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Effects *of Campylobacter jejuni* infection on serum level of IL-6, IL-8 and TNF-α

Maha H. Tawfeeq[*1], Hiba M. Nasir[2]

[1]*Department of Anatomy, College of Medicine, University of Al-Qadisiyah, Iraq*

[2]*Analytical Department, College of Medical and Health Technique, Middle Technical University, Iraq*

Abstract

Background: *Campylobacter jejuni* is the main cause of acute bacterial enteritis in human worldwide, and more common in developed countries

Aim: This study was designed to assess the effects of *Campylobacter jejuni* infected persons on serum concentration of I-L6, IL-8 and TNF-α.

Materials and Methods: Blood and stool samples were collected from 201 patients presented with diarrhea in Al-Diwaniyah Teaching hospital, Iraq with age ranged from 10-40 years old through period March 2017 to March 2018. Diagnosis of *Campylobacter jujeni* infected patients were done by using Rapid stool antigen test.

Results: the results were showed that 176 (87.56%) were positive infection by *Campylobacter jejuni* and 25 (12.44%) were negative for these bacteria. this study results were revealed that significant rising in TNFα (95.33±13.1) in serum of persons with +ve *Campylobacter jejuni* infection in comparison with -ve *Campylobacter jejuni* persons were serum TNFα (52.64±14.75). So, regard to the serum level of IL6 and IL8 were significantly raised in persons with +ve *Campylobacter jejuni* infection were (320.42±21.23, 282.5±24.14) respectively in comparison with non-infected persons were serum IL6 and IL8 (74.33±16.50 and **77**±18.30) respectively. The results also showed, that there is significant association between *Campylobacter jejuni* bacterial infection and age, and no significant difference between the incidence of infection and genders,

Conclusion: So the results indicated that higher infection with *Campylobacter jejuni* in rural resident area compared with urban resident area persons. Regarding to family history, showed that had significant relationship between it and prevalence of *Campylobacter jejuni* infection ($P \leq 0.05$), this supported by recent study showed that an outbreak with Campylobacter jejuni infection can be develop in several members of family.

Keywords: *Campylobacter jejuni*; IL6; IL8;TNFα

INTRODUCTION

Campylobacter jejuni is the main cause of acute bacterial enteritis in human worldwide, and more common in developed countries [1]. 49880 cases were reported of *Campylobacter jejuni* gastroenteritis in England and Wales in 2008 according to Health Protection Agency Center of Infection [2] and over 6000 cases were reported in United States 2009 [3]. *Campylobacter jejuni* are gram negative, non-spore form rods with comma or S shape bacteria. They are motile with single polar flagellum [4].

These bacteria are a component of intestinal microbiota of numerous birds and mammal species [5] and caused disease in human called Campylobacteriosis. In which disease raised after consumption of contaminated chicken products during processing [6]. Recent study shows that campylobacter contamination on up 88% of chicken carcasses [7]. In individuals infected with *Campylobacter jejuni* may asymptomatic and rate is varying widely by age and region [8]. While in individuals with symptoms, the onset of disease occurs 1-7 days after bacterial ingestion as acute diarrhea which common presentation in 98-99% of symptomatic patients [9,10]. Other symptoms, colicky abdominal pain, nausea, vomiting, headache, and myalgia [8]. Sever cases can develop chronic diarrhea, bacteremia with or without extraintestinal dissemination and post infectious syndrome [11]. The systemic complications included carditis, meningitis, abscesses, septic arthritis, cholecystitis, and urinary tract infection, which they are rare [12,13]. Post infectious complications included Guillian - Barre syndrome, reactive arthritis and post infection irritable bowel syndrome. Guillian - Barre syndrome is acute flaccid paralysis of peripheral nerves, involve 1 per 1000 – 3000 campylobacter infections [14] due to autoimmune molecular mimicry between terminal sugar molecules shared by human peripheral nerves and *Campylobacter jejuni* antigens [15].

The mechanism of disease on host caused by *Campylobacter jejuni* not fully understood, however, inflammatory responses of the host to bacteria are likely to contribute to a large extend to pathology observed [16, 17]. *Campylobacter jejuni* like other enteric pathogenic bacteria. They are infected human by colonizing the mucus layer of intestine, then adherence and invasion of intestinal epithelial cells [18,19]. Since, these intestinal epithelial cells act as 1st barrier facing bacterial pathogens and play major role in initiate host inflammatory response against any pathogens [20, 21, 22]. The intestinal epithelial cells release pro-inflammatory cytokines to infected site, which lead to recruit neutrophils, microphages and other cells involved in immune response to site of infection [23, 24, 25]. The aim of study is to evaluate the relationship between patient infected with *Campylobacter jejuni* and serum IL-6, IL-8 and TNF-α concentrations.

MATERIALS AND METHODS

Samples collection

Blood and stool samples were collected from 201 patients presented with diarrhea in Al-Diwaniyah Teaching hospital, Iraq with age ranged from 10-40 years old through period March 2017 to March 2018. And all data regard age, gender, residency and family history of the patients were recorded on answer prepared Questionnaire paper.

Diagnosis of *Campylobacter jujeni* infected patients by using Rapid stool antigen test.

After fecal sample was collected in a container, 200 mg of sample was moved by wooden stick in collection tube contain tri-Neutrat which is used to softening and dilution the sample. Then the tube shakes well to mix the liquid with sample. Couple drops of sample mixture squeezed in sample hole in the cassette test. The results will appear after ten minuts.

Human IL-6, IL-8 and TNF-α ELISA

All collected blood samples from patients were managed by separation of the serum and stored at -20 C °till time of using. ELISA Kit of human IL-6, IL-8 and TNF-α were used to determine of those cytokines serum concentration in blood

samples and done according to instructions of manufactured company (Diagnostic Automation, Inc., USA).

Statistical analysis

The statistical analysis of our results data was used to assess the significance of changes between IL-6, IL-8 and TNF-α at the level of probability (P-value <0.05) by using one and two-way analysis of variance. The result data had been expressed as Mean ± Standard Errors (SE) and P-value <0.05 and they were considered statistically significance.

RESULTS AND DISCUSSION

Rapid stool antigen diagnostic cassette test for *Campylobacter jejuni* infection:

176 (87.56%) stool samples were give positive results for this test with *Campylobacter jejuni* infection and 25 (12.44%) samples were give negative results from 201 collected samples from suspected individuals with diarrhea as Figure No.1. There is no study used rapid stool antigen test to compare our study results with it. This test depends on immune chromatography process to diagnosis the bacterial antigen in stool sample. The test strip containing antibodies in its outer membrane against *Campylobacter jejuni* bacteria, the interaction between antibodies and bacterial antigens results in a change color in the control area, which give red color always. In case negative sample, a red color at letter C (control line) only. While in sample of infected individual a red package at letter T (result line) will appear in addition to control line as in fig 2.

Stool antigen test has high accuracy in diagnosis *Campylobacter jejuni* bacterium over that is easy and give a result with in few minutes. So that it can identify the bacteria in infected persons directly.

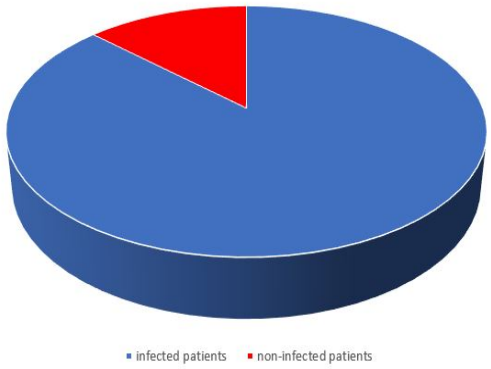

Figure 1: percentage of *Campylobacter jejuni* in non-infected and infected patients

Figure 2: positive results of rapid stool antigen test

In our study, we did not used bacteria culture test because this type of bacteria very sensitive to environmental stressful condition and loss during culturing and give false negative results [26]. Over that, non-fastidious bacterial contamination may be appearing that affect accurate identification of ***Campylobacter*** in culture test [27]. So that, any exposure the culture media to light toxic oxygen derivate such as peroxide, toxic products to Campylobacter are formed such as singlet oxygen and superoxide ions, because of Campylobacters are lack to enzymes peroxidase and superoxide dismutase [28]. There for we used in this study rapid stool antigen test because of it is a rapid and accessible diagnostic method to identify of Campylobacter jejuni isolates from clinical cases in laboratory. In this study found that the individuals with +ve *Campylobacter jejuni* was significant rising in serum TNF-α level (95.33±13.1) when compare them with -ve *Campylobacter jejuni* individuals (52.64±14.75) as in table No. 1. These results may be due to strong immune response to *Campylobacter jejuni*, which it is enhance the systemic inflammation and reflected by increasing in serum TNF-α level. TNF-α has an important role in host defense against *Campylobacter jejuni*, but a high serum level of TNF-α may lead to sever pathology. So that, in host immune response that induced by lipopolysaccharide (LPS) or bacterial super antigens from gram -ve bacteria in septic shock syndrome cause release of TNF-α [29]. IFN-γ can be enhanced TNF-α secretion from LPS-activated mononuclear phagocytes or antigen-stimulated T cells. Study showed that mice total lack of gastritis and atrophy with severely colonized with Campylobacter jejuni when there is defective or lacking interferon regulator factor [25]. The severity of the disease is correlated with some bacterial factors. Whoever's these factors alone not enough to explain divers' outcome of disease caused by *Campylobacter jejuni* [30]. The contact of *Campylobacter jejuni* with gastric cells is induce immune response of the body, which it is a key event in the development of the disease and followed by the stimulation of pro-inflammatory cytokine production such as TNFα [20].

So, this study showed significantly increased in serum concentration level of both IL-8 and IL-6 in infected individuals were (320.42±21.23, 282.5±24.14) respectively, compared with non-infected individuals (74.33±16.50 and 77±18.30) respectively.

Table (1) Serum level of IL-6, IL-8 and TNF-α infected and non-infected groups

	TNF-α. (Pg per ml)	IL-6 (Pg per ml)	IL-8 (Pg per ml)
Infected	95.33±13.10	320.42±21.23	282.5±24.14
Non-infected	52.64±14.75	74.33±16.50	77±18.30

Gastric acid secretion is inhibited by IL-6, IL-8 and TNF-α [31], result in distribution of *Campylobacter jejuni* to gastric corpus and causes gastric atrophy. So that, where the level of cytokines could be influence persistent infection [32]. These cytokines are essential components of inflammatory immune response to enteric bacterial pathogen. And furthermore, this information gives us sight into mechanism of tissue injury by Campylobacter and give more support the premise that the significant role of inflammation in pathogenesis of *Campylobacter jejuni* and that the intestinal epithelial tissue likely plays important roles in initiation the inflammatory response [33].

From the results of our study showed significant association between *Campylobacter jejuni* infection and age of the patients, where the higher prevalence rate in age group [10 – 20] years followed by [21 -30] age group and lowest in [31 – 40] years as in table 2. This result agrees with record by [34], which found that common causes of diarrhea in infants and young adults are by Campylobacter bacterial infection, especially during summer, where the bacteria found in row and undercooked chicken. So [35] mention that children and infant higher probability than adults for campylobacter infection, but other study [36] contradicted with documented finding, which found that campylobacter in all ages.

Table (2) Campylobacter jejuni prevalence in infected individuals depending on their demographic characteristics

		Total	*Campylobacter jujune* positive	Percentage (%)	X^2	P-value
Age group	Group 1 (10-20)	98	92	93.88	6.44	0.142
	Group 2 (21-30)	72	63	87. 5		
	Group 3 (31-40)	31	21	67.74		
		201	176	87.56		
Gender	Male	102	90	88.23	3.43	0.004
	Female	99	86	86.87		
		201	176	87.6		
Residence	Urban	90	73	81.11	4.321	0.253
	Rural	111	103	92.79		
		201	176	87.56		
History	+	104	94	90. 38	3.27	0.138
	-	97	82	84.54		
		201	176	87.56		

CONCLUSION

From the current study, the incidence of male and female in the table No. 2 have no significant difference and this go with results by [34, 37]. So that the results give us that the patients in rural residency were higher infected with *Campylobacter jejuni* (92.79%) compared with urban residency (81.11%). This is variation could due to many factors facilitate acquired infection such as inadequate living sources and poor sanitation. Recent study showed that *Campylobacter jejuni* developed outbreak in several family members of same infection, and there is significant relationship (P value $\leq$ 0.05) between prevalence of *Campylobacter jejuni* infection and family history. Also, the incidence rate was high (90. 38%) among patients have family history of *Campylobacter jejuni* with patients have no family history and from this finding explained that transmitted of Campylobacter between the family members.

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