Short paper

Toxigenic Clostridium difficile-mediated Diarrhoea in Hematopoietic Stem Cell Transplantation In-Patients: Rapid Diagnosis and Efficient Treatment

Running head: C. difficile in Leukemic patients

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Abstract

Allogenic hematopoietic stem cell transplant (HSCT) recipients are susceptible to any kind of infectious agents including *Clostridium difficile*. We studied 86 allogenic-HSCT patients who faced diarrhoea while receiving antibiotics. DNA from stool samples were explored for the presence of *C. difficile* toxin genes (*tcd*A; *tcd*B) by multiplex real-time PCR. Results showed nine toxigenic *C. difficile* amongst which seven were positive for both toxins and two were positive for *tcd*B. Six of toxigenic *C. difficile* organisms harbouring both toxin genes were also isolated by toxigenic culture. *C. difficile* infection was controlled successfully with oral Metronidazole and Vancomycin in the confirmed infected patients.

**Keywords:** *Clostridium difficile*; hematopoietic stem cell transplant; real-time PCR.
**Introduction**

Leukemic patients undergoing hematopoietic stem cell transplantation (HSCT) receive significant risk factors of graft-versus-host disease (GVHD), including immunosuppressant drugs, chemotherapy and broad-spectrum antibiotics (to avoid infectious complications). Empirical antibiotic therapy in HSCT patients increases the risk of developing *Clostridium difficile* infection (CDI) during hospitalisation (1). Considerable *C. difficile* infection rates of 4-27% in HSCT recipients forces the early detection and control of CDI in bone marrow transplanted patients (2). Accurate and quick detection of toxin genes in samples could be applied for patients with antibiotic-associated diarrhoea especially those whom undergo transplantation or chemotherapy. Conventionally, detection of *C. difficile* is based on a two-staged culture and PCR algorithms that confirms *C. difficile* specific gene *gluD* (glutamate dehydrogenase GDH); diagnosis may be completed by detecting toxins by either ELISA or tracing toxin genes with PCR-based methods. The time consuming procedure have been suggested in guidelines as the gold standard method for approaching CDI diagnosis (3).

*C. difficile* pathogenesis depends on producing toxins A and/or B (TcdA and TcdB, respectively) which are interesting targets for the detection of pathogenic types. The main objective of the present study was to evaluate the CDI rates in leukemic patients receiving stem cell transplantation by targeting *tcdA* and *tcdB* in a rapid multiplex real-time PCR method. This procedure may be suggesting a short-cut method for considerably rapid and accurate detection of toxigenic *C. difficile*.

**Patients**

During December 2017 to November 2018, 364 patients were admitted for hematopoietic stem cell transplantation. Isolation of the patients was fulfilled according to the CDC (Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Health
Care Settings) and the World Health Organisation (WHO Guidelines on Hand Hygiene in Health care). Eighty-six HSCT adult patients, receiving antibiotics and having diarrhoea (3< unformed stools a day) were investigated for toxigenic C. difficile infections. The Ethics Committee of Tarbiat Modares University (IR.TMU.REC.1395.403) and Digestive Disease Research Institute, Tehran University of Medical Sciences approved this survey.

Amongst 86 diarrhoeic patients, 42 had acute lymphoblastic leukemia (ALL; 48.8%) as underlying disease, followed by 26 chronic myelogenous leukemia (CML; 30.2%) and 18 acute myeloid leukemia (AML; 20.9%). According to the development of febrile periods mostly due to neutropenia, pulmonary and other relevant infections, the patients empirically received Imipenem (36%), Meropenem (24%), Ciprofloxacin (72%), and Cefixime (31%) as the most predominant antibacterial components. The history of antibiotic therapy starts from one day to one month before the diarrhoea happening.

**Multiplex Real-time PCR**

Stool samples were spiked with internal control of RealStar® C. difficile PCR Kit (altona) before extraction of total DNA by QIAamp® DNA Stool Mini Kit (Qiagen). DNA from stool samples were investigated for the presence of tcdA and tcdB by RealStar® C. difficile PCR Kit using both LightCycler®96 (Roche) and Rotor-gene Q (Qiagen) machines simultaneously to ensure the accuracy of Real-time PCR results. Multiplex real-time PCR assessment of the stool samples showed toxigenic C. difficile infection in nine patients (10.46%) amongst which 7 (8.1%) were positive for both tcdA and tcdB and 2 (2.32%) were only positive for tcdB.

**Toxigenic culture**
Stool samples were collected; one spike (~1g) of each specimen was treated by alcoholic shock for 1h. Another one was transferred into the Clostridium difficile Brucella broth (CDBB) for 1h under strict anaerobic condition (4). Treated specimens were inoculated onto the Cycloserine-Cefoxitin Fructose Agar, enriched by vitamin K1 (1µg/mL) and hemin (5µg/mL) and incubated in anaerobic condition for 2-5 days at 37° C. Recovered colonies were confirmed by microbiological characteristics and presence of C. difficile species-specific glutamate dehydrogenase (GDH) gene (gluD) by PCR (5). Presence of tcdA and tcdB genes were explored by RealStar® C. difficile PCR Kit as described above. Toxigenic culture yielded 10 isolates, which were confirmed as C. difficile by positive PCR results for gluD. Real-time PCR assessment of the C. difficile isolates for tcdA and tcdB showed 6 (6.97%) having both tcdA and tcdB. Four other isolated C. difficile were non-toxigenic.

Overall results showed that the culture method missed three toxigenic C. difficile and so the calculated sensitivity and specificity of the culture method is 76.92% (CI of 95%; 46.19% to 94.96%) and 100.00% (CI of 95%; 95.26% to 100.00%), respectively. Five patients whom diagnosed with the toxigenic C. difficile had ALL (55.5%), two had CML (22.2%) and the other two had AML (22.2%). Pairwise two-tailed regression showed no significant correlation between the antibiotics used nor leukemia type and the CDI in subject patients.

**Treatment**

After the development of diarrhoea in patients who were receiving antibiotics, a single dose of Vancomycin and Metronidazole were administered orally (following stool sample collection) (6). Oral treatment with Metronidazole and Vancomycin was continued for the individuals for at least seven days upon real-time PCR confirmation of the toxigenic C. difficile infection. C. difficile infection was successfully controlled and no mortality was recorded due to the toxigenic C. difficile during this study.
Discussion and Conclusion

Leukemic patients receiving allogeneic transplantation are amongst the most susceptible patient to all types of infections. Recent studies show the increasing rates of *C. difficile* infection from 5-20% and even rising higher to above 50% after the HSCT which may ruin the stem cell transplantation success (7). Allogeneic HSCT patients are exposed to many risk factors making them susceptible to the CDI such as broad-spectrum antibiotics, chemotherapy, immunosuppression, and proton pump inhibitor medications.

Our study showed that 10.4% of patient with bone marrow transplantation were infected with the toxigenic *C. difficile*; the infection rate will be increased to 15.11% if four non-toxigenic *C. difficile* isolates are included. History of antibiotic therapy for CDI patients was from one week to one month in the post-transplantation period. We did not detect or isolate *C. difficile* from any patient who received antibiotic therapy or being hospitalised less than a week and it is compatible with the known risk of prolonged antibiotic therapy as one of the most important predisposing factors for CDI. However, we could not correlate the antibiotics used in the subject patients with the incidence of *C. difficile* infection.

Toxins A and B —the *C. difficile* enterotoxin and cytotoxin, respectively— are the main pathogenicity factors of the organism. Toxigenic types of *C. difficile* cause the same clinical signs illnesses ranging from mild diarrhoea to life-threatening inflammation of colon, toxic megacolon, and deadly infectious colitis. (8).

Detecting *tcdA* and *tcdB* directly in fesses leads to diagnosis of pathogenic *C. difficile* rather than targeting *gluD* gene that only confirms the presence of *C. difficile* without distinguishing between pathogen and non-pathogen types. Timing of toxigenic culture and the isolation of *C. difficile* is unfavorable when the urgency of HSCT patient cases come to account.
However, in the current study, toxigenic culture results confirm the sensitivity and specificity of the multiplex-real-time PCR detection of the pathogen.

In addition, microbiological isolation and characterisation of the toxigenic *C. difficile* may fuel further investigations such as epidemiological tracing of the pathogen transmission and the infection control measurements. Antibiotic susceptibility testing of the *C. difficile* isolates also reported to be considered (9).

Oral Metronidazole and Vancomycin were administered after collecting stool samples from HSCT patients with diarrhoea (6). This blind treatment continued if the results approve the infection with toxigenic *C. difficile*. Multiplex real-time PCR results were obtained on the same day (fewer than four hours) and helped the precise decision on the proper use of antibiotics. Although, there are widely used serological methods for screening of the *C. difficile* toxins in stool samples, real-time PCR detection of the pathogen is significantly more sensitive, specific and rapid. It remarkably improves the proper management of critically infection-susceptible HSCT patients (10).

We assume that using real-time PCR for detection of the toxigenic *C. difficile* in leukemic/HSCT patients with antibiotic-associated diarrhoea significantly increases the accuracy of diagnosis in a short time. This leads to efficient management and control of the disease and reduces the mortality due to CDI.
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Conflict of interest

Authors are agreeing with content of the manuscript and declare there is no conflict of interest in all aspects of this work.

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Reference:


