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ORIGINAL ARTICLE

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.* ***Objectives:*** *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. Colonoscopical 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 detected 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.* ***Conclusion:*** *Higher detection rate of Sg in CRC tissues than that of normal controls indicates a strong association between Sg and CRC development, and higher level of IL-8 in Sg +ve tissues than Sg –ve tissues indicates that IL-8 has a role in the association between this bacterium and CRC.*

INTRODUCTION

Colorectal cancer (CRC) is one of the most commonly diagnosed tumors with a high mortality rate [1]. The mortality rates and incidence of CRC differ obviously all over the world. Generally, CRC represents the second diagnosed cancer in females and the third in males, with about 861,000 deaths and 1.8 million new cases in 2018 as reported by the World Health Organization 2019. By 2030, it is expected that the global burden of CRC to be increased by 60% to more than 1.1 million deaths and 2.2 million new cases [2].

CRC is a multi-factorial process that occurs over many years as the result of a combination of different genetic and environmental factors. Beside to genetic alterations, the microenvironment of the tumor has a role in the development of CRC and important factors are related to nutrition, inflammation, epigenetics modifications and gut microbiota [3].

The gut microbiota is currently considered to be an organ, and the symbiotic interactions between the microbiota and the digestive tract, under the surveillance of the immune system, are essential for maintaining homeostasis. Any disturbing imbalance can

change this specific ecosystem and induce diseases such as cancer and inflammatory bowel diseases [4].

Growing evidence indicates a correlation between CRC and gut microbiota changes (intestinal dysbiosis). Among the dysbiotic species of bacteria identified to have a role in carcinogenesis of colorectal cancer are *Bacteroides fragilis*, *Enterococcus faecalis*, *Streptococcus gallolyticus*, *Clostridium septicum*, *Fusobacterium* spp. and *Escherichia coli* [5]. Strikingly, however, *Streptococcus gallolyticus* is one of the very few opportunistic pathogens that has been clearly linked to malignant colonic diseases [6].

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 [7].

Despite a strong clinical association between *Streptococcus gallolyticus* and CRC, the causality link between them is still obscured and there is a growing need to understand the probable mechanisms that these bacteria may play in promoting colorectal cancer, if any. Moreover, the relationship of this organism with cell growth factors, oncogenic factors and pro-inflammatory cytokines has not yet been elucidated well [8].

Different works stated that cytokine-based consequence of long-lasting bacterial inflammation might be the chief mechanism of transformational changes in the colorectal mucosa. In *H. pylori* infections, the gastric levels of cytokines were found to correlate strongly with inflammation and the degree of gastritis [9]. It was also reported that in vitro exposure of colonic cells to toxin A of *Clostridium difficile* induced cytokines production [10]. Alike, cell wall antigens of *Streptococcus bovis* were found to increase the production of inflammatory cytokines in the colonic mucosa of rats [11].

IL-8 is one of the pro-inflammatory cytokines that acts as a leukocytes chemoattractant factor and is implicated in tumor 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. *H. pylori* [12].

To the best of our knowledge, no previous studies investigated the association between the *Streptococcus gallolyticus* (*Sg*) colonization and CRC in Egyptian patients. Also, very few works studied 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 General Surgery and Hepatology, Gastroenterology & Infectious Diseases 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 gastrointestinal disorder other than CRC or any major illness 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) were obtained from operative specimens from each patient. 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. Colonoscopical biopsies were obtained from control subjects for comparing them with excisional biopsies from CRC patients. In addition, fecal specimens were collected from control subjects as well as from CRC patients preoperatively for assessing fecal *Sg* bacteria. Written informed consent was gained from every participating individual. The work was approved by Ethics Committee for Human Research of Benha University.

Preparation of tissue specimens:

Each tissue biopsy (200 µl PBS for each 1gram of tissue) was homogenized using rotor-stator homogenizer (Art-Miccra D-8 Germeny) for 20 sec. and classified to 3 parts:

- One part processed for bacteriological isolation of *Sg*.
- The 2nd part processed for DNA extraction for molecular detection of *Sg*.
- The 3rd part processed for measurement of IL-8 levels by ELISA.

Bacteriological isolation of *Sg*:

Sg were subjected for isolation from fecal material and colorectal tissues. The isolation and identification of target bacteria were done according to the protocols of *Devriese et al.* [13].

Bacterial enrichment:

- For feces: 5 gram of feces were 1:5 diluted in brain heart infusion broth (Oxoid, UK) with 5% bovine blood (Oxoid, UK) and incubated for 18-24 h at 37°C.
- For bacteria infiltrating colorectal tissues: The tissue homogenates were incubated in brain heart

infusion broth and Columbia agar with 5% bovine blood (Oxoid, UK) for 18-24 h at 37°C.

Bacterial culture:

Enriched bacteria were then inoculated on selective media, Slantez and Bartley agar (Oxoid, UK), incubated at 42°C and supplemented with 5% CO_2 which increased growth. *Sg* was identified by colony morphology and color, any small pale pink colony diagnosed as *S*g. In addition, any suspected colonies were confirmed by the use of API (API 20 strep system, bioMérieux) following the manufacturer's instructions.

Molecular detection of *Sg*:

Genomic DNA extraction:

Genomic DNA was extracted from the homogenized tissues by DNeasy Blood & Tissue Kits (QIAGEN, Germany) according to the manufacturer's instructions. The DNA yields were determined from the concentration of DNA in the elute measured by absorbance at 260 nm. On the other hand, the purity of DNA was measured by calculating the A260/A280 ratio. Pure DNA had an A260/A280 ratio of 1.7-2.0. The extracted DNA was then stored at -20°C until further processing.

Amplification:

The primers designed for the current study are highly specific in targeting *SodA* gene for the identification of *Sg*[14]. The forward primer, 5'-CAATGACAATTCACCATGA-3' and the reverse primer, 5'-TTGGTGCTTTTCCTTGTG-3'. Amplification with these primers generated an amplicon of 408 bp. (Dream Taq green PCR master Mix 2x) supplied by (Fermentas, Germany) was used for amplification. The PCR mix contained 25 ul of Taq PCR master Mix 2x, 3 ul of each of the two primers, 6 ul of the template DNA and 13 ul of nuclease free water to reach a final volume of 50 ul. For amplification, ***G*** storm thermal cycler, UK was used based on the following protocol: an initial denaturation at 95°C for 5 min. with 30 cycles of denaturation at 94°C for 60 s, annealing at 52°C for 30 s, and extension at 72°C for 30 s; and a final extension at 72°C for 8 min.

Agarose gel electrophoresis:

10 µl of each amplified DNA & 100-1000 bp ladder (molecular weight marker) were separated on 2% agarose gel containing 0.3 mg/ml of ethidium bromide. The bands were visualized using UV transilluminator (254nm) & analyzed. PCR assay yielded an amplicon of 408 bp.

Assessment of Tissue level of IL-8 by ELISA:

The tissue homogenates were transferred to 1.5 ml Eppendorf tubes, centrifuged at 13.000 g for 10 minutes at 4°C. The supernatant stored at -80 °C until analysis. A human IL-8 level was measured in tissue samples from both patients and control subjects by quantitative sandwich ELISA technique using Quantikine® ELISA (D8000C; R&D Systems, Inc., Minneopolis, MN, USA). A monoclonal antibody specific for human IL-8 has been pre-coated onto a microplate. Samples and standards were added to the wells and any IL-8 will react to the captured antibody. After washing to remove any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-8 was added to the wells. After another wash to remove any unbound substances, a substrate was added and color developed which is proportional to the amount of IL-8 bound in the initial step. The reaction was stopped and the intensity of the color was measured.

Statistical analysis:

The collected data were tabulated and analyzed using SPSS version 16 soft ware (SpssInc, Chicago, ILL Company). Categorical data were presented as number and percentages using Fisher's exact test (FET), Chi square test (X^2) for their analysis. McNemer's test was used for matched categories. Continuous data were expressed as mean ± standard deviation, Median, inter quartile range (IQR) and range. They were tested for normality using Shapiro-Wilks test, assuming normality at P>0.05. Normally distributed data were analyzed by St"t" test and ANOVA for 2 and 3 independent groups respectively, while non parametric data were analyzed by Mann Whitney (Z_{MWU}) and Kruskal Wallis tests respectively. Significant ANOVA or Kruskal were followed by post hoc multiple comparisons using Bonferroni tests to detect the significant pairs. P ≤0.05 was considered significant.

RESULTS

The CRC patients were 21 males and 14 females with a mean age of 54.6±15.3 years. According to the WHO grading system, tumors were defined as well differentiated in 13 patients (37.1%), moderately differentiated in 15 patients (42.9%) and poorly differentiated in 7 patients (20%). Based on the standard for tumor-node-metastasis (TNM) stage, 10 patients (28.6%) were in stage I, 8 patients (22.9%) in stage II, 12 patients (34.3%) in stage III, and 5 patients (14.3%) in stage IV. The tumors were located in the proximal colon in 15 patients (42.9%) and in distal colon in 20 patients (57.1%). The healthy control subjects were 12 males and 8 females with mean age of 49.3±12.6 years.

The comparative results between the bacteriological and molecular methods of *Sg* detection found that the molecular method revealed more positive results than that of bacteriological method (Table 1, Figure 1).

According to the results of the bacteriological method, it was failed to isolate any fecal *Sg* within the control group, however, it was isolated in fecal sample of only one CRC case. *Sg* isolated from TU and NTU colorectal tissues of CRC patients were significantly higher than in control group *(P= 0.019, 0.04, respectively)*, however, the frequency of positively detected *Sg* in TU versus NTU tissues of CRC patients was not significantly different *(P= 0.63)* (Table 1).

According to the results of the molecular method, it was found that, the positive detection of fecal *Sg* was not significantly different between patients and control groups *(P=1.0)*. *Sg* isolated from TU and NTU colorectal tissues of CRC patients were significantly higher than in control group *(P= 0.008, 0.039, respectively)*, however, the frequency of positively detected *Sg* in TU versus NTU tissues of CRC patients was not significantly different *(P= 0.5)* (Table 1).

All CRC patients, whose fecal samples revealed positive isolation of *Sg*, showed also positive isolation of *Sg* from colorectal tumor tissues but the opposite was not true. There was no single case where NTU was positive for *Sg* while the corresponding TU was negative. All *Sg* detected by bacteriological methods were also detected by molecular methods but the opposite was not true.

Table 1: Bacteriological and molecular detection of *Sg* among the studied groups.

Variable		CRC Patients (n=35)		Controls (n=20)		P (Fisher's test)
		No.	%	No.	%	
Bacteriological method	**NTU**	7	20.0	0	0.0	0.04 (S)
	TU	9	25.7			0.019 (S)
P (McNemer's test)		0.63 (NS)				
Molecular method	**NTU**	11	31.4	1	5.0	0.039 (S)
	TU	13	37.1			0.008 (S)*
P (McNemer's test)		0.5 (NS)				
Fecal *Sg*	**Bacteriological method**	1	2.9	0	0.0	1.0 (NS)
	Molecular method	2	5.7	1	5.0	1.0 (NS)

***→ X^2 test was used S=significant NS=non-significant**

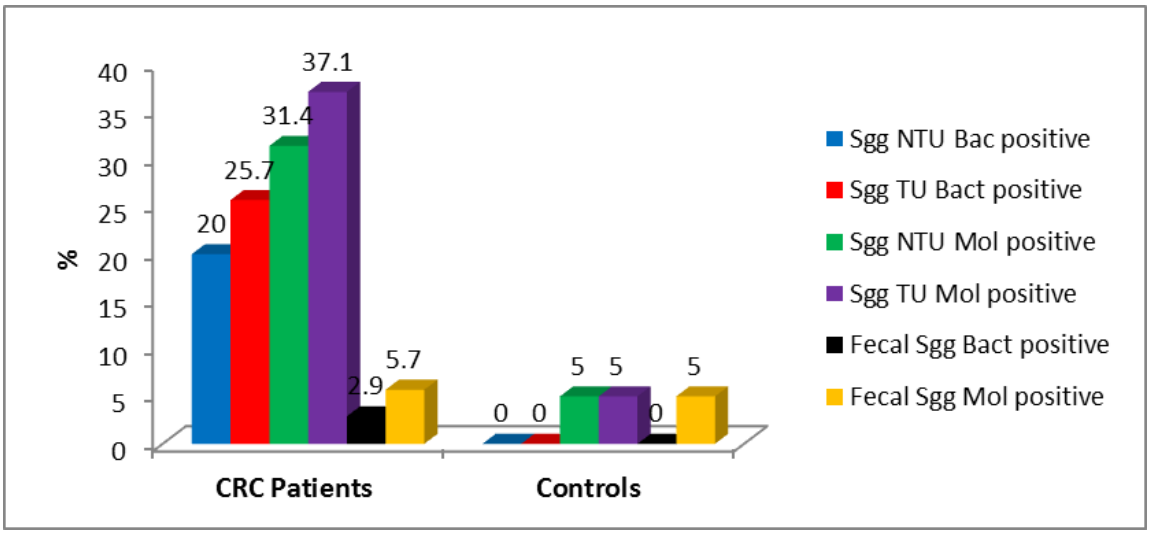


Figure 1: Bar chart showing the results of the bacteriological and molecular methods among the studied groups.

There was no significant association between the positive detection of *Sg* from TU tissues (as detected by molecular method) and each of age and sex of patients, stage, grade, and location of tumors *(P = 1.0, 1.0, 0.18, 0.31 and 1.0, respectively)* (Table 2).

Table 2: Relation between positive detection of tissue *Sg* and different demographic and clinical variables of the patients.

Variable		N	*Sg* Negative (n=22)		*Sg* Positive (n=13)		P
			No.	%	No.	%	
Age	**< 50**	10	6	27.3	4	30.8	**1.0**
	≥50	25	16	72.7	9	69.2	
Sex	**Male**	21	13	59.1	8	61.5	**1.0**
	Female	14	9	40.9	5	38.5	
Staging	**I**	10	9	40.9	1	7.7	**0.18**
	II	8	4	18.2	4	30.8	
	III	12	6	27.3	6	46.2	
	IV	5	3	13.6	2	15.4	
Differentiation	**Well**	13	10	45.5	3	23.1	**0.31**
	Moderate	15	9	40.9	6	46.2	
	Poor	7	3	13.6	4	30.8	
Localization	**Proximal colon**	15	9	40.9	6	46.2	**1.0**
	Distal colon	20	13	59.1	7	53.8	

Fisher's test was used

For IL-8, tissue level was significantly higher in TU and NTU tissues of CRC patients when compared with control tissues *(P <0.001 and P <0.001, respectively)*, and was significantly higher in TU than in NTU tissues of CRC patients *(P <0.001)*. Also, IL-8 tissue level was significantly higher in *Sg*+ve tissue samples than in *Sg*-ve tissue samples both in TU and NTU tissues *(P <0.001 and P <0.001, respectively)* (Table 3). There was a significant positive correlation between IL-8 tissue level and both stage and grade of the tumor *(P <0.001 and P <0.001, respectively)* (Table 4, Figure 2, Figure 3). There was no significant difference in tissue levels of IL-8 regarding age and sex of patients nor the location of the tumor *(P= 0.84, 0.62 and 0.56, respectively)* (Table 4).

Table 3: Tissue levels of IL-8 in different studied groups.

Group		IL-8 level (pg/ml)		Test & P
		Mean±SD (Range)	Median (IQR)	
Controls: (n=20)		77.8±30.8 (29-156)	76.5 (55.8-97.8)	Z_{MWU}1=6.12,P<0.001 (HS) St.'t'1=12.7 P<0.001 (HS)
CRC patients: **TU tissues** (n=35) **NTU tissues** (n=35)		 488.8±135.2 (311-845) 351.2±92.8 (178-537)	 466 (378-555) 343 (298-421)	 Wilcoxon test=5.14, P<0.001 (HS)
TU tissues	***Sg* +ve** (n=13)	612±129.1 (439-845)	588 (489.5-714.5)	St.'t'$_3$ =5.8, P<0.001 (HS)
	***Sg* –ve** (n=22)	415.9±71.5 (311-555)	408 (353.5-474)	
NTU tissues	***Sg* +ve** (n=11)	438.8±75.2 (323-537)	455 (371-512)	St.'t'$_2$ =4.89, P<0.001 (HS)
	***Sg* +ve** (n=24)	311±70 (178-430)	325 (250-355.2)	

St "t1 between NTU and Controls HS=highly significant

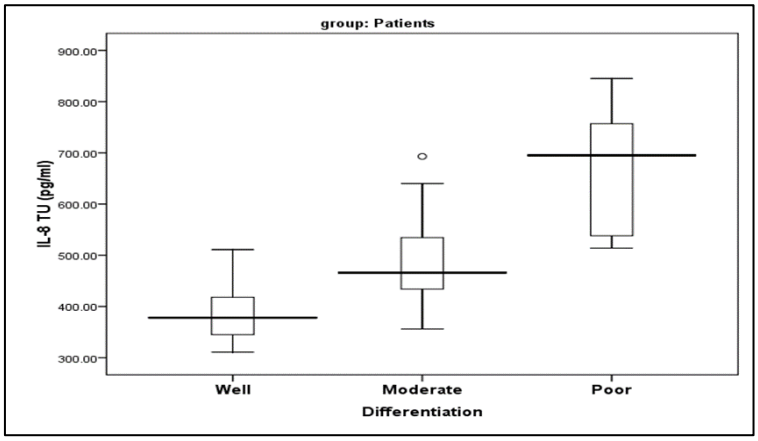
Z_{MWU1} between TU and controls

Wicoxon test between TU and NTU within the patients group

Table 4: Relation between IL-8 tissue levels and different demographic and clinical variables of the patients.

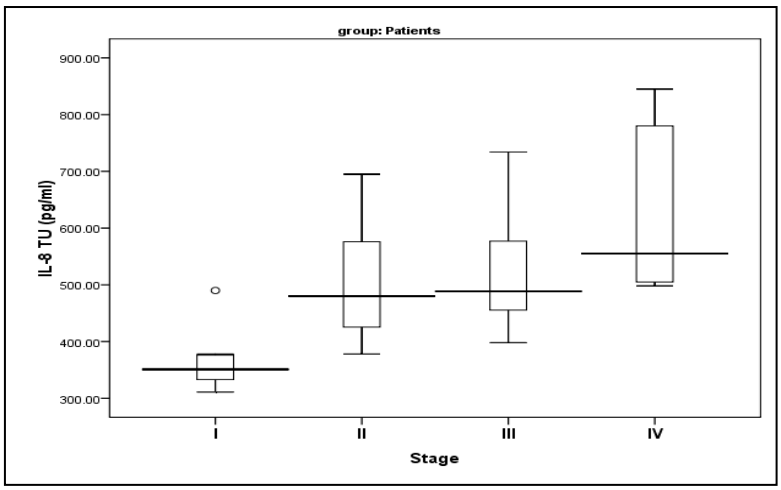
Variable		n.	IL-8 TU (pg/ml)			Z_{MWU} / KW test	P
			Mean	±SD	Range		
Age	**< 50**	10	474.1	126.1	345-780	0.21	0.84
	≥50	25	494.6	140.7	311-845		
Sex	**Male**	21	479.3	132.2	311-780	0.49	0.62
	Female	14	502.9	143.2	333-845		
Staging	**I**	10	362.6	49.8	311-490	19.1	<0.001 (HS)
	II	8	504.5*	106.8	378-695		
	III	12	521.8*	108.1	398-734		
	IV	5	636.6*	163.6	498-845		
Differentiation	**Well**	13	392.0	66.6	311-511	18.4	<0.001 (HS)
	Moderate	15	491.1†	94.8	356-693		
	Poor	7	663.4†‡	133.4	514-845		
Localization	**Proximal colon**	15	476.0	136.8	311-734	0.58	0.56
	Distal colon	20	498.3	136.7	324-845		

*→ significant In comparison with stage I, †→ significant In comparison with well differentiated
‡→ significant In comparison with moderately differentiated



Median: 378 466 695
IQR : 339-453 429-564 521-780

Figure 2: Box plot showing median and IQR of IL-18 according to differentiation of the tumor.



Median: 351 480 488 555
IQR : 330.8-376.5 423.8-582 455.3-608.5 501.5-812

Figure 3: Box plot showing median and IQR of IL-18 according to stage of the tumor.

DISCUSSION

The association between the *Sg* colonization and development of CRC were widely studied all over the world, however, no previous reports, to the best of our knowledge, about this association in Egyptian patients. The current work studied the prevalence of *Sg* in TU and NTU tissues as well as in fecal samples of 35 Egyptian CRC patients.

In the current study, the prevalence of *Sg* in our subjects was detected by using both bacteriological and molecular assays. Our results showed that the molecular method revealed positive results more than that of bacteriological method. The lower isolation rate detected by bacteriological method, in spite of the step of the pre-enrichment used, means that the bacteriological isolation of *Sg* without enrichment media was unsuccessful, in addition to the underestimated result about the prevalence of *Sg* in CRC patients. Therefore, the molecular method is recommended in this study, rather than the bacteriological method, for detection of *Sg* in CRC patients.

Our results showed that the positive detection of fecal *Sg* both in patient and control groups was 5.7% and 5% respectively, which is within the normal range (2.5 to 15%), and in agreement with the previous studies [7,14]. Unlike to the fecal isolation, *Sg* were remarkably isolated in TU (37.1%) and matched NTU (31.4%) tissues of our CRC patients, while very low percentage of positive *Sg* isolation (5%) was associated with control group, as detected by the molecular method.

There is wide range of the association rates between *Sg* and colorectal cancer reported by different studies. *Kumar et al.*[15] reported a much higher prevalence of *Sg* in CRC patients: up to 74% in tumor tissue and 47% in the matched normal colonic mucosa. Also, *Abdulamir et al.* [14] reported *Sg* detection rate of 49% in tumor tissue and 36% in the matched normal colonic mucosa. In contrast, a much lower prevalence of 3.2% was reported by *Andres-Franch et al.* [16] in their study.

This apparent discrepancy in *Sg* prevalence between different studies may be related to the different detection methods used and the fact that specimens were taken from groups of different ethnicities and from different geographical regions (Malaysia [14] ,United States [15], Spain [16] and Egypt (our study)). Moreover, differences in patient characteristics may also contribute to this discrepancy, in one study, the authors focused on CRC from a selected population with a history of *Sg* bacteremia [14], which is a quite different to the unselected frozen biopsies of CRC patients included in the other study [16].

Despite the difference, all these studies sharing our work in a common clue– the positive detection of *Sg* was confined to tumorous and adjacent safe margin tissues of CRC patients rather than colorectal tissues of control subjects and preferentially associates with tumor tissues. These results propose that there might be a definite kind of host-bacteria interaction and peculiar adhesive potential of *Sg* involving both malignant and premalignant colorectal tissues, suggesting a strong association between *Sg* and CRC [14].

Although, all previous accumulated data confirm the strong association between *Sg* and CRC, a big question is still frequently asked, is *Sg* a cause and plays an etiological role in the colorectal tumors development or it is just a passenger and merely a marker of the disease?

Some authors suggest that the higher presence of *Sg* in the tumor tissue is mostly a consequence and that bacteria represent a passenger that benefit from colon tumor microenvironment. Particular metabolites resulting from increased glycolysis in tumor cells, facilitate *Sg* multiplication and outgrowth, resulting in a significant growth benefit of *Sg* over other bacteria [17]. In addition, altered expression of surface colonic mucins such as MUC5AC and collagen type IV proteins on tumor cells may also facilitate *Sg* adherence mediated by both Pil3 and Pil1, respectively, and hence increase *Sg* preferential colonization of dysplastic tissues within the colon [18]. Finally, it was found that *Sg* produces a certain bacteriocin, named "gallocin", which suppresses the growth of closely related commensals, Enterococci, thus building a proper colonization niche for *Sg*. Gallocin is strongly activated in the existence of secondary bile acids which are considered important risk factors for CRC [19].

On the other hand, other authors suggest that the *Sg* association with colon cancer appears to be of etiological nature. A recent study reported that *Sg* strain TX20005 triggers development of colorectal tumor through increase of epithelial cell proliferation with upregulation of β-catenin levels and its oncogenic downstream targets (c-Myc, cyclin D) [15]. In addition, *Sg* was found to be involved in eliciting severe inflammatory response in colorectal mucosa, inducing inflammatory and angiogenic cytokines that are involved in the development or propagation of colon cancer [14].

In an attempt to clarify the possible inflammatory potential of *Sg* in promoting CRC, the current work selected the pro-inflammatory angiogenic chemokine, IL-8 and studied its relation to *Sg* colonization and CRC development. The result of our study, in agreement with another work [14,20], found that the tissue level of IL-8 was higher in CRC than in control groups, and higher in TU than in NTU groups, in addition to its positive correlation with the degree of differentiation and staging of the disease which all proving its association with the transformation process. Moreover, the current study reported that the tissue level of IL-8 was higher in

Sg+ve than in *Sg*-ve groups, which reflects its strong association with *Sg* colonization and rendering it as an important link between *Sg* and carcinogenesis of CRC.

Jung et al,[9] reported that IL-8 was the most significant chemokine expressed by epithelial cells of the colon when exposed to *H. pylori* bacteria. Moreover, it was found that production of IL-8 was induced by cells of CRC after in vitro exposure to *Clostridium difficile* toxin A [10]. Interestingly, *Streptococcus bovis* also increases the production of IL-8, which suggests direct interactions with the colonic mucosal cells via IL-8 [11]. Therefore, IL-8 appears to be greatly related to carcinogenesis when induced by microbial-driven inflammation. It was stated that the secretion of inflammatory cytokines in response to bacterial antigens including IL-1β, IL-6, IL-8 and TNF-α have an important role in the defense mechanisms of the host leading to the production of nitric oxide and free radicals such as superoxide, peroxynitrites and hydroxyl radicals with their remarkable mutagenicity which might participate in the tumorigenesis process by modifying cellular DNA [14].

Colorectal cancer is a prominent cause of cancer-related death. The realization that microbial agents can participate in the development of CRC increases hope for improving the diagnosis and treatment of CRC by incorporating both microbial and patient characteristics into clinical strategies. The early detection of colon carcinomas via detection of *Sg* DNA or their specific IgG antibodies might be of high value in screening high risk groups of colon cancer [14]. In a study done in Egypt, the authors evaluated the effect of mycosynthesized zinc nanoparticles (ZnNPs) on normal colon cells, colon carcinoma cells and its most associative pathogen (*Streptococcus gallolyticus*) and they concluded that ZnNPs could be an ideal drug for elimination of cancer colon and its associative microbe while posing no impact on normal colon cells and that may be due to the selective mechanism of antibacterial and anticancer induced by ZnNPs [21].

CONCLUSION

Higher detection rate of *Sg* in tissues of CRC patients than that of normal controls indicates a strong association between *Sg* and CRC development, and the higher level of IL-8 in *Sg* +ve tissues than *Sg* –ve tissues indicates that IL-8 has a role in the association between this bacterium and CRC.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M. Cancer incidence and mortality worldwide: sources, methods and major patterns in Globocan 2012. *Int. J. Cancer*. 2015; 136:359–386.
2. Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global patterns and trends in colorectal cancer incidence and mortality. *Gut*. 2017; 66: 683–691.
3. Fleming M, Ravula S, Tatishchev SF, Wang HL. Colorectal carcinoma: pathologic aspects. *J. Gastrointest. Oncol. 2012;* 3: 153–173.
4. Gao R, Gao Z, Huang L, Qin H. Gut microbiota and colorectal cancer. *Eur. J. Clin. Microbiol. Infect. Dis.* 2017; 36: 757–769.
5. Gagnière J, Raisch J, Veziant J, Barnich N, Bonnet R, Buc E. Gut microbiota imbalance and colorectal cancer. World Journal Gastroenterology. 2016; 22: 501–518.
6. Boleij A, Tjalsma H. The itinerary of *Streptococcus gallolyticus* infection in patients with colonic malignant disease. Lancet Infect Dis. 2013; 13: 719–724.
7. Dumke J, Vollmer T, Akkermann O, Knabbe C, Dreier J. Case-control study: determination of potential risk factors for the colonization of healthy volunteers with *Streptococcus gallolyticus* subsp. *gallolyticus*. *PLoS One*. 2017; 12(5):e0176515.
8. Abdulamir AS, Hafidh RR, Bakar FA. The association of Streptococcus bovis/gallolyticus with colorectal tumors: The nature and the underlying mechanisms of its etiological role. J Exp Clin Cancer Res. 2011; 30(1): 11.
9. Jung HC, Eckmann L, Yang SK, Panja A, Fierer J, Morzycka-Wroblewska E, Kagnoff MF. A distinct array of proinflammatory cytokines is expressed in human colon epithelial cells in response to bacterial invasion. J Clin Invest. 1995;95(1):55–65.
10. Mahida YR, Makh S, Hyde S, Gray T, Borriello SP. Effect of Clostridium difficile toxin A on human intestinal epithelial cells: induction of interleukin 8 production and apoptosis after cell detachment. Gut. 1996;38(3):337–347.
11. Ellmerich S, Djouder N, Scholler M, Klein JP. Production of cytokines by monocytes, epithelial and endothelial cells activated by Streptococcus bovis. Cytokine. 2000;12(1):26–31.
12. Strieter RM, Belperio JA, Phillis RJ, Keane MP. CXC chemokines in angiogenesis of cancer. Semin Cancer Biol. 2004;14:195–200.

13. Devriese LA, Vandamme P, Pot B, Vanrobaeys M, Kersters K, Haesebrouck F. Differentiation between Streptococcus gallolyticus strains of human clinical and veterinary origins and Streptococcus bovis strains from the intestinal tracts of ruminants. J Clin Microbiol. 1998;36(12):3520–3523.
14. Abdulamir AS, Hafidh RR, Bakar FA. Molecular detection, quantification, and isolation of *Streptococcus gallolyticus* bacteria colonizing colorectal tumors: inflammation-driven potential of carcinogenesis via IL-1, COX-2, and IL-8. Mol Cancer. 2010; 9: 249 10.
15. Kumar R, Herold JL, Schady D, Davis J, Kopetz S, Martinez-Moczygemba M. *Streptococcus gallolyticus* subsp. *gallolyticus* promotes colorectal tumor development. *PLoS Pathog.* 2017;13(7):e1006440.
16. Andres-Franch M, Galiana A, Sanchez-Hellin V, Ochoa E, Hernandez-Illan E, Lopez-Garcia P, Castillejo A. Streptococcus gallolyticus infection in colorectal cancer and association with biological and clinical factors. PLoS One. 2017; 12(3): e0174305.
17. Boleij A, Dutilh BE, Kortman GA, Roelofs R, Laarakkers CM. Bacterial responses to a simulated colon tumor microenvironment. Mol Cell Proteomics. 2012; 11: 851–862.
18. Martins M, Porrini C, Du Merle L, Danne C, Robbe-Masselot C, Trieu-Cuot P. The Pil3 pilus of *Streptococcus gallolyticus* binds to intestinal mucins and to fibrinogen. *Gut Microbes. 2016;* 7: 526–532.
19. Aymeric L, Donnadieu F, Mulet C, du Merle L, Nigro G, Saffarian A. Colorectal cancer specific conditions promote *Streptococcus gallolyticus* gut colonization. *Proc. Natl. Acad. Sci. U.S.A.* 2017;115: 283–291.
20. Abdulamir AS, Hafidh RR, Mahdi LK, Al-jeboori T, Abubaker F. Investigation into the controversial association of *Streptococcus gallolyticus* with colorectal cancer and adenoma. BMC Cancer. 2009;9:403.
21. Abdel-Aziz MM, Safwat NA, Amin BH. Selective toxicity of mycosynthesized zinc nanoparticles toward colon cancer and its most associative pathogen (*Streptococcus gallolyticus*). Egypt. J. Exp. Biol. 2018; 14(1): 133 – 142.