ORIGINAL PAPER

doi: 10.5455/medarh.2021.75.23-26
MED ARCH. 2021 FEB; 75(1): 23-26
RECEIVED: DEC 19, 2020
ACCEPTED: JAN 15, 2021

Interleukin– 17agene Polymorphism, Serum Level and Its Tissue Expression in Iraqi Patients Gastric Lesions

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ABSTRACT

Background: T-helper 17 plays a novel role in inflammation in gastritis by producing IL-17A, IL-17A gene polymorphisms that might be responsible for disease susceptibility and development of different gastric lesions. **Objective:** The aims of study was to determine the association of IL-17A (G197A) genotype and allele frequency with disease phenotype and risk with different gastric lesions. **Methods**: Case controlled study involved 30 gastroduodenal ulcer, 30 chronic gastritis and 30 subjects as a control group with negative endoscopic findings. After genomic DNA extraction, IL-17A (G197A)ARMS-PCR genotyping were done for all cases. Serum IL-17A was measured using ELISA method and tissue expression was visualized using immunohistochemistry staining method. **Results:** The results showed that allele A was significantly frequent in gastroduodenal ulcer more than that in healthy control odd ratio= 4 (1.42-10.46), and none significantly with chronic gastritis p=0.071. Serum IL-17A was significantly higher in gastroduodenal ulcer (116.45±48.09 pg/ml), chronic gastritis (78.02±30.17pg/ml) and healthy control 19.36±9.28 pg/ml).However, the serum IL-17A level is not related to the allele pattern of each group. The tissue expression was expressed as dense granular cytoplasmic and membranous of inflammatory cells. Interestingly, the percentage of IL-17A protein expression was significantly higher in gastroduodenal ulcer (38.2±16.55%), chronic gastritis (30.89±14.02%) and normal mucosa (2.8±3.02%). Furthermore, patients with strong intensity of IL-17A stained mucosa were frequently carrier for mutant allele (68%). **Conclusion:** IL-17A might predispose for aggressive inflammation of advanced lesions in stomach like ulcer.

Keywords: Interleukin-17A, gene polymorphism, gastritis and gastric ulcer.

1. BACKGROUND

Gastric mucosa damage is frequent due to various chemicals or microbial insults (1). T-helper 17 belongs to a group of effector T-lymphocytes that plays various roles in different diseases such as autoimmunity and infections (2) there is a clear need for administration of more potent, potentially more toxic, drugs. Alternatively, biopharmaceuticals may hold potential but require specialized protection from premature in vivo degradation. Thus, a paralleled need for specialized drug delivery systems has arisen. Although cell-mediated drug delivery is not a completely novel concept, the few applications described to date are not yet ready for in vivo application, for various reasons such as drug-induced carrier cell death, limited control over the site and timing of drug release and/or drug degradation by the host immune system. Here, we present our hypothesis for a new drug delivery system, which aims to negate these limitations. We propose transport of nanoparticle-encapsulated drugs inside autologous macrophages polarized to M1 phenotype for high mobility and treated to induce transient phagosome maturation arrest. In addition, we propose a significant shift of existing paradigms in the study of host-microbe interactions, in order to study microbial host immune evasion and dissemination patterns for their therapeutic utilization in the context of drug delivery. We describe a system in which microbial strategies may be adopted to facilitate absolute control over drug delivery, and without sacrificing the host carrier cells. We provide a comprehensive summary of the lessons we can learn from microbes in the context of drug delivery and discuss their feasibility for in vivo therapeutic application. We then describe our proposed "synthetic microbe drug delivery system" in detail. In our opinion, this multi-

disciplinary approach may hold the solution to effective, controlled drug delivery. Among group of interleukin 17 isoforms, interleukin 17A considered the most potent one in inducing inflammation by recruiting inflammatory cells to the lesion (3). Furthermore, the role of IL-17A in autoimmunity have been described in systemic lupus Erythematous (4), rheumatoid arthritis (5) and Sjögren syndrome (6). Th17 cells have been implicated in host responses to infections and in pathogenesis associated with autoimmune diseases. This cytokine is implicated in primary Sjögren's syndrome (pSS. Few studies have been conducted on interleukin 17A gene polymorphism and risk for development of gastric lesions. Polymorphism in interleukin-17A (rs2275913) have been studied in Iran (7) the interleukin 23 receptor (IL23R, Korea (8)IL-17F expression was increased in the T allele carriers, but was not associated with gastritis. In 300 gastric cancer patients, the frequency of the rs2275913 A/A genotype was significantly higher in intestinal-type gastric cancer, when compared to that of diffuse-type gastric cancer (P>0.05, and China (9, 10) gastric cancer patients and 800 healthy controls to assess the association between IL-17A G197A and IL-17F A7488G polymorphisms and risk of gastric cancer. Genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP. Furthermore, it have been reported that the individuals carrying variant allele were more likely to have gastritis, gastric ulcer(10) number of rs2275913 A allele was significantly associated with an increased risk for peptic ulcer (OR, 1.50; 95%CI, 1.11-2.01; p=0.0082 or gastric carcinoma (11,12) the G-197A genotype was determined by restriction fragment length polymorphism analysis of polymerase chain reaction-amplified fragments. Statistical analyses were performed to determine whether any demographic or behavioral factors, infection with Helicobacter pylori (H. pylori. IL-17A plays a pivotal role in host-pathogen interaction especially in *Helicobacterpylori* infected mucosa (13). It can induce atrophy mediated apoptosis in parietal cells (14), promoting chronic inflammation (15).

2. OBJECTIVE

This study aimed to determine the significance of rs2275913, serum IL-17A and gastric tissue expression in relation to different gastric lesions.

3. METHODS

Case controlled study involved 30 gastroduodenal ulcer, 30 chronic gastritis and 30 subjects as a control group with negative endoscopic findings. These samples were collected from hepatology and gastroenterology hospital and center in AL-Kadhemia teaching hospital. All participants were asked for participation in this study by taking them verbal approval.

Measurement of serum Interleukin-17A

After serum separation was performed, all serum samples were stored -20°C. Serum IL-17A was measured using sandwich ELISA kit purchased from Abbexa® abx152020, Cambridge, UK. The procedure was done according to manufacturer instructions.

Interleukin-17A (G197A) genotyping

Two milliliters of whole blood were stored in EDTAk3 tubes. Genomic DNA extraction was done using Genomic DNA Mini Kit (Blood/Cultured Cell) GB100; IL-17A (G197A) was genotyped with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using forward primer 5-AACAAGTAAGAATGAAAAGAGGACATGGT-3 and reverse primer 5-CCCCCAATGAGGTCATAGAAGAATC-3. 100 ng of extracted DNA and 1µM of each of forward and reverse primers with ready to use premix for conventional PCR 20µl reaction volume Accu Power PCR premix. The product incubated overnight at 37°C with XagI (10 units). The product of digestion was electrophoresed using polyacrylamide gel. The homozygous AA mutant left undigested (102bp), the heterozygous (AG/GA) were digested into 102, 68 and 34 bp (9, 10) gastric cancer patients and 800 healthy controls to assess the association between IL-17A G197A and IL-17F A7488G polymorphisms and risk of gastric cancer. Genotypes were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP.

Immunohistochemical staining of Interleukin-17A

Formalin fixed paraffin embedded punch biopsied were taken from histopathology laboratory. Tissue sections were placed on positive charged slide. Rabbit anti-IL17A (orb48920) Biorbyt ® UK was used to specify the reaction, then the expression was visualized using Rabbit IgG Super Vision Assay Kit (HRP) (orb76022) Biorbyt ® UK. The staining IL-17A was measured by calculation of percentage of positively expressed cells.

Statistical analysis

The statistical analysis was done using GraphPad Prism 8.0 San Diego, California, US,the independent sample T test used for comparison of numerical data while, Chi-square or fisher exact test used for comparison between categorical data. Odd ratio calculated to estimate the potential risk of mutant allele in disease group. P value considered as significant at the level of equal or below 0.05.

4. RESULTS

The results in Table 1 showed that comparable age groups and gender frequencies among three groups (p>0.05). Biopsies of 16 (53.33%) of gastric ulcer group were urease positive, while 20 (66.67%) found in gastritis group.

Interleukin 17A G197A as a risk factor of gastric ulcer

	Gastric ulcer n=30		Gastritis N=30		Control N=30	
Age (>50 years)	17	56.67%	15	50.00%	13	43.33%
Sex (Male)	20	66.67%	21	70.00%	22	73.33%
Urease positive*	16	53.33%	20	66.67%	0	0.00%

Table 1: Study groups characteristics involved in this study NS: none statistical significance (p>0.05). *: statistical significance (p<0.05).

Our study finds that allele A (mutant allele) was present in 20% in gastric ulcer patients and its significantly higher (p=0.024) than those of control (5%) and those have a 4.75 times risk to develop ulcers. While, gastritis patients carrying mutant allele A (13.33%) have none statistical association when compared with controls (p=0.253) as detailed in Table 2.

		Gastric ulcerN=30		GastritisN=30		ControlN=30	
IL-17A G197A genotype	GG	22	73.33%	25	83.33%	27	90.00%
	GA/AG	4	13.33%	2	6.67%	2	6.67%
	AA	4	13.33%	3	10.00%	1	3.33%
IL-17A G197A Allele	G	48	80.00%	52	86.67%	57	95.00%
	A	12	20.00%	8	13.33%	3	5.00%
P value		0.024*		0.253NS		-	
Odd ratio (CI)		4.75 (1.36-16.4)		2.92 (0.82-10.54)		-	

Table 2. IL-17A G197A genotype and allele distribution in study groups. NS: none statistical significance (p>0.05). *: statistical significance (p<0.05).

Serum and tissue IL-17A was higher in advanced gastric lesions

The results in Figure 1 A showed that patients with gastric ulcer have higher serum level of IL-17A (mean=116.45 pg/ml) than that gastritis group (mean=78.02 pg/ml) and control group (mean=19.36 pg/ml) and independent sample t-test showed that highly statistical difference was found between each two groups. Figure (1 B) describe the tissue expression of IL-17A in different study groups, gastric ulcer group (mean=38.2%) and gastritis group (mean=30.89%) showed highly statistical difference than control group (mean=2.8%).

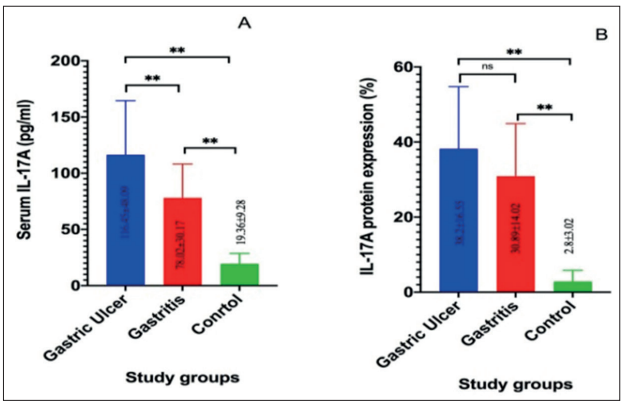

Figure 1. Bar chart represent mean and standard deviation of (A) serum IL-17A and (B) tissue IL-17A expression among study groups. NS: none statistical significance (p>0.05). **: Highly statistical significance (p<0.001)

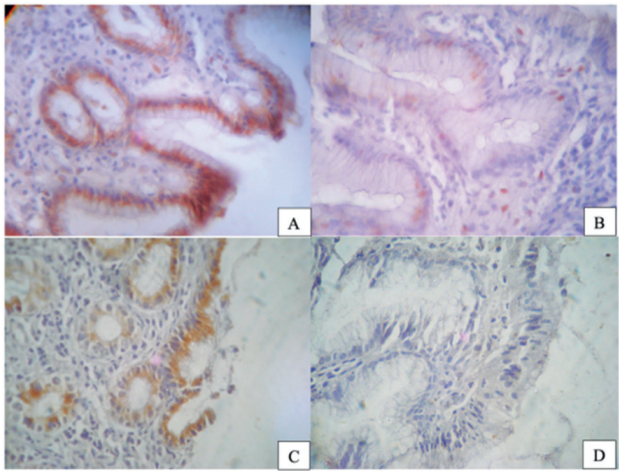

Figure 2. Immunoperoxidase staining of IL-17A (6pg/ml) of Urease positive gastritis (A), Urease positive negative (B), Urease positive ulcer (C) and Negative control omitting primary antibody (D)

The immunoreactivity of IL-17A visualized by peroxidase staining method illustrated in Figure 2 showing dense epithelial and inflammatory cells expression in Urease positive gastritis lesion (Figure 2A) and lower in urease negative (Figure 2B). Urease positive gastric ulcer lesion (Figure 2C) showing dense immunoreactivity of IL-17A in damaged epithelia.

5. DISCUSSION

Interleukin-17A plays an important role in coordination of local inflammatory reactions through activation of pro-inflammatory activity and chemotactic factors (16). Here in this study, the possible association of IL-17A G197A gene polymorphism with gastric lesionsin addition to the evaluation of IL-17A was measured in serum and tissue of gastric ulcer and gastritis patients. The results showed that individuals carrying mutant allele (A) were likely to develop advanced gastric lesions like ulcer. Our results in agreement with Hayashi, et al. whom found that risk allele (A) was 1.5 times risk of inducing gastro-duodenal ulcer (10) number of rs2275913 A allele was significantly associated with an increased risk for peptic ulcer (OR, 1.50; 95%CI, 1.11-2.01; p=0.0082. Furthermore, they found that G197A is associated with the severity of gastric mucosal atrophy in atrophic and metaplastic lesions suggesting an important role of this gain of function mutation in induction of local inflammation (8) IL-17F expression was increased in the T allele carriers, but was not associated with gastritis. In 300 gastric cancer patients, the frequency of the rs2275913 A/A genotype was significantly higher in intestinal-type gastric cancer, when compared to that of diffuse-type gastric cancer (P>0.05. Higher percentage of gastric IL-17A protein expression was found in both ulcer and less in gastritis lesion, this in turn reflecting an aggressive local inflammatory response. *Helicobacter pylori* considered as the main inducers of gastric inflammation that might develops into ulcer or tumor by different mechanisms (17). Several studies have been reported an association between local IL-17A protein expression in the presence of *H. pylori* in different gastric lesions (15, 18, 19). Th17 plays an important role in the defense against H. pylori and may cause gastritis and peptic ulcer due to the increased activation of Th17 and cytokine changes. Aim: To find a relationship between Th17 and IL-17A, IL-21,

IL-22, IL-23, TGF-β in the patients with H. pylori infection having signs including gastritis and peptic ulcer.

In total of 36 samples from the patients [24 Hp+ and 12 Hp- cases] with dyspepsia symptoms were collected. The percentage of Th17 was measured by flow cytometry. The levels of Th17-associated cytokines in the sera and supernatants of peripheral blood mononuclear cells (PBMCs and mRNA expression (20). Interleukin-17A induce atrophy in gastric parietal cell in chronic gastritis in mice model (14). This provide an explanation about the mechanism how IL-17A higher concentration associated with the severity of gastritis and gastric cancer (21). IL-17A plays a protective role against *H. pylori* in mouse model and IL-17A deficient mouse developed less severe gastric pathology upon infection (22).

IL-17A plays important role in acute disease but the chronicity of gastritis might be related to continuous inflammatory reactions. We suggest that IL-17A G197A gene polymorphism might increase the level of IL-17A in local response to *H. pylori* and gives higher rate of chronic gastritis and or more advanced lesions such as ulcers or tumors.

6. CONCLUSION

In conclusion, IL-17A G197A gene polymorphism might be responsible for presence of gastric ulcer under uncontrolled production of IL-17A.

- **Acknowledgment**: Authors acknowledge the staff of gastroenterology and hepatology hospital for their cooperation in sample collection.
- **Author's contribution**: All authors were involved in all steps of the preparation this article. Final proofreading was made by the first author.
- **Conflict of interest**: Authors declares that there is no conflict of interest.
- **Financial support and sponsorship**: Nil.

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