Histopathological Study of *Escherichia coli* O157:H7 Isolated from Children with Bloody Diarrhea in Mice

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Abstract

*Escherichia coli* O157:H7 is well documented with Shiga toxin–producing serotypes of *E. coli*. Infection with this type of pathogenic bacteria may lead to hemorrhagic diarrhea and kidney failure. This study was carried out to detect the pathogenicity of *E. coli* O157:H7 isolated from patients with bloody diarrhea. A total of 200 bloody diarrhea samples were collected from children of both sexes, with age between 3 to 10 years in the period from beginning of September to the end of December 2016 in Al-Eskan Pediatrics Hospital and Children Safe Hospital (Baghdad/Iraq). The samples were cultured aerobically on enrichment and selective media, then the isolates were identified by Vitek 2 and they were confirmed by latex agglutination test. Eight isolates were diagnosed and identified as *Escherichia coli* O157:H7. The pathogenicity of the stool recovered isolates were study to recognize the alterations in some organs of mice after experimentally infected with this pathogen. Twelve mice provided by animal house of AL-RAZI center in Baghdad, divided into two groups each group consist of 6 mice. The first group injected with 0.2 ml of de-ionized water and left as control group, whereas the second group infected orally with 0.2 ml of 1.8x10⁶ cfu/ml. The animals of two groups sacrificed 24-48 hours post infection. The histopathological examination of intestine for infected mice showed infiltration of inflammatory cells, focal lining epithelial stratification, with multiple layer basal lamina degeneration, then distention of villi appeared with increase inflammatory cells infiltration. The liver showed accumulation of lymphocyte, hemorrhage in central vein with sinuses expansion and, hepatocyte cells showed, degeneration, increase nuclear size and increase hyperchromasia with irregular chromatin distribution. The histopathological examinations of the control group were naïve.

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Introduction

The *Escherichia coli* serotype O157:H7 is a rare variety of *E. coli* that produces large quantities of one or more related, potent toxins that cause severe damage to the lining of the intestine, [1]. The bacteria do not invade mucosal cells as readily as *Shigella*, *Yersinia* or *Aeromonas*, which are armed with fimbrae, [2; 3]. It is an emerging pathogen that causes acute human gastroenteritis and hemorrhagic colitis [4] and a major causative agent of severe UTI in children [5]. *Enterohemorrhagic Escherichia coli* as a subgroup of Shiga-toxin (Stx)-producing *E. coli* (STEC) are characterized by certain serotypes that are frequently occurring in outbreaks and are associated with severe clinical illnesses such as hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS), [6].

There are several virulence factors that contribute to *E. coli* pathogenicity, such as pilli, enterotoxins (LT, ST), shiga-like toxins, endotoxin (lipopolysaccharide), hemolysin, aerobactin, cyt necrotizing factor, intimin, and biofilm formation [7;8]. Endotoxin lipopolysaccharide (LPS) and Vero toxin (VT) are released and absorbed across the gut epithelium. LPS, VT and maybe other virulence factors lead to an increase in pro inflammatory cytokines from host cells and subsequent release of chemokines from inflammatory cells. The capability for VT in the intestine micro vascular endothelial cells is exacerbated by this pro inflammatory response, [9]. LPS and VT activate the
endothelial injuries and thrombocytes, activation of the coagulation cascade and inhibition of fibrinolysis lead to formation of thrombi that lead to block capillaries and small arterioles. The intestinal vascular damage leads to ischaemia and necrosis that together with the inflammatory response may be cause the bloody diarrhea, [10]. LPS and VT can also enter the blood stream where bound to thrombocytes (LPS) and polymorph nuclear leukocytes (VT), [11; 10].

Histopathological examination of tissue biopsies for the identification of infectious organisms is a very important diagnostic tool specifically, in clinical medicine; histopathology has contributed to advances in our understanding of disease leading directly to new and more effective treatment. Some new infection involving histopathology in their discovery, have also led to fresh diagnostic challenges. Histopathology has become increasingly common in recent decades, [12].

Material and Methods

Isolation and Identification

A total of 200 bloody diarrhea samples were collected from children of both sexes aged of 3 - 10 years. They were all suffered from bloody diarrhea, in the period extended from beginning of September to end of December 2016 in Al-Eskan pediatrics hospital and children safe hospital.

Full loop of each sample was cultured aerobically on enrichment and selective media, as modified tryptic soy broth (mTSB) supplemented with Vancomycin (4 mg/L) according to [13]. Then full loop was streaked onto CT-SMAC to seek sorbitol non-fermenting bacteria (colorless colonies) after that streaked onto Hicrome media and Eosin methylen blue (EMB) as differential Media. The culture incubated at 37°C for 24 h., [13] The isolates were identified by Vitek 2 and they were confirmed by latex agglutination test depend on. [3].

Histopathological study

Animals grouping:

Twelve mice of 22-25 grambody weight with age of 9-15 weeks were provided by animal house of Al-RAZI center in Baghdad, housed in previously sterilized and cleaned plastic cages. Mice were transferred to animal house of biotechnology research center/ Al-Nahrain University and stay for seven days before administration started. The animals divided into two groups of mice each group consist of 6 mice.

1- The first group used as control group was administrated with 0.2 ml of de-ionized water.
2- The second group infected orally with 0.2 ml of 1.8×10⁶ cfu/ml.

The animals placed under observation and the symptoms daily recorded, then animals of two groups sacrifice after 24-48 hours.

Histological examination

The intestine removed from control and infected mice and cut in long pieces, then fixed in 10% neutral buffered formalin. Further routine processing for hematoxylin2 and eosin staining was performed in the biotechnology research center at the Al-Nahrain University according to reference, [14].

Results and Discussion

Results:

Isolation and Identification

Results revealed that 120 out of 200 stool sample were positive to *E. coli*. Only 8 isolates were diagnosed as *E. coli O157:H7* that appeared on SMAC as small, circular and colorless colonies with smoky center and (1-2) mm in diameter. This bacteria is identified by classical microbiological diagnostic procedures based on its inability to ferment sorbitol [15], which aids in the initial recognition of suspicious colonies isolated from bloody stools.

Culture on Hicrome media that appeared dark purple to magenta colored moiety colonies. Then, the isolates were identified by Vitek 2 and they were confirmed by latex agglutination test as mentioned by [16].

Pathogencity investigation of *E coli O157: H7* isolates by experimental Infection of Mice (*invivo* study):

The symptoms appeared in all mice with morbidity (100 %) after 24-48 hrs. post infection. The clinical sings represented by bloody diarrhea, raised fur, shivering labored
breathing, Fig.(1). This finding also described by [17] and [18]. No signs of disease or mortality were observed in animals of control group that administrated with normal saline.

**Histopathological Examination**

The pathogenicity of the stool recovered isolates were appeared in the infected animals as different lesions in different organs especially intestine and liver that attributed to virulence factors of this isolates which leads to the development of these histological alterations.

**Histological Examination of Intestine**

The macroscopic and microscopic examination of intestinal section of control group animals showed normal intestinal architecture, Fig.(4).

Histopathological examination of Intestine of experimentally infected mice showed that intestine was affected with variable lesions with infiltration of inflammatory cells, focal lining epithelial stratification and basal lamina degeneration, Fig.(5) at one day post infection. This agreement with [19] who found symptoms appeared on all mice after one day post infection with *E. coli O157:*H7 that administered orally and changes appeared in the intestine when they examined histopathology. On the other hand [20] recorded sticky, considerably empty, flimsy, of mice intestine when macroscopic examination after 1 day post infected with *E. coli O157:*H7.

At 2\textsuperscript{nd} day post infection, intestine of infected mice showed distention of villi with increase inflammatory cell infiltration, Fig.(6) multiple areas show stratification with mixed inflammatory cell (lymphocyte, plasma and eosinophil cell). Stratification of lining epithelial cell with destruction of basal lamina, reactive epithelial atypia secondary to inflammation with multiple layer, Fig.(7).
At second day post infection, liver sections showed accumulation of lymphocyte, hemorrhage in central vein with sinuses and, Hepatocyte cells appeared, degenerative cells with sinuses expansion, Figs.(9) and (10). Increase nuclear size and increase hyperchromasias with irregular chromatin distribution), with abnormal shape of some nuclei and some have prominent nucleoli. Increase N/C ratio with area of necrosis, Fig.(11). The congestion and hemorrhage noted in this study accounted for activation of endothelial cells by inflammatory mediators.

**Histological Examination of Liver**

Microscopic examination of liver section of mice in control groups presented in Fig.(8).

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**Fig.(5)** Histopathological section in the intestine of infected mice at one day post infection shows focal lining epithelial stratification (A), basal lamina degeneration (B) (H&E stain 40X).

**Fig.(6)** Histopathological section in the intestine of infected mice at day 2nd post infection show distention of villi (H&E stain 40X).

**Fig.(7)** Histopathological section in the intestine of infected mice at day 2nd post infection shows reactive epithelial atypia secondary to inflammation (A) with multiple layer (B) (H&E stain 40X).

**Histological Examination of Liver**

Microscopic examination of liver section of mice in control groups presented in Fig.(8).
Histopathological section in liver of infected mice at second day post infection shows degeneration cells and sinuses expansion (H&E stain 40X).

Histopathological section in liver of infected mice at second day post infection shows area of necrosis (H&E stain 40X).

Histopathological section in kidney of mice shows Normal looking tubules and glomeruli figure (H&E stain 40X).

Histological Examination of kidney
Normal looking tubules and glomeruli, Fig.(12).

In this study, intestinal sections showed lymphocyte aggregation below the mucosa and distention of villi with increase inflammatory cell infiltration. Multiple areas shows stratification with mixed inflammatory cell (lymphocyte, plasma and eosinophil cell) and destruction of basal lamina. Most of these finding are described by other researchers as [21] and [22].

The intestinal histopathological changes observed in this study could in part be explained by the effect of endotoxin (lipopolysaccharides), the destruction of epithelial layer of mucosal glands and infiltration of inflammatory cells observed in this study also mentioned by [23] who inject lipopolysaccharides of E. coli O157:H7 intra peritoneally into mice.

Histopathological changes of liver observed in this study were similar to that mention by [24] who found congestion in liver of mice that infected by E. coli O157:H7 that isolated from rectal swab of pet dogs and cats in west Bengal.

[25] showed necrosis in liver of pigeons after infected by E. coli O157:H7 in India. Hepatic parenchymal necrosis has been reported a consequence of tissue anoxia resulting from vasoconstriction or by extracellular bacterial toxin [26].

Conclusions
Current study revealed distinguish percentage of E. coli O157: H7 isolation recovered from bloody diarrhea and urinary tract infections in children. The results of experimental infection in mice (in vivo) indicated the pathogenicity of E. coli O157:H7 isolate through sever lesions scattered in intestine and liver.

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