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TUMOR NECROSIS FACTOR-± (TNF-±) 308 G/A PROMOTER POLYMORPHISMS IN GASTRIC CANCERS PATIENTS IN IRAQ

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Abstract

Gastric cancer (GC) is the fourth most frequent cancer and the second most frequent motive of cancer demise world huge after lung cancer. Genetic polymorphisms in the promoter place of the tumor necrosis factor-± (TNF-±) gene are involved in the regulation of expression levels and have been related with a number of inflammatory and malignant conditions. We have investigated two polymorphisms in the promoter area of the TNF-± gene (-308 G/A two) for their function in the susceptibility to means of an allelic association study. Using a case–control find out about design, lung cancer patients (n = 70) and fabulous age- and sex-matched controls recruited from the fitness check-up unit (n = 40) were subjected to genotype analysis for these polymorphisms, using a high-throughput allelic discrimination method. Genotype used to be analyzed the use of amplification refractory mutation device polymerase chain response (ARMS PCR) method two technique with genomic DNA isolated from peripheral blood two . The distribution of TNF ± genotypes at 308 (G → A) have been GG 41.4%, GA 14.4% and AA 44.2% in GC sufferers and GG 67.5%, GA 15% and AA 17.5% in control subjects. A sizeable association between the 308 G/A two polymorphisms in the promoter location of TNF-± and the susceptibility to Gastric most cancers was once demonstrated. Also, two polymorphisms had been associated with the severity of Gastric cancer. The -308 A allele has a promotive effect for Gastric cancer improvement and progression.

Key words : TNF-±polymorphisms**,** ARMS PCR, Gastric cancers.

Introduction

Gastric cancer (GC) is one of the most generally recognized malignancies and stays a sizeable public fitness hassle worldwide (Parkin *et al.,* 1997). The incidence of GC indicates marked version amongst countries (Parkin *et al.,* 1999). Incidence charges are particularly excessive in Japan and other East Asian countries, Eastern Europe and parts of Latin America (Inoue and Tsugane, 2005). Marked differences in GC incidence among unique ethnic corporations dwelling in the identical geographical area have been observed, pointing to host genetic factors or socio environmental factors peculiar to a precise racial group. GC stays one of the most frequent cancers in Asia (Bae *et al.,* 2002). It is the third most frequent most cancers in India and the 2d main website of most cancers occurrence worldwide. The incidence fee of GC is 4 instances greater in Southern India compared to Northern India (Singh and Ghoshal, 2006).

TNF ± is a cytokine triggered by way of Hp and inhibits gastric acid secretion (Gao *et al.,* 2008). The TNF A gene on chromosome 6p21.3 encoding TNF ± is known to have five biallelic single nucleotide polymorphisms in the promoter region; G 238A, G 308A, C 857T, C 863A, and T 1031C (Bidwell *et al.,* 2001). HP is accountable for triggering a pathological progression in the gastric mucosa that starts off evolved with persistent gastritis and progresses to atrophic gastritis, intestinal metaplasia, dysplasia, and in the end GC. The TNF-± 308 promoter polymorphism is a bi allelic G to A polymorphism, and the TNF a. A allele is related with extended ranges of TNF in plasma (Israa *et al.,* 2016).

Two TNF can regulate the chance of GC, the genuine role of TNF as a gastric carcinogen is nevertheless controversial. In the current study, we

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investigated the association between the TNF-± 308 G/A polymorphism and susceptibility to GC in Iraq populace

Materials and Methods

Sampling and data collection

This a case-control learn about consisted of 70 sufferers with RA and 50 healthy men and women 5 ml of blood was accrued from each subject in vacutainers with anticoagulant ethylene diamine tetra acetic acid (EDTA). Genomic DNA was removed from total blood samples of all patients and control subjects by means of the salting out procedure.

DNA extraction and purification

Genomic DNA was once extracted from complete blood accrued in EDTA-tubes from all subjects (patients and control individuals) the usage of Genomic DNA Extraction Blood DNA Mini Kit (FAVORGENE). The concentration (ng/ml) and purity (260/280 nm) of the DNA extracts have been measured at 260 nm and 280 nm with a Nano Drop spectrophotometer (OPTIZEN POP – Korea).

Genotyping

Genotyping of TNF-± 308 G/A polymorphism used to be carried via tetra primer amplification refractory mutation machine polymerase chain reaction (ARMS PCR) approach as described with the aid of Shu et al. [19] PCR primers sequences are given in table 1. two [8].

Each PCR response was once carried out in a complete volume of 10 µl, containing 30 ng of genomic DNA, 10 pmol of every inner primer, 1 pmol of each outer primer, 200 µM of each deoxy nucleotide triphosphates dNTPs, 1X reaction buffer, 2 mM MgCl2 and 0.5 U Taq polymerase. After amplification, the PCR products were separated by using electrophoresis on an agarose gel (1.5%) stained with ethidium bromide. The gel was visualized below ultraviolet light with 100 hundred bp ladder. All the accrued samples have been successfully genotyped. 10% of the samples had been randomly taken and the assay used to be repeated and determined no bias in the genotyping

The following program was set in the thermo cycler after determination of the optimum annealing temperature to amplify *TNF-α* (G-308A) shown in table 2.

Statistical analysis

The two sided Pearson's 2 check was adopted to observe the variations between the cases and the manage group with recognize to sex, age, smoking, alcoholism, and family history. Odds ratio (OR) and corresponding 95% self assurance intervals (CI) had been calculated by way of Open Epi software, A P fee □ 0.05 used to be regarded as significant (Kelley *et al.,* 2003).

Results and Discussion

A total of 70GC patients and 40 controls were enrolled in this case control study. Table 3 shows the distribution of allele frequency and genotype of (*TNF*-±)308 G/A between GC patients and controls. However, significant difference was observed between cases and controls.as shown in (Table 3).

A gene polymorphism of different individuals based on polyacrylamide gel electrophoresis 2% stained with ethidium bromide. Left lane: 100 bp DNA ladder.

Details of Fig. 2: Lanes 1, 2, 5, 6, 13, 15, 16, 19, 22, 23, 27, 28, 29, 35 and 36: We used both primers which

Table 1: The PCR primers and conditions for TNF α (-308 G/A) polymorphism.

Descriptive	Sequence	Tm	Amplicon size
Forward inner primer (A allele)	5 ,-ATAGGTTTT GAGGGGCATGG	68°	484bp (A allele)
AntisenseG allele	5,-AATGGTT TTGAGGGGCATGA- 3'		
AntisenseA allele	5,-TCTCGGTTTCTTCTCC ATCG-3,	63℃	186bp (G allele)

TNF- α: Tumor necrosis factor alpha, PCR: Polymerase chain reaction 65°C (from two outer primers)

Table (3-7): The program used for*TNF-α (G-308A)*amplification sequence.

Stage	Temp. (C^O)	Time (min)	Function	Cycles
1	94	5:0	Initial denaturation	1
2	94	0:15	Denaturation	10
	65	0:50	Primer annealing	
	72	0:40	Template elongation	
3	94	20	Denaturation	25
4	59	0:50	Annealing	
5	72	0:50	Extension	

Table 3: Distribution of allele frequency and genotype of(*TNF*-±)308 G/A in case-control study.

Genotype	Patients N% 70 (100%)	Control N% 40 (100%)	TEST X^2	Odd ratio	CI 95%
GG	29(41.4 %)	27(67.5%)	00 .001*		
GA	10(14.4%)	6(15%)		0.013	1, 55
AA	31(44.2%)	7 (17.5%)		4, 12	4.123 1.55 to 10.91
Allele Frequency					
G	68(49%)	54(67%)	1.8	2.4119 1.3530 to 4.2996	
A	72(51%)	26(33%)			

OR: Odd Ratio.

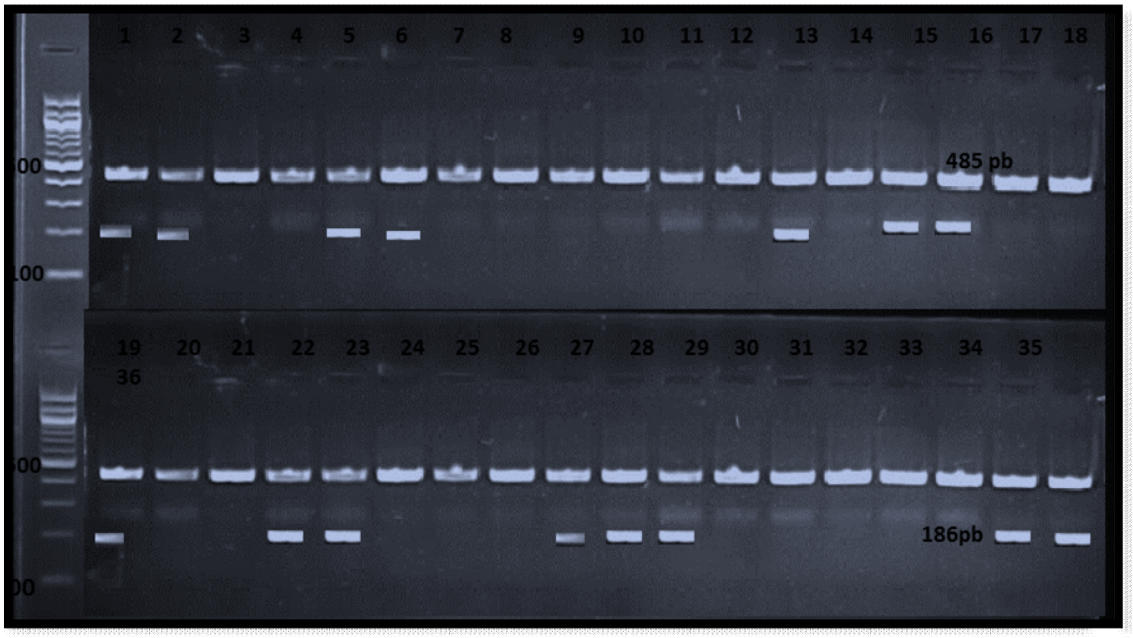

Fig. 2: ARMS-PCR of the TNF-a-308 G/A gene polymorphism of different individuals based on polyacrylamide gel electrophoresis 2% stained with ethidium bromide. Left lane: 100 bp DNA ladder.

revealed the G and A alleles. So, this pattern belongs to an individual who has GA heterozygote genotype. Lanes 3&4, 7, 8, 9, 10, 11, 12, 14, 17, 18, 20, 21, 24, 25, 26, 30, 31, 32, 33, 34: We applied a primer which showed the G allele. So, they are homozygote for GG genotype, the same as lane 5 & 6. Lanes 7 and 8: we used a primer which showed the A allele. So, they are homozygote for AA genotype. Both alleles have the same molecular weight. Genetic pre disposition is an necessary contributor in the pathogenesis of GC. Genetic elements finding out most cancers chance have been postulated for the final a long time and appear to be more apparent for GC (Correa 1992). A learn about by way of Wilson *et al.,* suggests that the much less frequent allele of TNF ±-308 A induces a greater environment friendly transcription of TNF ± and hence intensifies the inflammatory response against the infection (Howell and Rose Zerilli (2007). High TNF ± manufacturing inhibit gastric acid secretion which may additionally reason the spread of the organism into the corpus. However, the correlation between TNF ± 308 A allele and higher production of TNF ± has now not been proven in different studies (McCarron *et al.,* 2002).

TNF ±-308 AA genotype was related with an multiplied risk of non cardia gastric carcinoma especially in Hp seropositive folks (OR = 2.6) (Ishraq 2020). A good sized increased hazard of GC used to be found among TNF ±-308 GA heterozygotes compared to TNF ±-308 GG homozygotes (OR = 1.81;

95% CI: 1.04 3.14) in a study (BealesI and Calam 1998) Some different case control studies, which have been performed in China (Kamizono *et al.,* 2002). Iraq (Rababandlshraq 2018) did not find any extensive affilation between TNF a-308 polymorphism and the danger of GC. Our consequences are also in agreement with the latter reports displaying no association between TNF a 308 G/A polymorphism and GC and indicate that the 308 polymorphism is either generally functionally silent or does no longer play a purposeful position in TNF a expression.

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