The clinical utility of tumor necrosis factor alpha 308 gene polymorphism in hepatocellular carcinoma patients with or without helicobacter pylori infection

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Abstract: **Background:** Contribution of Single Nucleotide Polymorphism (SNP) in hepatocellular carcinoma (HCC) development is widely studied. Tumor Necrosis alpha (TNF α) 308 gene polymorphism and HCC risk is still controversial. It was hypothesized that helicobacter pylori infection (H. pylori) has systemic impact via cytokines, our purpose was to assess the association between TNF alpha 308 polymorphisms and HCC and to investigate H. pylori infection as a possible associated risk factor of HCC. **Methods:** This study includes 110 HCC patients and 126 healthy control. The study was conducted at Mansoura University for 2 years. H. pylori stool Ag and SNP of TNF alpha 308 gene have been investigated in each group. **Results:** A higher positive H. pylori stool antigen was found in HCC cases. A higher 'A' allele was found in HCC cases. A participant with 'A' allele has 2.5-times higher odds to exhibit HCC. A higher G/A – A/A genotypes was found in HCC cases than control. A participant with G/A or A/A genotype has 2.8-times higher odds to exhibit HCC. Also participants with positive H. pylori, and G/A-A/A genotypes have 5.3-, and 2.8-times higher odds, respectively to exhibit HCC. **Conclusion:** TNF alpha 308 single nucleotide gene polymorphism and positive H. pylori stool Ag could be valuable markers for prediction of HCC development.

1. Introduction:

Primary liver cancer has been ranked as the seventh most frequently occurring cancer in 2020 after breast, lung, colorectal, prostate, skin and stomach cancers and the third most common cause of cancer mortality after lung and colorectal cancer[1]. Globally, HCC is the dominant type of liver cancer, accounting nearly for 75% of all liver cancers[2]. Regarding the number of new cases in 2020 (both sexes and all ages), liver cancer was the most common cancer in Egypt in 2020[3]. This could be attributed to the improvement in screening programs[4]. HCC was estimated to be responsible for nearly 9.1% of the total deaths in 2012[5]. In Egypt, It is the most common cause of mortality- and morbidity-related cancer[6].

The development of HCC depends on some factors such as viral infection, environmental, behavioral, metabolism, and genetics[7]. TNF-α is an important inflammatory cytokine in the development of liver disease. This cytokine can cause hepatic injury, cirrhosis and eventually promote hepatocellular carcinoma[8]. SNPs of TNF-α 1031 T/C, 863C/A, 857C/T, 308G/A, and 238G/Aare SNPs in the TNF-α promotor site that have often been investigated regarding their association with HCC in several previous studies[9]. It was also said that those SNPs could affect TNF-α production at the transcription level[10]. High production of TNF-α is related to the increase of pro-inflammatory cytokine secretion, the activation of proto oncogenes and several genes associated with cell growth, invasion, and cancer cells metastasis[11]. TNF-α 308 G/A is the most studied SNP which is also correlated with the risk of other cancers, such as breast cancer and gastric cancer[12]. However, those studies are still

limited and yield conflicting results. Thus, the main aim of the present study was analysis of TNF-α 308 polymorphism as a diagnostic tumor marker for HCC. On the other hand, findings concerning the influence of H. pylori on various extra-gastric organs have accumulated including metabolic, allergic, autoimmune and neurodegenerative as well as hepatobiliary, pancreatic and colorectal diseases[13]. Consequently, the relationship between H. pylori and liver diseases has been discussed and still remains controversial[14]. Therefore, another aim for our study was to investigate the possible association between H. pylori infection and the risk of HCC in addition to the conventional risk factors of the disease.

2. Methods:
Study design:
The present study was case control study.

Settings:
The study was conducted at Mansoura Specialized Medical Hospital outpatient HCC clinic for 2 years (from April 2019 to May 2021).

Sample and sampling technique:
Sample size was calculated by using An Online Sample Size Estimator (OSSE) http://osse.bii.a-star.edu.sg/index.php. Based on data from three Egyptian studies: This study included 110 HCC patients (case group) and 126 control persons (control group) and all of them were from Mansoura Specialized Medical Hospital outpatient HCC clinic for 2 years. H. pylori stool Ag and SNP of TNF-α 308 by polymerase chain reaction (PCR) restriction fragment length polymorphism assays have been investigated in each group after thorough history and physical examination.

Data collection:
All patients were subjected to thorough history taking and physical examination. Investigations have included radiology in the form of abdominal ultrasound and triphasic abdominal computed tomography (CT). Laboratory investigations have been done in the form of complete blood picture, liver function tests as ALT (Alanine aminotransferase)and AST (Aspartate transaminase) Serum total bilirubin, albumin, also Hemoglobin serum creatinine, H. pylori stool Ag and TNF-α genotyping at position 308 using restriction fragment length polymorphism (RFLP)

Inclusion criteria:
Cirrhotic patients with HCC having or not H. Pylori infection coming to Mansoura Specialized Medical Hospital outpatient HCC clinic .

Exclusion criteria:
1-Evidence of extrahepatic malignancies.
2-Hepatic secondaries.
3-History of recent antibiotics or PPI in last 2weeks.
4-Known drug abuse .
5-Pregnant females.
6-Organ failure: heart failure, respiratory failure, renal failure..

Statistical analysis:
The collected data were entered and analyzed using IBM-SPSS software (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp).
All cases (N=236), HCC cases (N=110) and control subjects (N=126) were in Hardy-Weinberg equilibrium (HWE).

3. Results:
The main findings were:
Table 1 shows no statistically significant difference in sex distribution between HCC cases vs control subjects. A statistically significantly higher proportion of ex-smokers and a statistically significantly lower proportion of non-smokers were observed in HCC vs control. A statistically significantly higher proportion of DM and positive H. pylori stool antigen test were found in HCC cases vs control subjects.

Table 2 shows no statistically significant difference in age distribution and serum creatinine levels between HCC cases vs control subjects. A statistically significantly higher ALT, AST, and serum total bilirubin were observed in HCC vs control. A statistically significantly lower BMI, hemoglobin, platelet count, and serum albumin were found in HCC cases vs control subjects.

Table 3 shows a statistically significantly higher 'A' allele in HCC vs control (risk allele). This association is of low strength. A participant with 'A' allele has 2.5-times higher odds to exhibit HCC.

Table 4 shows a statistically significantly higher G/A – A/A genotypes in HCC vs. control (risk genotypes). This association is of low strength. A participant with G/A or A/A genotype has 2.8-times higher odds to exhibit HCC.

Table 5 shows that the best inheritance model is the dominant model. Participants with G/A or A/A genotype have 2.8-times higher odds (adjusted for age and sex) to exhibit HCC

Table 6 shows the results of binary logistic regression, which was run to ascertain the effects of diabetes, positive H. Pylori, current or ex-smoker, and G/A-A/A genotypes of TNF-α 308 on the likelihood of occurrence of HCC. All 4 variables

were statistically significant on univariate analysis. However, on multivariate analysis, diabetes was no longer statistically significant predictor. The model correctly classifies 69% of cases, with 71% sensitivity and 67% specificity. The model was

statistically significant (χ^2 [4] = 57.034, P <0.001). Participants with history of smoking (ex-smoker and current), positive H. pylori, and G/A-A/A genotypes have 2.5-, 5.3-, and 2.8-times higher odds, respectively to exhibit HCC.

Table (1): Comparisons of categorical parameters between HCC and control groups

Parameter	Control	HCC	χ^2	φ / Cramer's V	P value
Sex			2.476	0.102	0.116
Male	114 (90.5%)	92 (83.6%)			
Female	12 (9.5%)	18 (16.4%)			
Smoking			15.212	0.254	**<0.001**
Never smoker	102 (81%) a	68 (61.8%) b			
Ex-smoker	3 (2.4%) a	16 (14.5%) b			
Current smoker	21 (16.7%) a	26 (23.6%) a			
Diabetes	27 (21.4%)	38 (34.5%)	5.063	0.146	**0.024**
H. pylori	33 (26.2%)	70 (63.6%)	33.481	0.377	**<0.001**

Notes: Data is N (%). Measures of the strength of association are φ (phi) for 2X2 table and Cramer's V for 2X3 table (smoking). Test of significance is Chi-Square test.

Table (2): Comparisons of quantitative parameters between HCC and control groups

Parameter	Control	HCC	Z value	P value
Age (years)	60 (55 – 64)	60 (55 – 65)	-0.448	0.654
BMI (kg/m^2)	29.7 (27.1 – 31.9)	25.9 (24 – 30.2)	-5.918	**<0.001**
Hemoglobin level (g/dl)	13.3 (12.5 – 14)	11.2 (10.3 – 12.8)	-7.166	**<0.001**
Platelet count	290 (251 – 350)	135 (88 – 194)	-11.740	**<0.001**
Serum albumin (g/dl)	3.8 (3.6 – 4)	3.5 (3.2 – 3.9)	-4.535	**<0.001**
ALT (IU/L)	24.5 (15 – 31)	42 (30 – 63)	-8.593	**<0.001**
AST (IU/L)	21.5 (15 – 30)	55.4 (41 – 74)	-11.806	**<0.001**
Serum total bilirubin (mg/dl)	0.9 (0.8 – 1.0)	1.0 (0.8 – 1.3)	-5.496	**<0.001**
Serum creatinine (mg/dl)	1.0 (0.9 – 1.1)	0.9 (0.8 – 1.1)	-1.005	0.315

Notes: Data is median (Q1 – Q3). Test of significance is Mann-Whitney U-test.

Table (3): Comparisons of TNF-α 308 alleles between HCC and control groups

Allele	Control	HCC	Chi-square test			Binary logistic regression	
			χ^2	φ	P value	COR	95% CI
'G' allele	222 (88.1%)	164 (74.5%)	14.473	0.175	**<0.001**	r(1)	r(1)
'A' allele	30 (11.9%)	56 (25.5%)				2.5	1.6 – 4.1

Notes: Data is N (%). φ (phi) is a measure of the strength of association. r(1) = reference category. COR = crude odds ratio. CI=confidence interval.

Table (4): Comparisons of TNF-α 308 genotypes between HCC and control groups

Genotype	Control	HCC	Chi-square test			Binary logistic regression	
			χ^2	φ	P value	COR	95% CI
G/G	99 (78.6%)	62 (56.4%)	13.360	0.238	**<0.001**	r(1)	r(1)
G/A – A/A	27 (21.4%)	48 (43.6%)				2.8	1.6 – 5.0

Notes: Data is N (%). φ (phi) is a measure of the strength of association. r(1) = reference category. COR = crude odds ratio. CI=confidence interval.

Table (5): TNF-α 308 SNP association with HCC (adjusted by age and sex).

Model	Genotype	Control	HCC	AOR	95% CI	P value	AIC	BIC
Co-dominant	G/G	99 (78.6%)	62 (56.4%)	r(1)	r(1)	**0.001**	318.8	336.1
	G/A	24 (19.1%)	40 (36.4%)	2.7	1.5-5.0			
	A/A	3 (2.4%)	8 (7.3%)	4.1	1.1-16.4			
Dominant	G/G	99 (78.6%)	62 (56.4%)	r(1)	r(1)	**0.0002**	317.1	331.0
	G/A-A/A	27 (21.4%)	48 (43.6%)	2.8	1.6-5.1			
Recessive	G/G-G/A	123 (97.6%)	102 (92.7%)	r(1)	r(1)	0.084	327.6	341.4
	A/A	3 (2.4%)	8 (7.3%)	3.1	0.8-12.0			
Overdominant	G/G-A/A	102 (81%)	70 (63.6%)	r(1)	r(1)	**0.0026**	321.5	335.3
	G/A	24 (19.1%)	40 (36.4%)	2.5	1.4-4.5			
Log-additive	-	-	-	2.4	1.5-4.0	**0.0003**	317.2	331.1

AOR = adjusted odds ratio. AIC = Akaiki information criterion BIC = Bayesian information criterion. CI = confidence interval.

Table (6): Predictors of the likelihood of occurrence HCC

Predictor	Univariate			Multivariate		
	P value	COR	95% CI	P value	AOR	95% CI
Diabetes	**0.025**			0.364		
Absent		r(1)	r(1)		r(1)	r(1)
Present		1.9	1.08-3.45		1.4	0.695-2.7
H. pylori test	**<0.001**			**<0.001**		
Negative		r(1)	r(1)		r(1)	r(1)
Positive		4.9	2.8-8.6		5.3	2.9-9.6
Smoking	**0.001**			**0.009**		
Never smoke		r(1)	r(1)		r(1)	r(1)
Ex- or current-smoker		2.6	1.5-4.7		2.5	1.3-4.9
TNF-α 308 genotype	**<0.001**			**0.002**		
G/G		r(1)	r(1)		r(1)	r(1)
G/A – A/A		2.8	1.6-5.0		2.8	1.5-5.3

Notes: COR=crude odds ratio. AOR=adjusted odds ratio. CI=confidence interval. r(1)=reference category. Test of significance is binary logistic regression.

4. Discussion:

Our study showed, regarding smoking, 23.6% of HCC patients were current smokers in comparison with 16.7% in control group. A statistically significant higher proportion of past history of smoking (ex-smoker) was found in HCC patients (14.5%) vs (2.4%) in control group and a statistically significant higher proportion of never smoker (81%) was observed in control group vs HCC group (61.8%) with a P value <0.001 as shown in table 1.

These findings are in agreement with the study of **Abdel-Rahman et al, 2017**[15] who reported smoking as a relative risk factor for development of HCC cirrhosis.

A statistically significant higher proportion of DM was found in HCC patients (34.5%) vs (21.4%) in control group with a P value of 0.024 (Table 1).

This finding is in agreement with **Ohkuma et al., 2018**[16] who reported that DM was associated with a 2 to 3 fold increased risk of HCC, with a significantly greater relative risk among men than women.

A statistically higher proportion of positive H. pylori stool antigen test in HCC patients (63.6%) vs (26.2%) in control group with a P value <0.001 (Table 1). This finding is in agreement with **Mekonnen et al., 2018**[17].

A statistically significantly higher ALT, AST, and serum total bilirubin were observed in HCC patients vs control group with a P value of <0.001 each. A statistically significantly lower BMI, hemoglobin, platelet count, and serum albumin were found in HCC cases vs control group with a P value of <0.001 each (Table 2). These findings are in parallel to **Gopal et al., 2014**[18].

Regarding TNF-α 308 alleles, Table 3 shows a statistically significantly higher 'A' allele in HCC cases (25.5%) vs. control group (11.9%) with a P value of <0.001 (risk allele). A participant with 'A' allele has 2.5 times higher odds to exhibit HCC.

Regarding TNF-α 308 genotypes, a statistically significantly higher G/A – A/A genotypes in HCC cases (43.6%) vs. control group (21.4%) with a P value of <0.001 (risk genotypes). A participant with G/A or A/A genotype has 2.8 times higher odds to exhibit HCC (Table 4).

Table 5 shows the different inheritance models for TNF-α 308 SNP and their association with HCC. All inheritance models showed significant relationship between the SNP and HCC risk except the recessive model. However the best inheritance model was the dominant model (P value 0.0002). Participants with G/A or A/A genotype have 2.8-times higher odds (adjusted for age and sex) to exhibit HCC.

These findings are in line with **Xiao et al., 2016**[19]. Also in line with **Hu et al., 2014**[20] and **Tavakolpour and Sali, 2016**[21] on allele models and dominant model analyses. **Wungu et al., 2020**[22] showed a significant relationship between the SNP and HCC risk in all five genetic analysis models.

However, **Wei et al., 2011**[23] reported that SNP 308 AA was associated with an increased risk of HCC in Asian ethnicities, but not for Caucasian. On the other hand, a study conducted on South Korean population showed that TNF-α 308 SNP alone was not significantly associated with HCC, but when several genotypes were combined (e.g. 1031 / 308 / 238), there was a significant association with the incidence of HCC[24]. Those findings suggest that TNF-α 308 SNP seems to be variable within ethnicities

Binary logistic regression was run to ascertain the effects of diabetes, positive H. pylori, current or ex-smoker, and G/A-A/A genotypes of TNF α 308 on the likelihood of occurrence of HCC (Table 6). All 4 variables were statistically significant on univariate analysis. However, on multivariate analysis, DM was no longer a statistically significant predictor. participants with history of smoking (ex-smoker and current), positive H. pylori stool Ag, and G/A-A/A genotypes have 2.5-, 5.3-, and 2.8-times higher odds, respectively to exhibit HCC and these findings are in line with **Abdel-Rahman et al., 2017**[15]**, Mekonnen et al., 2018**[17] **and Tavakolpour and Sali, 2016**[21] respectively.

Limitations:

First limitation of the present study is that it was hospital-based case-control study, and patients were selected at a single institution (Mansoura Specialized Medical Hospital) and thus may have been unrepresentative of hepatocellular carcinoma patients in the general population. Also, the relatively small number of patients was due to the difficulty in acceptance by patients to be included in a research study in addition to the high expense of the kits.

Conclusion:

H. pylori stool Ag and TNF-α 308 single nucleotide gene polymorphism could be valuable markers for prediction of HCC.

Recommendations:

This was a pilot study with small sample size so further large scale studies are warranted to confirm the possible role of analysis of H. pylori stool Ag and TNF-α 308 SNP in the prediction of HCC development.

Author Contributions:

All authors contributed to the study conception and design. Manuscript review, editing, publishing and final revision. were performed by Prof. Nancy Abdel-Fattah Ahmed, idea of the study and data collection by Prof. Ashraf Ahmed Omar, laboratory investigations by Prof. Mohammed Hosamel Deen Zaghloul and literatures, clinical and statistics by Dr.Ghada Mostafa Badawy. All authors read and approved the final manuscript.

Data Availability:

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval:

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Medical Ethics Research Team, Faculty of Medicine, Mansoura University code number: MD/17.07.102

Consent to participate:
    Informed consent was obtained from all individual participants included in the study**.**

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