Research Article

Chemotherapy Could Induce Antibiotic Resistance in *E. Faecalis* in Patients with Colorectal Cancer

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Abstract | Background: Colorectal cancer is one of the most common cancers in Iran. There are many effective methods of treatment of it. As a conventional treatment, chemotherapy has become a part of treatment scheme for patients with colorectal cancer. Enterococci are intestinal commensals. They are opportunistic pathogens which cause millions of human and animal infections annually. The aim of this study was to investigate the side effects of chemotherapy of sufferers from colon cancer on the antibiotic resistance of microflora. **Methods:** In this study, participants were divided into three groups: Group A: 300 colorectal cancer patients before the start of the cancer chemotherapy, group B: 300 healthy people living with patients at least for recent 12 months and group C includes 300 patients with colorectal cancer after six weeks chemotherapy. RNA was extracted from the stool of all the participants of the study. Following the RNA extraction from stool samples, cDNA libraries were constructed. Eight virulent genes (*vanA*, *vanB*, *gelE*, *esp*, *asa1*, *aggA*, *efaA* and *enlA*) of E. faecalis were evaluated by real-time qPCR. **Results:** The results were showed the expression level of the virulent genes in the group of the patients after chemotherapy was significantly higher than the two groups of B and C ($P<0.05$). Although the expression of these genes in the group of patients before chemotherapy was higher than that of the control group, this increase was not significant ($P>0.05$). **Conclusions:** It seems that chemotherapy could change the balance of mRNA expression of microflora such as antibiotic resistance genes. These could be responsible for infections arisen after ending the chemotherapy of cancer.

Keywords | Colorectal cancer, Chemotherapy, Antibiotic resistance, *Enterococcus faecalis*

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INTRODUCTION

Colorectal cancer has become predominant cancer and now accounts for approximately 10% of cancer-related mortality in western countries (Kuipers et al., 2015). Colorectal cancer is the second- and third-most common cancer in women and men, respectively (Kuipers et al., 2015, Kuipers et al., 2013). Colorectal cancer (CRC) is the third most common mortality factor in Iran (Dolatkhah et al., 2015). The rate of an outbreak of CRC depended on the geographical regions (Khosravi Shadmani et al., 2017, Dolatkhah et al., 2015, Johnson et al., 2013). A high incidence of CRC has been observed in developed nations, such as the US, and Canada, and developing countries also continue increasing outbreak rates (Jemal et al., 2010, Khosravi Shadmani et al., 2017). In Iran, the prevalence of the CRC has experienced the same growth as those of other Asian nations, with CRC accounting for 8.4% of all

cancers in the country (Khosravi Shadmani et al., 2017, Mohagheghi et al., 2009). Several chemotherapy regimens are used in the treatment of this disease, the common basis of which is the 5-FU (5-fluorouracil) (Tang et al., 2017, Fang et al., 2016).Chemotherapy aims at shrinking primary tumors, slowing the tumor growth, and killing cancer cells that may have metastasized to other parts of the body from the original (WELCH, 1959). An injectable 5-FU drug has been used as an effective drug in the first line of colorectal cancer metastasis treatment. 5-FU has a finite therapeutic dose range and its usage indicate remarkable individual difference (Baker et al., 2002, Gamelin and Boisdron-Celle, 1999).

One of the most pivotal side effects of every cancer treatment method in human is dysregulation of microbial flora functions (Tayebe Talebzade, 2017, Soha Sadeghi, 2018). That is related to decrease in the number and diversity of microbiota and susceptibility of under treatment's patients to infections such as gastrointestinal mucositis had reported in patients and rat models (Fijlstra et al., 2015, Tayebe Talebzade, 2017, Nicolatou-Galitis et al., 2006, Soha Sadeghi, 2018). Post treatment infections in cancer patients is a significant problem; and most reported infection in these patients which is commonly induced by *E.Coli* and *Enterococcus faecalis* (Seo and Lee, 2013, Liss et al., 2011, Hakim et al., 2018, Soha Sadeghi, 2018). Several studies have been conducted on the association between microbial flora isolated from feces and colorectal cancer. In some researches were reported arate of the microbial flora (such as *Streptococcus bovis*, *Enterococcus faecalis, bacterioidophagylis,* and *Clostridium*) isolated from stool of people with colorectal cancer had much more than healthy people (Michaux et al., 2014, Verneuil et al., 2005). an array of research had been done on the mechanism and function of these bacteria. Generally, these bacteria produce their metabolites in the cycle of proliferation, which may stimulate and proliferate colon mucous membrane cells and, in the long run, maybe lead to cancer (Montalto et al., 2004, Trenkner et al., 1987).

The Gram-positive Commensal *Enterococcus faecalis* is a pivotal member of the human and other animals intestinal core microbiota. Also, they are isolated from environmental sources (Qin et al., 2010, Ocvirk et al., 2015). The ability to acquire several virulence genes (such as the antibiotic resistance) and the emerging importance in nosocomial infections (such as gastroenteritis, intestinal infections and endocarditis) clearly indicate its role as an opportunistic pathogen (Comerlato et al., 2013, Arias and Murray, 2012, Pinholt et al., 2014, Sievert et al., 2013). Several regulatory proteins with known functions were identified as pathogenicity factors in clinical and commensal Enterococcus isolates. Attendance and increase in expression levels of the virulence genes are considerably associated with infection, biofilm construction and antibiotic resistance in Enterococcus isolates (Semedo et al., 2003, Tayebe Talebzade, 2017). Some environmental and biological changes may be responsible for altering mRNA level of virulence genes of microbial flora (Lenz et al., 2010).

The purpose of this research was to scrutinize the side effects of the chemotherapy on the microflora of body as well as investigation of the mechanism of the antibiotic resistance of bacteria in cases with colon cancer undergoing chemotherapy.

MATERIALS AND METHODS

Subject Selections and Stool Sampling

The Patient group includes 300 patients (168 males and 132 female) with early diagnosed adenocarcinoma of the colon which were candidates for chemotherapy with different protocols based on 5-FU or capecitabine that have been investigated for six period of cancer chemotherapy. The criteria for entering the study included age range of 40 to 60 years, hematologic status, and function of renal and hepatic were at normal levels. Patients should not have other cancer history and any chemotherapy. 300 healthy subjects selected from individuals which were lived with patients at least for recent 12 months due to microbial flora is strongly related to the life style of individuals. Also, age range, BMI and socioeconomic situation of the normal group were matched with patients group. The subjects with current and history of the severe medical condition including any infection or allergy were omitted from the study. All subjects were explained on the purpose of the study and next, written informed consent has been provided. The demographic characteristics of the subjects participating in the study are presented in Table 1.

Gene Expression Study by Quantitative Real-Time Pcr

Total RNA was directly extracted from fecal specimens using Viral RNA Mini Kit (catalog number: 52906, QIAGEN®, USA). The quantity of extracted RNA examined by Denovix Nano drop device (Model Ds-11). The cDNA was synthesized according to the protocol of Transcription first strand cDNA synthesis kit (Revert Aid Premium First Strand cDNA Synthesis Kit #K1652, Thermo Scientific, Latvia). 16srRNA was selected as a housekeeping gene, then Specific primers for all genes was designed by "primer3" software. Quantitative RT-PCR in atriplicate method performed by using Rotor-Gene 6000 (Corbett Research, Mortlake, NSW, Australia), and 2X RealQ_PCR master mix with Green DNA I dye (Ampliqon, Denmark) according to manufacturer's protocol.

Statistical Analysis

Descriptive data are expressed as mean ± SD (range) and

Table 1: Demographic and clinical data for subjects participating in the study

Age	Sex	Cancer stage	Karnofsky Performance Status	Infection
50±10	Patient Group 168 males (56%) 132 female (44%)	Stage I = 0 Stage II= 76 (25.33%) Stage III = 107 (35.66%) Stage IV= 117 (39%)	90% [a]=224(74.66%) 80% [b]=53(17.66%) 70% [c]= 11(3.66%) 60% [d]=12(4%)	no infection: 172 patients treated by Vancomycin: 27 patients treated by ciprofloxacin: 101
	Normal Group 154 males (51.33%) 146 female (48.66%)			

a= capable of normal activity, few symptoms or signs of disease; b= normal activity with some difficulty, some symptoms or signs; c= caring for self, not capable of normal activity or work; d= requiring some help, can take care of most personal requirements

level of statistical significance was set at P < 0.05.Gene expression changes between studied groups were examined by paired t test and independent sample t test. SPSS (version 22) was used for statistical assessments.

RESULTS

Demographic, clinical and pathological data of patients are presented at table1.The patients were in stage II, III and IV.

Gene Expression Results

The results of the expression level of the virulent genes in the group of the patients after chemotherapy was significantly higher than the two groups of healthy subjects and Patients before chemotherapy (P<0.05). However, the expression of studied genes in the group of patients before chemotherapy was higher than that of the control group, this increase was not significant (P>0.05) (Table 2, 3 and 4).

From 300 treated patients, 128 patients were shown symptoms of infection between 3 and 5 chemotherapy periods. 101 patients showed Vancomycin (Van) antibiotic resistance. Therefore 27 patients were treated with vancomycin and 101 patients treated by ciprofloxacin (Cipro).

Table 2: *P-values* of *E. faecalis* microflora genes in patients before chemotherapy treatment vs. related normal subjects (*P-values*<0.05)

Gene	$2^{-\Delta CTCT}$	*P-values*
vanA	1.02	0.41
vanB	1.013	0.33
gelE	1.19	0.31
esp	1.15	0.32
Asa1	1.11	0.53
aggA	1.06	0.11
efaA	1.05	0.39
enlA	1.006	0.44

Table 3: *P-values* of *E. faecalis* microflora genes in after chemotherapy treatments vs. before chemotherapy treatments (*P-values*<0.05)

Gene	$2^{-\Delta CTCT}$	*P-values*
vanA	2.17	0.0005
vanB	2.34	0.002
gelE	1.75	0.004
esp	1.49	0.001
Asa1	1.55	0.001
aggA	1.48	0.002
efaA	1.75	0.014
enlA	1.79	0.026

Table 4: *P-values* of *E.faecalis* microflora genes in after chemotherapy treatments vs. related normal subjects (*P-values*<0.05)

Gene	$2^{-\Delta CTCT}$	*P-values*
vanA	1.55	0.002
vanB	1.45	0.001
gelE	1.34	0.002
esp	1.47	0.005
Asa1	1.65	0.004
aggA	1.36	0.003
efaA	1.80	0.013
enlA	1.47	0.024
efaA	1.55	0.001
enlA	1.45	0.001

DISCUSSION

Regarding the growth of the use of chemotherapy drugs in the treatment of various cancers in hospitals in Iran, the number of recipients of immunosuppressive medications is dramatically increasing daily. Unfortunately, this group of patients is continuously at risk of various types of infections (Hoos, 2012, Tayebe Talebzade, 2017, Soha Sadeghi, 2018).

Results of the present study have been showing chemotherapy could change virulence gene expression of microbial flora. Moreover, it could be responsible for significant over expression of antibiotic resistance genes (*vanA* and *vanB*) in the group of the patients after treatment compared to normal subjects and patients before chemotherapy (P<0.05).This outcome has confirmed some result of the previous study in the side effect of the cancer therapy ways on germs and viruses. Tayebe Talebzade et al. (2017) and Soha Sadeghi et al. (2018) have claimed immunotherapy might have side effects such as increasing the pathogenicity risk of microflora in patients. Maybe these side effects could cause further infections after ending the immunotherapy of cancer (Tayebe Talebzade et al., 2017, Soha Sadeghi, 2018).

Also, the results of researches of the Nicolatou-Galitis et al. (2006) and Fijlstra M et al. (2015) on side effects of the head and neck cancer radiotherapy and side effects of the chemotherapy respectively were shown radiotherapy and chemotherapy might change number and diversity of microflora. The changes may be responsible for bacterial and viral infection of cancer patients under those treatment ways (Nicolatou-Galitis et al., 2006, Gafter-Gvili et al., 2012, Fijlstra et al., 2015).

Nevertheles, our result was shown increased negligible expressionin patients group in comparison with related normal individuals which mean cancer ostensibly would not change gene expression pattern of virulence genes in microflora (Pvalue>0.05). This result was inconsistent with some results of previous studies. Some of the previous studies have indicated the rate of the prevalence of the microbial flora (such as *Streptococcusbovis, Enterococcus faecalis, bacterioidophagylis*, and *Clostridium*) isolated from the stool of people with colorectal cancer had much more than healthy people (Michaux et al., 2014, Verneuil et al., 2005) Also, several researchers claimed microbial flora could lead to colorectal cancer in their host's body by some their mechanism (Montalto et al., 2004, Trenkner et al., 1987). The difference between these results can be due to the difference in the studied method, the statistical society and the select of the community studied. Striking over-expression of all eight virulence genes in *E. faecalis* from treated patients' flora was detected. It seems six-period chemotherapy triggered the of the gene expression alterations of *E. faecalis* in patients which may increase the ability of microflora of body to infection. 101 participants with colorectal cancer after chemotherapy were observed vancomycin antibiotic resistance. It may be proofed the causing of the over expression of the *vanA* and *vanB* genes in the group of the patients after chemotherapy in compared with patients before chemotherapy.

A pivotal question could be asked is that how cancer chemotherapy could affect microflora. The normal microflora acts as a barrier against overgrowth of opportunistic microorganisms as well as against colonization of potentially pathogenic microorganisms. Control of growth of opportunistic microorganisms is termed colonization resistance. Administration of antimicrobial agents, therapeutically such as chemotherapy, causes disturbances in the ecological balance between the host and the normal microflora (Sullivan et al., 2001). It is known that chemotherapy reduced or altered white blood cells production. That may be responsible for the development of bacteremia caused by Gram-positive and Gram-negative bacteria. A well-balanced microflora prevents the establishment of resistant microbial strains. An antimicrobial agents cause the risk of emergence and spread of resistant strains between patients due to they do not disturb colonization resistance. It is possible to presume that reduced number of the white blood cells could set up a natural selection in microflora population like what antibiotics effect do (Nowak et al., 2002).Therefore, if preventive measures are not done, an infection will be caused.

The most primary of this study is the investigation of side effects of chemotherapy on antibiotic resistance genesand pathogenic genes of microbial flora in CRC cancer patients. Therefore, level of the expression of virulence genes and *VanA* and *VanB* as two antibiotic resistances which is essential genes in *E. faecalis* were investigated. In order to reduce error in this study, patients were scrutinized before and after chemotherapy as well as healthy individuals were selected from familiar member of patients. Our result was shown chemotherapy might cause temporary antibiotic resistance and change balance microflora. Therefore, it is possible that measuring and evaluating viral and antibiotic-resistant genes of the body's natural microflora can be useful for patients under chemotherapy as well as it may contribute to prognosticate the onset of infection and select the appropriate antibiotic to treat the patient.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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AUTHORS CONTRIBUTION

Soha Sadeghi conceived of the presented idea and supervised the project. Soha Sadeghi provided the samples as well as she reviewed the existing journals' policy. Tayebe Talebzade, Donya Altafi and Hamed Hojatian helped oversee the project,and they had participated in study de-

sign. Soha Sadeghi and Donya Altafi performed the statistical analysis. Soha Sadeghi, Tayebe Talebzade, Donya Altafi and Hamed Hojatian discussed the results and contributed to the writing of the final version of the manuscript. They carried out all experiments. RNA Extraction was done by Farnaz Rahbarzare, Niloofar Ahmadi, Shabnam Naderifar, Elham Eslahi and Mona Mirgeloybayat. All authors, participate in revising the article critically for important intellectual content.

Percentage contributions are Soha Sadeghi: 35%, TayebeTalebzade: 15%, Donya Altafi:15%, Hamed Hojatian: 10%, Farnaz Rahbarzare (%5), Niloofar Ahmadi (%5), Shabnam Naderifar (%5), Elham Eslahi (%5) and Mona Mirgeloybayat (%5).

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