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Immunological investigation of *Clostridium difficile* infection in inflammatory bowel disease patients

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ABSTRACT

Background: The pathogenesis of Inflammatory bowel disease (IBD) is complex and remains undetermined being although to result from an interaction between susceptibility genes, bacteriology and environmental factor and immune response.

Aim: the purpose of this research is to prove the role of *Clostridium difficile* infection in contribution in inflammatory bowel disease onset.

Materials and methods: The current study includes 30 samples from the Ulcerative colitis (UC) and 30 samples from Crohn's disease (CD) patients. and 25 healthy persons. After endoscopy diagnosis of IBD patients by physician, 3 ml of venous blood from patients and healthy control were collected, the samples were used for immunological detections of *Clostridium difficile* toxin B level by ELISA method

Results: the result of the current study showed that there was highly significant concentration of toxin B of *Clostridium Difficile* bacteria in UC and CD plasma patients, means were (18.42 and 20.95) respectively compared with healthy control was 13.04

Conclusion: the elevated levels of C.difficile toxin B as a marker for infection with virulent strain of this bacteria considered a main cause linked with IBD occurrence

Keywords: *Clostidium difficile*, inflammatory bowel syndrome (IBS)

INTRODUCTION

Inflammatory bowel disease (IBD) is a term that encompasses several intestinal conditions of chronic inflammation in the gastrointestinal (GI) tract. The pathogenesis of IBD is a complex process, involving environmental, genetic, microbial and immune factors. Crohn's disease (CD) and Ulcerative colitis(UC) there are two types of this disease (Von Stein, 2009).

Naturally intestinal epithelium that maintain segregation between the gut lumen and the mucosal immune system represent the first physical and chaemical barrier encountered by the intestinal bacteria, pathogens and the food antigens (Peterson and Artis, 2014). Mucosal barrier defects and alterations, possibly caused by environmental factors and/or infections (Sartor, 2006), result in increased intestinal permeability that has been observed in patients with IBD in addition, a preexisting injury to the patient mucosa might predispose bacterial infection like with *Clostridium difficile* (Mallina *et al.,* 2018).

Under Gram staining, *C. difficile* cells are Gram-positive and show optimum growth on blood agar at human body temperatures in the absence of oxygen. When stressed, the bacteria produce spores that are able to tolerate extreme conditions that the active bacteria cannot tolerate. Clostridium difficile may become established in the human colon; it is present in 2–5% of the adult population. The most well-characterized are enterotoxin (*C. difficile* toxin A) and cytotoxin (*C. difficile* toxin B) which associated with mucosal ulcer, both of which may produce diarrhea and inflammation in infected patients, although their relative contributions have been debated (Peterson et al., 2008).

Pathogenic *C. difficile* strains produce multiple toxins. *C. difficile* is transmitted from person to person by the fecal-oral route. The organism forms heat-resistant spores that are not killed by alcohol-based hand cleansers or routine surface cleaning. Thus, these spores survive in clinical environments for long periods. Because of this, the bacteria may be cultured from almost any surface (Sokol et al., 2008) .Once spores are ingested, their acid-resistance allows them to pass through the stomach unscathed. Upon exposure to bile acids, they germinate and multiply into vegetative cells in the colon. In 2005, molecular analysis led to the identification of the *C. difficile*. The incidence of *C. difficile* infection (CDI) among hospitalized IBD patients increased from 1% in 1998 to 3% in 2007 with an increase in disease severity (Rodemann et al., 2007), in IBD patients has also been associated with a significant increase in need for colectomy and even mortality with an effect that can persist up to 1 year after the primary infection (Papadakis et al., 2001). However, while a wealth of literature supports this adverse impact of CDI on IBD patients, few have attempted to identify (Wang et al., 2014 b).

MATERIALS AND METHODS

1-Study design

This study were planned in college of science for women in university of Babylon and, The places where samples collected were AL-Hussaein hospital in Karbala city, marjan, and Al-Sadek hospitals in Hilla city during the period from September 2018 to February 2019.

The population of study was consist of 85 subjects, 25 healthy individual apparently no disease are shown, they do not any medication, and non-smokers and considered the control group, while the other group are sick and include Ulcerative Colitis (30) and Corhn's Disease (30) patients

2-Specimens collection and methods

After endoscopy diagnosis of IBD patients by physician, 3 ml of venous blood collected from patients and control, the specimens were centrifuged for 10 min at 3300 rpm to obtain plasma layer which was used in evaluation

The levels of *C. difficiale* toxin B by using quantitative sandwich ELISA kit (MyBiosource, USA).The procedure was done according to the guideline provided by the manufactured scientific institute. The toxin B level was determined in plasma by plotting the absorbance of each sample against standard curve of typical concentrations supplied by the kit.

3-typical data

To determine the concentration of *C.difficile* toxin B in plasma we need standard curve correlate optical density at 450 nm (primary wavelength) of prepared typical concentration of the toxin as provided by the supplied company

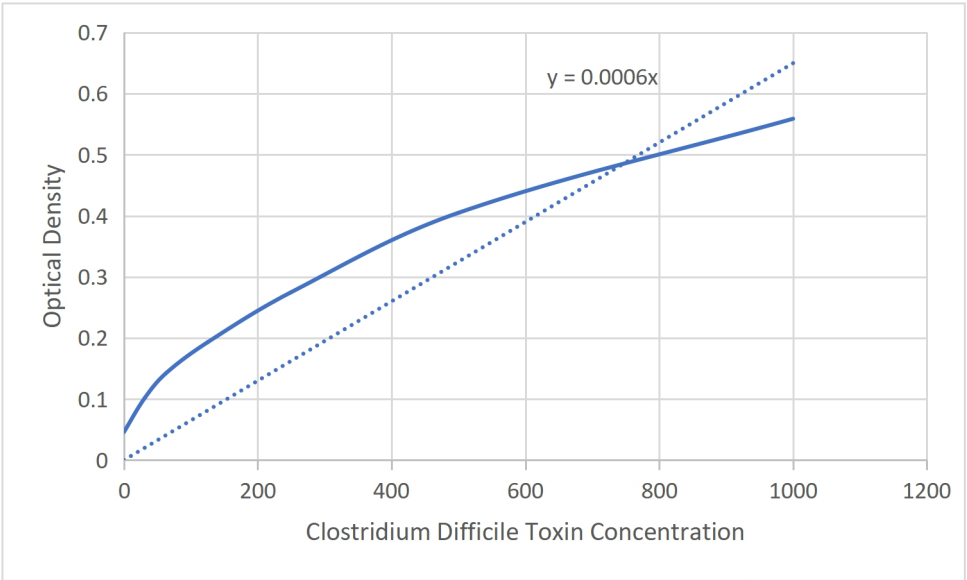

Figure1. standard curve of *C.difficile* toxin B

RESULTS

This results showed that the concentration of plasma *Clostridium Difficile* in both CD and UC patients were high significant, the means (18.42 and 20.95 pg/ml) respectively, compared with control, It's means was 13.04 pg/ml, as is shown in the table (4-1). The statistical analysis for estimation the difference between the concentration in patients and control was significant P≤0.05.

Table 1. Concentration and P value of *Clostridium Difficile* Toxin B in Patients and Control

Groups	Mean and ± standard deviation(pg/ml)	P value Significant P≤0.05
Crohn's Disease (No. 30)	18.42 ± 7.45	0.01
Ulcerative colitis (No. 30)	20.95 ± 11.82	0.02
Control, No. 25	13.04 ± 2.65	

DISCUSSION

This results agree with (Barkin, 2008) who shown that the Level of *C.difficile* infection increased from 1.8% of IBD patients in 2004 to 4.6% in 2005 (P < .01) in North American

As well as matched with (Pascarella *et al.*, 2009) who

explained that the kids with IBD were associated with a rise prevalence of *C. difficile* infection compared with healthy group in California

Other study in Belgium and Canada (D'Aoust *et al*., 2017) who found *C. difficile* related with IBD patients compared with control.

This study in Iraq agree with (Zhang *et al.,* 2016) who found the prevalence of *Clostridium difficile* infection (CDI) in patients suffering from inflammatory bowel disease (IBD) has increased rapidly over the past several decades in North America and Europe countries. However, the exact global epidemiology remains unclear because of insufficient data from developing countries. The specimens were obtained and used for toxin B of *Clostridium difficile* detection. The incidence of CDI in Crohn's disease (CD) patients was significantly lower than that in Ulcerative disease (UC) patients. Length of stay, hospitalization frequency and bowel surgery rate were significantly higher in the CDI than in the non-CDI group in CD or UC patients. More, antibiotics and infliximab usage likely increased the CDI rate in CD patients, Infliximab treatment was considered a risk factor in UC patients. CDI is an exacerbating public health issue that may influence IBD course, increase expenditures, and delay the remission of IBD patients

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