J Appl Biotechnol Rep. 2022 June;9(2):645-650

Journal of
Applied Biotechnology Reports

doi 10.30491/JABR.2022.330055.1499

Original Article

The Association of -1031 T/C TNF-α Gene Promoter Polymorphism with the Incident of Gastric Carcinoma Among Iraqi Patients

Qatar Al-nada Mohammed Azeez [1], Zeena Hadi Obaid Alwan [1*]

[1] Department of Biology, College of Science, University of Babylon, Babylon, Iraq

Corresponding Author: Zeena Hadi Obaid Alwan, PhD, Assistant Professor, Department of Biology, College of Science, University of Babylon, Babylon, Iraq. Tel: 07816655430, E-mail: zeenaalwan2017@gmail.com

Received February 17, 2022; **Accepted** April 26, 2022; **Online Published** June 18, 2022

Abstract

Introduction: Among all the different types of cancer, Gastric Carcinoma (GC) has become the most frequently diagnosed and has continued to be a major public health issue in the last few decades worldwide. Association between the polymorphisms of the (T/C) -1031 TNF-α promoter gene sequence (rs1799964) and the incidence of gastric carcinoma was tested in Iraqi patients undergoing 5-FU plus Cisplatin, patients without chemotherapy, and healthy controls.

Materials and Methods: Blood samples were collected from patients and control groups to carry out the molecular and immunological diagnostic tests. Two ml of blood were collected in EDTA tubes for (T/C) -1031 TNF-α genotyping by using the RFLP technique. The serum part was used for the purpose of immunological tests via ELISA technique.

Results: Findings revealed that the homozygous wild genotype (T/T) was more abundant than other genotypes (T/C and C/C) in different groups of this study (82, 73, and 72% in control, patients under treatment, and patients without treatment respectively). From the Chi-square (data of the *p* value), there was no significant differences between genotypes in the different groups. TNF-α concentration increased significantly in heterozygous (T/C) and homozygous (T/T) genotypes in patients without treatment ($p = 0.0143$) and was highly significant in healthy control samples ($p = 0.0003$). The results showed there were non- substantial differences ($p = 0.1083$) in the TNF-α concentrations between different genotypes in patients treated with chemotherapy.

Conclusions: The genotyping study through the RFLP Technique and allele frequency measurement revealed that the homozygous wild genotype (T/T) was more frequent compared with the other genotypes in different groups of this study. However, TNF-α concentration significantly increased in heterozygous (T/C) genotypes. Non-significant differences in TNF-α concentration were detected among different genotypes in gastric carcinoma patients under treatment of chemotherapy.

Keywords: -1031 T/C, TNF-α Promoter, Polymorphism, Gastric Carcinoma, Iraqi Patients

Citation: Azeez QANM, Alwan ZHO. The Association of -1031 T/C TNF-α Gene Promoter Polymorphism with the Incident of Gastric Carcinoma Among Iraqi Patients. J Appl Biotechnol Rep. 2022;9(2):645-50. doi:10.30491/JABR.2022.330055.1499

Introduction

Gastric cancer (GC) has become the second cause of death around the world, affecting approximately one million people each year. Despite the significant geographical variations in incidence, GC is now the fourth most common cancer worldwide. Many factors including gastric precursor lesions, *Helicobacter pylori* infection, and genetic polymorphisms, have been linked to GC.[1] The heterogeneity of genetic and malignant phenotypes within each type of cancer makes cancer diagnosis and treatment incredibly challenging.[2] Furthermore, in clinical practice, variability in the efficacy and toxicity of anticancer agents in patients is a major issue.[3,4] Nonspecific therapeutic approaches can lead to chemo-resistance, which results in either increasing chemotherapy dosage with increasing the chance of toxicity or rendering patients intolerable for treatment. In consequence, a stronger inflammatory response by the host may alter the risk of GC.[3,5] Furthermore, 5-fluorouracil (5-FU) has long been used to treat gastrointestinal cancers. According to the research, low-dose Cisplatin (CDDP) and continuous venous infusion of 5-FU (low-dose FP therapy) have additive or synergistic antitumor effects in experimental case.[6] Previous research revealed more information about the links between the polymorphism of various proinflammatory factors and GC. Tumour necrosis factor-α (TNF-α) gene is a powerful immuno-modulator and pro-inflammatory cytokine that mediates a variety of pathological processes that have been linked to the severity of various immuneregulated diseases such as autoimmune diseases and transplantation. This gene is found on chromosome 6p21.3 in the MHC region, which contains highly polymorphic sequences. TNF-α has a huge number of polymorphisms.[7] The entire human (TNF-α) gene size is 2.76 kb of DNA and contains four exons sequences and three introns[8,9] as well as five balletic single-nucleotide polymorphisms (SNPs) in the promoter region: G-238A, G-308A, C-857T, C-863A, and T-1031C.[10]

SNPs within TNF-α have the capacity to cause changes in

the structure of the regulatory sites, which can directly alter the production, function, and regulation of TNF-α.[9] TNF- expression significantly increased in the sera of people with advanced GC.[11] The aim of this study was to investigate the effect of -1031 T/C polymorphism in the TNF-α concentration, distribution of -1031 genotypes within patients under treatment with 5-FU plus Cisplatin, patients without treatment as well as healthy control, and with the association between different genotypes and allele frequency on the incidence GC disease.

Materials and Methods

Groups of Study

Blood samples were collected from patients with GC from November 2019 until July 2020 under the supervision of an oncologist. The plan and sampling protocol were reviewed and approved by the ethical committee of the university of Babylon/ College of Science along with the Department of Middle Euphrates Cancer Center in Kufa. The sampling was carried out by collecting 108 blood samples: 36 samples from patients with a confirmed diagnosis of gastritis carcinoma, under treatment of a 5-FU drug plus Cisplatin. Twenty samples from patients without treatment and (52) samples of control group from healthy individuals with no previous history or clinical confirmation of gastritis carcinoma or any other type of cancer were considered in the present study. The age of the patients admitted to the Middle Euphrates Cancer Center were in the range of 23-80 years, whereas healthy control groups were in the range of 20-76 years old.

Sample Preparation

About 5 ml of whole blood were collected from all study participants and then were subdivided into two groups. In the first group, 2 ml of blood was collected into EDTA tube and kept in a freezer until the onset of gDNA extraction.[12,13] For the second group of samples, 3 ml of fresh blood were centrifuged for 15 min at 6000 rpm and the serum was frozen at -20 °C until use for the immunological tests.[13]

TNF-α Quantity Assay by ELISA

Samples were quantified by using the Human TNF-α ELISA Kit, (Bioassay Technology Laboratory- China Company, Shanghai) according to the manufacturer's instructions.

Genotyping Analysis

Extraction of Genomic DNA

DNA extraction kit (Wizbiosolution, Korea) was used to extract the genomic DNA from 1 ml of the frozen whole blood in EDTA tubes.

Genotyping of -1031 Sequences Through TNF-α Promoter Region

The target sequence of the -1031 TNF-α gene promoter region was amplified by Polymerase Chain Reaction (PCR) with primers: forward (5'-TATGTGATGGACTCACCAGG-3) and reverse (5'-CCTCTACATGGCCCTGTCTT-3). Amplification conditions were set as following: an initial denaturation at 94 °C for 3 min followed by 30 cycles of denaturation at 94 °C for 30 sec, annealing at 61 °C for 1 min, and extension at 72 °C for 1 min. PCR product was visualized by electrophoresis in 2% agarose gel containing 0.2 ul ethidium bromide. The amplified product was digested by using *BbsI* restriction enzyme (300 units) for 2 h, then analyzed in 3% agarose gel electrophoresis via UV transilluminator compared with uncut PCR product.

Statistical Analysis

Statistical analysis was carried out using SPSS software version 26. Deviations in the distribution of categorical variables of allelic and genotypic frequencies in rs1799964 polymorphism were evaluated by chi-square test between patients and controls. Odds ratio identified the danger factors of GC with 95% confidence interval (CI), and *p* values under 0.05 were considered statistically significant in all calculations.

Results

DNA Extraction and Genotyping

Details for PCR products are shown in Figure 1. Digestion product for -1031 region by using *BbsI* distinguished two fragments with sizes 251 and 13 bp for the (T/T) wild type. However, three band sizes (180, 71, 13 bp) were achieved in the (C/C) homozygous SNP and bands with sizes 251, 180, 71, and 13 bp were identified for the heterozygous genotype (T/C) (Figure 2).

The genotypes of T>C rs1799964 for all participants are shown in Table 1. The frequency of wild (T/T) genotype was 82, 73 and 72% in the samples of healthy control, patients under treatment, and patients without treatment, respectively compared with the other genotypes (T/C and C/C) and it was more abundant in the control group. From the Chi-square (data of the *p* value and odds ratio), there was no significant association between the rs1799964 genotypes and increased risk of GC. However, high risk (OR.) was detected within different models (codominant heterozygous, recessive, and dominant) among different groups of samples. In addition, no relationship was found between T, C alleles and the risk of GC.

Relation Between TNF-α (-1031) Concentration Level and Genotypes Variation

This study included a comparison for the effects of different genotypes (T/T, T/C, and C/C) on the TNF-α concentration, collectively or within different groups of samples. The rates of TNF-α concentration increased significantly in heterozygous (T/C) and homozygous (T/T) genotypes in patients without treatment ($p = 0.0143$) (Table 2) and it was significantly high in healthy control samples ($p = 0.0003$). In contrast,

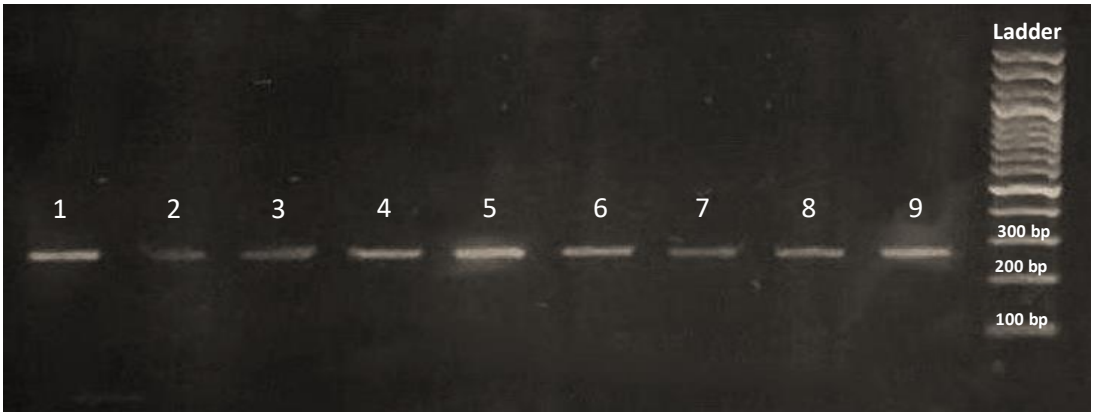

Figure 1. Gel Electrophoresis of PCR Products for -1031 TNF-α Gene Promoter Region. DNA fragments (264 bp) were analyzed compared with 100 bp ladder. Samples run in 3% agarose, at 85 V for 1 h.

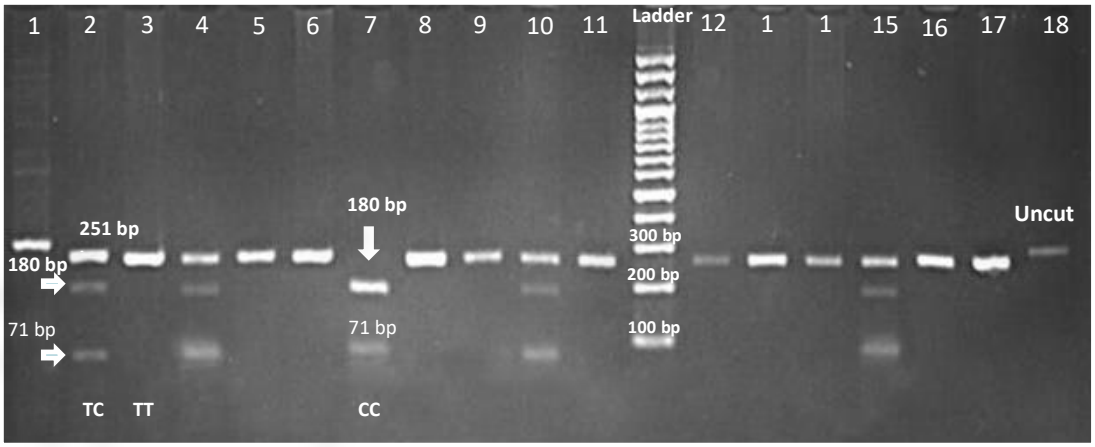

Figure 2. Gel electrophoresis for the -1031 TNF- α after digestion with BbsI restriction enzyme. Band size 251 bp are T/T wild type including 3, 5, 6, 8, 9, 11, 12, 13, 14, 16, and 17 lanes. Lane 7 with 180 and 71 bp bands sizes represent C/C homo SNP. T/C was achieved bands with 251 ,180, and 71 bp sizes including 2, 4, 10, and 15 lanes. The digested products were compared with uncut (264 bp) PCR product in 1 and 18 lanes. Band 13 was lost during electrophoresis from all digested PCR product. DNA fragments size were analyzed comparing with 100 bp ladder. Samples were ran at 3% agarose and 85 V for 1 h.

there were non- substantial differences (p = 0.1083) in the TNF-α concentrations between different genotypes in patients under treatment with chemotherapy. TNF-α concentrations were also compared in different genotypes among three types of samples. Results revealed that the dominant effect was for the T/C heterozygous genotype in healthy controls compared with the other groups. Table 2 reveals the average and standard error for the TNF-α concentrations within three genotypes in different groups.

Discussion

The findings of different studies on the association between -1031 TNF-α gene polymorphism and disease incidence are variable in conclusions due to geographical variation besides the difference in the volume of the collected samples.[14] In this study, the homozygous wild genotype (T/T) was more frequent compared with other genotypes (homozygous mutant (C/C) and heterozygous (T/C), among different groups of samples. These observations are highly agreed with the genotyping results of -1031 promoter region by Sakamoto.[15] The findings of this study are consistent with the results achieved by Zhang[14] and Baradaran Ghavami[11] that showed non-significant association between TNF-α -1031 polymorphism and GC when patients and the healthy control groups were compared. However, controversial findings have been reported on different types of cancers: TNF-α -1031 C allele is relevant with an increased risk for gastric ulcer development.[16] Moreover, Yang[17] revealed that no significant relation were achieved among the polymorphisms in TNF-α -308, -238, -857, -863, -1031, -1210 sequences and the incident of Breast Cancer (BC) in overall population.[17,18] Polymorphism in -1031 T/C TNF-α promoter region has been approved in many previous studies to influence in TNF-α concentration in different ways depending on the size of their effect. In this study, protein concentration was evaluated among different genotypes (T/T and T/C) associated with different samples. Enzyme-Linked Immunosorbent Assay (ELISA) technique has been adapted for all samples to quantify TNF-α level in serum of patients and healthy control as a pro-inflammatory factor leading to induce immunological response and thus

Table 1. Value of Odd Ratio and *p* value via Chi-square Test to TNF-α (-1031) Promoter Region of Patients with Treatment, Without Treatment, and Healthy Control

Variables	Genotype	Percentage of Patients (with/without)	Percentage of Control (+/-)	Comparison	OR (95% CI)	*p* value	Chi–Square
A- TNF-α 1031 Patients treated vs. positive control		(T/T) 73	72%	T/T			
	Codominant model	(C/C) 2	0	T/T VS. C/C	0.68 (0.55-0.84)	0.69	0.45
		(T/C) 23	27%	T/T VS. T/C	1.19 (0.33-4.28)	0.51	0.07
	Recessive model	26 2	27% 0	T/C+ C/C VS. C/C	1.50 (1.04-2.14)	0.68	0.48
	Dominant model	26 73	27% 72%	T/C+C/C VS. T/T	0.92 (0.26-3.27)	0.57	0.13
	Allele frequency	65 11	31% 5%	T C	0.95 (0.30-2.98)	0.59	0.07
B-TNF-α 1031 Patients treated with chemotherapy vs. negative control		(T/T) 73	82%	T/T			
	Codominant model	(C/C) 2	0	T/T VS. C/C	0.50(0.38 – 0.65)	0.50	0.98
		(T/C) 23	17%	T/T VS. T/C	0.66(0.20 – 2.21)	0.34	0.47
	Recessive model	26 2	17% 0	T/C+ C/C VS. C/C	1.60(1.09 – 2.33)	0.64	0.58
	Dominant model	26 73	17% 82%	T/C+C/C VS. T/T	1.66(0.53 – 5.20)	0.27	0.78
	Allele frequency	65 11	62% 6%	T C	0.57(0.19 – 1.64)	0.21	1.10
C-TNF-α 1031 Patient untreated with chemotherapy vs. negative control		(T/T) 72	82%	T/T			
	Codominant model	(C/C) 0	0	T/T VS. C/C	0	0	0
		(T/C) 27	17%	T/T VS T/C	0.55 (0.14-2.16)	0.30	0.72
	Recessive model	27 0	17% 0	T/C+ C/C VS. CC	0	0	0
	Dominant model	27 72	17% 82%	T/C+C/C VS. T/T	1.79 (0.46-6.97)	0.31	0.72
	Allele frequency	31 5	62% 6%	T C	0.60 (0.17-2.12)	0.31	0.63
D-TNF-α 1031 Total patients with gastric carcinoma vs. healthy (negative) control		(T/T) 73	82%	T/T			
	Codominant model	(C/C) 1	0	T/T VS. C/C	0.59 (0.48-0.72)	0.60	0.67
		(T/C) 25	17%	T/T VS T/C	0.62 (0.21-1.83)	0.27	0.73
	Recessive model	1 26	17% 0	T/C+ C/C VS. C/C	0.71 (0.54-0.93)	0.72	0.39
	Dominant model	26 73	17% 82%	T/C+C/C VS. T/T	1.70(0.59-4.93)	0.23	0.98
	Allele frequency	96 16	62% 6%	T C	0.58 (0.21-1.56)	0.19	1.17

Table 2. Means and Standard Divisions for TNF-α Concentration (pg/ml) Among Different Genotypes (T/T, T/C, and C/C) in Patients Treated with Chemotherapy, Patients without Treatment and Healthy Group

Genotype		Mean ± Std. Error	95% Confidence Interval for Mean		*p* value
			Lower Bound	Upper Bound	
Patient with treatment	T/T	146.0408 ± 14.65528	115.7240	176.3575	0.1083
	T/C	170.5632 ± 28.22595	103.8195	237.3070	
Patients without treatment	T/T	131.9815 ± 13.33480	102.9275	161.0356	0.0143
	T/C	182.9546 ± 26.95149	108.1253	257.7839	
Healthy control	T/T	289.4069 ± 13.15789	262.4091	316.4046	0.0003
	T/C	348.5838 ± 30.58387	269.9655	427.2022	
All groups		203.3898 ± 11.43347	180.6608	226.1188	<0.0001

may lead to the development of GC. On the other hand, the TNF-α concentration may be affected by chemotherapy usage for curing patients with stomach cancer. The level of TNF-α can be related to the stage of disease, as it increased in patients with the stage III and IV of cancer when compared with the stages I, and II,[19,20] or increased due to the tumor size.[21] The rates of TNF-α concentration increased significantly in heterozygous (T/C) genotypes in patients

without treatment and it was highly significant in healthy control samples compared with the other groups. The results were incompatible to some degree with previous studies. In 2017, Nourian[22] reported an increased TNF-α mRNA expression level observed in the C/C genotype of the -1031 TNF-α gene polymorphism compared with the T/C and T/T genotypes (p<0.05).[22] In 2018, results from Powrózek's study[23] indicated that C/C individuals had the highest TNF-α plasma level among the studied cases and showed a higher significant risk of the early death incidence compared to other genotype carriers.

Conclusion

The genotyping study through RFLP Technique and allele frequency measurement revealed that the homozygous wild genotype (T/T) was more frequent compared with other genotypes in different groups of this study. However, TNF-α concentration significantly increased in heterozygous (T/C) genotypes. Also, non-significant differences in TNF-α concentration were detected among different genotypes in gastric carcinoma patients under treatment of chemotherapy.

Authors' Contributions

The study was designed by ZHOA. The experiments and analyzed the data statistically was performed by QANMA. ZHOA and QANMA were written the manuscript together and proofreading was achieved by ZHOA.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

Acknowledgment

The authors would like to thank all the participants who agreed to participate in genetics studies related to GC. Many thanks to the Oncologist Dr. Imad Kareem Alwan Alsabri, in the Department of Middle Euphrates Cancer Center in Kufa for supporting this study.

References

1. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process—first American Cancer Society award lecture on cancer epidemiology and prevention. Cancer Res. 1992;52(24):6735-40.
2. Su X, Dong C, Zhang J, Su L, Wang X, Cui H, et al. Combination therapy of anti-cancer bioactive peptide with Cisplatin decreases chemotherapy dosing and toxicity to improve the quality of life in xenograft nude mice bearing human gastric cancer. Cell Biosci. 2014; 4(1):7. doi:10.1186/2045-3701-4-7
3. Lecomte T, Ferraz JM, Zinzindohoue F, Loriot MA, Tregouet DA, Landi B, et al. Thymidylate synthase gene polymorphism predicts toxicity in colorectal cancer patients receiving 5-fluorouracil-based chemotherapy. Clin Cancer Res. 2004;10(17):5880-8. doi:10.1158/1078-0432.CCR-04-0169
4. Van Kuilenburg AB. Dihydropyrimidine dehydrogenase and the efficacy and toxicity of 5-fluorouracil. Eur J Cancer. 2004;40(7):939-50. doi:10.1016/j.ejca.2003.12.004
5. Van Kuilenburg AB, Meinsma R, Zoetekouw L, Van Gennip AH. Increased risk of grade IV neutropenia after administration of 5-fluorouracil due to a dihydropyrimidine dehydrogenase deficiency: high prevalence of the IVS14+ 1g> a mutation. Int J Cancer. 2002;101(3):253-8. doi:10.1002/ijc.10599
6. Tsuji A, Morita S, Horimi T, Takasaki M, Takahashi I, Shirasaka T. Chemotherapy with low-dose CDDP and continuous 5-FU for the treatment of advanced gastric cancers. Gan to Kagaku ryoho. Cancer Chemother. 1999; 26(7):933-8.
7. Hajeer AH, Hutchinson IV. TNF-α gene polymorphism: Clinical and biological implications. Microsc Res Tech. 2000;50(3):216-28. doi:10.1002/1097-0029(20000801)50:3<216::AID-JEMT5>3.0.CO;2-Q
8. Mandal RK, Khan MA, Hussain A, Akhter N, Jawed A, Dar SA, et al. A trial sequential meta-analysis of TNF-α–308G> A (rs800629) gene polymorphism and susceptibility to colorectal cancer. Biosci Rep. 2019;39(1):BSR20181052. doi:10.1042/BSR20181052
9. Tu Y, Fan G, Zeng T, Cai X, Kong W. Association of TNF-α promoter polymorphism and Graves' disease: an updated systematic review and meta-analysis. Biosci Rep. 2018;38(2):BSR2018014. doi:10.1042/BSR20180143
10. Kamizono S, Hiromatsu Y, Seki N, Bednarczuk T, Matsumoto H, Kimura A, et al. A polymorphism of the 5′ flanking region of tumour necrosis factor α gene is associated with thyroid-associated ophthalmopathy in Japanese. Clin Endocrinol. 2000;52(6):759-64. doi:10.1046/j.1365-2265.2000.01011.x
11. Ghavami SB, Chaleshi V, Derakhshani S, Aimzadeh P, Asadzadeh-Aghdaie H, Zali MR. Association between TNF-α rs1799964 and RAF1 rs1051208 MicroRNA binding site SNP and gastric cancer susceptibility in an Iranian population. Gastroenterol Hepatol Bed Bench. 2017;10(3):214-9.
12. Abdulla AA. Association between Genetic Polymorphism of 5-HTTLPR, and SGOT, SGPT and Catalase with Alcoholism of Iraqi People. Indian J Forensic Med Toxicol. 2020;14(2):2154-60. doi:10.37506/ijfmt.v14i2.3334
13. Alwan ZH, AL-Gelawi RA, AL-Hilli NM. The relation of PROZ1 polymorphism and risk of early fetal loss in Iraqi patient? s diagnostic with Antiphospholipid Syndrome. Syst Rev Pharm. 2020;11(12):1312-7. doi:10.31838/srp.2020.12.192
14. Zhang C, Sturgis EM, Zheng H, Song X, Wei P, Jin L, et al. Genetic variants in TNF-α promoter are predictors of recurrence in patients with squamous cell carcinoma of oropharynx after definitive radiotherapy. Int J Cancer. 2014;134(8):1907-15. doi:10.1002/ijc.28512
15. Sakamoto K, Takeda S, Kanekiyo S, Nishiyama M, Kitahara M, Ueno T, et al. Association of tumor necrosis factor-α polymorphism with chemotherapy-induced oral mucositis in patients with esophageal cancer. Mol Clin Oncol. 2017;6(1):125-9. doi:10.3892/mco.2016.1081
16. Sugimoto M, Furuta T, Shirai N, Nakamura A, Xiao F, Kajimura M, Sugimura H, Hishida A. Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcer and gastric cancer. J Gastroenterol Hepatol. 2007;22(1):51-9. doi:10.1111/j.1440-1746.2006.04442.x
17. Yang Y, Feng R, Bi S, Xu Y. TNF-alpha polymorphisms and breast cancer. Breast Cancer Res Treat. 2011;129(2): 513-9. doi:10.1007/s10549-011-1494-2
18. Azeez QANM, Alwan ZHO. Association between -308 G/A TNF-A promoter region gene polymorphism and the incidence of gastric carcinoma in Iraqi patients under

treatment of 5-FU plus cisplatin chemotherapy. Biochemical and Cellular Archives. 2020;20(2):6667-73.
19. Forones NM, Mandowsky SV, Lourenзo LG. Serum levels of interleukin-2 and tumor necrosis factor-alpha correlate to tumor progression in gastric cancer. Hepatogastroenterology. 2001;48(40):1199-201.
20. Al Obeed OA, Alkhayal KA, Al Sheikh A, Zubaidi AM, Vaali-Mohammed MA, Boushey R, et al. Increased expression of tumor necrosis factor-α is associated with advanced colorectal cancer stages. World J Gastroenterol. 2014;20(48):18390-6. doi:10.3748/wjg.v20.i48.18390
21. Nikiteas NI, Tzanakis N, Gazouli M, Rallis G, Daniilidis K, Theodoropoulos G, et al. Serum IL-6, TNFα and CRP levels in Greek colorectal cancer patients: prognostic implications. World J Gastroenterol. 2005;11(11):1639-43. doi:10.3748/wjg.v11.i11.1639
22. Nourian M, Chaleshi V, Pishkar L, Azimzadeh P, Baradaran Ghavami S, et al. Evaluation of tumor necrosis factor (TNF)-α mRNA expression level and the rs1799964 polymorphism of the TNF-α gene in peripheral mononuclear cells of patients with inflammatory bowel diseases. Biomed Rep. 2017;6(6):698-702. doi:10.3892/br.2017.908
23. Powrozek T, Mlak R, Brzozowska A, Mazurek M, Gołębiowski P, Małecka-Massalska T. Relationship between TNF-α− 1031T/C gene polymorphism, plasma level of TNF-α, and risk of cachexia in head and neck cancer patients. J Cancer Res Clin Oncol. 2018;144(8): 1423-34. doi:10.1007/s00432-018-2679-4