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The Role Of Salmonella In Progression Of Colorectal Cancer

Olfet Jabbar Makki Al-Hassani*, Assistant Professor Dr.Saad Abdul Azeez Atiyah, Assistant Professor Dr. Hameed Naeem Musa*****

*Dept. of Microbiology\college of medicine\University of Thi-Qar,

** Dept. of Microbiology\college of medicine\University of Thi-Qar,

*** Dept. of Pathology\college of medicine\University of Thi-Qar

Abstract:

Background: Colorectal cancer constituting one of the most frequently occurring malignancies. Previous studies exhibiting the cells of the host signaling manipulation by the salmonella, where chronic or carriage of the salmonella infection can triggering the cancer development among the predisposed genetically persons,

Aim: The assessments of the role of salmonella in colorectal cancer grading and staging and whether if it had a role in poor progression or good prognosis of the colorectal cancer.

Method: A 76 cases compared with nearly similar number of control by selecting their histo-pathologic specimens most of the control where excluded because of the insufficient sample size. Comparison done in Thi-Qar province- Southern of Iraq, extended from September 2020 the end of June 2021. Salmonella DNA was detected by PCR technique, and the PCR results were linked with demographic parameters such as age and gender. The data were examined by SPSS (26), P value <0.05 consider significant.

Result: Universal bacterial primer gave a positive results in 58(57.4%) of cases and only 8 (7.8%) cases of this where positive for Salmonella primers. Most cases of colorectal cancer grade were grade II -A 36.8% followed by grade II-B 19.7%. The distribution of bacterial DNA universal primer was highly amounted in Stage III (56.8%) followed by Stage II, and the salmonella DNA were also detected in higher proportion in stage III of colorectal cancer followed by Stage II and there was no case of salmonella species were detected among the cases of stage IV or stage I.

Conclusion: Bacteria including salmonella present in an important percent in the colorectal cancer tissues, which indicates the role of bacteria in the developments of cancer as confirmed by other studies. Salmonella were highly distributed among male , retired, resident in Al- Nasiriya, and married but with no significant statistical difference in their distribution within the colorectal cancer cases.

Key word: Colon, Rectal Cancer, Salmonella

Introduction:

The gastrointestinal tract is a natural habitation for a microbial community, that contacts with the intestinal epithelial cells.The direct link of intestinal bacteria to human sporadic colorectal cancer is still limited[1,2].Infectious agent contribute to about 1/5 of the Global cancer burden [3]. In comparison to cancersinducedvirally[4-6], bacteria largely neglected as attributer factors for cancer[7], only a few bacterial infections that may be linked to cancer development to date [8-9]. This is superlative proven for *Helicobacter pylori* linked with gastric cancer[10,11], for *Salmonella typhi* and chronic typhoid carriers in gallbladder carcinoma and colorectal cancer[12-15].

During inflammation, bacteria may contribute to cancer development, initiation DNA damage by metabolites, toxins, and/or influence of host cell signaling pathways throughout their infection

cycle[16,17]. Bacteria similarly may modify the cell biology of the host for the duration of infection cycle, by way of showed via Salmonella species which control host cell signaling pathways to impose bacterial uptake, intracellular survival [18]. *Salmonella* secretes effector proteins into host cells which activate the host by different pathological pathways, furthermore, These pathways are activated in many cancers, and are essential for transforming pre transformed cells[15]. Another *Salmonella* effector Avr -A activates host β-catenin signaling as well as promotes colon carcinogenesis[19,20]. If by feature of altered host cell signaling bacteria offer one step towards cancer development[21],it is possible that bacterial infections increase cancer risk.

In 2012With 694,000 deaths, colon cancer is a major cause of cancer morbidity and mortality in the world[22], mainly for older patients, where colon cancer incidence increases evidently after the age of 60 years [10,23]. Factors disposing for colon cancer are Inflammatory Bowel Disease (IBD) [24,25] and genetic mutations [26,27].Over the years Colon cancer incidence increases as a function of largely unknown risk factors[28,29]. To report whether *Salmonella* infections institute yet another risk factor for colon cancer, researcher linked the frequency salmonella species DNAs among colon cancer . Additionally, we examined potential effects of gender, age, other socio-demographic factors as intervening or predisposing factor for the two diseases of interest, and try to find the role of *salmonella* species in the progression of colorectal cancer inform of grading and staging.

Method and Materials:

Type & design of the study: Diagnostic type of study that using molecular diagnostic tools for detection of the presences of the *salmonella*in colorectal cancer, Comparative analytical cross sectional study that extending from 1st September 2020 till 1st week of June 2021.

Study population: Inclusion criteria: Cases: colorectal cancerous (CRC) casesof the years 2019-2021, that entitled by histo-pathological exam as a cases of CRC, which taken from Al-Husain teaching hospital –ThiQar governorate/Iraq for the previous three years mentioned above.

Control: colonic polyp or other pathologies of the colon rather than colorectal cancer taken form patients under went excisional surgeries and sent for histo-pathological exam for the same time: years (2019-2021).

Exclusion criteria: 1-Not fully informative patients (either lack of their phone number, or not responding during communication, or answer of surrogate about main questions of risk factors where the patients died, and finally those who not accepted to engage within the study)

2- Poor efficacious histopathological slide and or material for DNA extraction for salmonella.

Sampling and sample size: A 76 cases fitting to inclusion criteria, where registered formally from Al-Hussain teaching hospital main laboratory in the histo-pathological unit, from the 1st of January of 2019- till the end of March of the 2021. A 76 FFPE tissues of other colon pathologies rather than colorectal cancer taken form patients as controls.

Tools of the study:

Questionnaire forma: including the following two items:

1- Personal information: that include (age, gender, residence, occupation, marital status)
2- Histo-pathological information: concerned with grading , staging of the tumor .

Sectioning step:

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Pieces of tissue collected within Eppendorf tube and labeled according by sectioning. The samples were cut 4–5 Mm-thick sections from paraffin blocks done by microtomes and transfer tissue to Eppendorf tubes.

DNA extraction:

Tissue in Eppendorf tube was crash 10-20 times using pestle then washed by xylene (1 ml) to remove paraffin from tissues (the step repeated 5 times), then the sample was washed by 1 ml of absolute ethanol five times, then it put in oven at 50 ˚C for 15 min to evaporated ethanol. The DNA were extracted from FFPE tissues according to manufacturer instruction (Genaid-Taiwan).

Polymerase Chain Reaction (PCR) procedure:A . Primers

The presence of bacteria and salmonella in colorectal FFPE tissues was determined by PCR technique using a primers listed in table (1).

Table (1):Primers used for detection of bacteria and salmonella in this study

No.	Name	Sequence	Annealing Temp.	M.wt. bp	Ref.
1	SdiA1	AAT ATC GCT TCG TAC CAC	52 °C / 40 sec.	274	Halatsi *et al.*, 2006
	SdiA2	GTA GGT AAA CGA GGA GCA G			
2	341F	CCT ACG GGA GGC AGC AG	55 °C / 40 sec.	566	Muyzer *et al.* 1997
	907R	CCG TCA ATT CCT TTG AGT TT			
3	16S rDNA F	TGT TGT GGT TAA TAA CCG CA	56 °C / 60 sec.	574	Ziemer&Steadham, 2003
	16S rDNA R	CAC AAA TCC ATC TCT GGA			

B. The reaction mixture and conditions

PCR amplifications were achieved in a final volume of 25µL in PCR tubes. The reaction mixtures contained of 12 µL of master mix (Promega-USA), 5 µL of the DNA template, 1 µl (50 pmol) from the forward and reverse primers (Macrogen-South Korea), of the primers pairs of interest (Table 1), and 6 µL of deionized distilled water. PCR reactions was done in the thermal cycler (Eppendorf-Germany) which adjusted as follows: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing as mentioned in Table 2 and extension at 72 °C for 1 min. Final extension was carried out at 72 °C for10 min, then the PCR products were cooled at 4 °C. Amplified products were detected by the electrophoresis in 1.5 % agarose gel containing 2 µL ethidium bromide. Visualizations were done by using a UV transilluminator (Lab Tech-Germany), and the 100 bp DNA ladders were used as molecular markers.

Statistical analysis:

SPSS version 26, has been used for the data analysis, where quantitative variables assessment done through estimation of frequency , percentages, mean, standard deviation, standard error, ANOVA, while qualitative variables presented inform of figures that drown by Excel sheet 2010, while analysis including chi-square, . P value lesser than 0.05 consider as significant, while lesser than 0.001 consider as highly significant.

Results:

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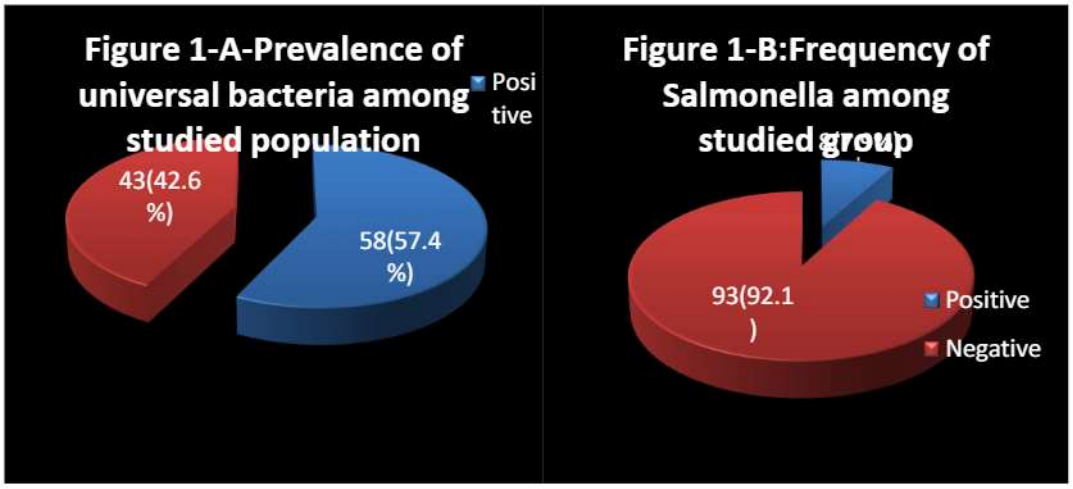
Figure 1-A expressing the prevalence of total universal bacteria that detected by universal bacterial primer resulting in 43.6% of population show positive type of bacteria while only 7.9% show positive result for the *Salmonella* species as shown in figure 1-B.



Even though there was some sort of difference in the mean age of positive and negative cases of *Salmonella* with high similarity in mean and standard deviation but there was no significant statistical difference between the mean age of the positive and negative age group as shown in table 2:

Table 2. Distribution of bacteria (universal and Salmonella) primer according to age

Universal Primer	Salmonella Primer	Mean Age	N	Std. Deviation	ANOVA, P
Positive	**Positive**	63.7500	8	15.08784	0.11
	Negative	55.0600	50	16.37371	0.919
Negative	**Positive**	0	0	0	
	Negative	56.5814	43	14.59816	
Total	**Positive**	63.7500	8	15.08784	
	Negative	55.7634	93	15.51325	
	Total	56.3960	101	15.55769	

The seven cases of *Salmonella*were distributed in a highest proportion among male, while only one detected on female with colorectal cancer; there was no significant statistical difference in the distribution of the cases among different gender within the colorectal cancer cases but had four fold odds ratio which might give a hint regarding the distribution of *Salmonella* in different gender groups.

As regards the distribution of *Salmonella*in different occupational groups the retired group carrying the higher person of *Salmonella*, there was three cases in comparison to employer only two cases housewife one case other group representing the last one case of *Salmonella* this distribution also reflecting no significant statistical difference between the *Salmonella* distribution and occupational group.

On the topic of the residence of patients with colorectal cancer *Salmonella* where mainly distributed among those who are inhabiting Al-Nasiriya, those who were habit in Al-Nasiriya where 5 cases were

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detected, the remainder 2 cases were resident in Rifaee, also there was no statistical difference and the distribution of the cases and the control residence status.

Marital status show also no differences that is statistically but distribution also differ among different groups where most of the cases on the married while only two widows and widowers, single registered no case of *Salmonella.*

Table 3 : Distribution of *Salmonella* according demography of studied population

		Salmonella in cases			X^2	Salmonella in control			X^2
		Positive	Negative	Total	P	Positive	Negative	total	P
Gender	**Male**	6	41	47	1.862[e],0.172, 1.098**	1	16	17	0.490[g],0.680, 0.941**
		12.8%	87.2%	100.0%		5.9%	94.1%	100.0%	
	Female	1	28	29		0	8	8	
		3.4%	96.6%	100.0%		0.0%	100.0%	100.0%	
Occupation	**Employer**	2	15	17	3.262 ,0.499[b]	0	13	13	6.341, 0.438[b]
		11.8%	88.2%	100.0%		0.0%	100.0%	100.0%	
	Self – employer	0	6	6		0	1	1	
		0.0%	100.0%	100.0%		0.0%	100.0%	100.0%	
	house wife	1	20	21		0	5	5	
		4.8%	95.2%	100.0%		0.0%	100.0%	100.0%	
	Retired	3	26	29		1	4	5	
		10.3%	89.7%	100.0%		20.0%	80.0%	100.0%	
	Other	1	2	3		0	1	1	
		33.3%	66.7%	100.0%		0.0%	100.0%	100.0%	
Address	**Nasiriya**	5	32	37	4.428 ,0.140[b]	1	12	13	2.133 ,0.811[b]
		13.5%	86.5%	100.0%		7.7%	92.3%	100.0%	
	Suq-alsheyokh	0	15	15		0	4	4	
		0.0%	100.0%	100.0%		0.0%	100.0%	100.0%	
	Shatra	0	14	14		0	5	5	
		0.0%	100.0%	100.0%		0.0%	100.0%	100.0%	
	Rifaee	2	8	10		0	3	3	
		20.0%	80.0%	100.0%		0.0%	100.0%	100.0%	
Marital state	**Married**	5	55	60	1.154, ,0.681[b]	0	18	18	4.352, ,0.287[b]
		8.3%	91.7%	100.0%		0.0%	100.0%	100.0%	
	Single	0	2	2		0	3	3	
		0.0%	100.0%	100.0%		0.0%	100.0%	100.0%	
	Widows	2	12	14		1	3	4	
		14.3%	85.7%	100.0%		25.0%	75.0%	100.0%	
		7	69	76					
		9.2%	90.8%	100.0%					

The distribution of bacterial DNA universal primer was highly amounted in the Stage III 56.8% followed by Stage II minimal proportion where detected on stage IV, while stage 1 register no cases of

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bacterial DNA, on other hand the*Salmonella* DNA they we're also detected and higher proportion on the stage III of colorectal cancer followed by Stage II, an d there is no case of *Salmonella* were detected among the cases of stage IV or stage I also there was no significant statistical association between the distribution of universal bacterial primer DNA or *Salmonella* DNA according to the stage of colorectal cancer in table.4:

Table 4: Distribution of universal bacteria primer and *Salmonella* according stages of colorectal cancer-details

Stage	Universal Primer		Total	X^2, P value	Salmonella primer		Total	X^2, P value
	Positive	Negative			Positive	Negative		
One	0	1	1	1.872, 0.666	0	1	1	1.297, 0.999
	0.0%	100.0%	100.0%		0.0%	100.0%	100.0%	
Tow	16	11	27		3	24	27	
	59.3%	40.7%	100.0%		11.1 %	88.9%	100.0%	
Three	25	19	44		4	40	44	
	56.8%	43.2%	100.0%		9.1%	90.9%	100.0%	
Four	3	1	4		0	4	4	
	75.0%	25.0%	100.0%		0.0%	100.0%	100.0%	
Total	44	32	76 100.0%		7	69	76 100.0%	
	57.9%	42.1%			9.2%	90.8%		

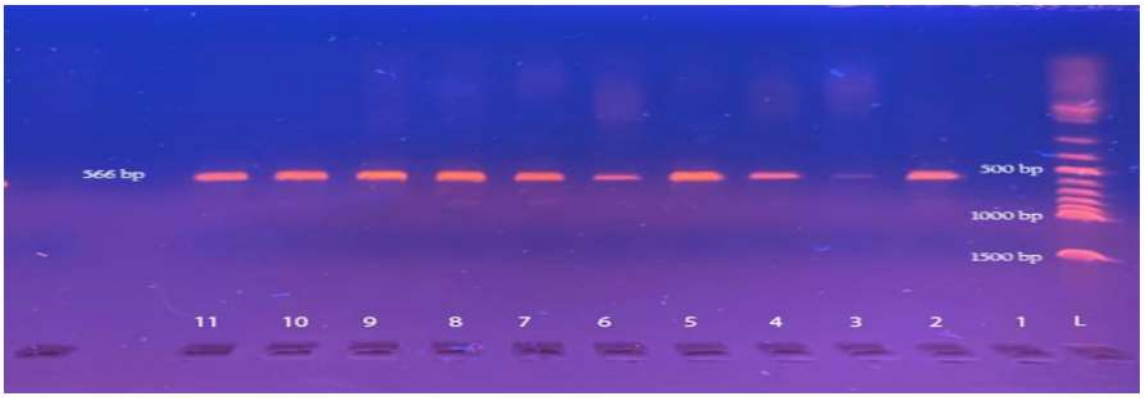

Photo 1: Agarose gel electrophoresis of PCR products of (341F-907R) universal primer which showed a product of (566 bp) . L: DNA ladder, Lanes: 2,4,5,6,7,8,9,10,11 showed a positive PCR products for a useful primer

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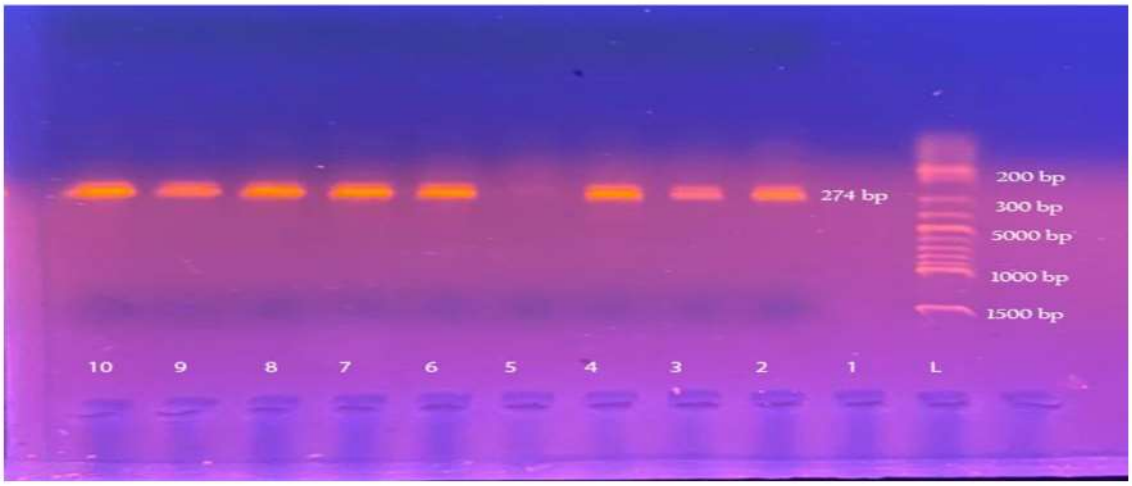

Photo2 : Electrophoresed PCR products for identification of *Salmonella* by using quorum sensing specific sdiA amplicon in agarose gel that gives an amplification products of (274) bp . L: ladder; Lane 2,3,4,6,7,8,9,10 showed a positive results for *Salmonella* sdiA gene.

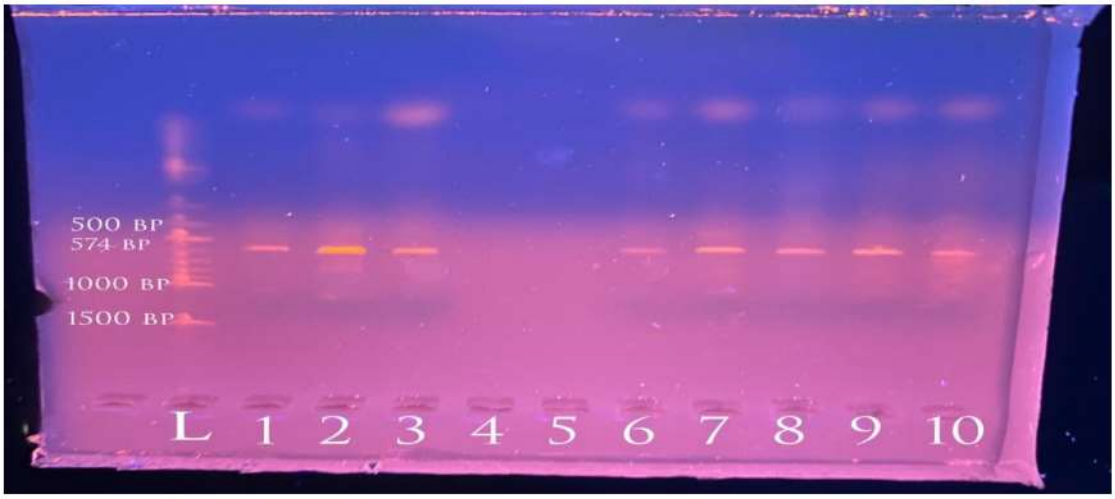

Photo3: PCR products of specific 16S rRNA amplicon for identification of *Salmonella* in an agarose gel that gives an amplification products of (574) bp . L: ladder; Lane 1,2,3,6,7,8,9,10 reveals a positive bacterial signals to the used primer

Discussion:

Universal bacteria and *Salmonella* in Colorectal cancer:

General findings:

The current study carrying main objectives for the assessments of the rule of salmonella in colorectal cancer prognosis. The distribution of bacterial DNA universal primer was highly amounted in Stage III (56.8%) followed by Stage II ; minimal proportion were detected on stage 4 while stage I register no cases of bacterial DNA, also detected in higher proportion in stage III of colorectal cancer followed by Stage II and there is no case of *Salmonella*were detected among the cases of stage IV or stage I and there was no significant statistical association between the distribution of universal bacterial primer DNA or *Salmonella* species DNA according to the stage of colorectal cancer.

According to the grading of colorectal cancer the highest grade of bacterial distribution that detected by universal bacterial primer grade II it is also representing the highest grade of *Salmonella* DNA, other

grades were of nearly equal distribution regarding the universal bacterial DNAs while for *Salmonella*, grade 1-B registered no case of *Salmonella* also there was no significant statistical difference in the distribution of universal bacterial DNA and or *Salmonella* DNA according to the colorectal grading which was inconsistent with Lapo Mughini *et al*[25].Where noticed rise in colon cancer risk among patients with *positive* history for infection by salmonella.

Various factors may lead to carcinogenesis in the colon, containing inflammation, dysbiosis and continuous growth of epithelial cells with pretransforming mutations. Accordingly, pre-malignant forms (i.e. polyps) of colon cancer are observed, specifically at higher age. If *Salmonella* infection provides one step, so in the multistep process resulted cancer [26,27] , infection of premalignant colon polyps give full transformation.

In the current study also difficult to assess the past history of nutrition, smoking, alcohol, fruit and vegetables intake while other studies compare these potential factor with control group especially that the designed as a cohort study, where potential confounders of tumor pathological features we can assessed . demography is an alternative for many factors, as diet,smoking behavior, general health status,obesity and physical activity [28] significantly, did not contribute to salmonellosis-related colon cancer risk. Only irritable bowel diseases (IBD) incident differed significantly between colon cancer patients with or without reported salmonella infection, though the small number of IBD patients (n = 5) comparative to the total number of colon cancer patients with a history of salmonellosis for that IBD status was known (n = 65), suggests that IBD impact is minimal. So, IBD may predispose to longer periods of salmonella infection, by increasing the risk of developing colon cancer.

The observed data that tumors of patients with a history of salmonella were of low grade .these tumors are different from those rising without a contribution from *Salmonella* infection, as observed for the colorectal carcinomas with a history of *S. typhi* infection [26] .The tumors that associated with salmonellosis started from pre-transformed cells, state would usually take around 4 years[26] . Yet, it is unknown how long this transformation would take in humans. so, other researcher recurring the analyses setting that getting similar increased risks of colon cancer after *S .enteritidis* infection. patients diagnosed with CRC at 4 years from salmonella infection that had already premalignant colon adenomatous polyps or transforming to cancer was accelerated by the salmonella infection itself, thereby simulating the situation observed under laboratory conditions[26] while patients diagnosed with colon cancer afterwards have had a different contribution fromsalmonellainfection, hold induction of the pre-transformed state itself.

As compared to the general population, *S. enteritidis* infection showed a three-fold increased risk of CRC, in spite of that it was not different from the effects salmonellosis cases only (i.e. within the cohort). Accordingly, acquired immunity against S. Enteritidis is less possible to occur andtherefore infection be more severe or persistent.

Laboratory experimentations show that *Salmonella (typhi* and *typhimurium*) can contribute multistep process to oncogenic transformation [26] . A coincidental infection of a pre-transformed cell may therefore suffice in motivating cancer development.then Persistent or severe salmonella infections may increase the risk of developing cancer, where the chances of infecting a pre-transformed cell are higher under these circumstances.

The data have to be definite in the human colon cancer samples in patients with a history of severe Salmonella infection. Independent verification of the relationship between *S. enteritidis* and colon cancer in independent data sets will additional strengthen these points, as needed to convince health authorities in permitting early participation of patients diagnosed for this pathogen into National Colon Cancer Screening Programs.

Prevalence of total bacterial colonization was 43.6% in the samples under this study, while only 7.9% show positive result for the salmonella , where all cases of salmonella species were detected also by universal primer of bacteria this is done when a cross-tabulation of the cases that are detected by both

primer for salmonella species and universal bacteria give a hint for the sensitivity of universal bacterial primer which might reach 100%.

Also inconsistent with other studies that talk about the effect of bacteria as anticancer, that focusing on the inflammatory processes prevention by bacterial change of colonic mucosa change, which might use in the future.Prevalence of total bacterial colonization was 43.6% in the samples under this study, while only 7.9% show positive result for the salmonella , where all cases of salmonella species were detected also by universal primer of bacteria this is done when a cross-tabulation of the cases that are detected by both primer for salmonella species and universal bacteria give a hint for the sensitivity of universal bacterial primer which might reach 100%.Nowadays, thorough qualitative and quantitative assessment of all environmental issues is not conceivable. Numerous environment- inductive illnesses had branded, based on microbiota (colon microflora), analyses. Aggregated data exhibit special microflora changes (dysbiosis) in patients stools with CRC that adhere to the mucosa of colon. Thus, microbiota seems to be considered as an offering host platform in environment communications for CRCs study. A hypothesis that CRC might be a disease of bacteria-relation[29].

Also inconsistent with other studies that talk about the effect of bacteria as anticancer, that focusing on the inflammatory processes prevention by bacterial change of colonic mucosa change, which might use in the future. So that, dietary fibers fermentation of microbes pointes to short-chain fatty acid acetate, butyrate and propionate production. These are absorbed by colonocytes and used as a primary source of energy. They provide protection during the early stages of tumourigenesis .(30)

Conclusions:

Bacterial colonization including salmonella present in an important percent in the colorectal cancer tissues, which indicates the role of bacteria in the pathological events of colorectal cancer. Salmonella were highly distributed among male , retired, resident in Al- Nasiriya, and married but with no significant statistical difference in their distribution within the colorectal cancer cases.The topic requires to perform another studies to detects the salmonella associated factors that contributes with colorectal cancer.

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