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Relationship between *H. pylori* infection and IL-1β polymorphism in pregnant women

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ABSTRACT

A study was performed on 100 pregnant women in the outpatient department of gynecology and obstetrics of Maternity and Children Hospital in Al-Diwaniya City during the period between (March to September 2016). One hundred blood samples (50 for patients and 50 for control) were collected under the supervision of the treating gynecologist**.** *The detection of Helicobacter. pylori was done by the use of the* serum antibody Rapid test. The results showed that 50(100%) were positive and 50(100%) were negative for *H. pylori* in above method. All blood of patients and control samples were used for the extraction of genomic DNA, where the 304 bp PCR product size. Genotyping of the IL-1β -511 (C/T) SNP was performed by restriction fragment length polymorphism PCR (RFLP-PCR). PCR products were digested with *AvaI* restriction enzyme. Individuals with the IL-1β-511(CC) homozygote produced digested DNA bands at 190 bp and 114 bp. A heterozygous genotype of IL-1β-511 (CT) produced 304 bp, 190 bp, and 114bp bands. Individuals with the IL-1β-511(TT) homozygote genotype had no amplicon digested and generated only one band of 304 bp. There was a significant difference in the frequency of the (IL-1β -511) CC genotype between *H. pylori* positive group and *H. pylori* negative group (22%, 8% respectively). Also for CT genotype, there was a significant difference between *H. pylori* positive group and *H. pylori* negative group (44%, 66% respectively). Concerning the frequency of the (IL-1β -511) TT genotype between *H. pylori* positive group and *H. pylori* negative group, there was no significant difference between the two groups.

Keywords: *H. pylori*, IL-1β polymorphism

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INTRODUCTION

Helicobacter pylori (*H*. *pylori*) is the most common chronic bacterial infection, and it has been demonstrated worldwide and in individuals of all ages. *Helicobacter pylori* (*H. pylori*) infection is investigated in gastric diseases even during pregnancy; in particular, this Gram-negative bacterium seems to be associated with hyperemesis gravidarum, a severe form of nausea and vomiting during pregnancy (Karaer *et al*., 2008).

Several studies have reported a relationship between *H. pylori* infection and an increase in the inflammatory gene response characterized by the up-regulation of several genes such as *IL-1β* and tumor necrosis factor-alpha (*TNF-α*). These cytokines are considered to be important mediators of gastric pathophysiology and could play a critical role in the etiology of gastric cancer (GC) (Person *et al*., 2011; Bhagat *et al.*, 2008).

MATERIALS AND METHODS

Samples collection

Fifty blood samples were collected from each pregnant women suspected to be infected with *H. pylori* who visited Maternity and Children Teaching Hospital during the period from (March to September 2016). And 50 blood samples were collected from pregnant healthy women (control group).The samples were collected under the supervision of the treating gynecologist. Serum Antibody Rapid Test was used for detection of *H. pylori* in (100) blood samples.

Serum Antibody Rapid test:

The test is used to diagnose the bacteria in blood samples, where the interaction results in a change of color through the sample movement of the membrane test strip in the package control area which gives the red color always. In the case of absence of infection with the bacterium red color appears only on the letter C (control line).While in the case of infection in addition to the package control in red color, a red package will appear on the letter T (result line).

Molecular study

Genomic Blood DNA Extraction

Genomic DNA from blood samples was extracted by using Genomic DNA Mini kit extraction kit (Frozen Blood) Geneaid. USA, and done according to company instructions.

Genomic DNA estimation

The extracted blood genomic DNA was checked by using Nanodrop spectrophotometer (THERMO. USA), which measured DNA concentration (ng/µL) and checked the DNA purity by reading the absorbance at (260 /280 nm).

Primers

IL-1β-511 gene polymorphisms primers were designed by (Santos *et al.,* 2012) and these primers were provided from (Bioneer Company, Korea) as in the following tables:

Table (1): The Multiplex PCR primers with their sequence and amplicon size

Primer	Sequence	Amplicon
IL-1β-511	TCCTCAGAGGCTCCTGCAAT	304bp
	TGTGGGTCTCTACCTTGGGTG	

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RFLP-PCR Technique

RFLP-PCR technique was performed for genotyping and detection IL-1β single-nucleotide polymorphism in blood samples of *Helicobacter pylori* gastric diseases patients and in healthy blood samples. This method was carried out according to that described by (Santos *et al*, 2012)

RFLP-PCR master mix preparation

PCR master mix was prepared by using (Accu Power PCR PreMix Kit) and this master mix has done according to company instructions as in the following table (2):

Table (2): PCR Master Mix with their volume for IL-1β gene

PCR Master mix	Volume
DNA template	5µl
IL-1β Forward primer (10pmol)	1.5µl
IL-1β Reveres primer (10pmol)	1.5µl
PCR water	12µl
Total volume	20µl

After that, these PCR master mix component mentioned in the above table placed in standard AccuPower PCR PreMix Kit that contains all other components which were needed to PCR reaction such as (Taq DNA polymerase, dNTPs, Tris-HCl pH: 9.0, KCl, $MgCl_2$, stabilizer, and loading dye). Then, all the PCR tubes transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler (Mygene. Korea).

PCR Thermocycler Conditions

PCR thermo cycler conditions were done for each gene independently as in the following table (3)

Table (3): PCR thermocycler conditions for IL-1β

PCR step	Temp.	Time	Repeat
Initial denaturation	95°C	5min.	1
Denaturation	95°C	30 sec.	35cycle
Annealing	55°C	30 sec.	
Extension	72°C	30 sec.	
Final extension	72°C	5min	1
Hold	4°C	Forever	-

PCR product analysis

The PCR products were analyzed by agarose gel electrophoresis as in the following steps:

1. A 1% Agarose gel was prepared by using 1X TBE and dissolving in a water bath at 100 °C for 15 minutes, after that, left to cool 50°C.
2. Then 3µL of ethidium bromide stain were added into agarose gel solution.
3. Agarose gel solution was poured into the tray after fixing the comb in proper position after that, left to solidify for 15 minutes at room temperature, then the comb was removed gently from the tray and 10µl of PCR product were added into each comb well and 10ul of (100bp Ladder) in first well.
4. The gel tray was fixed in electrophoresis chamber and fill by 1X TBE buffer. Then an electric current was performed at 100 volts and 80 AM for 1hour.
5. A 304 bp PCR product for *IL-1β* was visualized by using UV trans illuminator.

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RFLP mix for (*IL-1β* gene)

RFLP-PCR mix was prepared by using *AvaI* restriction enzyme (New England Biolabs.UK), and this master mix has done independently according to company instructions as in the following table (4)

Table (4): RFLP master mix with their volume for IL-1β gene

RFLP-PCR Master mix	Volume
PCR product	10µl
AvaI Restriction enzyme buffer 10X	2 µl
AvaI (10 unit)	1 µl
Free nuclease water	7 µl
Total volume	20 µl

After that, this master mix placed in exispin vortex centrifuge at 3000rpm for 2 minutes, then transferred into incubation at 37°C for overnight. After that, RFLP-PCR product was analyzed by 2% agarose gel electrophoresis method that mentions in PCR product analysis. The genotype IL-1β -511 C/C produced digested DNA bands of 190/114 bp, the IL-1β -511 T/T genotype had no amplicon digestion and generated only one band of 304 bp. A heterozygous genotype of IL-1β -511 C/T produced 304/190/114-bp bands.

RESULTS

Detection of *H. pylori* by Serum Antibody Rapid Test

The test is used to diagnose the bacteria in blood samples. In the case of absence of infection with the bacterium red color appears only on the letter C (control line), while in the case of infection in addition to the package control in red color, a red package will appear on the letter T (result line) as shown in (Figure 1). The result of this study revealed that (50) (100%) of patient blood samples was positive for this test and all control blood samples (50) (100%) gave a negative result for this test**.**

Fig(1): Serum antibody rapid test A: In the case of infection in addition to control red color, red color will appear on the letter T. B: In the case of no infection red color appears only on the letter C

RFLP-PCR for IL-1β Gene

All blood specimens of patients and control were used for extraction of genomic DNA to detect IL-1β gene which appears in 304bp PCR product size, figure (2). This result is in accordance with Santos work (2012).

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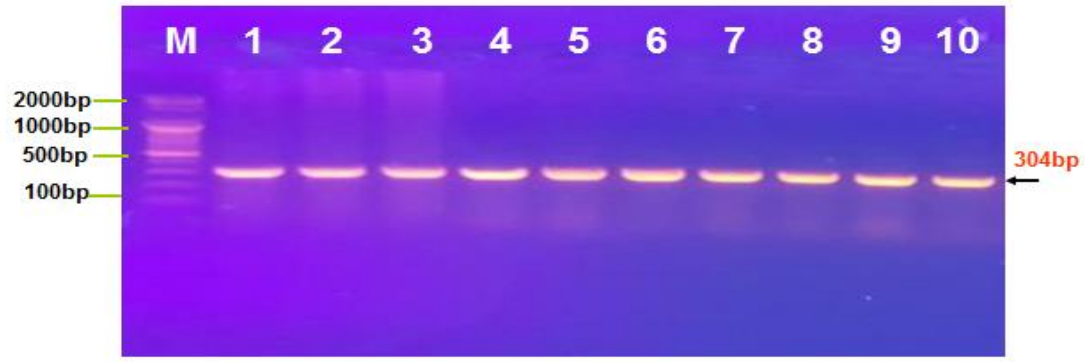
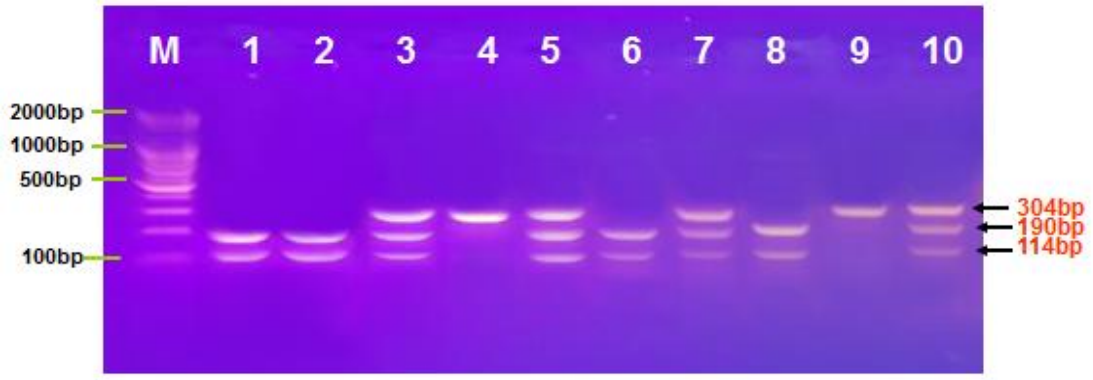


Figure(2): Agarose gel electrophoresis image that shows the PCR product analysis of *IL-1β* gene from some blood patient samples and healthy control sample. Where M: marker (2000-100bp), lane (1-10) patient samples that show 304bp PCR product size. All samples exposed to same thermocycler conditions at initial denaturation 95°C for 5min, followed by 35 cycles at 95°C for 30 sec.denaturation,55° C for 30 sec. annealing ,and 72° C for 30 sec. extension ,and followed by final extension at 72° C for 5min.The PCR product was separated on 1% agarose gel.

Our results demonstrated that the IL-1β -511C/C homozygote produced digested DNA bands at 190bp and 114bp, and heterozygous genotype of IL-1β -511(CT) produced digested DNA band at 304bp, 190bp, and 114bp.While individuals with IL-1β -511(TT) homozygote genotype had no amplicon digested and generated only one band of 304bp as shown in the figure (3) and (4). This result demonstrated that IL-1β -511 (C/C) and IL-1β -511 (C/T) alleles were associated in *H. pylori* positive patients.



Figure(3): Agarose gel electrophoresis image that shows the RFLP-PCR product analysis of IL-1β gene by using *AvaI* restriction enzyme of some blood patient samples. Where M: marker (2000-100bp), lane (1, 2, 6, and 8) as (CC) homozygote at 190bp and 114bp. Lane (3, 5, 7, and 10) as (CT) heterozygote at 304bp, 190, and 114bp. Lane (4 and 9) as (TT) homozygote at undigested 304bp.RFLP-PCR product was separated on 2% agarose gel.

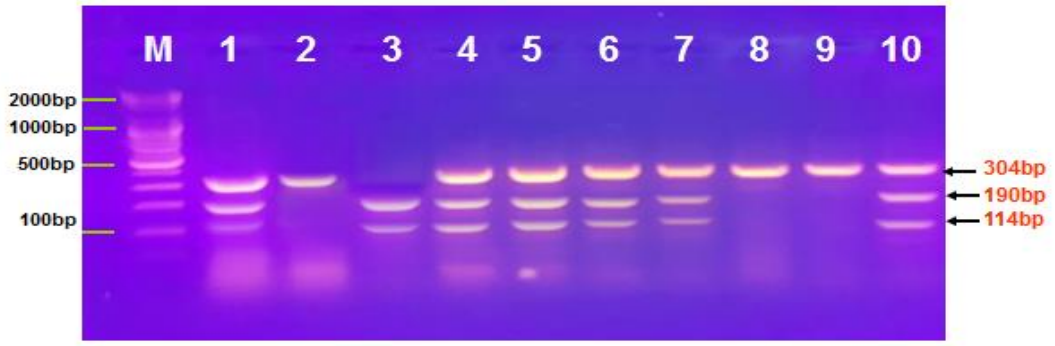


Figure (4): Agarose gel electrophoresis image that shows the RFLP-PCR product analysis of IL-1β gene by using *AvaI* restriction enzyme of some blood control samples. Where M: marker (2000-100bp), Lane (1, 4-7, and 10) as (CT) heterozygote at 304bp, 190, and 114bp. Lane (2, 8, and 9) as (TT) homozygote at undigested 304bp. Lane (3) as (CC) homozygote at 190bp and 114bp. RFLP-PCR product was separated on 2% agarose gel.

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RFLP-PCR for Genotyping of IL-1β SNP (C/T)

The genotype frequencies of polymorphisms studied are shown in the table (5). The frequency of IL-1β (CC) polymorphism differs significantly (p<0.001) between *H.pylori* positive group 11/50 (22%) and uninfected group 4/50 (8%), also the frequency of IL-1β (C/T) polymorphism differs significantly (p<0.001) between *H.pylori* positive group 22/50 (44%) and high percent 33/50 (66%) of uninfected group. While there is no significant different in frequency of IL-β (T/T) polymorphism between *H.pylori* positive group and uninfected group .This results is in agreement with the result of Santos *et al* (2012), Rad *et al*., (2003) and Martinez-Carrilo *et al*.,(2010) .

Table 5: The genotype frequencies of the polymorphism (IL-1B-511 C/T)

Genotype	*H.pylori +*	*H.pylori -*	Statistics
CC	11 (22%)	4 (8%)	X^2cal= 3.310 X^2 tab= 2.61 Significant
CT	22 (44%)	33 (66%)	X^2cal= 5.621 X^2 tab= 4.11 Significant
TT	17 (34%)	13 (26%)	X^2cal= 4.11 X^2 tab= 4.22 Non-Significant

DISCUSSION

Our results disagree with the results of Nassir *et al.*, (2011) which shows 85/115 (73.91%) from patients were positive for anti *H.pylori* IgG antibodies and 7/10 (70%) from individuals who represented the control group.

Both the sensitivity and specificity of the serum antibody rapid test was (100%).So our result is in agreement with Nassir *et al.*, (2011) for sensitivity which was (100%) but specificity was (55.3%).

Extensive studies have found uniformly high sensitivity (90-100%) but variable specificity (79-96%) (Monteiro *et al*., 1996).

Diagnostic testing for *H. pylori* can be divided into invasive and non-invasive techniques based upon the need for endoscopy. The techniques may be direct (culture, microscopic demonstration of the organism) or indirect (using urease or antibody response as a marker for the disease).

The choice of test depends on issues such as cost, availability, clinical situation, the population prevalence of infection, pre-test probability of infection, and factors such as the use of proton pump inhibitors and antibiotics which may influence certain test results.

Different studies in Iraq studied the prevalence of *H.pylori* either in normal population or diseased people using ELISA technique to detect IgG, but they didn't use the serum antibody rapid test which is considered a good non-invasive test for all age groups. However serology dose not distinguish reliably between active and past infection. Furthermore, the positive predictive value of serology is poor in areas where the prevalence of *H. pylori* infection is low thus, the stool testing is a better alternative to serology (Sidhu *et al*., 1997) .

IL-1β is a pro-inflammatory cytokine induced by *H. pylori* infection and powerful inhibitor of gastric acid secretion. Its effects promote hypochlorhydria, favoring further colonization of *H. pylori* and more severe gastritis (Camargo *et al*., 2006). It is shown that IL-1β -511polymorphisms are associated with increased production of IL-1β and are therefore related to the development of gastric cancer (El-Omar *et al.,*2000).Thus, persons harboring IL-1β polymorphisms who are also colonized by an *H. pylori* toxigenic strain have an increased risk of developing gastric cancer (Feteih *et al*., 2009).

Several studies have reported a relationship between *H. pylori* and increase in the inflammatory gene response characterized by up-regulation of several genes such as IL-1β ,IL-8 and IL-10. These cytokines are considered to be important mediators of gastric pathophysiology (Ponzetto.,1998 ; Shahin *et al*.,2001).

In our study we found polymorphism in IL-1β gene, this polymorphism may affect protein expression of mRNA translation (Bidwell *et al*., 1998). Polymorphism in cytokine genes may influence the development of several diseases (Bidwell *et al*., 1999). Susceptibility to many diseases is associated with a particular pro-inflammatory profile that can be explained by individual genetics determinants. Host genetic factors that affect cytokine polymorphisms may determine why some individual infected with *H. pylori* develops gastric cancer while other do not (El-Omar *et al*., 2000). Furthermore, cytokine polymorphisms may play an important role in *H. pylori* pathogenicity.

Several studies have evaluated the direct association of *H. pylori* infection with cytokine gene polymorphism, these genes include IL-1R, IL-1β, IL-1A, IL-6, IL-13, IL-12, IL-2,IL10 and TNF-α. They found clear polymorphisms in this gene such as studies of (Ando *et al*.,2006 ; Hartland *et al*.,2004 ; Mayerle *et al*.,2013; Queiroz *et al*.,2013).

Our results indicate that association was observed for IL-1β-511 (C/C) and IL-1β-511 (C/T) where two product DNA was 190bp and 114bp.These result are in agreement with results of (Santos.,2012).

Two diallelic polymorphisms at positions-511 in IL-1β have been extensively studied in several diseases. El-Omar *et al.,* (2000) reported that interleukin -1 gene cluster polymorphisms are associated with an increased risk of both hypochlorhydria induced by *H. pylori* and gastric cancer, but there are no available studies in Iraq that have examined the relationship of IL-1β polymorphisms with *H. pylori* infection in pregnant women to compare our study with it.

In the present study, we found an association between the IL-1B-511 CC genotype and the presence of *H. pylori* in patients infected with this bacteria(risk factor) .This result is similar to those of previous study conducted by Martnez-Carrillo *et al*., (2010).

IL-1β is an important factor for initiating and amplifying the inflammatory response. It acts as a potent inhibitor of acid secretion and its expression levels are increased in *H. pylori* infected patients (Basso *et al*., 1996 ; Jung *et al*.,1997) .Some investigators have shown that IL-1β -511 polymorphisms are associated with increased IL-1β production and are therefore related to the development of the disease (Figueiredo *et al*., 2002). There are no available studies about IL-1β gene polymorphisms in pregnant women to compare our results with it, but there are studies about IL-1β gene polymorphism and the relationship with gastric cancer patients, these studies have shown that gastric carcinoma and ulcer disease have a distinct effect on gastric secretions .Gastric cancer is associated with low gastric acid production, whereas ulcer disease is associated with high levels of gastric acid secretion (Hansson *et al*.,1996 ; Broutet *et al*.,2002). IL-1β is also a potent inhibitor of gastric secretion (Hansson *et al*., 1996; Tiwari *et al*., 2011) and is up regulated in the presence of *H. pylori* and play an important role in initiation and amplification of the inflammatory response to infection (Broutet *et al*., 2002; Persson *et al*., 2011).

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