Pathological Study of Campylobacter jejuni in adult mouse model

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Introduction

The Gram-negative, spiral, microaerophilic bacterium, Campylobacter jejuni, has established itself as the leading cause of food- and water-borne human gastroenteritis in both developed and developing countries, The spectrum of disease may range from mild, self-limiting, non-inflammatory diarrhea to severe, inflammatory, bloody diarrhea with fecal leukocytes, pyrexia, abdominal cramps and bacteraemia (33).and this gastroenteritis is sometimes followed by unprecedented complications, ranging from localized peritonitis, pericarditis, hepatitis and encephalopathy to generalised neuropathy and bacteraemia. (1).

Although C. jejuni is an important cause of diarrhea throughout the world, the pathogenic mechanism associated with Campylobacter enteritis remains ill-defined (22, 19). The mechanism by which C. jejuni causes diarrhea have been postulated from studies of clinical syndromes. Toxin production is proposed mechanism in patients with acute watery diarrhea. Another mechanism, involved penetration and proliferation within the intestinal epithelium and clinically the stool contain blood and inflammatory cells. A third mechanism, termed translocation, the organism penetrates the mucosa, resulting in minimal damage (24,37). Much effort has been invested to elucidate the pathogenic mechanism of C. jejuni, four major virulence properties were recognized: motility, adherence, invasion, and toxin production (36).

In addition there are few attempts to find appropriate animal model, the following animals have been tested as model for studies on C. jejuni pathogenesis: cattle, poultry, monkey, swine, and none is completely satisfactory as a model of Campylobacteriosis due to their size, cost and they are impractical for use in most laboratories. RITARD method is useful for studying pathogenesis and immune response but not suitable for screening large numbers of strains for difference in virulence factors (9). While Humphy et al. suggested that hamster might extremely valuable small animal model for Campylobacter infection (16), contrary to the reports by Aguero-Reseafeld et

al. were unable to induce diarrhea or colitis in hamster (2). Blaser et al. showed that oral infection of adult mice induce infection in 100% of the animals but without diarrhia (6), where's Stanfield et al. was able to induce diarrhea in adult mouse model (35), Fauchere et al. demonstrated that gnotobiotic mice are better model than holoxenic animals (13). Baqar et al found BALB/c mice as preferred host for studying of pathogenesis and immunity of C. jejuni (5). Lastly, AL-Juboori found that intragastric inoculation of swiss mice with C. jejuni resulted in clinical symptom of infection.

According to as mentioned above, the aims of present study are to isolation of C. jejuni from children as well as studying the pathology of local virulent isolate by using Balb/c mice as animal model.

Material and Methods

A- Sample collection

The study included 340 children who were admitted to the Babylon hospital for maternity and children and General AL-Qasim hospital for the management of diarrhea, their age range from 1-5 years. A total of 250 watery stool specimens and ٩٠bloody stool specimens were collected from these children during a period of nine months (from the first July 2010 till the end of March 2011).

B- Direct staining method

A thin smear of stool was prepared. Staining was performed by covering the smear with 1% carbol fuchsin for one minute. The presence of recognized shapes of Campylobacter cells were registered (8).

C- Isolation of Campylobacter isolates using selective media

All specimens were inoculated on selective media (Campylobacter agar) within two hours by mixing loopful of a sample with 2 ml of normal saline, after that it was spread out by a swab; the plates were then incubated at 42ºC for 48-96 hour under microaerophilic condition using CO_2 gas generating kits.

D- Identification of Campylobacter isolates

Identification of the isolates depends on cultural and morphological characteristics of the colonies, also using biochemical and physiological tests according to Cheesbrough (8). Confirmative serological diagnosis was made by using Hicampylobacter latex test.

E- Experimental protocol :

a- Laboratory animals: Thirty female BALB/c mice were divided into sixth groups, each group contained 5 mice and the 6th group was left as a control. All animals were checked to be free of pathogens before beginning of experiment.

b- Determination of LD_{50}: Pure C. jejuni isolate that isolated from watery diarrhea stool, was grown over night at 42C° under microaerophilic condition in brucella broth followed by centrifugation at 3500 r/m for 10 minutes and suspended in sterile phosphate buffered saline to give the suspension ranging from 10^6 to 10^{10} cell / ml. Five groups of mice (5 mice for each group) were injected via intraperitoneal route with serial 10 fold bacterial dilution and as follow 10^6, 10^7, 10^8, $10^9$and 10^{10} bacterial cells /ml.

F- Histopathological studies

The pre-marked mouse which exhibited a sign of infection (like weakness or loss of appetite) were sacrificed. Before sacrificing, its general appearance was noted and any soiling in perineal region. After sacrifice, the abdomen and the result of any distension of the gut with fluid or blood were noted, and the intestine, liver and spleen were separated in different petri dishes. The formalin-fixed tissue samples were undergone a series of histopathological preparations and routine staining by hematoxylin and eosin stains according to Humason, (17).

Results & Discussion

1- Isolation and identification of Campylobacter jejuni

As a result, eight Campylobacter isolates were isolated from 340 diarrheal stool specimens of hospitalized children with percent 2.35%; six isolates from 250 watery stool specimens with percent 2.4%, and 2 isolates from 90 bloody stool specimens with percent 2.2% (table-1).

This result match with Al-Sibahee (4) who isolate four Campylobacter isolates from 186 bloody stool specimens of hospitalised children.

The age of our children from which the Campylobacter isolated from their stool, were range from 1- 5 years, and this is in agreement with available data from the Arabian Gulf countries in Kuwait, Saudi Arabia and Bahrain (3, 9, 34) which found that significantly higher incidence among children, particularly those under the age of four years.

Of 340 stool specimens processed, 15 were positive for campylobacters by Direct smear examination (Fig.-1). Only 8 of these smear-positive specimens yielded Campylobacter colonies by using selective media. This result was reasonable because these seven cases received treatment for two days before the collection of stool specimen, previous study reported that early antibiotic treatment can reduce the fecal shedding period effectively (37), therefore the result of bacterial culture became negative while the detection of the small number of the stained Campylobacter cells which shedded with the fecal normal flora still is possible.

Rapid diagnosis of fecal smear with 1% carbol fuchsin is simple, inexpensive and sensitive method which can be available in our local laboratories, but the success of this method depends upon examination of fresh stool, thin smear preparation, gentle fixation, and the examination must be done by trained eyes of a skillful observer. Early diagnosis of Campylobacter enteritis allows rapid institution of proper therapy, obviating costly and unnecessary diagnostic procedures to rule out other possible clinical enteritis such as Crohns disease and ulcerative colitis (28).

The classical methods for the identification of Campylobacter species was used in this study, such as oxidase, catalase, hippurate, motility, growth at 25 and 42 ºC, TSI, H2S, salt tolerant test, urease, cephalothin and nalidixic acid (table-2). The most useful tests for the identification of Campylobacter species are growth on selective media, temperature requirements, Gram stain morphology, and production of oxidase and catalase. C. jejuni and C. coli are very similar biochemically, with the exception of the hydrolysis of sodium hippurate. If the isolate is hippurate positive, the organism can be reported as C. jejuni. The occurrence of hippurate negative strains necessitates the use of other tests to identify the organism (12).

For confirmed C. jejuni diagnosis, Slide Agglutination Test was made with specific C. jejuni antibody, All eight C. jejuni isolates were positive.

2- Pathological study on lab animal

According to reed and Munch (30), LD_{50} dose was evaluated through intraperitoneal route in balb/c mice. Five serial concentrations of live bacteria were used and the results showed that the LD_{50} was 3.16×10^8 cell/mouse. Previous study recorded different value for LD50 of C. jejuni suspension were Hossain et al. (15) revealed that the LD50 of C. jejuni was about 1.7×10^9 cell/mouse, and Dasti (11) was observed that

1×10^{11} cell/mouse was a lethal dose for all mice tested. The difference in the LD50 values of bacterial suspension between different studies may be according to differences in the strains of mice used, and differences in their intestinal flora or differences in the C. jejuni strains used and differences in the their potential virulence factors like enterotoxin, cytotoxin, outer membrane proteins, LPS and other virulence factor (11).

The post mortal gross examination of viscera of mice group injected with 10^8 cell/ml showed abnormal appearance in comparison with the control mice (Fig.2), this group showed accumulation of fluid on the small intestine in (3 day) after injection (Fig2-A), while later in infection (7day) the large intestine appeared distended and full of the accumulation of fluid, also there was obvious abnormal appearance of liver which appeared enlarged with yellow spots. (Fig2-B)

In this study, the gross examination of the small intestine of the mice gave evidence of possessing multiple pathogenic properties, it is well known that the accumulation of fluid is due to the action of enterotoxin, while the mottled yellow liver surface indicates the presence of an invasive C. jejuni or due to the action of cytotoxin (7). Previous study reported these signs of gross lesions of Campylobacter infection in a C57BL/6 IL-$10^{-/-}$ mice and showed colon from the Campylobacter jejuni-infected mouse was enlarged and pale, with a thickened wall and watery contents (25).

Also the results of this study showed the enlargement of liver which also agree with dasti (11) which recorded the occurrence of hepatomegaly after intraperitoneal injection of Balb/c mice with 10^8 C. jejuni. In previous studies the isolation of some strains which have the potential to colonize the liver and gall bladder for a long period after intra-gastric inoculation and cause hepatitis by expressing a hepatotoxin was recorded (20, 21, 22).

There is a suggestion that some, but not all, campylobacters may indeed produce more than one toxin (31., 32, 18, 10, 14, 29). Previous studies, although unable to provide genetic data on toxin elements, have described various putative Campylobacter toxins active on several different cell lines, ranging from Shiga-like toxin activity, to a number of different cytotoxins, enterotoxin and a hepatotoxin. Strain variation may account for some of these differences in toxin production, which may become clearer as new evidence of the genetic heterogenicity of the campylobacters emerges (29).

Histopathological examination was done, The small intestinal lesions were characterized by desquamation and destruction of villi and mild infiltration of inflammatory cells within the lamina propria of the villi (Fig.3-1), while the examination of small intestine later at (7day) showed obvious villi atrophy (Fig.3-3). Liver histology at (3day) after injection C. jejuni showing multiple foci of inflammatory cell infiltration and congestion of central vein. Thereafter, in (7day) the pathological change was showed increased cytoplasmic vaculation and pyknotic nuclei of hepatocyte and foci of infiltrative lesion (Fig.4). The spleen histology showed red pulp hyperplasia, and hemorrhage compared with normal tissues of control (Fig.5).

Although this experimental infection does not produce overt illness in mice, examination of small intestine shows mucosal lesions resembling those found in human infections, and this lesion was similar to that reported by previous study (4). Also our results recorded hepatic lesions and this agrees with previous study reported that C. jejuni can induce hepatitis in mice (11, 20, 21). There is a possibility that organisms may spread systematically via the bloodstream to liver during the early phase of infection. This is supported experimentally by the fact that C. jejuni was recovered from the blood and liver of infected mice within 60 min of oral inoculation (11).

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