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ROLE OF IL-1B GENE POLYMORPHISM in DISEASE SUSCEPTIBILITY in *HELICOBACTER PYLORI* ASSOCIATED GASTRITIS and GASTRIC CANCER

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Abstract:
Background: Interleukin-1β gene Polymorphisms might be associated with individual variations in the levels of cytokine messenger RNA resulting in different risk of inflammation of the gastric mucosa in response to *H. pylori* infection.
Objective: The aim of present study a role for IL-1B31, IL-1B-511 gene polymorphism (SNP) in *Helicobacter pylori* associated gastritis and gastric cancer that could have an effect on disease susceptibility.
Patients and methods: A case control study have been conducted and based on three groups, 20 patients with *Helicobacter pylori* infection associated gastric cancer, Second group was including 20 patients with Helicobacter pylori infection associated Gastritis and Third group was including 40 healthy volunteers. Two milliliter of blood Directly collected in a sterile tube with EDTA for DNA extraction ,then uses (RFLP PCR) technique application to IL-1B31, IL-1B-511 gene polymorphisms, Such these samples will be stored at -20C right away.
Results: present study show that IL-1β-511 TT genotype significantly higher in gastric cancer patients than control group (P=0.005). Concerning IL-1B-31 genotype, current result found that, the Frequency distribution of IL-1B-31 CT and CC genotypes show, no significant difference in gastric cancer patients than control (P=0.173) and (P=0.076) respectively. Moreover, IL-1B-31 (C/T), show no significant difference in the frequency of CT and CC genotype between patients with gastritis than control group (P=0.522) and (P=0.291) respectively. IL-1B-31 (C/T), show no significant difference in the frequency of heterozygous (CT), and homozygous (CC) genotype between gastric cancer patients in comparison with gastritis (P = 0.516), and (P = 0.510) respectively.
Conclusion: current study has shown that IL-1B 31 C > T (rs1143627) genotype have no impact on individual susceptibility to H. pylori associated gastric cancer and gastritis. Additionally, IL-1B 511 T > C (rs16944) gene polymorphisms act as a risk factor to H. pylori-related diseases including gastric cancer.

Keywords: IL-1β, Helicobacter pylori associated gastritis and gastric cancer.

INTRODUCTION

Gastric cancer results from numerous factors including genetic predisposition and environmental factors. Between such, H. pylori infection inducing chronic inflammation Has a crucial role to play in causing carcinogenesis. According to a multi-stage process first

described by Correa, H. pylori-driven inflammation triggers the development of intestinal type gastric cancer leading to typical stages of mucosal alterations such as chronic gastritis, glandular atrophy, intestinal metaplasia, dysplasia, before reaching the final stage of invasive gastric cancer [1]. Accumulating data indicate the gastric cancer is a complex multi-stage and multifactorial process and the product of interactions between the genes and the environment. Genetic variations in certain main genes which alter the ability of the host to respond to environmental stimulation may help to explain inter individual variability in the clinical outcome related to H. pylori [2]. IL-1β was of particular interest among them, because it closely influences the gastric physiological behavior in response to an infection of H. pylori [3]. These cytokines play a key role in deciding the etiology of gastric disease. Several studies have shown that the degree of IL1B expression in the promoter region of the gene can affect two allelic variants at positions -511 (T / T, rs16944) and -31 (C / C, rs1143627). An increased IL1B transcription is associated with such an allelic variant. Interleukin 1 beta is a strong regulator of gastric acid secretion. Decreased acidity and effective avoidance of gastric acid secretion in the stomach helps to reduce gastric acid secretion to inhomogeneous resettlement of H. Pylori up to the stomach body from the pylorus. Therefore, such polymorphisms may be regarded as a possible genetic factor in the predisposition of gastritis that determines the risk of malignant transformation [4].

MATERIALS AND METHODS

Patient group and sample collection

This case control study comprised of 3 groups, 1st consisted of20 patients with H. pylori-associated Gastric Cancer who were observation in Oncology Hospital in Diwaniya in the period for March 2018 to February 2019. Under professional supervision, Lesions observed by endocytoscopy underwent endoscopic biopsy or endoscopic resection. Second group was 20 patients with H. pylori-associated Gastritis who visited Endoscopy Department of Gastroenterology and Hepatology of al- diwanyia Teaching hospital. Individuals with gastritis were recruited consecutively from health checkup examinees that had undergone gastro copy and serologic analysis as part of a screening program for gastritis under the supervision of specialist in internal medicine. While, 3rd group was include 40 healthy volunteers .Venipuncture was used to collect samples from all groups, Two milliliter of Blood directly obtained from a sterile tube containing EDTA for DNA extraction, then uses (RFLP PCR) technique application to IL-1B31, IL-1B-511 gene polymorphisms, Such samples should be frozen at -20C right away.

PCR RFLP method

PCR RFLP was performed for detection of IL-1B 511, IL-IB 31 gene polymorphism in Gastritis, Gastric Cancer patients and blood samples of healthy control. This method was carried out as defined in the [5].

Genomic DNA Extraction

Gene aid's (Frozen Blood) Genomic DNA mini extraction kit (USA) was used to extract the genomic DNA from samples of blood conferring to the instruction of company. Moreover, the Nano-drop spectrophotometer (THERMO, USA) was utilized to extract the genomic blood DNA. It further checked the purity of DNA through absorbance reading at 260/280nm and calculated the concentration of DNA in (ng/µL).

RFLP-PCR Primers

RFLP Primers for detection of The **IL-1B 31, IL-1B 511** polymorphism and were and designed according (5**).** These primers were provided from (Macrogen Company, Korea) as following table (1).

Table (1): RFLP Primers for detection

Primer	Sequence		Amplicon
IL-1B 31 **c** > T	F	GCCTGAACCCTGCATACCGT	155 bp
	R	GCCAATAGCCCTCCCTTCT	
IL-1B 511 **c**> T	F	AGAAGCTTCCACCAATACTC	152 bp
	R	ACCACCTAGTTGTAAGGAAG	

NCBI-SNP: IL-1B 31 C > T (rs1143627), IL-1B 511 T > C (rs16944)

RESULTS

IL-1β-511 (C/T) genotype and allele study among control and study groups

The frequency of heterozygous (CT) genotype showed no significant difference between gastric cancer and control group (P = 0.509), 20.0 % versus 32.5 %, respectively, as shown in table (2), thus heterozygous (CT) genotype is neither a risk factor nor a protective factor. However, the frequency of homozygous (TT) genotype was significantly higher in gastric cancer patients in comparison with control group (P = 0.005), 65.0 % versus 25.0 %, respectively, as shown in table 2. The odds ratio was 7.37 with 95 % confidence interval of 1.68 -32.32 making homozygous (TT) genotype a risk factor for gastric cancer with an etiologic fraction of 0.49, as shown in table (2).

Moreover, there was highly significant difference in frequency distribution of patients with gastric cancer according to IL-1β (C/T) alleles in comparison with control subjects in such a way that allele C was less frequent and allele T was more frequent in gastric cancer patients in comparison with control, 25 % versus 58.8 % and 75 % versus 41.3 %, respectively, (P < 0.001), as shown in table (2).

Table (2): Frequency distribution of patients with gastric cancer according to IL.1β-511 and IL-1B-31 genotypes and alleles in comparison with control subjects

		Gastric cancer n = 20	Control n = 40	P	OR	95% CI	EF	PF
IL-1β-511	CC genotype	3 (15.0 %)	17 (42.5 %)	Reference				
	CT genotype	4 (20.0 %)	13 (32.5 %)	0.509 ¥ NS	1.74	0.33 -9.19	0.1	---
	TT genotype	13 (65.0 %)	10 (25.0 %)	0.005 ¥ HS	7.37	1.68 -32.32	0.49	---
	C allele	10 (25.0 %)	47 (58.8 %)	<0.001 ¥	0.23	0.10 -0.54	---	0.36
	T allele	30 (75.0 %)	33 (41.3 %)	HS	4.27	1.84 -9.93	0.36	---
IL-1B-31	TT genotype	4 (20.0 %)	17 (42.5 %)	Reference				
	CT genotype	9 (45.0 %)	15 (37.5 %)	0.173 ¥	2.55	0.65 -10.01	0.23	---
	CC genotype	7 (35.0 %)	8 (20.0 %)	0.076 ¥	3.72	0.84 -16.47	0.34	---
	T allele	17 (42.5 %)	49 (61.3 %)	0.052 ¥	0.47	0.22 -1.01	---	0.23
	C allele	23 (57.5 %)	31 (38.7 %)	NS	2.14	0.99 -4.63	0.23	---

N: number of cases; OR: ratio of odds; CI: interval of confidence; EF: fraction of the etiology; PF: preventive fraction; yen: chi-square test; NS: non-significant $P > 0.05$; HS: Highly significant at $P \leq 0.01$

The Heterozygous Frequency (CT) genotype showed no significant difference between gastritis and control group ($P = 0.853$), 20.0 % versus 32.5 %, respectively, as shown in table 3, thus heterozygous (CT) genotype is neither a risk factor nor a protective factor. In addition, the frequency of homozygous (TT) genotype showed no significant difference between gastritis and control group ($P = 0.106$), 50.0 % versus 25.0 %, respectively, as shown in table (3), thus homozygous (TT) genotype is neither a risk factor nor a protective factor.

Regarding IL-1β-511 (C/T) alleles, There was no significant difference between gastritis patients and the control group in the frequency distribution of those alleles $P = 0.053$), as shown in table (3); thus neither C nor T allele is a risk factor or a protective factor.

Table (3): Frequency distribution of patients with gastritis according to IL.1β-511 and IL-1B-31 genotypes and alleles in comparison with control subjects

		Gastritis $n = 20$	Control $n = 40$	*P*	OR	95% CI	EF	PF
IL-1β-511	CC genotype	6 (30.0 %)	17 (42.5 %)	Reference				
	CT genotype	4 (20.0 %)	13 (32.5 %)	0.853 ¥	0.87	0.20 -3.74	---	0.03
	TT genotype	10 (50.0 %)	10 (25.0 %)	0.106 ¥	2.83	0.79 -10.18	0.32	---
	C allele	16 (40.0 %)	47 (58.8 %)	0.053 ¥	0.47	0.22 -1.01	---	0.22
	T allele	24 (60.0 %)	33 (41.3%)	NS	2.14	0.99 -4.63	0.22	---
IL-1B-31	TT genotype	6 (30.0 %)	17 (42.5 %)	Reference				
	CT genotype	8 (40.0 %)	15 (37.5 %)	0.522 ¥	1.51	0.43 -5.36	0.12	---
	CC genotype	6 (30.0 %)	8 (20.0 %)	0.291 ¥	2.13	0.52 -8.70	0.23	---
	T allele	20 (50.0 %)	49 (61.3 % %)	0.240 ¥	0.63	0.29 -1.36	---	0.14
	C allele	20 (50.0 %)	31 (38.7 %)	NS	1.58	0.73 -3.40	0.14	---

N: number of cases; OR: ratio of odds; CI: interval of confidence; EF: fraction of the etiology; PF: preventive fraction; yen: chi-square test; NS: non-significant $P > 0.05$; HS: Highly significant at $P \leq 0.01$

The frequency of heterozygous (CT) genotype showed no significant difference between gastric cancer and gastritis group ($P = 0.486$), 20.0 % versus 20.0 %, respectively, as shown in table 4, thus heterozygous (CT) genotype is neither a risk factor nor a protective factor. In addition, the frequency of homozygous (TT) genotype showed no significant difference between gastric cancer and gastritis group ($P = 0.238$), 65.0 % versus 50.0 %, respectively, as shown in table (4), thus homozygous (TT) genotype is neither a risk factor nor a protective factor.

Regarding IL-1β (C/T) alleles, there was no significant difference in frequency distribution of those alleles between patients with gastritis and gastric cancer group ($P = 0.152$), as shown in table (4); thus neither A nor T allele is a risk factor or a protective factor.

IL-1β-31 (C/T) genotype and allele study among control and study groups
Regarding IL-1B-31 (C/T), there was no significant difference in the frequency of heterozygous (CT) genotype between gastric cancer patients in comparison with control group (P = 0.173), 45.0 % versus 37.5 %, respectively, thus heterozygous (CT) genotype is neither a risk factor nor a protective factor. The frequency of homozygous (CC) genotype No significant difference was noted between gastric cancer and control group (P = 0.076), 35.0 % versus 20.0 %, respectively, as shown in table (2), thus homozygous (CC) genotype is neither a risk factor nor a protective factor.

Regarding IL-1B-31 (C/T) alleles, there was no significant difference in frequency distribution of those alleles between patients with gastric cancer and control group (P = 0.052), as shown in table (2); However, this is consider as a border line P value, and according to table 2.

Moreover, IL-1B-31 (C/T), show no significant difference in the frequency of heterozygous (CT) genotype between patients with gastritis in comparison with control group (P = 0.522), 40.0 % versus 37.5 %, respectively, thus heterozygous (CT) genotype is neither a risk factor nor a protective factor, table 3. The frequency of homozygous (CC) genotype showed no significant difference between gastritis and control group (P = 0.291), 30.0 % versus 20.0 %, respectively, as shown in table (3), thus homozygous (CC) genotype is neither a risk factor nor a protective factor.

In addition IL-1B-31 (C/T) alleles, show no significant difference in frequency distribution of those alleles between patients with gastritis and control group (P=0.240), as shown in table (3) thus neither C nor T allele is a risk factor or a protective factor.

Regarding to comparison between Gastric cancer and gastritis, IL-1B-31 (C/T), show no significant difference in the frequency of heterozygous (CT) genotype between gastric cancer patients in comparison with gastritis (P = 0.516), 45.0 % versus 40.0 %, respectively, thus heterozygous (CT) genotype is neither a risk factor nor a protective factor, table (4). The frequency of homozygous (CC) genotype showed no significant difference between gastric cancer and gastritis group (P = 0.510), 35.0 % versus 30.0 %, respectively, as shown in table (4), thus homozygous (CC) genotype is neither a risk factor nor a protective factor.

In addition to that, IL-1B-31 (C/T) alleles, demonstrated that there was no significant difference in frequency distribution of those alleles between patients with gastric cancer and gastritis group (P = 0.501), as shown in table (4); thus neither C nor T allele is a risk factor or a protective factor.

Table (4): Frequency distribution of patients with gastric cancer according to IL.1β-511 and IL-1B-31 genotypes and alleles in comparison of gastritis group

		Gastric cancer n = 20	Gastritis n = 20	P	OR	95% CI	EF	PF
IL-1β-511	CC genotype	3 (15.0 %)	6 (30.0 %)	Reference				
	CT genotype	4 (20.0 %)	4 (20.0 %)	0.486 ¥	2	0.28 -14.20	0.25	---
	TT genotype	13 (65.0 %)	10 (50.0 %)	0.238 ¥	2.6	0.52 -13.04	0.35	---
	C allele	10 (25.0 %)	16 (40.0 %)	0.152 ¥	0.5	0.19 -1.30	---	0.28
	T allele	30 (75.0 %)	24 (60.0 %)	NS	2	0.77 -5.20	0.28	---

	TT genotype	4 (20.0 %)	6 (30.0 %)	Reference				
IL-1B-31	CT genotype	9 (45.0 %)	8 (40.0 %)	0.516 ¥	0.59	0.12 -2.89	---	0.22
	CC genotype	7 (35.0 %)	6 (30.0 %)	0.510 ¥	0.57	0.11 -3.04	---	0.23
	T allele	17 (42.5 %)	20 (50.0 %)	0.501 ¥	0.74	0.31 -1.78	---	0.14
	C allele	23 (57.5 %)	20 (50.0 %)	NS	1.35	0.56 -3.27	0.14	---

N: number of cases; OR: ratio of odds; CI: interval of confidence; EF: fraction of the etiology; PF: preventive fraction; yen: chi-square test; NS: non-significant $P > 0.05$; HS: Highly significant at $P \leq 0.01$

DISCUSSION

The findings of the current research are supported by previous studies, as such studies have shown that H. The synergistic effect of pylori infection on the production of GC with IL-1β gene polymorphisms (6). And the IL-1β-511 T allele is more commonly observed at H. Pylori-positive, rather than H, patients. Non-GC patients with Pylori-negative [7]. Interestingly, infection with H. pylori alone has only a marginal impact on the production of GC, yet in combination with the genotype IL-1β-511 T / T[5], the risk of GC is substantially increased. Likewise, an IL-1β-511TT genotype patient with an active H. pylori Infection with raises the risk of developing GC [8]. In particular, the highest risk carriers are patients with both high-risk bacterial and host genotypes (vacuolating cytotoxin gene A s1 region (vacAs1)/IL-1β-511*T carrier, vacAm1 / IL-1β-511*T carrier, and cytotoxin-related gene A (cagA)-positive / IL-1β-511*T carrier). [9]. In South China, children with IL-1β-511TT/-31CC have an increased risk of relatively significant histological changes in the gastric mucosa, where H. pylori The prevalence of infection is (10). Likewise, in patients with IL-1β-511T/-31CC there is a greater occurrence of intestinal metaplasia and atrophic gastritis. [11]. Furthermore, In comparison to H, pylori-related atrophic gastritis was shown as the more malignant phenotype. In patients with H. pylori- negetive atrophic gastirtis IL-1β-31CC/-511TT genotype d, [12]. In addition, patients with both host and bacterial high-risk genotypes (cagA(+)/vacAs1(+)/IL-1β-511T) Serious gastric disorders have the highest incidence (severe lymphocyte and granulocyte infiltration, atrophic gastritis, and intestinal metaplasia). Mechanistically, H. Pylori IL-1β-induced infection, which, in turn , promotes gastric carcinogenesis by affecting both inflammatory and epithelial cells. [13]. In the Hamajima study, the T allele IL-1β-31 polymorphism was found to be correlated with susceptibility to recurrent H pylori infection.(14), wheraes IL-1β-31 CC may be associated with The risk of developing relatively seriuos gastritis in South China [14]. In other meta-analysis study, IL-1β-31 C allele or CC genotype could increase the gastritis risk in the Caucasian population, while it could decrease the gastritis risk in the population of Mexican. The contradictory results between the Caucasian population and the Mexican population can be due to constitutions of the population and distributions of the genotype. Separate constitutions of the population may also account for the difference between the Asian and Caucasian populations. The lack of interaction between IL-1β polymorphisms and the incidence of gastritis in the Indian population is suspected to be attributed to the relatively lower level of cytokines secreted in the mucosa than in the Caucasian population. [15]. As IL-1β polymorphisms are entirely unrelated to the risk of gastritis in the Asian population, the level of cytokine expression can also affect the epidemiological outcomes of the entire Asian population. However, in the present analysis, there is no association of IL-1β-31 polymorphism with the risk of gastritis. One potential explanation is that IL-1β-31 is located in the TATA box, which may directly affect the expression of IL-1β, while IL-1β-511 is not. The latest results of IL-1β-31 polymorphism have been clear with the previous study [16].

CONCLUSION

Current study has shown that IL-1B 31 C > T (rs1143627) genotype have no impact on individual susceptibility to H. pylori associated gastric cancer and gastritis. Additionally, IL-1B 511 T > C (rs16944) gene polymorphisms act as a risk factor to H. pylori-related diseases including gastric cancer.

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