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Research Article

Effects of Smoking and Body Weight on the Presence of *E. Coli* Harbouring Colibactin Genes in Patients with Colorectal Polyps

Ali Abdul Hussein S. AL-Janabi, Zahraa Falah Al-Fatlawi

Department of Microbiology, College of Medicine, University of Karbala, Karbala, Iraq

Abstract

Objectives: Smoking and weight of the human body were investigated as factors affecting the presence of *E. coli* harbouring colibactin genes.

Methods: A case control study was performed, including 50 patients with colorectal polyps and 50 healthy individuals. Smoking condition and body weight of those subjects were determined. Rectal swabs were collected from subjects for isolation of *E. coli* and the presence of *clbA* and *clbP* genes as the main colibactin genes was determined.

Results: *E. coli* isolated from smoker patients with colorectal polyp, especially in males with neoplastic polyps, revealed a significantly higher content of colibactin genes, while these genes were found in one non-smoker female with neoplastic polyps. Genes were also detected in three smoker healthy individuals and one non-smoker female.

Colibactin genes were found more often in *E. coli* isolated from overweight and obese males with neoplasm. Four healthy individuals had also colibactin genes, two healthy obese females, and two males with overweight and obese condition.

Conclusion: Colibactin genes were frequently found in *E. coli* of smoking and heavy-weight patients with colorectal polyps, especially in those with neoplastic polyps. The frequency of these genes in smokers and obese healthy individuals was raised.

Keywords: Body weight, colibactin, colorectal polyp, smokers

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Colorectal polyp is a final step of overgrowth of cells in tissues of the large intestine, which can convert later into colorectal cancer.[1] It can take on multiple shapes and sizes. On the basis of morphological appearance, a polyp can be classified into polypoid and non-polypoid.[2] Also, it can be histologically classified into neoplastic and non-neoplastic.[3]

Escherichia coli, a member of the family Enterobacteriaceae, is naturally found on the mucosa of the human intestine, especially in the large bowel.[4] It is a prevalent facultative anaerobic, Gram-negative, rod, motile and non-spore forming bacterium.[5] Four phylogenetic groups of *E. coli*, including A, B1, B2, and D are described by Clermont and his colleagues (2000) after using the triplex PCR technique which was updated later to eight groups using a quadruplex PCR method.[6] *E. coli* B2 is the common type and it's represented 60% of resident *E. coli* and 21% of transient strains of intestinal lumen of infants.[7]

Colibactin is a secondary genotoxic metabolite of the cyclomodulins group which is created by intestinal and extra-intestinal pathogenic *E. coli*.[8] Its structure is unidentified until now due to its difficult purification from polyketide

Address for correspondence: Ali Abdul Hussein S. AL-Janabi, MD. Department of Microbiology, College of Medicine, University of Karbala, Karbala, Iraq
Phone: +9647811411260 **E-mail:** aljanabi_bio@yahoo.com
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synthase (pks)-*E. coli*.[9] Colibactin is mainly encoded by the genes of the pks cluster as first identified in 2006 by Nougayréde and his colleagues.[10] *E. coli* pks that encodes for colibactin is mostly related to phylogenetic group B2 which is considered a more virulent strain of clinical isolates *E. coli*.[11] The pks island consists of a total of 19 genes (*clbA* to *clbS*).[9] From this pks island of *E. coli*, *clbA* and *clbP* are the most important genes required for synthesis of colibactin.[12]

Several factors may have a potential contributory role in the development of colorectal polyps. Smoking and alcohol had been found a stronger association with the development of colorectal polyp than with colorectal cancer.[13] Several studies proved that obesity also plays an important role promoting formation of colorectal polyps. Obese individuals are recorded to be 7.8 times more susceptible to have a small adenomatous polyp (tubular adenoma) than the normal group, especially at age 50-65 years.[14] In Japanese adult patients, an association was demonstrated between obesity and colorectal adenomas.[15] This was also noted in African-American patients who smoked and had BMI ≥25.0.[16]

The effect of smoking and the weight of the human body on the presence of *E. coli* with colibactin genes in patients with colorectal polyps were investigated.

Methods

Patients

Case control study was performed, including 100 subjects distributed between 50 patients with colorectal polyps in age range 3-80 years and 50 healthy individuals at age range 5-75 years who represented a control group. The patient group included 33 males (age range 5-80 years) and 17 females (age range 3-65 years), while those in the control group included 30 males (age range 5-75 years) and 20 females (age range 15-82 years). All patients were volunteers and signed a consent form. Colorectal polyps were investigated in both patients and controls by the histopathologist staff of the Centre for the digestive system of Al-Sadder hospital in Al-Najaf province from December 2017 to January 2018. Colonoscopy and histopathological examination of biopsy specimens were performed during attendance by the involved subjects at the Centre. Development of polyp into neoplastic stages was also determined by histopathological examination. A healthy group was chosen after obtaining a negative result for polyps or other intestinal diseases. Body weight was indicated as body mass index (BMI) which is defined as lean (BMI <25), overweight (25≤ BMI <30) and obese (BMI ≥30).[14] Subjects considered as smokers are those who consumed an average of 2-4 packets of cigarettes per day. Patients under antibiotic treatment and those suffering from other types of colorectal diseases were excluded from the study.

Isolation of Bacteria

Rectal swabs were collected from the involved subjects after insuring that they had not taken any antibiotic for at least three days before sample collection. Collected specimens were cultured on Eosin methylene blue (EMB) media (Himedia, India) as a specific medium for *E. coli* and incubated at 37 °C for 24 h. Primary diagnosis of bacteria depended on the visual evidence of green metallic sheen colour of growing colonies and on morphological characters of isolated bacteria. Complete diagnosis of *E. coli* was performed by using Api 20E system for Enterobacteriaceae (BioMérieux, France).

Colibactin Genes and Amplification Conditions

Isolated bacteria were sub-cultured in Mueller-Hinton broth (Himedia, India) and incubated at 37 °C for 24 h. Bacterial DNA was extracted by PrestoTM Mini g DNA Bacteria Kit (Geneaid Biotech Ltd., USA). *ClbA* and *clbP* genes were chosen as the main colibactin genes in *E. coli*. Primers and PCR conditions for amplifying these genes were performed as mentioned by McCarthy et al. (2015) with some modification in PCR conditions.[12]

Statistical Analysis

The data of all tests were expressed as mean±SD. The values were analyzed statistically by one-way ANOVA through using Microsoft Excel. The level of (p) equal to or lower than 0.05 considered as a significance level.

Results

Histopathological examination of colonoscopical specimens from patients with colorectal polyps showed the presence of neoplastic polyps in 23 patients and non-neoplastic polyps in 27 patients. The relationship between smoking and the presence of colibactin genes of *E. coli* was investigated among patients with these types of colorectal polyps. *E. coli* with colibactin genes were observed more often in males than in females, but without a significant difference at $p<0.05$. More of them were significantly detected in smoker males with neoplastic polyps than in females. Meanwhile, the presence of such bacteria in females was more clearly detected in non-smokers compared to the males with similar conditions (Table 1). In the healthy group, two smoker females and one smoker and non-smoker males had colibactin genes in their colorectal area (Table 1).

Table 1. Association of colibactin genes with smoking in patients with types of colorectal polyp

Subjects group	Smoking Condition	Male No (%) Colibactin genes		Female No (%) Colibactin genes		Total No
		Positive	Negative	Positive	Negative	
Neoplastic polyp	Smoker	11* (22)	5 (10)	1 (2)	1 (2)	18
	Non-smoker	0	3 (6)	1 (2)	1 (2)	5
Non-neoplastic polyp	Smoker	0	10 (20)	2 (4)	6 (12)	18
	Non-smoker	0	4 (8)	4 (8)	1 (2)	9
Control	Smoker	1 (2)	20* (40)	2 (4)	2 (4)	25
	Non-smoker	1 (2)	8 (16)	0	16* (32)	25
Total No.		13	50	10	27	100

*Significant difference at p<0.05.

Negative results of the presence of colibactin genes in isolated *E. coli* from patients with two types of colorectal polyps was clearly observed in smoker males with neoplastic polyps (10%). Meanwhile, such absence among the healthy group was significantly found in 20 (40%) smoker males and 16 (32%) non-smoker females (Table 1).

The effect of weight of patients with colorectal polyps on the presence of *E. coli* with colibactin genes was also determined. Greater numbers of such bacteria were significantly found in overweight and obese males with neoplastic polyps compared to females. Otherwise, such bacteria were also found in one lean male patient (Table 2). On the other hand, overweight females with non-neoplastic polyps showed more content of colibactin genes in isolated *E. coli* than in males. In healthy individuals, two obese females showed the presence of colibactin genes, while this type of genes was found in one male with overweight and one with an obese condition (Table 2).

Discussion

A colorectal polyp is simply defined as any abnormal growth of tissue or mass prominent on the surface mucosa of the large intestine, especially colon and/or rectum.[17] Neoplastic as a malignant form and non-neoplastic as a benign form are the main types of colorectal polyps based on histological characters.[3] The prevalence of colorectal polyp may depend on its type. Histological and immunohistochemical examination of 896 polyps showed the presence of 177 adenomas and 202 non-neoplastic polyps.[18] Colonoscopy specimens from 7.795 patients showed that non adenomatous polyps were found in 263 of them, non advanced adenomas in 104 and advanced adenomas in 142 patients.[19] Another study demonstrated that 9% of colorectal polyps were non-neoplastic, while a neoplastic type was found in 91%.[20]

E. coli is an important member of coliform bacteria in the

Table 2. Association of colibactin gene with weight of patients with types of colorectal polyps

Subjects group	BMI (Kg/m²)	Male No, Mean±SD (%) Colibactin genes		Female No, Mean±SD (%) Colibactin genes		Total No
		Positive	Negative	Positive	Negative	
Neoplastic polyp	<25	1±0.2 (2)	2±0.5 (4)	0	0	3
	25-30	5±1* (10)	2±0.4 (4)	1±0.3 (2)	2±0.6 (4)	10
	>30	5±1.3* (10)	4±1.8 (8)	1±0.2 (2)	0	10
Non-neoplastic polyp	<25	0	4±2 (8)	0	3±1.5 (6)	7
	25-30	0	4±0.6 (8)	3±0.9 (6)	1±0 (2)	8
	>30	0	6±2.1 (12)	3±1.7 (6)	3±1.7 (6%)	12
Control	<25	0	12±3* (24)	0	2±1 (4)	14
	25-30	1±0.3 (2)	8±2.3 (16)	2±0.7 (4)	10±4* (20)	21
	>30	1±0.2 (2)	8±2 (16)	0	6±1.8 (12)	15
Total No.		13	50	10	27	100

*significant difference at p<0.05.

large intestine. Its first colonization in the gastrointestinal tract of the human newborn starts within a few hours of life and it exists in commensalism relationship with its human host for a considerable length of time.[21] B2 group of *E. coli* are considered one of four groups based on phylogenic classification of Clermont and his colleagues (2000).[6] This group of *E. coli* contains a specific genomic island of 54-kb polyketide synthases (pks) which has the capacity to encode the synthesis of a hybrid peptide-polyketide genotoxin called colibactin.[10] About 9.5% of *E. coli* has the pks island with an ability to produce colibactin, especially extraintestinal pathogenic *E. coli* (ExPEC), while intestinal isolates could not harbor colibactin.[22] *clbA* and *clbP* genes are the most important pks genetic group for producing colibactin.[9,11] It was found that the product of *clbA* gene has the ability to induce DNA double strand breaks and chromosome abnormalities in the cells of other organisms.[23] Furthermore, the main function of *clbP* gene, which plays a role as fmtA peptidase, is intermediate accessory protein for maturing of colibactin through transport of percolibactin components from the cytoplasm to the periplasm.[9]

From our results, *E. coli*-containing colibactin genes were demonstrated with more frequency in smoker patients with colorectal polyps compared with the control group, especially among males. Neoplastic polyps and advanced adenomas occurred much more frequently in smoker individuals.[1] A higher risk to develop adenomatous polyps was also found by another study among cigarette smoking patients than in normal healthy individuals.[24] This type of risk among smokers appeared to be dose-related.[25] Adenomatous polyps were noted to be more frequent in patients with a more than 15 pack-year smoking history than those who smoked for less than 15 pack-years.[26] However, about 25% of smokers remain at increased risk for development of colorectal cancer compared to non-smokers.[1]

An association between obesity and colorectal polyps is suggested by several studies. The *E. coli* bacteria with colibactin genes were found in a high percentage in our patients with neoplastic polyp of heavy weight with a significant difference from those with non-neoplastic polyp of the same weight. Obesity contributes to increase the risk of the presence of adenomas and cancer.[1] Obese individuals are recorded to be 7.8 times more likely to have a tubular adenoma than the normal group, especially at age 50-65 years.[14] Bird et al. (1998) found that patients who gained weight over 10 years were strongly under the risk to develop large polyp size and adenomas, while the risk was decreased in patients who gained weight over 5 years.[27] They also mentioned that there was no difference for the effects of obesity on the location of polyps in rectum or in colon. An association between obesity and colorectal adenomas was also demonstrated among Japanese adult patients.[15] Higher body weight (BMI ≥25.0) can interact with smoking to increase the risk of colon polyp as noted with African-American patients.[16] Smoking could also be associated with obesity to significantly increase the risk of adenomas and colorectal cancer.[28] Moreover, a multivariate analysis revealed that high body mass index (BMI >25) and current smoking were independent predictors for hyperplastic and adenomatous colorectal polyps, especially at age over 60 years.[29] Thus, control of weight in adulthood could prevent occurrence of colorectal adenoma and colorectal cancer.[30]

Conclusion

Colibactin genes were frequently found in *E. coli* of smoking and heavy-weight patients with colorectal polyps, especially in those with neoplastic polyp. Although healthy individuals showed lower presence of colibactin genes, the frequency of these genes in *E. coli* from smokers and obese were raised.

Disclosures

Ethics Committee Approval: The Ethics Committee of University of Karbala College of Medicine provided the ethics committee approval for this study (167 in 23 July 2017).

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