Association between *Streptococcus gallolyticus* and Colorectal Cancer In Egyptian Patients

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ABSTRACT

**Background:** Growing evidence indicates a correlation between colorectal cancer (CRC) and intestinal dysbiosis or colonization by single bacterial species such as *Streptococcus gallolyticus* (Sg), yet a causality link remains to be established. IL-8 is one of the pro-inflammatory and angiogenic cytokine which is frequently related to carcinogenesis. **Aim of the work:** This study was designed to assess the association between Sg colonization, IL-8 tissue levels and CRC development in Egyptian CRC patients. **Methodology:** Both tumour tissue (TU) and adjacent normal mucosa (NTU) were obtained from each patient of a total 35 CRC patients undergone surgical resection of CRC. Colonoscopy biopsies from normal colonic mucosa were also taken from 20 control subjects. Detection of Sg were done by both bacteriological and molecular (conventional PCR) methods from all tissue samples from both patients and control subjects. In addition, fecal samples were collected from both CRC patients and control subjects for assessing fecal Sg bacteria. Moreover, tissue level of IL-8 was measured by ELISA in all tissue samples from both patients and control subjects. **Results:** The molecular method revealed more positive results than the bacteriological method. The positive detection of fecal Sg was not significantly different between patients and control groups. Sg isolated from TU and NTU colorectal tissues of CRC patients in a significantly higher rate than control group, however, the frequency of positively detected Sg in TU versus NTU tissues of CRC patients was not significantly different. There was no significant association between the positive detection of Sg and each of age and sex of patients, stage, grade, and location of tumors. For IL-8, tissue level was significantly higher in TU and NTU tissues of CRC patients when compared with control tissues, and was significantly higher in TU than in NTU tissues of CRC patients. Also, IL-8 tissue level was significantly higher in Sg+ve tissue samples than in Sg-ve tissue samples. There was a significant positive correlation between IL-8 tissue level and both stage and grade of the tumor. There was no significant difference in tissue levels of IL-8 regarding age and sex of patients nor the location of the tumor. **On conclusion:** There is a strong association between CRC development and Sg colonization through cytokine dependent inflammation via induction of the pro-inflammatory and angiogenic cytokine, IL-8.

**Key words:** Colorectal carcinoma, Streptococcus gallolyticus, IL-8.
INTRODUCTION:

Colorectal cancer (CRC) is one of the most commonly diagnosed tumors with a high mortality rate. CRC incidence and mortality rates vary markedly around the world. Globally, CRC is the third most commonly diagnosed cancer in males and the second in females, with 1.8 million new cases and almost 861,000 deaths in 2018 according to the World Health Organization 2019. The global burden of CRC is expected to increase by 60% to more than 2.2 million new cases and 1.1 million deaths by 2030.

CRC development is a complex multi-factorial process occurring over many years as the result of a combination of different genetic and environmental factors. In addition to genetic alterations, the tumor microenvironment plays a critical role in CRC development and important contributing factors are linked to nutrition, inflammation, epigenetics modifications and gut microbiota.

The gut microbiota is currently considered to be an organ, and the symbiotic interactions between the gut microbiota and the digestive tract, under the surveillance of the immune system, are essential for maintaining homeostasis. Any disruptive imbalance can alter this particular ecosystem and promote diseases such as inflammatory bowel diseases and cancer.

Growing evidence indicates a correlation between CRC and gut microbiota changes (intestinal dysbiosis). Among the dysbiotic bacterial species identified and suspected to play a role in colorectal carcinogenesis are Streptococcus gallolyticus, Bacteroides fragilis, Enterococcus faecalis, Clostridium septicum, Fusobacterium spp. and Escherichia coli. Strikingly, however, Streptococcus gallolyticus is one of the very few opportunistic pathogens that has been clearly linked to malignant colonic diseases.

Streptococcus gallolyticus (previously known as Streptococcus bovis biotype I) belongs to the Group D streptococci, a large group of phenotypically diverse bacteria known as the S. bovis/S. equinus complex (SBSEC). Streptococcus gallolyticus is a member of intestinal flora in 2.5 to
15% of individuals. It is a Gram-positive, opportunistic pathogen, which is considered the main causative agent of septicemia and infective endocarditis in elderly and immune-compromised persons\textsuperscript{7}.

Despite a strong clinical association between \textit{Streptococcus galloyticus} and CRC, the causality link between them is still obscured and there is a growing need to highlight the possible mechanisms that these bacteria might play in triggering or promoting colorectal cancer, if any. Moreover, the relationship of this bacterium with oncogenic factors, cell growth factors, and pro-inflammatory cytokines has not yet been clarified well\textsuperscript{8}.

Different studies showed that cytokine-based sequel of long-standing bacterial inflammation might be the main mechanism of transformational changes in normal colorectal mucosa. In \textit{H. pylori} infections, the gastric levels of cytokines were found to correlate strongly with inflammation and the degree of gastritis\textsuperscript{9}. It was also reported that colonic cells exposed in vitro to \textit{Clostridium difficile} toxin A showed induced cytokines production\textsuperscript{10}. Alike, \textit{Streptococcus bovis}, especially their cell wall antigens, were found to increase remarkably the production of inflammatory cytokines in the colonic mucosa of rats\textsuperscript{11}.

IL-8 is one of the pro-inflammatory cytokines that acts as a chemoattractant factor for leukocytes and is involved in tumour growth, metastasis and survival in colon cancer. Moreover, IL-8 is a very potent angiogenic factor which is frequently related to bacterial carcinogenesis e.g. \textit{H. pylori}\textsuperscript{12}.

To the best of our knowledge, no previous studies investigated the association between the \textit{Streptococcus galloyticus} (Sg) colonization and CRC in Egyptian patients. Also, very few studies investigated the association between Sg colonization and tissue level of IL-8 in CRC. Therefore, this study was designed to assess the association between Sg colonization, IL-8 tissue levels and CRC development in Egyptian CRC patients.
METHODOLOGY:

A total of 35 CRC patients undergone surgical resection of colorectal cancer were involved in this study. The cases were attending the Departments of Hepatology, Gastroenterology & Infectious Diseases and General Surgery at Benha University Hospitals during the period from October 2016 to August 2018. All had sporadic CRC, proved by colonoscopy and histopathology. The patients who revealed no major illness or gastrointestinal disorder other than CRC and did not receive any antibiotic for the last 3 months, were only recruited in the current study. Both tumor tissue and adjacent normal mucosa (> 5 cm away from cancer tissues) from each patient were obtained from operative specimens and immediately frozen at −70°C until analysis. Clinico-pathological characteristics of the patients were received from surgical and pathological records. On the other hand, 20 age- and sex- matched apparently healthy control subjects were involved, they were referred to hospitals for doing colonoscopy for unexplained abdominal pain and/or altered bowl habits, in whom normal colonic mucosa was confirmed and no other gastrointestinal disease or history of gastrointestinal diseases and ulcerations were found. Colonoscopy punch biopsies were taken from control subjects for comparing them with excisional biopsies of CRC patients. The excisional biopsies from CRC patients and the colonoscopy punch biopsies from control group were prepared for different processing pathways: bacteriological isolation of Sg, DNA extraction for molecular detection of Sg and measurement of IL-8 levels by ELISA. In addition, fecal samples were collected from control subjects as well as from CRC patients preoperatively for assessing fecal Sg bacteria. Written informed consent was obtained from every participating individual. The work was approved by Ethics Committee for Human Research of Benha University. It was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans.