8-OHdG.

Accumulation of P53 proteins has been reported to be involved in the carcinogenesis, progression, and metastasis of many human cancers **(Lee et al., 2013)**. Serum TP53 was highly expressed in HCC patients in the current study compared to LC (p=0.001) and a successive increase was recorded with HCC progression. P53 protein accumulation was associated with transaminases (AST, ALT) and significant elevation was noticed related to poor tumor differentiation (P<0.0001) reliable to other reports **(Abdou et al., 2021; El-Emshaty et al., 2014)**. Thus poorer cellular differentiation is usually late stages of hepatocarcinogenesis **(Wong et al., 2001)** which may explain the higher expression of TP53 at higher grades of HCC. The proportion of serum TP53-positive HCC cases was 68.1% of existing HCC patients compared to 50% and 55.6% positivity by others **(Cengiz et al., 2003 and El-Emshaty et al., 2014,** respectively). Several immunohistochemical studies have also recorded the proportion of p53-positive HCC cases fluctuates between 22% and 81% **(El-Emshaty et al., 2014 and Mitselou et al., 2010)**. These discrepancies could be accounted for as the interpretation of staining intensity is highly subjective **(Mitselou et al., 2010)**. Yet, **Chai et al. (2017)** reported that P53 expression was not related to cancer characteristics.

Increased 8-OHdG levels are closely related to the accumulation of mutations triggering carcinogenesis **(Korkmaz et al., 2018)**. 8-OHdG levels were strongly associated with TP53 in existing HCV-related liver diseases patients and both were highly correlated with disease progression and poor tumor differentiation suggesting their potential role in HCV hepatocarcinogenesis as the accumulated oxidative DNA damage induced by HCV could increase the carcinogenic potential of HCC through activation of TP53. However, the generation of ROS and 8-OHdG could play a critical role in the abnormal methylation of TSGs, which in turn should play an important role in the early stages of HCV-related human hepatocarcinogenesis **(Nishida et al., 2013)**.

The role of AFP in the diagnosis of HCC is controversial. AFP can be elevated in other primary malignancies of the liver as well as metastasis and is within normal limits in a large proportion of patients with known HCC **(Bruix et al., 2011)**. The low sensitivity of serum AFP (69.6%) in the current study comparable to 69.3% **(Raedle et al., 1998)** and 58.46% **(Gadelhak et al., 2009)** makes its value restricted in HCC diagnostic and prognostic approaches. Accordingly, some studies have reported that combined analysis improved the diagnostic and prognostic value of AFP in HCC **(Wang et al., 2017; Kamiyama et al., 2017)**. Thus, the diagnostic performance of 8-OHdG and TP53 for discriminating HCC from LC by ROC curve in the current study showed the best AUC was recorded for 8-OHdG (0.745) followed by TP53 (0.667) with accuracy 87.2% and 82% respectively. Further, TP53 and 8-OHdG expression levels simultaneously with AFP were reliably predicted for disease progression and HCC development in the current study as previously reported **(Zhu et al., 2020)** that joint analysis of AFP, P53, and VEGF might be performed for the prediction of the clinical outcome of patients with HBV-related HCC in Guangxi. **In conclusion:** Serum 8-OHdG and TP53 might play a role in the pathogenicity of HCV-related liver diseases and may be useful for tumor assessment as early predictors.

***Acknowledgements***

We thank the Late Prof. Nabil A. Gadelhak, Professor of Digestive Surgery at Gastrointestinal Surgery Center, Mansoura University for providing the facilities and support.

***Ethics***

The study originates from a master thesis. Its protocol was approved by the Ethics Committee of GISC, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

***Availability of Data***

All the authors declare that they read and agreed to the content of the submitted article and are

responsible for the validity and originality of the data contained therein. The original data is available on request from the corresponding author (email: elemshaty_h@yahoo. com).

***Conflict of interest***

All the authors declare that they have no conflict of interest regarding this study.

**References**

**Abdel-Atti E. (2015).** HCC burden in Egypt. Gastroenterol Hepatol; 2: 00045.

**Abd-Elsalam S, Elwan N, Soliman H. Ziada D, Elkhalawany W, Salama M, Hawash N, Arafa M, Badaw R, Shehata WM, Khalil HS, Elmashad N. (2018)**. Epidemiology of liver cancer in Nile delta over a decade: a single-center study. South Asian J Cancer; 7: 24-26.

**Abdou AG, Holah NS, Elazab DS, El-Gendy WG, Badr MT, Al-Sharaky DR. (2021).** Hepatocellular carcinoma score and subclassification into aggressive subtypes using immunohistochemical expression of p53, B-Catenin, CD133 and K-67. Appl Immunohistochem Mol Morphol; 29:20-33.

**Bruix J, Sherman M. (2011).** Management of hepatocellular carcinoma: an update. Hepatology; 53(3):1020-1022.

**Cadet J, Loft S, Olinski R, Evans MD, Bialkowski K, Wagner JR, Dedon PC, Moller P, Greenburg MM, Cooke MS. (2012).** Biologically relevant oxidants and terminology, classification and nomenclature of oxidatively generated damage to nucleobases and 2-deoxyribose in nucleic acids. Free Radical Research; 46: 367-381.

**Cengiz C, Akarca US, Goker E, Yuce G. (2003).** Detection of mutant p53 in hepatocellular cancer from turkey and its correlation with clinicopathologic parameters. Dig Dis. Sci; 48(5): 865- 869.

**Chai Y, Xiaoyu L, Haiyan W. (2017).** Correlation between expression levels of PTEN and p53 genes and the clinical features of HBsAg-positive liver cancer. *Journal of BUON: official journal of the Balkan Union of Oncology*; 22:942–6.

**El-Emshaty HM, Saad EA, Toson EA, Abdel Malak CA. Gadelhak NA. (2014).** Apoptosis and Cell Proliferation: Correlation with BCL-2 and P53 Oncoprotein Expression in Human Hepatocellular Carcinoma. Hepato-Gastroenterology; 61:103-111.

**Foulkes WD. (2007).** P53-master and commander. N Engl J Med; 357: 2539-41.

**Gadelhak NA, Gadelhak SA, El-Wahab El-Morsi DA, Abdelaziz MM, Abbas AT, El-Emshaty HM. (2009).** Prognostic Significance of Three Hepatitis Markers (p53 Antibodies, Vascular Endothelial Growth Factors and Alpha-Fetoprotein) in Patients with Hepatocellular Carcinoma. Hepato-Gastroenterology; 56: 1417-1424.

**Inoue K, Kurabayashi A, Shuin T, Ohtsuki Y, Furihata M**. **(2012).** Overexpression of p53 protein in human tumors. Med Mol Morphol; 45:115-123.

**Kamiyama T, Orimo T, Wakayama K, Shimada S, Nagatsu A, Yokoo H, Kamachi H, Yamashita K, Shimamura T, Taketomi A. (2017)**. Survival outcomes of hepatectomy for stage B Hepatocellular carcinoma in the BCLC classification. World journal of surgical oncology; 15:156.

**Korkmaz KS, Butuner BD, Roggenbuck D. (2018).** Detection of 8-OHdG as a diagnostic biomarker. Journal of Laboratory and Precision Medicine; 3:95-102.

**Lee YC, Wu WJ, Li WM. Lin H-H, Huang C-N, Chai C-Y, Chang L-L, Lin H-L, Ke H-L. (2013).** Prognostic value of p53 protein overexpression in upper tract urothelial carcinomas in Taiwan. Anticancer Res; 33(3):1091-1098.

**Li S, Wang X, Wu Y, Zhang H, Zhang L, Wang C, Zhang R, Guo Z. (2012).** 8-Hydroxy-2'-deoxyguanosine expression predicts hepatocellular carcinoma outcome. Oncol Lett; 3(2):338–42.

**Lujambio A, Akkari L, Simon J, Grace D, Tschaharganeh DF, Bolden JE. (2013).** Non-Cell-Autonomous Tumor Suppression by P53. Cell; 153:449–60.

**Ma-on C, Sanpavat A, Whongsiri P, Suwannasin S, Hirankarn N, Tangkijvanich P, Boonla C. (2017).** Oxidative stress indicated by elevated expression of Nrf2 and 8-OHdG promotes

hepatocellular carcinoma progression. Med Oncol; 34:57.

**Mitselou A, Karapiperides D, Nesseris I, Vougiouklakis T, Agnantis NJ. (2010).** Altered expression of cell cycle and apoptotic proteins in human liver pathologies. Anticancer Research; 30:4493-4502.

**Nishida N, Goel A. (2011).** Genetic and epigenetic signatures in human hepatocellular carcinoma: a systematic review. Curr Genomics; 12:130–137.

**Nishida N, Kudo M. (2013).** Recent Advancements in Comprehensive Genetic Analyses for Human Hepatocellular Carcinoma. Oncology; 84:93–7.

**Pawlotsky JM. (2003).** Hepatitis C virus genetic variability: pathogenic and clinical implications. Clin Liver Dis; 7:45-66.

**Raedle J, Oremek G, Truschnowitsch M, Lorenz M, Roth WK, Caspary WF, Zeuzem S. (1998).** Clinical evaluation of autoantibodies to p53 protein in patients with chronic liver disease and hepatocellular carcinoma. Eur J Cancer; 34 (8): 1198 - 1203.

**Rastogi A. (2020).** Pathomolecular characterization of HCC in non-cirrhotic livers. Hepatoma Res; 6:47.

**Tanaka H, Fujita N, Sugimoto R, Urawa N, Horiike S, KobayashiY, Iwasa M, Ma N, Kawanishi S, Watanabe S, Kaito M, Takei Y. (2008).** Hepatic oxidative DNA damage is associated with increased risk for hepatocellular carcinoma in chronic hepatitis C. Br J Cancer; 98: 580-586.

**Tinke CL, Haas-Kogan D. (2012).** Hepatocellular carcinoma: natural history, current management, and emerging tools. Biologics: Targets and Therapy; 6:207-219.

**Van Dyke T. (2007).** P53 and tumor suppression. N Engl J Med; 356: 79-81.

**Vousden KH, Lane DP. (2007).** P53 in health and disease. Nat Rev Mol Cell Biol; 8:275-83.

**Wang X, Zhang W, Liu Y, Gong W, Sun P, Kong X, Yang M, Wang Z. (2017).** Diagnostic value of prothrombin induced by the absence of vitamin K or antagonist-II (PIVKA-II) for early stage HBV related hepatocellular carcinoma. Infectious agents and cancer; 12:47.

**Wong IH, Yeo W, Leung T, Lau WY, Johnson PJ. (2001).** Circulating tumor cell mRNAs in peripheral blood from hepatocellular carcinoma patients under radiotherapy, surgical resection or chemotherapy: a quantitative evaluation. Cancer Lett; **167**:183–191.

**World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. (2013).** JAMA; 310(20), 2191-4.

**Zahran F, El-Emshaty H, Gouida M, Hussien M. (2017).** Assessment of Transforming Growth Factor β1 and DNA Cell Cycle in Egyptian Patients with Chronic Hepatitis-C Virus and Hepatocellular Carcinoma**.** IJSRSET; 3(2): 769-774.

**Zhang Y, Wang C, Xu H, Xiao P, Gao Y. (2019).** Hepatocellular carcinoma in the noncirrhotic liver: a literature review. Eur J Gastroenterol Hepatol; 31:743-738.

**Zhao P, Malik S, Xing S. (2021).** Epigenetic mechanisms involved in HCV-induced hepatocellular carcinoma (HCC). Frontiers in Oncology; 11: 677926.

**Zhu G-Z, Liao X-W, Wang X-K, Gong Y-Z, Liu X-G, Yu L, Han C-Y, Yang C-K, Su H, Huang K-T, Yu T-D, Huang J-L, Li J, Zeng Z-M, Qin W, Liu Z-Q, Zhou X, Liu J-Q, Lu L, Han Q-F, Shang L-M, Ye X-P, Peng T. (2020).** Comprehensive investigation of p53, p21, nm23, and VEGF expression in hepatitis B virus-related hepatocellular carcinoma overall survival after hepatectomy. J Cancer; 11(4): 906–918.