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Original article

Association Between Interleukin-22 Genetic Polymorphism and Colon Cancer Risk in Egyptian Patients

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Abstract:

Background: The cytokine interleukin-22 (IL-22) is produced by T cells and natural killer cells and is associated with tumorigenesis and tumor progression. Unlike most cytokines, the main impact of IL-22 is on non-hematopoietic epithelial cells and fibroblasts in tissues as lung, liver, kidney, thymus, pancreas, breast, gut, skin and synovium. Several (SNPs) have previously been identified in the IL-22 gene as (−429C/T, +1995 A/C and +1046 T/A). **Aim:** This study aimed to investigate the association of IL-22 polymorphisms with the colon cancer risk in Egyptian population in relation to tumor staging. **Subject and method:** A prospective center-based case-control studies comprising 100 patients with pathologically proven colon cancer attending Mansoura Oncology Center from Jan 2017 till Jan 2019 and 100 age and gender-matched healthy controls. Real time PCR with TaqMan probes was used to determine the genotypes of -429 C/T. **Results:** A significant association was found between CT, TT, CT+TT genotypes, and T allele with the risk of cancer colon. Unfortunately, we did not find any statistically significant difference between IL 22 different genotypes of cases regarding sex, gender, tumor size, albumin, CA 19.9 (P=0.570) or age (P 0.993). **Conclusion:** up to the authors' knowledge, this is the first report recording that the IL-22 -429 C/T gene polymorphism is associated with colon cancer risk in Egyptian patients. Also, our findings suggested that the IL-22 −429C/T gene polymorphisms might be associated with colon cancer in Egyptian population. Further studies are necessary to confirm our results.

Keywords: Interleukin- 22, -429C/T, rs2227485, single nucleotide polymorphism, cancer colon.

Introduction

Colorectal cancer is the 4th most common cancer in the world. Studies had shown different tumor behavior according to the site, pathology, and stage. However, the characters of Egyptian colon cancer patients are not well addressed (1). Colorectal cancer is a major cause of morbidity and death. Various environmental and persistent factors have been associated with the colon cancer risk (2). In Egypt, high Colon cancer percentage occurs in children and appearing in adults below 40 years of age. Among the main causes of increased cancer risk, the loss of biological enzymatic as result of smoking and pollution exposure (3).

Interleukin-22 (IL-22) which is produced by T cells and natural killer cells is associated with tumorigenesis and malignant progression. Not like most cytokines, which target hematopoietic cells, the main impact of IL-22 is on non-hematopoietic epithelial cells and fibroblasts in tissues as varied as lung, liver, kidney, thymus, pancreas, breast, gut, skin, and the synovium (4). The human IL-22 gene is located on the long arm of chromosome 12, on 12q15 (5). Some single nucleotide polymorphisms (SNPs) have previously been identified in the IL-22 gene locus as (−429C/T, +1995 A/C and +1046 T/A) (6, 7). Several case-control studies predominantly in European descent was done to assess the association of IL-22 SNPs in colorectal carcinoma CRC (8).

Consequently, it is important to explore the association of IL-22 polymorphisms with the colon cancer in the Egyptian population in relation to tumor risk. The process by which the IL-22 –429_rs2227485, TT genotype and T allele might be associated with colorectal cancer risk remains to be demarcated. It has been indicated that IL-22 induced inflammatory responses in colonic sub-epithelial myofibroblasts in patients with inflammatory bowel disease (9).

IL-22 plays a role in controlling tumor growing and tumor advance by multiple signaling pathways such as ERK1/2 and AKT phosphorylation (10). As the IL-22 signaling pathway including pro-survival signal cell drive, angiogenesis, and dysplasia. These functions can stop initial tumor growth, but they can also be blocked by advanced cancers to enhance tumor growth and metastasis. Thus, the IL-22/IL-22R1 axis has a dual role in cancer development.

IL-22 has circumscribed tissue specificity as its tissue specific receptors are solely expressed on epithelial and tissue cells, but not immune cells. Targeting IL-22 is important for cancer therapy. IL-22 is most clearly pro-tumor cytokine in gastrointestinal tract cancers and its level was positively correlated with staging of tumor (11). IL-22 enhances inducible nitric oxide synthase production and activity induced by interferon (IFN) in colon carcinoma cells converting it into nitrites, accompanying tumorigenic inflammation in the colon (12). It has been verified that SNP IL-22 −429C/T was linked to CRC in European descent only (8). Thus, it is probable that the IL-22 −429C/T gene polymorphisms might be linked with colon cancer in Egyptian population too.

Aim of work

Was to study the association between -429 C/T of the IL-22 gene and CRC inte r-relation to tumor staging and cancer risk.

Subjects and methods

Study population:

This study is a prospective center-based case-control study comprising 100 patients with pathologically proven CRC attending Mansoura Oncology Center from Jan. 2017 till Jan. 2019 and 100 age- and gender-matched apparently healthy controls. All participants were Egyptians from the same geographic region. Inclusion criteria were: Egyptian ethnicity, age > 25 years, and both genders are allowed. Exclusion criteria were patients with inflammatory bowel disease, familial polyposis, non-CRC-related previous bowel surgery, history of neoplastic disease other than CRC or adenoma, previous history of colorectal cancer and those previously treated with colorectal cancer with chemotherapy or immunotherapy or targeted therapy. The study was approved by IRB of Mansoura University IRB R/19.07.556 and informed consent (I.C) according to the Declaration of Helsinki was obtained from all participants. Real time-polymerase chain reaction (RT-PCR) with TaqMan probes was used to determine -429 C/T genotypes.

Patient information were retrieved from Mansoura Oncology Center data base which included, name, age, gender, smoking status, occupational and other exposure history. Also, tumor stage, grade, and histological type were recruited.

DNA extraction and genotyping

Whole blood (3–6 ml) obtained by venipuncture using standard EDTA collection tubes. DNA was extracted using the QIAGEN Gentra Puregene blood kit (QIAGEN Inc., Valencia, CA, USA). Genotypes of -429 C/T, IL-22 genes were determined by TaqMan assays. PCR was performed using a total volume of 5 µl containing 2.5 µl of Universal-Master Mix, 0.125 µl 40x Assay-by- Design mix, 0.375 µl H2O and 2 µl DNA. Reactions were overlaid with 15 µl of mineral oil. Cycling parameters were: 10 mins at 94°C for primary denaturation, followed by 40 cycles of 20 s at 92°C and 1 min at 60°C (13).

Statistical analysis

Continuous variables were analyzed by *t*-test and presented as mean ± standard deviation (SD). Categorical variables were presented as percentages and compared by chi-squared test using SPSS (SPSS Inc., Chicago, Illinois, USA) version 16 and Epi-info statistical program.

Results

Mean age of cases was (55.4) table (1). The studied sample involved 43 male and 57 female patients There was no statistically significant distinction among cases and controls concerning sex (*P*=0.570) or age (*P* =0.993) (table 1).

Lab finding of studied cases showed significantly higher CA 19-9 and significantly lower hemoglobin table (1).

As regard studying the different genotypes of IL 22 in relation to control, we found that Colon cancer cases had a considerably higher occurrence of IL-22–429 CT genotype [odds ratio (OR)= 1.903, 95% confidence interval (CI) =1.015; 3.567; P =0.045] and −429 T allele (OR =2.132, 95% CI =1.418, 3.203; P<0.001) than healthy controls. Both cases and control groups were in Hardy Weinberg (HW) equilibrium CT, TT, CT+TT genotypes, T allele were significantly associated with risk of cancer colon table (2).

When stratifying cases in relation to the tumor location, tumor size, growth pattern, we found no statistical relationship table (3). CT, TT, CT+TT genotypes and T allele were significantly associated with risk of cancer colon.

Table 1: Lab finding of studied cases.

	Groups, N		
Parameters (Unit)	**Control, 100**	**Cases, 100**	**p**
Age (years)	55.4+6.4	55.4+10.4	NS
Males/females	47/53	43/57	NS
Albumin (mg/dl)	3.8+0.2	3.6+0.6	NS
Hemoglobin (g/dl)	13.5+2.2	10.1+1.7	<0.001
CA19-9 (U/mL)	4.0+1.1	111.7+36.2	<0.001

Data are mean± S.D, NS: non-significant.

Table 2: Comparison of IL-22 -429 C/T genotypes and alleles between CRC cases and apparently healthy control group.

		Control (N=100)		**Cases (N=100)**		**p**	**OR**	**95% CI**	
-429 C/T Genotype	**CC**	47	47	27	27		**1**	**Reference**	
	CT	43	43	47	47	0.045	1.903	1.015	3.567
	TT	10	10	26	26	0.001	4.526	1.897	10.797
	CT+TT	53	53	73	73	0.004	2.398	1.328	4.329
Alleles frequency	**C**	137	68.5	101	50.5		**1**	**Reference**	
	T	63	31.5	99	49.5	<0.001	2.132	1.418	3.203
HW p		0.971		0.549					

HW: Hardy Weinberg.

Table 3: Comparison of studied groups data among IL-22 -429 C/T genotypes.

	-429 C/T Genotype, N					
Parameters	CC, 27	CT, 47	TT, 26	CT+TT, 73	*P1*	*P2*
Age (years)	54.7±10.0	56.2±10.9	54.7±10.2	55.7±10.6	0.766	0.686
Males n, %	12, 44.4%	16, 34.0%	15, 57.7%	31, 42.5%	0.146	0.859
Females n, %	15, 55.6%	31, 66.0%	11, 42.3%	42, 57.5%		
Albumin	3.5±0.6	3.7±0.6	3.7±0.7	3.7±0.6	0.436	0.208
Hemoglobin	10.4±1.6	9.8±1.6	10.4±2.0	10.0±1.8	0.219	0.340
CA 19-9	75.3±21.6	128.9±34.3	118.7±39.4	125.2±29.1	0.682	0.389
Tumor size	5.6±1.1	6.1±1.4	5.2±1.2	5.8±1.4	0.270	0.815

Data are mean ±S.D, *p*1: comparison between CC, CT, TT; *p*2: comparison between CT+TT versus CC.

Discussion

The IL-22 −429C/T gene polymorphisms might be associated with CRC in Egyptian population, but further studies are required to validate these findings.

Some researchers genotyped Seven tagging IL22 SNPs using Q PCR, in colon cancer in European descent patients and found not statistically significantly associated with colon cancer risk (8).

Others studied IL-22 −429C/T in Chinese population with CRC and found a superior frequency of IL-22–429 TT genotype and proposed an association between this gene polymorphism and CRC (14). In line to our findings, they did not find any statistical association between IL-22 SNP and CRC when stratifying results according to the tumor location, tumor size, and growth pattern or TNM stage.

At 2010, Cheryl L Thompson *et al*, in their review concluded that IL-22 SNPs are risk factors for colon cancer and encouraged more research to study IL-22 expression in the context of CRC pathogenesis (8).

Unfortunately, at 2018, a meta- analysis of the rs2227485 polymorphism that was done by Zhang *et al*, concluded that with a whole of five case–control studies involving 1020 cancer cases and 1213 controls the polymorphism was not associated with risk of cancer colon risk (15).

Our results come in agreement with (13) who studied IL- 22 -429 in patients with bladder cancer and found a significantly higher frequency of the IL-22 -429 TT genotype and -429 T allele than the healthycontrols.

Also, in a case-controlled study on patients with multifocality of papillary thyroid cancer, they found that the IL-22 -429 C/T gene polymorphism might be associated with cancer risk (13).

CRC is a multifactorial disease which is induced by the interaction of several genes with environmental factors, which were not revealed in the present case control study. As a cancer center-based case control study, so the selection bias cannot be avoidable, and the subjects may not be demonstrative of the general Egyptian population.

Recommendation

Further research involving the biological mechanisms of the association between the IL- 22 −429C/T gene polymorphism and the CRC are necessary. Finally, the small-scale sample size did not report all variations in the gene (limitation).

Conclusion

The present conclusions suggested that the IL-22 −429C/T gene polymorphism might be associated with CRC in Egyptian population. Further studies are necessary to confirm these results.

Conflict of interest

None of the contributors declared any conflict of interest.

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