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Association of ATG16L1 T300A Genetic Variant with *H. pylori* and None *H. pylori* Atrophic Gastritis

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Abstract

Background *Helicobacter pylori* persistence may develop atrophic gastritis, gastric ulcer or cancer.

Objectives To determine the association between the presence of autophagy related gene 16 like 1 and thrionin 300 alanine mutation and *H. pylori* infection among atrophic gastritis patients.

Methods Gastric biopsy was taken from eighty patients and tested for urease, and blood samples were taken for serum separation for detection of Anti-*H. pylori* IgG by ELISA and DNA extraction from whole blood were used for sequence specific primer – polymerase chain reaction for autophagy related gene 16 like 1 and thrionin 300 alanine mutation allelic discrimination.

Results Among 40 *H. pylori* positive cases, the carriers of mutated allele were 62.5% compared with 36.25% in *H. pylori* negative cases (p = < 0.001, OR = 1.72, CI = 1.23-2.42).

Conclusions Among Iraqi atrophic gastritis, there is an association between *H. pylori* infection and mutated autophagy related gene 16 like 1 and thrionin 300 alanine mutation allele. Thrionin 300 alanine may confer higher risk for infection with *H. pylori*.

Key words Autophagy, *H. pylori*, atrophic gastritis.

List of abbreviations: *H. pylori* = *Helicobacter pylori*, Vac-A = Vaculatinig cytotoxin-A, SNP = single nucleotide polymorphism, ATG16L1 =, OR = odd ratio, CI = Confidence interval.

Introduction

Helicobacter pylori (*H. pylori*) infection remains the most common cause for chronic gastritis in humans that may develop to gastric ulcer or cancer [1]. The precise mechanisms by which *H. pylori* exploit host cell machineries for intracellular survival are poorly understood [2]. However, *H. pylori* able to enter and embed in the mucus, attach to gastric epithelium, evade immune responses, and persistently colonize there [3,4].

Among several virulence factors, Vaculatinig cytotoxin-A (Vac-A) can modulate disease pathogenesis. It has been found that Vac-A can contribute to increase persistence of infection through inactivation of cellular autophagy and promoting *H. pylori* survival inside gastric epithelia and macrophage [3]. Studies described that Vac-A can induce the formation of large autophagic-like vesicles. Over the last decade, several research groups have independently reported that infection by *H. pylori* can inhibit autophagy [5].

Autophagy is an intracellular mechanism by which host cells can eliminate microbes [6]. However, some pathogens have the capacity to escape from autophagy processes as a strategy for increasing intracellular survival [7]. It has been proposed that *H. pylori* once internalized and sequestered in double-membrane autophagosomes, can use these compartments as a replicative niche. Genetic studies have confirmed that a single nucleotide

polymorphism (SNP) in the autophagy related gene 16 like 1 (ATG16L1) confer an increasing risk for intracellular survival of several microbes due to impaired phagosome-lysosome fusion and secretion of antimicrobial peptides [8].

Considering the capacity for colonization, and the persistence in gastric tissue, it is theoretically plausible that ATG16L1 thrionin 300 Alanine (T300A) genetic variant, that the resultant protein reduce autophagic responses to Vac-A and increased susceptibility to infection with an *H. pylori* Vac-A suggesting that it facilitates chronic inflammation.

This study aims to investigate the association between the presence of mutated ATG16L1 allele and infection with *H. pylori* among gastritis patients.

Methods

Study subjects

This cross-sectional study involved eighty patients suffering from gastritis were recruited from the gastroenterology units in Gastroenterology and Hepatology Teaching Hospital and Al-Emamain Al-Kadhemain Medical City during the period of September 2013- August 2014. Patients were undergone gastroscopy due to clinical indications and the IgG anti-*H. pylori* antibody examination and rapid urease test was performed in order to identify the presence of *H. pylori*.

Genotyping of ATG16L1 T300A by Sequence Specific Primer-Polymerase Chain Reaction (SSP-PCR)

The steps of DNA extraction and genotyping were done in molecular biology laboratory / department of Microbiology/ AL-Nahrain University. DNA was extracted from 300µl peripheral blood EDTA containing tubes using DNA isolation kit (Wizard®, Promega, USA) following manufacturer information. The DNA concentration ranged from (85-120) and purity (1.7-1.84). Allelic discrimination of substitution mutations of Adinin with Guanine was checked by SSP-PCR. DNA from study groups individuals were amplified by using two sequence specific primers in two separated reaction mixtures, to give a PCR products of 201bp in positive reaction for allele A or allele G, allowing discrimination of homozygous or heterozygous genotype.

The sequence of primers customized as Forward allele A: $^{5'}$CCCCAGGACAATGTGGATA$'^{3}$, Forward allele G $^{5'}$CCCCAGGACAATGTGGATG$'^{3}$ and common reverse $^{5'}$AGGTGGAAAGGCTTGATATAAG$'^{3}$. Detection of β-globin gene considered as internal control. For each reaction of allele A or G or internal control 0.3 µl of each primer (forward and reverse) added to pre-mix PCR tube (Promega, USA) and 0.5-3 µl of genomic DNA and complete reaction volume to 20 µl by DNAse free water (Fig. 1).

PCR reaction tubes were transferred into thermal cycler (eppendroff-thermal cycler, Germany), that was programmed as following in (separated PCR-runs-for each allele): 96^{o}C for 1min (X1), (96^{o}C 20s, 72^{o}C) for 1min 10s (X5), 96^{o}C for 25s, 69^{o}C for 50s, 72^{o}C for 30s (X21), 96^{o}C for 30s, 59^{o}C for 1min and 72^{o}C for 1 min and 30s (X4) then PCR products were electrophoresed in 2% agarose gel.

Statistical analysis

The statistical analysis was done by using Graphpad PRISM ® version 6. Crosstab model used to estimate association of allelic variant among study groups and relative risk (RR) and corresponding 95% confidence intervals were estimated.

Results

Demographic, anthropometric and serologic data of enrolled patients

As shown in Table 1, the age and gender type were distributed between patients from two groups without statistical significant difference ($p > 0.05$). The results in Table 1 showed that there are no statistical significant difference in the mean of age Likely, smoking habit, vomiting, antibiotic therapy, anti-acid therapy, H_2 blocker and NSAID all of then does not reach the statistical significance value. Proton pump inhibitor showed a statistical significance in

which *H. pylori* negative cases were 21(52.5%) using PPI ($p < 0.05$).

Association ATG16L1 Thr300Ala allelic variant with H. pylori infection

The allelic frequencies were presented in Table 2. The carriage of mutated allele was statistically significant higher in *H. pylori* positive cases 62.5% compared with 36.25% in *H. pylori* negative ($p = < 0.001$, OR = 1.72, CI = 1.23-2.42) and it was associated with the increased risk for *H. pylori* gastritis.

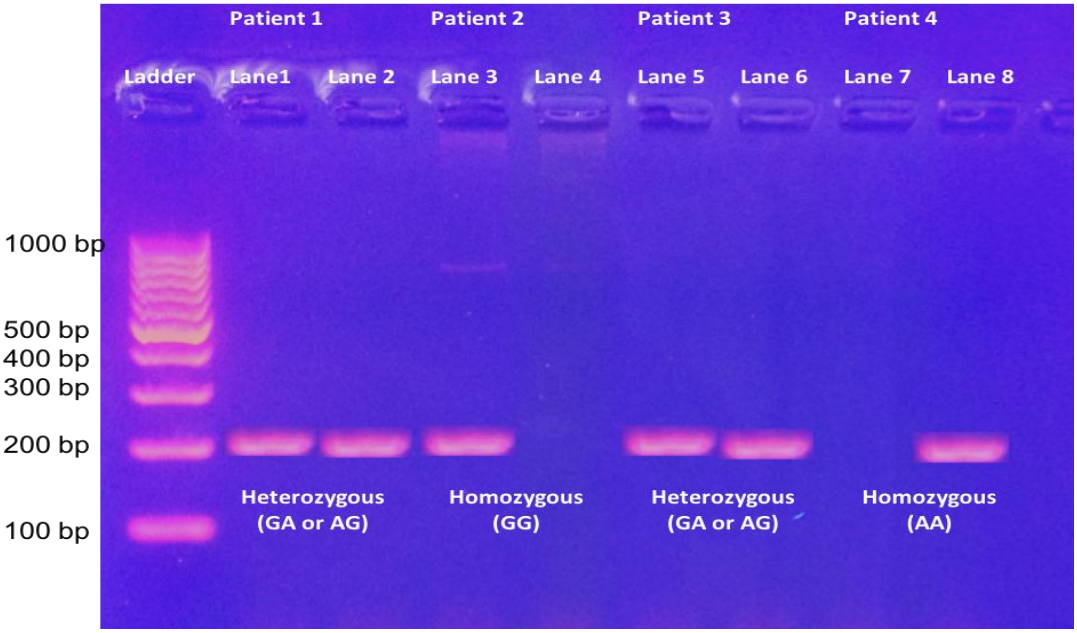

Fig. 1. Agarose gel electrophoretic profiles corresponding to SSP-PCR products, Lane 1 and Lane 2: heterezygous genotype (GA). Lane 3 and Lane 4: homozygous for allele G, Lane 5 and Lane 6: were heterozygous for allele GA, Lane 7 and Lane 8: Homozygous genotype AA. Molecular size of PCR product of allele A or allele G=201bp. Lane 1,3,5 and 7 represents allele G reactions, Lane 2,4,6 and 8 represents allele A reactions.

Table 1. Summary of demographic and clinical description for study groups

Feature	*H. pylori*	
	Negative N = 40	Positive N = 40
Age Mean±SD	38.7±9.2	41.3±12.4
Gender (Male)	15 (37.5)	17 (42.5)
Fresh blood*	4 (10)	12 (30)
Smoking habit	12 (30)	9 (22.5)
Vomiting	19 (47.5)	18 (45)
Antibiotic therapy	10 (25)	10 (25)
Anti-acid therapy	6 (15)	12 (30)
H_2 blocker	11 (27.5)	18 (45)
NSAID	6 (15)	6 (15)
Proton Pump Inhibitor*	21 (52.5)	12 (30)
Steroid therapy	0 (0)	3 (7.5)
Anti-*H. pylori* (IgG)	32 (40)	48 (60)

* = Significant difference ($p < 0.05$), NSAID = non-steroidal anti-inflammatory drugs..

Discussion

The chronic atrophic gastritis state could contributes to the development of gastric ulcer as well as gastric cancer [9]. Several lines of evidence supports that *H. pylori* can invade, survive and multiply in both epithelial cells and professional phagocytes *in vitro* and *in vivo* [10]. However, after induction of autophagy by *H. pylori*, itcan evade autophagy by down regulating autophagic proteins; alternatively the bacterium can exploit autophagosomes as their intracellular niche or be degraded in autolysosomes [5,11,12].

Table 2. Allelic frequencies of ATG16L1 T300A in atrophic gastritis patients

H. pylori	Allele G	Allele A	p value
Positive	50 (62.5%)	30 (37.5%)	0.001
Negative	29 (36.25%)	51 (63.75%)	
Relative risk		1.72	
Confidence interval		1.23-2.42	

The study presents an association between *H. pylori* incidence with the presence of T300A allelic variant (Table 2). However, the substitution mutation in the ATG16L1 gene at the position 300 considered to be as a loss of function mutation with reduced selective autophagy against invading microbes [8]. Results in this study have been supported by Raju et al. 2012; when they found that after 24 hrs of VacA exposure to the gastric epithelia would disrupt autophagy by impairing of autophagy and reduction of tissue lysosomal enzymes production [13]. Studies demonstrated that susceptibility to *H. pylori* infection were increased in the presence of T300A genetic variant [14]. So, the presence of risk allele will modulate autophagy [15] resulting in reduced clearance of bacterium in the mucosa or phagocytic cells.

The impaired autophagy could exacerbate chronic gastritis by different mechanisms. First, the microenvironment undergoes secondary necrosis in the gastric mucosa due to release pro-inflammatory cytokines as a compensatory mechanism [8]. These events will release of pro-inflammatory cytokines, including TNF-α, by mononuclear phagocytes, which may further affect tissue pathology. It can be explored by the fact that virulent bacterium could impair dendritic cell response against it [12]. This impairment occurs due to autocrine effect and paracrine effect of pro-inflammatory cytokine [16].

The up-regulation of autophagy has been an attractive approach in treatment and prevention of *H. pylori* infection. Several papers have highlighting this address, the using of combination sialic acid and catechins in dose dependent manner by prevention of infection and reducing bacterial number after treatment [17]. Thus, identification of patients whom have mutated ATG16L1 allele may benefit from the using of these compounds in addition to antibiotics. It may be helpful to treat *H. pylori* gastritis and reducing the risk of development to ulcer or even cancer of stomach.

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Conflict of Interest

There is no conflict of interest.

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