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# Long Noncoding RNA H19 rs3741219 Polymorphism and Risk of Hepatocellular Carcinoma in Egyptian Population

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## ABSTRACT

**Background:** RNA molecules which are longer of 200 nucleotides that are unable for protein coding are known as long non-coding RNAs or LncRNAs. They play a vital role in different biological activities and cancer biology. Numerous long non-coding RNAs with tumor-suppressive and carcinogenic functions have been found in human hepatocarcinogenesis.

**Objective:** Exploring the role if lncRNA H19 (rs3741219) gene polymorphism plays in raising the hepatocellular carcinoma (HCC) risk in Egyptian patients.

**Patients and Methods**: We performed this case-control study upon95 participants, 40 HCC patients diagnosed by Computed Topography (according to EASL guidelines), 35 cirrhotic patients, and 20 healthy subjects as control.

**Result:** A significant statistical difference between the three groups under study was found regarding child score (p = 0.028). The results of a univariate and multivariate analysis on the risk variables for HCC showed that liver size and child score were linked to a higher risk of HCC when compared with the cirrhosis group (P<0.05 and P=0.024, respectively). Comparing the HCC group versus the cirrhotic group, variant genotypes of rs3741219 were not significantly associated with the risk of HCC when compared with the wild genotype (AA) (P=0.230). Non-significant correlations were found between the variant genotypes AG/GG of rs3741229 (adjusted OR = 608, 95%CIs = 0.162–2.28, P = 0.679) and the risk of HCC.

**Conclusion:** Our research showed a non-significant correlation between the risk of HCC and the H19 (rs3741219) genotype variants.

**Keywords:** Hepatocellular carcinoma, long non-coding RNA, H19, Real-time PCR.

## INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the major causes of cancer related deaths among many countries as it ranks fourth worldwide [1]. Factors that raise the risk of HCC include being exposed to aflatoxin, having persistent infections of HBV and HCV, drinking alcohol, and having non-alcoholic fatty liver disease (NAFLD) [2]. The two main risk factors for HCC in Egypt are chronic hepatitis brought on by HBV and HCV infection [3]. Early detection of HCC greatly predicts the course of the illness and the death rate. Individuals who receive a diagnosis at an early stage can receive curative treatments, such as liver transplantation, ablative therapies, and resection; patients who receive a late-stage diagnosis can only receive palliative treatments, which have a greater death rate. Patients whose cancer is detected early have a 5-year survival rate of over 70%, whereas those whose cancer is detected later have a rate of less than 5% [4].

One of the RNA molecules that do not code for functional proteins, is long non-coding RNAs, are more than 200 nucleotides long. It has been established that long noncoding RNAs (lncRNAs) are essential for a wide range of biological activities, for example, cell differentiation, apoptosis, cell division, and regulation of gene expression [5]. Researchers have discovered that LncRNAs affect gene expression both during transcription and after transcription has taken place. More and more data suggests that LncRNAs play an important regulatory role in cancer biology. Numerous LncRNA subtypes have been identified as having both tumor-suppressive and carcinogenic functions in human hepatocarcinogenesis [6].

Of the several LncRNAs that have been linked to HCC, the most studied ones are H19, MALAT1, TUC338 HULC, and HOTAIR. H19 is a lengthy noncoding RNA that is highly expressed before birth, silenced in the majority of tissues upon birth, and then re-expressed in some cancers [7].

Recent studies reported that lncRNA-H19 polymorphisms play roles in HCC development, much research revealed a tumor-suppressive function whereas others revealed its oncogenic role. It might be a valuable biomarker in determining the risk and prognosis of HCC and a potential target for HCC chemotherapeutics [8].

## SUBJECTS AND METHODS

**Subjects:** This case-control research was performed during the period from January to September 2023, 95 participants were incorporated in the study, divided into three groups: 20 healthy individuals served as the control group, 35 patients with cirrhosis, and 40 patients with proven HCC. The National Liver Institute, Menoufia University, and inpatient and outpatient clinics were the sources of the patients. Patients with HCC were diagnosed according to EASL practice guidelines for HCC diagnosis [9], Cirrhotic patients were diagnosed based on clinical pictures, radiological findings, and laboratory evidence of liver cell damage. All HCC patients and cirrhotic



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patients were post-HCV infection with HCV positive Ab and negative HbsAg. The control subjects were healthy individuals with matched age and gender. Clinically, they were fully free, having no history of liver or renal illness, normal laboratory tests, and normal abdominal ultrasonography, with negative HCV Ab and HbsAg. Individuals under the age of 18 and those with long-term inflammatory conditions were not allowed to participate in the study. Additionally, those with liver tumors other than HCC and those with other cancers were not included in our sample. Cirrhotic patients are free from focal lesions based on abdominal ultrasonography.

Every research participant had a thorough history taking, clinical examination, radiological Study (Pelvi-abdominal ultrasound and Triphasic CT scan or MRI according to conventional diagnostic criteria [9] and routine laboratory investigations.

**Routine laboratory investigations:** Laboratory investigations included a CBC conducted on an Automated Hematology Analyzer (Sysmex XT-1800i, Sysmex Corporation, Kobe 651-0073, Japan), to assess Liver functions: total and direct bilirubin, albumin, and prothrombin time (PT) and INR using an automated analyzer by Sysmex Cs-1600, (Sysmex Corporation, Germany). Liver enzymes; ALT, AST, ALP, GGT, (Cobas c501 Auto analyzer, Hitachi, High technologies cooperation, Tokyo, Japan). Alfa fetoprotein (AFP) by Auto analyzer (Cobas e601, Hitachi, High technologies cooperation, Tokyo, Japan).

**Genotyping Assay:** Using the QIAmp DNA blood Mini kit (Cat. No./ID: 51104) and the manufacturer's instructions, total genomic DNA was isolated from whole blood. TE buffer 10 mM Tris (pH 7.8) and 1 mM EDTA was used to dissolve DNA. The finished product was kept at a temperature of -20°C and utilized as templates for PCR.

**SNP Genotyping assay:** Rotor-Gene real-time PCR system (QIAGEN, GmbH) using fluorescently labeled probes was used for LncH19 rs3741219 genotyping assay. TaqMan® Genotyping Master Mix Catalogue No. 4371353. TaqMan predesigned SNP, SNP ID: rs3741219, catalog number: 4351379, Assay IDC__27492510_10, (Applied Biosystems, Carlsbad, CA). The reaction is prepared in a total volume of 20 µl divided as follows (10µl Master Mix(2X), 0.5µl TaqMan assay 20k, 5µl DNA Template, 4.5 µl Water, nuclease-free). PCR cycling conditions: according to TaqMan master mix assay protocol, 1 min (pre-read) cycle at 60°C and 40 cycles (Denaturation takes place for 15 seconds at 95°C in each cycle, followed by annealing and extension, which each take 1 minute at 60°C). The sequence was as follows [VIC/FAM]CAGATGGAGGGCGGCCGGGCCCTG C**[A/G]**CAGGCACTTGCCAAGGTGGCTC ACAC(VIC dye for A allele, FAM dye for G allele).

**Ethical approval: The Ethical Review Board of National Liver Institute, Menoufia University approved the study's procedures. Every single subject supplied his written informed consent. The Helsinki Declaration was followed throughout the study's conduct.**

***Statistical analysis***

Using IBM SPSS version 25.0, the data analysis was carried out (IBM Corp., 2017). Statistical information was offered in the form of numbers and percentages. The distribution was confirmed to be normal by the Kolmogorov-Smirnov test. Displayed quantitative data included means, standard deviations, minimums, maximums, medians, and interquartile ranges (IQR). A significance level of 5% was used to evaluate the outcomes. The ANOVA test for analysis of variance. The statistical methods that were employed included the Chi-square test, the Mann-Whitney test, the Kruskal-Wallis test, and the Odd Ratio (OR). A significant p-value was considered when it is ≤ than 0.05.

**RESULTS**

Demographic as well as clinical statistics of the study participants were shown in table (1). Mean age of HCC patients was 59.30 ± 7.71 years with 31 males and 9 females, while the range of age in cirrhotic patients was 60.0 ± 8.38 years old with 30 males and 5 females. The age range in control members was 56.10 ± 7.72 years (18 males and 2 females). Regarding age and gender, there was no discernible difference between the three groups ($p>0.05$) with male predominance in the three groups (77.5%, 85.7%, and 90% respectively). Between the groups under study, there was a significant difference in terms of DAAs, smoking, DM, and hypertension ($P<0.05$).

**Table (1):** Demographic characteristics and clinical history among the study participants

| | Variable | HCC patients (No = 40) | Cirrhotic patients (No= 35) | Control (No = 20) | P-value | Post HOC test |
|---|---|---|---|---|---|---|
| Age (years) | Mean± SD | 59.30± 7.71 | 60.0± 8.38 | 56.10± 7.72 | 0.204 | |
| Gender No. (%) | Male<br>Female | 31 (77.5%)<br>9 (22.5%) | 30 (85.7%)<br>5 (14.3%) | 18 (90.0%)<br>2 (10.0%) | 0.320 | |
| Hypertension No. ( %) | NO | 22 (55.0%) | 16 (45.7%) | 20 (100.0%) | <0.001 | p1=0.568, p2=0.001, p3<0.001 |
| | Yes | 18 (45.0%) | 19 (54.3%) | 0 (0.0%) | | |
| DM No. (%) | No | 20 (50.0%) | 23 (65.7%) | 20 (100.0%) | 0.001 | p1=0.255 p2<0.001 p3=0.009 |
| | Yes | 20 (50.0%) | 12 (34.3%) | 0 (0.0%) | | |
| Smoking No. (%) | No | 24 (60.0%) | 17 (48.6%) | 17 (85.0%) | 0.028 | p1=0.448 p2=0.050 p3=0.017 |
| | Yes | 16 (40.0%) | 18 (51.4%) | 3 (15.0%) | | |
| History of DAAs No. (%) | No | 2 (5.0%) | 0 (0.0%) | 20 (100.0%) | <0.001 | p1=0.534 p2, p3<0.001 |
| | Yes | 38 (95.0%) | 35 (100.0%) | 0 (0.0%) | | |

SD: standard deviation DM: diabetes mellitus DAAs: direct acting antiviral drugs

Regarding Hb and platelet counts, a statistically significant difference was revealed among the three groups. Hb and platelets showed lower values in cirrhotic patients than in HCC patients. Regarding albumin, AST, GGT, and alkaline phosphatase, statistically significant differences were revealed between the three studied groups under investigation. However, a pairwise comparison showed no differences in albumin levels between patients with HCC and those with cirrhosis. AFP was considerably higher in the HCC group as compared to cirrhotic patients and control subjects. Bilirubin, ALT, INR, and creatinine values did not significantly differ from one group to another (Table 2).

**Table (2):** Laboratory Results among the Study participants

| | HCC group (No.= 40) | Cirrhotic group (No.= 35) | Control group (No.= 20) | P-value | Post HOC test |
|---|---|---|---|---|---|
| HB (gm/dl), Median (IQR) | 12.0 (9.8- 13.4) | 9.5 (8.9-10.2) | 14.85 (14.0- 15.4) | <0.001 | P1, P2, P3<0.001 |
| WBC ($\times 10^9$/L) Median (IQR) | 5.85 (4.45-6.90) | 5.7 (4.6 -7) | 6.3 (5.1 - 8.20) | 0.087 | |
| Platelets($\times 10^9$/L) Median (IQR) | 123.0 (89.50 - 152.5) | 102.0 (85 - 130) | 274.0 (233.0 - 303) | <0.001 | P1 NS, P2, P3<0.001 |
| ALT (U/L), Median (IQR) | 26 (20 -32) | 29 (22- 36) | 16.5(13- 20) | 0.146 | - |
| AST (U/L) Median (IQR) | 26 (21- 33) | 28 (21- 35) | 18 (15-19) | <0.001 | P1 NS P2, P3<0.001 |
| GGT (U/L) Median (IQR) | 48.0 (40.5-55.0) | 43.0 (35.0-52.0) | 17.98 (13.40-22.0) | <0.001 | P1 NS P2, P3<0.001 |
| ALP (U/L), Median (IQR) | 93 (63 -110) | 98 (72-123) | 65 (53 -73) | <0.001 | P1 NS, P2 0.002, p3 <0.001 |
| Albumin (gm/dl) Median (IQR) | 3.1 (2.80-3.80) | 2.9 (2.70-3.20) | 4.35 (4.0-4.65) | <0.001 | P1 NS P2 , p3 <0.001 |
| Bilirubin T (mg/dl) Median (IQR) | 1.35 (0.80-1.95) | 1.4 (0.90-2.10) | 0.56 (0.50-0.60) | 0.551 | - |
| Bilirubin D (mg/dl) Median (IQR) | 0.67 (0.25-1.15) | 1.1 (0.50-1.50) | 0.05 (0.02-0.10) | 0.133 | - |
| INR, Median (IQR) | 1.3 (1.17-1.35) | 1.2 (1.10-1.40) | 1.02 (1.01-1.04) | 0.872 | - |
| Creatinine (mg/dl) Median (IQR) | 1.05 (0.80 -1.2) | 1.01 (0.90-1.30) | 1.0 (0.8-1.1) | 0.262 | - |
| AFP (ng/ml) Median (IQR) | 261.0 (118.5-480) | 17 (8.5-26.0) | 2.0 (1.95-2.45) | <0.001 | P1, P2, P3<0.001 |

Median and IQR: non parametric test, ALP: Alkaline phosphatase, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase INR international normalized ratio, AFP alpha fetoprotein, P1: P-value between HCC group Vs cirrhotic group, p2: P-value between HCC group Vs control group, P3: P-value between cirrhotic group Vs control group.

Regarding ascites, there was significant difference (p<0.001) between the three groups under study. The cirrhotic group showed a significant decrease in liver size when compared to the HCC group (p=0.019). No difference was found between HCC and cirrhotic patients regarding spleen size. In the meantime, the HCC group showed a considerable higher rate of portal vein thrombosis than the cirrhotic group. Regarding the Child score, a high statistically significant difference (p < 0.001) was revealed between the HCC patients and the cirrhotic group (Table 3 and figure 1).

**Table (3):** Comparison between the studied groups regarding clinical and US findings.

| | | HCC group (No.= 40) | | Cirrhotic group (No.= 35) | | Control group (No.= 20) | | Chi-Square test | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % | Test of sig. | P-value |
| Ascites | Absent | 20 | 50.0% | 8 | 22.9% | 20 | 100.0% | X2= 36.52 | <0.001<br>p1=0.029<br>p2=0.002<br>p3<0.001 |
| | Minimal | 5 | 12.5% | 4 | 11.4% | 0 | 0.0% | | |
| | Mild | 12 | 30.0% | 11 | 31.4% | 0 | 0.0% | | |
| | Moderate | 3 | 7.5% | 10 | 28.6% | 0 | 0.0% | | |
| | Marked | 0 | 0.0% | 2 | 5.7% | 0 | 0.0% | | |
| Child score (grade) | A | 31 | 77.5% | 9 | 25.7% | - | | < 0.001 | |
| | B | 5 | 12.5% | 19 | 54.3% | | | | |
| | C | 4 | 10% | 7 | 20.0% | | | | |
| Liver size (cm) | Median (IQR) | 14.8 (14.2- 15.6) | | 14.0 (13.5- 15.4) | | 14.7 (14.3- 15) | | Kw= 7.907 | 0.019<br>p1=0.021<br>p2=0.725<br>p3=0.177 |
| | Range | 12.7- 17 | | 11- 18.5 | | 14.2- 15.2 | | | |
| Spleen size (cm) | Mean± SD | 15.83± 2.35 | | 16.11± 2.17 | | 12.25± 1.67 | | F= 7.907 | 0.019<br>p1=0.860<br>p2<0.001<br>p3<0.001 |
| | Range | 11- 22 | | 12.7- 24 | | 10- 14.8 | | | |
| PVT | No | 29 | 72.5% | 35 | 100.0% | 20 | 100.0% | X2= 17.11 | <0.001<br>p1=0.002<br>p2=0.025<br>p3=NA |
| | Yes | 11 | 27.5% | 0 | 0.0% | 0 | 0.0% | | |

PVT: portal vein thrombosis.

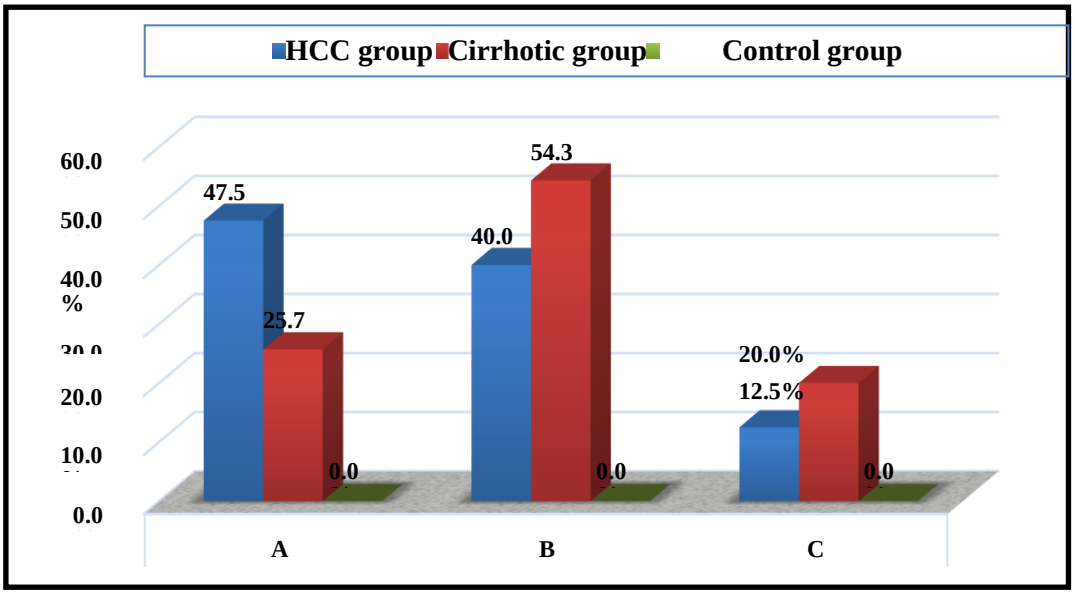


**Figure (1):** Comparing the study groups as regards Child score.

Concerning cases with hepatocellular carcinoma, the most frequently affected liver segment was segment VI (32.5%) followed by segment VII (27.5%) then segment III (25%). The mean size of the focal lesion was 4.25 ± 2.02 cm. 15 (37.5%) cases had positive lymph nodes affection. Metastasis was not reported in any case. Regarding TNM staging,

T3.N0.M0 was the most common stage found (30%). Regarding BCLC score, stage A was the most prevalent (45%) followed by stage B (30%) (Table 4).

**Table (4):** Tumor characteristics among HCC group

| | | HCC group (No.= 40) | |
|---|---|---|---|
| | | No. | % |
| Focal lesions | Segment I | 1 | 2.5% |
| | Segment II | 9 | 22.5% |
| | Segment III | 10 | 25.0% |
| | Segment IV | 2 | 5.0% |
| | Segment V | 9 | 22.5% |
| | Segment VI | 13 | 32.5% |
| | Segment VII | 11 | 27.5% |
| | Bilobar hepatic focal lesions | 2 | 5.0% |
| Focal Lesion size (cm) | Mean± SD | 4.25± 2.02 | |
| | Median (Range) | 4 (1.2 – 10.0) | |
| Lymph nodes | Negative | 25 | 62.5% |
| | Positive | 15 | 37.5% |
| Metastasis | Negative | 40 | 100.0% |
| | Positive | 0 | 0.0% |
| TNM staging | T1.N0.M0 | 4 | 10.0% |
| | T1.N1.M0 | 1 | 2.5% |
| | T2.N0.M0 | 3 | 7.5% |
| | T3.N0.M0 | 12 | 30.0% |
| | T3.N1.M0 | 5 | 12.5% |
| | T4.N0.M0 | 8 | 20.0% |
| | T4.N1.M0 | 7 | 17.5% |
| BCLC score | Stage A | 18 | 45.0% |
| | Stage B | 12 | 30.0% |
| | Stage C | 5 | 12.5% |
| | Stage D | 5 | 12.5% |

The genotype distribution and allele frequency among the study participants were presented in table (5) and figure (2). Non statistically significant variance was revealed between HCC patients, cirrhotic patients, and the control group regarding LncH19 rs3741219 genotypes either in the recessive genetic model or the dominant genetic model as well as allele frequencies ($p > 0.05$).

Comparing HCC patients and Cirrhotic patients, the GG genotype of rs3741219 revealed a non-significant increased risk of HCC compared to the wild AA genotype (OR = 1.524, at 95% CIs = 0.25–9.3). When comparing the HCC group and control group, rs3741219 variant genotypes were linked to a non-significantly higher chance of developing HCC.

**Table (5):** Distribution of LncH19 rs3741219 genotype and allele frequencies among the studied groups

| | **HCC group (No.= 40)** | | **Cirrhotic group (No.= 35)** | | **Control group (No.= 20)** | | **HCC group vs. Cirrhotic group** | | **HCC group vs. control group** | | **Cirrhotic group vs. control group** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotypes | N | % | N | % | N | % | P-value | OR (95% CI) | P-value | OR (95% CI) | P-value | OR (95% CI) |
| AA | 7 | 17.5 % | 4 | 11.4% | 5 | 25.0% | | Ref. | 0.29 | Ref. | | Ref. |
| AG | 25 | 62.5 % | 28 | 80.0% | 14 | 70.0% | 0.23 | 0.510 (0.13-1.95) | | 1.28 (0.34-4.78) | 0.40 | 2.5 (0.58-10.8) |
| GG | 8 | 20.0 % | 3 | 8.6% | 1 | 5.0% | | 1.524 (0.25-9.3) | | 5.714 (0.53-61.4) | | 3.75 (0.27-51.4) |
| Recessive genetic model | | | | | | | | Ref. | | Ref. | | Ref. |
| AA/ AG | 32 | 80.0 % | 32 | 91.4% | 19 | 95.0% | 0.29 | 2.67 (0.65-10.97) | 0.25 | 4.75 (0.55-40.98) | 0.96 | 1.966 (0.19-20.32) |
| GG | 8 | 20.0 % | 3 | 8.6% | 1 | 5.0% | | | | | | |
| Dominant genetic model | | | | | | | | Ref. | | Ref. | | Ref. |
| AA | 7 | 17.5 % | 4 | 11.4% | 5 | 25.0% | 0.68 | 0.608 (0.16-2.28) | 0.73 | 1.57 (0.43-5.77) | 0.35 | |
| AG/ GG | 33 | 82.5 % | 31 | 88.6% | 15 | 75.0% | | | | | | |
| Alleles | | | | | | | | Ref. | | Ref. | | Ref. |
| A | 39 | 48.75% | 36 | 51.4% | 24 | 60% | 0.87 | 1.113 (0.59-2.28) | 0.33 | 1.577 (0.73-3.40) | 0.50 | 1.00 (0.41-2.45) |
| G | 41 | 51.25% | 34 | 48.6% | 16 | 40% | | | | | | |

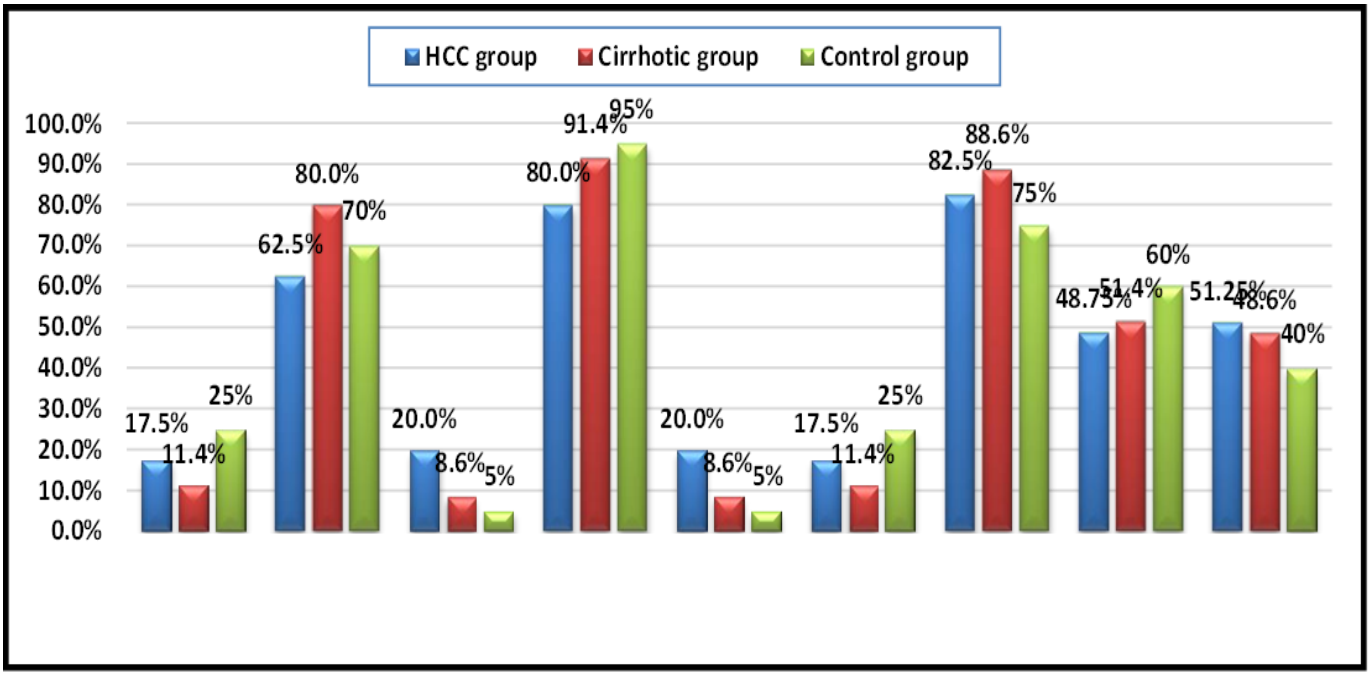


**Figure (2):** Comparing the study groups as regards LncH19 rs3741219 gene polymorphism

The univariate and multivariate analyses performed on putative risk variables of HCC revealed that liver size and child score were substantially linked to an increased risk of HCC relative to the cirrhosis group (Table 6).

**Table (6):** Univariate and multivariate logistic regression analysis for the parameters distinguish HCC group from cirrhotic group.

| Parameters | Univariate | | | | Multivariate | | | |
|---|---|---|---|---|---|---|---|---|
| | P-value | Odds ratio (OR) | 95%CI | | P- value | Odds ratio (OR) | 95%CI | |
| | | | Lower limit | Upper limit | | | Lower limit | Upper limit |
| Age | 0.703 | 1.011 | 0.955 | 1.071 | | | | |
| Gender (male) | 0.366 | 0.574 | 0.172 | 1.912 | | | | |
| HTN | 0.546 | 1.346 | 0.513 | 3.532 | | | | |
| DM | 0.154 | 0.496 | 0.189 | 1.302 | | | | |
| Smoking | 0.362 | 1.561 | 0.599 | 4.067 | | | | |
| Child Score | 0.028 | 1.321 | 1.03 | 1.694 | 0.047 | 1.295 | 1.004 | 1.671 |
| Liver size | 0.024 | 0.623 | 0.413 | 0.941 | 0.045 | 0.660 | 0.440 | 0.991 |
| Spleen size | 0.580 | 1.065 | 0.851 | 1.333 | | | | |
| LncH19 rs3741219 genotypes (AG+GG) | 0.461 | 0.438 | 0.438 | 6.17 | | | | |

B: Regression coefficient; HTN: Hypertension, DM: Diabetes mellitus.

## DISCUSSION

Hepatocellular carcinoma is more common in Egyptians with a history of chronic hepatitis C virus infection. HCC is one of the major cancer mortality globally causes [10]. Liver cancer is a complex disease that starts and spreads due to several genetic and environmental causes [11]. Overall mortality is largely predicted by the stage of HCC diagnosis. Patients in the later stages of the disease are usually only candidates for systemic palliative treatment, which has poor response rates, whereas those in the earlier stages are eligible for curative treatments [12]. Because long non-coding RNAs (lncRNAs) are implicated in a diversity of biological processes, they have gained attention in genetic research [13]. lncRNAs are suitable diagnostic markers or therapeutic targets in systemic therapies because different investigations on them have discovered that their expression is disrupted in a variety of tumors. SNPs play a crucial impact in carcinogenesis by influencing the expression and function of several important lncRNAs [14]. The oncofetal lncRNA H19 exhibits bipolar behavior, acting as an oncogene or a tumor suppressor contingent on the tumor's kind, stage, and genetic background. The 2.5 kb long H19 gene region has five exons that code for an RNA that controls the activity of other RNAs [15].

H19 single-nucleotide polymorphisms (SNPs) are linked to clinicopathologic characteristics and cancer susceptibilities in many types of cancer [16]. The SNP database contains more than 2200 H19 SNPs. Of these polymorphisms, the six significant SNPs linked to tumor susceptibility are rs3741219, rs217727, rs2839698, rs2735971, rs3024270, and rs2107425. Despite conflicting results, multiple studies in the last several years have investigated the link between H19 SNPs and cancer risk [17]. However, further research is necessary to validate these findings because of the small sample size and restricted number of studies [18].

Although numerous studies have examined the link between the LncH19 rs3741219 gene variant and various malignancies, there has been a dearth of research into the potential link between these variants and HCC. Therefore, we here investigated the connection between H19 (rs3741219) gene Polymorphism and HCC in the Egyptian population. When we compared the LncH19 rs3741219 genotypes of HCC patients, cirrhotic patients, and the control group in terms of allele frequencies and recessive or dominant genetic models, we found no statistically significant differences. Also, there was no role of H19 rs3741219 gene polymorphisms in the occurrence of HCC. Variant genotypes of rs37412219 were linked to a non-significantly lower risk of HCC when compared to the wild-type AA genotype in the HCC group vs. the Cirrhotic group. **Wu *et al.*** [11] conducted a study to ascertain whether the polymorphism in the H19 gene was linked to the elevated risk of HCC. Five SNPs in the H19 gene (rs3024270, rs3741219, rs2839698, rs217727, and rs2107425) were examined in the study. In this investigation, we found no evidence that any of the SNPs were associated with HCC serological marker levels, while two H19 SNPs, rs2839698 and rs37412219, were linked to an increased risk of liver cancer. Contrary to our findings, heterozygotes for the H19 minor alleles rs3741219 (G) and rs2839698 (T) were more susceptible to HCC [11]. In agreement with our study, **Li *et al.*'s** [18] meta-analysis offers evidence that polymorphisms of H19 rs3741219 may have no contribution to genetic predisposition to the risk of cancer. Moreover, **Liu *et al.*** [19] in their metanalysis investigated the relationship between lncRNA H19 polymorphisms and cancer susceptibility, which included 25 studies found no considerable correlation

was observed between H19 rs3741219, rs2735971, and rs3024270 polymorphisms and susceptibility to cancer.

Another metanalysis was carried out by **Yang *et al.*** [20] who studied the association between the polymorphism in H19 rs3741219 and the risk of cancer, using data from ten studies involving 6974 controls and 5305 patients. Overall research showed a correlation between cancer susceptibility and the GG allele of the rs37412219 polymorphism when compared to the AA + GA genotype. Further analyses showed that rs3741219 mutation significantly increased the risk of ovarian and hepatocellular carcinoma, but also reduced the risk of glioma. Another study conducted by **Abdollahzadeh and Ghorbian** [21] on rs3741219 T > C in breast cancer patients showed no correlation between different genotypes and clinicopathological data.

The univariate analysis of possible risk variables for HCC in our study revealed that liver size and Child score were substantially linked to an elevated risk of HCC compared to the cirrhosis group. Additionally, multivariate analysis revealed that liver size and Child score were substantially linked to an elevated risk of HCC relative to the cirrhosis group.

However, this study had a little weakness. One is the small sample size; also, the genetic mutation under study in this work might only apply to a certain ethnic group. The study may need a larger sample size and replication experiments. In addition, the experiment was limited to the effect of this polymorphism on the disease with ignorance of numerous variants within the same gene or other lncRNA genes that may affect the expression level of this polymorphism or gene. Another weakness is only one SNP was investigated in this research. As far as we know, we are the initial research to exclude the presence of a significant association between H19 (rs3741219) variant genotypes and HCC.

## CONCLUSION

Our research showed that the H19 (rs3741219) variant was not significantly correlated with the incidence of HCC. To confirm our findings, more research with a bigger sample size and across other ethnic groups is required.

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