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The efficacy and adverse effects of nivolumab and lenvatinib in the treatment of advanced hepatocellular carcinoma

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ABSTRACT

This study intends to investigate nivolumab's efficacy and adverse effects in combination with lenvatinib in treating advanced hepatocellular carcinoma (HCC). For this purpose, ninety-two patients with unresectable advanced HCC admitted were enrolled and were divided into the control group (N=46) and the observation group (N=46) according to the random number table. The control group was treated with lenvatinib while the observation group was treated with nivolumab combined with lenvatinib. The efficacy, adverse effects, liver function, completion rate, interruption and discontinuation of treatment, drug reduction, serum tumor markers, and immune function were compared between the two groups. Also, changes in the expression of some genes that regulate the cell cycle (P53, RB1, Cyclin-D1, c-fos, and N-ras) were investigated in the development of this cancer. According to the results, ORR and DCR (45.65%, 78.26%) in the observation group were higher than those (23.91%, 54.35%) in the control group (P<0.05); The incidence of adverse reactions in the observation group was slightly higher than that of the control group, but the difference was not significant (P>0.05); The rate of completion, interruption, discontinuation of treatment and drug reduction did not differ significantly between two groups (P>0.05); After treatment, the serum ALT, AST, TBIL, and GGT levels decreased and were lower in observation group than in control group (P<0.05); The serum tumor markers AFP, ENO1, GPC3, CEA levels decreased in both groups after treatment, and were lower in the observation group than in control group (P<0.05); CD3, CD4, CD8, and NK levels were improved in the observation group and worsened in the control group, and CD3, CD4, and NK levels were higher in the observation group and lower in the control group after treatment (P<0.05). All in all, nivolumab combined with lenvatinib for advanced hepatocellular carcinoma can improve tumor control, reduce tumor load, and improve liver function and immune function. Common adverse reactions include fatigue, loss of appetite, elevated blood pressure, hand-foot skin reaction, diarrhea, and rash, which should be controlled during treatment.

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Introduction

Hepatocellular carcinoma (HCC) is the most common primary malignant liver tumor and the fourth cause of cancer-related death worldwide (1). This malignancy is the seventh most common cancer in men and the ninth in women. The prevalence of this malignancy is higher in men than in women in communities where the risk of developing a tumor is increased than in communities with a low or moderate risk (3.7 to 1 compared to 2.4 to 1) (2). HCC has a diverse geographical distribution (3, 4). Countries with areas with a high incidence of HCC (50 to 120 cases per 100,000 people per year) include China, Taiwan, Korea, and other Southeast Asian countries, plus sub-Saharan Africa. The incidence of HCC is directly related to increasing age (5). Of course, this ratio is different in different countries. In areas where the incidence of HCC is high, this malignancy is mainly seen in young adults, while in low-risk areas, this malignancy often occurs in elderly patients (6). Several epidemiological studies have shown that the main risk factors for HCC are: age, sex, and cirrhosis of any etiology. However, the leading cause of HCC is excessive consumption of alcohol or chronic infection with hepatitis B or hepatitis C virus and contact with aflatoxin (7).

HCC treatment includes surgery and other standard methods such as radiation therapy and chemotherapy. However, limiting conditions such as central liver tumors, involvement of lobes or hepatic veins, cirrhosis, and low remaining active liver tissue have reduced the number of candidates for surgery (30-20%)(1). Also, the 5-year life expectancy of patients after complete mass surgery was about 33%. Various types of chemotherapy drugs that are effective on HCC have been reported. But unfortunately, none of them have a response rate of more than 10-15%; combined chemotherapy has also not been able to affect increasing life expectancy (8). To increase the effect of chemotherapy drugs, the method of drug injection through the hepatic artery has been used. Still, this method did not have much effect on increasing life expectancy. Another treatment method is taking medicine (2, 9). Two essential drugs in this field are nivolumab and lenvatinib (10, 11).

Nivolumab is a drug that is used in the treatment of some types of cancer (12). These cancers include melanoma, lung cancer, kidney carcinoma, Hodgkin's lymphoma, head and neck, colon, and liver cancer (10, 13). This drug

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is prescribed by "slow intravenous injection." Nivolumab is an IgG4 monoclonal antibody that inhibits PD-1. This drug is a type of immunotherapy and immune checkpoint inhibitor, and it blocks cell messages that inhibit the activity of T lymphocytes that prevent the attack of cancer cells (14).

Lenvatinib is an anticancer drug used to treat some types of thyroid cancer, liver cancer, and several other cancers (11). This drug is a protein kinase inhibitor against VEGFR2, VEGFR1, and VEGFR3. Some of these receptors and protein molecules play an influential role in cell signaling pathways in cancer development (15). Inhibition of the VEGFR2 receptor is one of the main reasons for the main side effect of this drug (high blood pressure) (16). Lenvatinib is rapidly absorbed from the gut and reaches peak plasma levels within 1 to 4 hours (3 to 7 hours if taken with food) (17). The bioavailability of lenvatinib is about 85%. This drug is almost entirely (98-99%) bound to plasma proteins, most notably to albumin. Lenvatinib is metabolized in the liver by the CYP3A4 enzyme (18). The half-life of this drug is 28 hours, and two-thirds of it is eliminated through feces and a quarter through urine (6, 15, 19)

They play an essential role in causing liver cancer (17). It seems that liver cirrhosis, via causing early genetic changes in the cells creates a suitable ground for malignant changes. Of course, very few studies have been done in this field (20). Chand *et al*. (21) have reported the absence of heterozygosity in both HCC and cirrhosis as high as 60 in 139 (RB1 gene locus). The lack of heterozygosity and disruption in the structure and function of the P53 gene is often present in HCC (22). A specific p53 mutation has been found in more than 50% of HCCs in India, China, and South Africa, where dietary aflatoxin is a major cause of liver cancer (23). Of course, this particular mutation occurs less often in patients with HCC in Western countries. Activation of ras family oncogenes has been observed during the chemical induction of HCC in rodents (20). But there is very little evidence of their activation in human tumors. Activation of ras family oncogenes has been observed during the chemical induction of HCC in rodents (24). But there is very little evidence of their activation in human tumors. Cyclin-D1 overexpression may be an early event in the development of liver malignancy, which plays a vital role in tumor differentiation (25). Also, the overexpression of the c-Fos gene in tumoral tissues has been seen compared to non-tumoral tissues (22).

This study aimed to investigate nivolumab's efficacy and adverse effects in combination with lenvatinib in treating advanced hepatocellular carcinoma (HCC). Also, changes in the expression of some genes that regulate the cell cycle (P53, RB1, Cyclin-D1, c-fos, and N-ras) were investigated in the development of this cancer.

Materials and Methods

Patients

This study was a prospective clinical trial. Patients with unresectable advanced HCC were admitted used to continue the work. The conditions for patients to enter the study were advanced HCC, which was proven in histological examination. The only condition for patients not to enter the study was a positive history of sensitivity to lenvatinib, nivolumab, or one of the aromatase inhibitor drugs. The conditions for the withdrawal of patients from the study included the recurrence of the disease during the treatment and the development of severe and intolerable side effects caused by drug use.

In general, ninety-two patients with unresectable advanced HCC admitted were enrolled and were divided into the control group (N=46) and the observation group (N=46) according to the random number table. The control group was treated with lenvatinib while the observation group was treated with nivolumab combined with lenvatinib. The efficacy, adverse effects, liver function, completion rate, interruption and discontinuation of treatment, drug reduction, serum tumor markers, and immune function were compared between the two groups.

Assessment of liver enzymes and serum tumor markers

Liver enzymes were determined in plasma that was collected in heparin plasma tubes. Vitros assays (Vitros 250, Ortho-Clinical Diagnostics; Johnson & Johnson, Rochester, USA) were used to determine GGT, ALT and AST levels in units per liter (U/L). Reference values provided by the laboratory were as follows: for GGT < 50 U/L (males) and <35 U/L (females); for ALT<45 U/L (males) and <40 U/L (females); for AST <40 U/L (both sexes). These reference values were based on the guidelines of the Dutch Association of Clinical Chemistry and Laboratory Medicine (NVKC). Enzyme levels were compared to the reference values.

We also analyzed preoperative tumor markers level of different biomarkers AFP, ENO1, GPC3, CEA, CD3, CD4, CD8, and NK. The normal reference values used for the three different biomarkers under study were as follows: AFP ≤20 ng/L CEA 5ng/L and CA19-9 ≤ 37 U/Mr. Patients with serum AFP > 20ng/L, serum CA 19-9 >37 U/mL and a serum CEA >5ng/L were considered positive.

Tissue preparation

The samples were cut and stained with hematoxylin and eosin. The definitive diagnosis of HCC and the degree of differentiation of the tumors were again performed by the pathologists of the Gastrointestinal and Liver Diseases Research Center, using the criteria proposed by the World Health Organization. The tumor was considered sufficient for immunohistochemical study when the size of the blocks was adequate (the cross-sectional area of the slices was more than 4 square centimeters and the tumor occupied more than 10% of the block's cross-sectional area). Immunohistochemical staining of the study technique was based on the avidin-biotin-peroxidase method using sections fixed in 10% formaldehyde and paraffin:

1. We deparaffinized the tissues by passing them through different concentrations of xylol and alcohol.
2. For antigen recovery, we heated the tissues at different temperatures and pressures in the environment containing citrate solution with different pH. After that, tissue peroxidase was inhibited by incubating tissues in the vicinity of the three hydrogen peroxide solutions.
3. To reduce non-specific staining, tissues were incubated in contact with high concentrations of bovine serum albumin.

Incubation with primary antibody was done (primary monoclonal mouse anti-human protein from DAKO Company, Lot 108 [p53], Lot 012[Cyclin-D1], Lot 019[RBI]; and Santa Cruz Biotechnology Inc. Lot I 251 [N-ras], Lot

J 251 [c-fos].

Incubation with primary antibody was done in different titers, and finally, we achieved a specific concentration for each gene. Then incubation with secondary antibody and labeling with Universal, HRP, Lot 10106 (HRP) was done. After staining the samples with chromogenic solutions such as AEC and DAB and background staining with hematoxylin solution, we examined the samples under a light microscope. It should be noted that between each of the above steps, the tissue was washed with the necessary buffers. In addition, to increase the sensitivity and specificity of the above method and to control the quality of the samples in each staining period, one positive control sample and one negative control sample were also stained. Our positive control sample was a specific tissue sample that we were sure was positive for antibodies. The negative control sample was the same patient's tissue sample that was not contacted with the antibody.

Evaluation of immunohistochemical staining

N-ras c-Fos RBI, Cyclin-Dl P53 staining was examined under a standard light microscope with 400 magnification. Nuclear staining was considered positive when uniform nuclear staining or non-uniform staining was seen in more than 10% of cancer cells.

The statistical analysis

The results of this study are expressed as the frequency of expression of genes and the odds ratio to find the relationship between the changes in the expression of these five genes. All statistical tests were performed using the Statistical Software program for the Social Sciences (SPSS 11 Chicago, IL).

Results

Overall Response Rate (ORR) and Disease Control Rate (DCR) (45.65%, 78.26%) in the observation group were higher than those (23.91%, 54.35%) in the control group (P<0.05); The incidence of adverse reactions in the observation group was slightly higher than that of the control group, but the difference was not significant (P>0.05); The rate of completion, interruption, discontinuation of treatment and drug reduction did not differ significantly between two groups (P>0.05).

After treatment, the serum ALT, AST, TBIL, and GGT levels decreased and were lower in the observation group than in the control group (P<0.05).

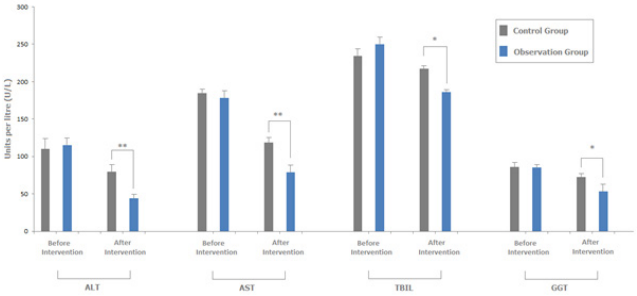

Figure 1. Serum ALT, AST, TBIL, and GGT levels in the observation group and control group; *: P<0.05; **: P<0.01.

The serum tumor markers AFP, ENO1, GPC3, and CEA levels decreased in both groups after treatment and were lower in the observation group than in the control group (P<0.05). CD3, CD4, CD8, and NK levels were improved in the observation group and worsened in the control group, and CD3, CD4, and NK levels were higher in the observation group and lower in the control group after treatment (P<0.05).

We found high levels of Cyclin D1 protein in 5 cases, while 20 cases of tumoral cells were negative for Cyclin D1 protein expression. The frequency of positive staining for c-fos and N-ras was 12 cases and 2 cases, respectively. In this study, the expression of the P53 gene was compared with the expression of Cyclin D1 c-Fos RB1 and N-ras genes (Table 1). In all cases (5 cases) of Cyclin D1 positive, lack of RB1 gene expression was reported, while in Cyclin D1 negative cases, 3 cases of RB1 positive and 17 cases of RB1 negative were reported. When the overexpression of P53 was observed in the cells, there were 4 cases of lack of RB1 gene expression. Finally, in all cases of overexpression of N-ras (2 cases), a lack of RB1 gene expression was observed. In cases of non-expression of the RB1 gene, 11 cases of c-Fos overexpression were detected.

Using the odds ratio, comparing P53 positive cases with negative cases showed that the first group had a higher chance (9 times) for RB1 gene positivity. This chance for c Fos, N ras, and Cyclin D1 was 6.3, 75.2, and 66.2, respectively. In Cyclin D1 positive cases, the chance of N Ras being positive was calculated to be 75.4 times higher than the possibility of this gene being negative.

Discussion

Since in the review of the available literature related to

Table 1. Comparison of p53 gene expression in two observation and control groups with the expression of RB1, Cyclin-D1, c-fos, and N-ras genes.

Gene Expression		P53 Expression (Observation Group)	P53 Expression (Control Group)
RB1	Positive	2 (4.34%)	1 (2.17%)
	Negative	4 (8.69%)	8 (17.39%)
Cyclin-D1	Positive	2 (4.34%)	3 (6.52%)
	Negative	4 (8.69%)	16 (34.78%)
c-fos	Positive	4 (8.69%)	8 (17.39%)
	Negative	2 (4.34%)	11 (12.79%)
N-ras	Positive	1 (2.17%)	1 (2.17%)
	Negative	5 (10.86%)	18 (39.13%)

the study, the project organizers did not find any similar study that compared the side effects of nivolumab and lenvatinib, in practice, it is not possible to compare the results of this study with previous literature. Our results illustrated that nivolumab combined with lenvatinib for advanced hepatocellular carcinoma can improve tumor control, reduce tumor load, and improve liver function and immune function. Common adverse reactions include fatigue, loss of appetite, elevated blood pressure, hand-foot skin reaction, diarrhea, and rash, which should be controlled during treatment.

Many studies have been conducted on the changes in the expression of oncogenes and tumor suppressor genes involved in the development of hepatocellular carcinoma caused by HBV/HCV or chemical carcinogens and other factors. In some regions, the mutation of the suppressive gene P53 has been observed in patients with hepatocellular carcinoma at a rate of 50-30% (1). The study of Liu et al. (26) showed that cases of hepatocellular carcinoma were accompanied by overexpression of the P53 gene. The overexpression of the P53 gene has been reported in 37.5% of Japanese and 62.5% in Indonesian residents (27). Of course, the rate of P53 gene mutation in hepatocellular carcinoma in India is very low, and it may not play an essential role in creating cancer in this region (28).

Cao et al. (29) study mentioned that P53 gene mutation might play a role in the development of hepatocellular carcinoma of the hepatitis B virus; however, due to the low rate of P53 gene mutation in this study, the authors concluded that other factors also play a role in hepatogenesis. Recently, Luo et al. (30) have completed that P53 gene mutation is more common in areas of China where hepatocellular carcinoma is more prevalent than in areas where the prevalence of this disease is low. More than 95 cancer samples showed significant intranuclear accumulation of P53 protein, which was detected by immunohistology. However, in the study of Sanaei et al. (31) and Oin et al. (32), the point mutation of the P53 gene in hepatocellular carcinoma was either absent or very low. Therefore, overexpression of the P53 protein in hepatocellular carcinoma samples can be considered a pathological sign in tumoral tissues, which the results of the current study proved it.

Acknowledgments
None

Declaration of conflict of interest
None.

References

1. Radwan NH, Abdelkhalik HA, Elgayar DF, Elsharkawy MM. Study the Role of Cell Free DNA in the Diagnosis of Hepatocellular Carcinoma, an Egyptian Study. Asian Pac J Cancer Biol 2022; 7(1): 3-8.
2. Hosen MB, Khaleque N, Chakraborty S, Al Mahtab M, Kabir Y. MDM2 (T309G) Gene Polymorphism Determines the Susceptibility of Hepatocellular Carcinoma in Bangladesh. Asian Pac J Cancer Biol 2021; 6(3): 213-217.
3. Uchendu OJ, Ikponmwosa O. Epidemiological and Pathologic Characteristics of Cancer Morbidity in Elderly Nigerians: A Call for Action. Asian Pac J Cancer Care 2020; 5(4): 295-302.
4. Navabi M, Darvishi I. The incidence and mortality of cancer in Eastern Mediterranean Regional Office (EMRO) and its relationship with Human Development Index (HDI): An Ecological Study. Asian Pac J Environ Cancer 2018; 1(1): 59-67.
5. Nguyen TT, Vu NP, Tran TS, Van Hoang H, Bui HT-T. Screening and Identification of Key Genes in Hepatitis B Virus-Related Hepatocellular Carcinoma Through an Integrated Bioinformatics Approach. Asian Pac J Cancer Biol 2022; 7(2): 143-149.
6. Ahmad W, Rehman I, Majeed O, Niazi IK, Mufti KA, Iqbal A. Survival benefit of transarterial chemoembolization for hepatocellular carcinoma. Asian Pac J Cancer Biol 2020; 5(2): 43-47.
7. Sofyan RF, Shalmont G, Tjiptoningsih UM. Management of Lymphoepithelial Carcinoma of the Parotid Gland with Liver Metastasis: A Case Report. Asian Pac J Cancer Care 2022; 7(3): 593-596.
8. Ahmad B, Marwa ME, Turkmani K et al. Liver Resection in a Tertiary University Hospital in Damascus-trends Related to the Political Strife and Social Crisis. Asian Pac J Cancer Care 2020; 5(2): 79-82.
9. Jafarov K, Melnik I. Immediate Results of Surgical Treatment of Patients with Strangulated of Ventral Hernia of the Anterior Abdominal Wall. Asian Pac J Environ Cancer 2021; 4(S1): 13-14.
10. Cascone T, William WN, Weissferdt A et al. Neoadjuvant nivolumab or nivolumab plus ipilimumab in operable non-small cell lung cancer: the phase 2 randomized NEOSTAR trial. Nat Med 2021; 27(3): 504-514.
11. Zhang D, Zhang Y, Cai Z, Tu Y, Hu Z. Dexamethasone and lenvatinib inhibit migration and invasion of non-small cell lung cancer by regulating EKR/AKT and VEGF signal pathways. Exp Ther Med 2020; 19(1): 762-770.
12. Forde PM, Spicer J, Lu S et al. Neoadjuvant nivolumab plus chemotherapy in resectable lung cancer. New En J Med 2022.
13. Ercisli MF, Kahrizi D, Aziziaram Z. Environmental factors affecting the risk of breast cancer and the modulating role of vitamin D on this malignancy. Centr Asian J Environ Sci Technol Innov 2021; 2(4): 175-183.
14. Wang L, Zhao D, Qin K, Rehman FU, Zhang X. Effect and biomarker of nivolumab for non–small-cell lung cancer. Biomed Pharmacother 2019; 117: 109199.
15. Liu Y-C, Huang B-H, Chung J-G, Liu W-L, Hsu F-T, Lin S-S. Lenvatinib inhibits AKT/NF-κB signaling and induces apoptosis through extrinsic/intrinsic pathways in non-small cell lung cancer. Anticancer Res 2021; 41(1): 123-130.
16. Jin H, Shi Y, Lv Y et al. EGFR activation limits the response of liver cancer to lenvatinib. Nature 2021; 595(7869): 730-734.
17. Ogasawara S, Mihara Y, Kondo R, Kusano H, Akiba J, Yano H. Antiproliferative effect of lenvatinib on human liver cancer cell lines in vitro and in vivo. Anticancer Res 2019; 39(11): 5973-5982.
18. Yamashige D, Kawamura Y, Kobayashi M et al. Potential and clinical significance of 18F-fluorodeoxyglucose positron emission tomography/computed tomography for evaluating liver cancer response to lenvatinib treatment. Oncology 2021; 99(3): 169-176.
19. Natanael G, Hardini N, Lardo S, Suzanna E. Associations between Nonalcoholic Fatty Liver Disease and Histopathological Features of Patients with Adenocarcinoma of Colon in Dharmais National Cancer Hospital, National Cancer Center, Indonesia. Asian Pac J Cancer Biol 2022; 7(1): 37-41.
20. Hindson J. Lenvatinib plus EGFR inhibition for liver cancer. Nat Rev Gastroenterol Hepatol 2021; 18(10): 675-675.
21. Chand V, Liao X, Guzman G, Benevolenskaya E, Raychaudhuri P. Hepatocellular carcinoma evades RB1-induced senescence by activating the FOXM1–FOXO1 axis. Oncogene 2022; 41(30): 3778-3790.
22. Duan X, Cai Y, He T et al. The effect of the TP53 and RB1 mutations on the survival of hepatocellular carcinoma patients with

different racial backgrounds. J Gastrointest Oncol 2021; 12(4): 1786.
23. Zhao P, Malik S, Xing S. Epigenetic mechanisms involved in HCV-induced hepatocellular carcinoma (HCC). Front Oncol 2021; 11: 677926.
24. Atif M, Mustaan MA, Falak S, Ghaffar A, Munir B. Targeting the effect of sofosbuvir on selective oncogenes expression level of hepatocellular carcinoma Ras/Raf/MEK/ERK pathway in Huh7 cell line. Saudi J Biol Sci 2022: 103332.
25. Alqahtani A, Khan Z, Alloghbi A, S. Said Ahmed T, Ashraf M, M. Hammouda D. Hepatocellular carcinoma: molecular mechanisms and targeted therapies. Medicina 2019; 55(9): 526.
26. Liu Y, Yan J, Wang F. Effects of TACE combined with precise RT on p53 gene expression and prognosis of HCC patients. Oncol Let 2018; 16(5): 5733-5738.
27. Li S, Gao M, Li Z et al. p53 and P-glycoprotein influence chemoresistance in hepatocellular carcinoma. Front Biosci 2018; 10(3): 461-468.
28. Ghanta MK, Hussain MF, Karnalkar A, Nagabhishek SN, Nuthalapati P, Bhaskar L. Pharmacogenomics and outcomes for hepatocellular cancer treatment. Theranostics and Precision Medicine for the Management of Hepatocellular Carcinoma, Volume 3: Elsevier; 2022: 401-414.
29. Cao H, Chen X, Wang Z, Wang L, Xia Q, Zhang W. The role of MDM2–p53 axis dysfunction in the hepatocellular carcinoma transformation. Cell Death Discov 2020; 6(1): 1-14.
30. Luo Y-D, Fang L, Yu H-Q et al. p53 haploinsufficiency and increased mTOR signalling define a subset of aggressive hepatocellular carcinoma. J Hepatol 2021; 74(1): 96-108.
31. Sanaei M, Kavoosi F, Amin Moezzi M. Effect of 5'-fluoro-2'-deoxycytidine and sodium butyrate on the genes of the intrinsic apoptotic pathway, p21, p53, cell viability, and apoptosis in human hepatocellular carcinoma cell lines. Iran J pediatr Hematol Oncol 2021; 11(4): 216-230.
32. Qin G, Tu X, Li H et al. Long noncoding RNA p53-stabilizing and activating RNA promotes p53 signaling by inhibiting heterogeneous nuclear ribonucleoprotein K deSUMOylation and suppresses hepatocellular carcinoma. Hepatology 2020; 71(1): 112-129.