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ASSOCIATION OF BLOOD GROUP A WITH COLORECTAL CANCER PREVALENCE IN CROATIAN POPULATION

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Summary

Purpose: The aim of our study was to investigate the association of ABO phenotypes and genotypes with colorectal cancer prevalence in Croatian population.

Methods: Study included 80 patients (51 men) with colorectal cancer and 303 healthy volunteer blood donors (180 men) in the control group. Using the PCR-SSP method, genotyping was performed on 5 main alleles (O1, O2, A1, A2, B), resulting in 15 ABO genotypes.

Results: There was no difference in the prevalence of colorectal cancer between men and women. There was no difference in a specific blood group phenotype between patients and healthy controls. However, there was a statistically significant higher odds ratio for individuals to carry blood group A than O in patients with colorectal cancer. There was no significant higher odds ratio for non-O genotypes compared to all genotypes constituting O blood group in patients with colorectal cancer.

Conclusion: Our study has shown that there are higher odds for blood group A in colorectal patients than O when compared to healthy controls, suggesting blood group A could be a potential risk factor for colorectal cancer. This is accordant with some previously published studies. Further studies with larger group of patients and controls are needed to confirm this observation.

KEYWORDS: *colorectal neoplasms; ABO blood-group system; blood group antigens*

INTRODUCTION

After the discovery of the ABO blood group system, which can lead to a life-threatening condition due to a mismatch between the donor and the recipient of blood, examinations of the association between the ABO system and diseases followed(1). ABO antigens are not only found on the cell surface of red blood cells but are also expressed in tissues, especially on the epithelium of the gastrointestinal tract(2). They are considered to function as adhesion and signaling molecules. It has been

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shown that in certain cancers, there is a decrease in their expression in tissues where they are generally present but increased in tissues where they are ordinarily absent(3). The first proven correlation of blood type with cancer was described in 1953 by Aird *et al*., who discovered a higher rate of stomach cancer in individuals with blood type A and lower in carriers of type O in the United Kingdom(4). Later, it was found that carriers of the A blood group are more prone to H. pylori infection than others. Observations of colorectal cancer (CRC) association with ABO blood groups are contradictory. Some researchers have found that certain ABO blood phenotypes could be associated with a higher incidence of CRC(5–9), while other researchers have found no association(10–13).

In the Croatian population, CRC is the second leading cause of death, right after cardiovascular disease. In the overall incidence of cancer, cancers of the colon, rectum, rectosigma and anus participate with 17 % in men and 12 % in women(14). The prevalence of CRC is very similar worldwide, standing as the third most common cancer in men and the second in women(15,16). Since the literature data about the association of blood groups and CRC are contradictory, and bearing in mind the burden of the CRC, we conducted a study to investigate the association of ABO blood phenotypes and genotypes with CRC in a sample of the Croatian population. This study represents the first analysis of blood group genotypes and phenotypes in the Croatian population of patients with CRC.

MATERIALS AND METHODS

The case-control study included 80 patients (51 men) with CRC and a control group of 303 (180 men) voluntary blood donors at the Croatian Institute of Transfusion Medicine (CITM), all of whom were Caucasians. This study was performed in line with the principles of the Declaration of Helsinki. Genomic DNA samples in this study originate from the Bank of Tumor and DNA from the Department of Molecular Medicine of the Ruđer Bošković Institute. These samples were collected from patients at Merkur University Hospital and were previously used for the research project on the molecular genetics of CRC in the Laboratory for Personalized Medicine. The Ethics Committee of Merkur University Hospital approved using these samples for the current research. The Ethics Committee of CITM in Zagreb approved the case-control study conduction.

ABO genotyping

Genomic DNA was isolated using QIAamp DNA Blood Mini Kit on the QIA cube device (QIAGEN, Germany). ABO genotyping was performed using the PCR-SSP method, according to Gassner *et al*., in the Department of Molecular Diagnostics of CITM(17). The method allows the differentiation of 5 main ABO alleles: A1, A2, B, O1, and O2, to distinguish 15 ABO genotypes with coamplification of a fragment of human growth hormone as an internal control of amplification.

Statistical analysis

Statistical data analysis was performed by MedCalc® Statistical Software, version 20.009(18). The normality of data distribution for all variables was assessed using the Shapiro-Wilk test. Since the data distribution was not normal, nonparametric tests were used. Descriptive data were presented by median and interquartile range (IQR). The difference between the groups for the nominal variables was examined with the Chi-squared test (or Fisher's exact test, as appropriate) and for the numerical variables with the Mann-Whitney test. Associations between different blood group phenotypes or genotypes with CRC were analyzed using the odds ratio (OR) with a 95 % confidence interval (CI). Statistical significance was set at $p < 0.05$.

RESULTS

The median age of patients with CRC and the control group was 66 years (interquartile range, IQR 59 to 73 years) and 39 years (IQR 30 to 50 years), respectively. The age difference between the two groups was statistically significant ($p < 0.001$, $Z = 12.067$). There was no statistically significant difference in the prevalence of CRC between men and women ($\chi 2 = 0.498$, $p = 0.481$).

ABO blood group phenotypes distribution of patients and control group is shown in Table 1. Comparison of proportions showed no statistically significant difference in particular ABO phenotype among patients and control group.

Table 1.

Distribution of ABO blood group phenotypes in patients and control group; comparison of proportions

ABO phenotype	Patients		Control group		*p*	χ^2	95 % CI
	n	%	n	%			
O	23	28.75	122	40.26	0.059	3.555	0.47 – 21.92
A	39	48.75	115	37.95	0.08	3.063	1.22 – 22.78
B	13	16.25	50	16.5	0.957	0.003	10.05 – 8.21
AB	5	6.25	16	5.28	0.735	0.115	3.76 – 8.79

n number, *CI* confidence interval

Table 2.

Odds ratio for colorectal cancer of non-O phenotypes compared to O phenotype

ABO phenotype	CRC patients (n)	Healthy controls (n)	Total (n)	OR for CRC compared to O phenotype	95 % CI	*p*
A	39	115	154	1.79	1.01 – 3.2	0.045
B	13	50	63	1.38	0.65 – 2.94	0.404
AB	5	16	21	1.66	0.55 – 4.97	0.367
O	23	122	145			

CRC colorectal cancer, *n* number, *OR* odds ratio, *CI* confidence interval

Table 3.

Odds ratio for colorectal cancer of non-OO genotypes compared to OO genotypes

ABO genotype		CRC patients (n)	Healthy controls (n)	Total (n)	OR for CRC compared to OO genotype	95 % CI	*p**
Non-OO	A^1A^1	3	12	15	1.33	0.35 – 5.07	0.713
	$A^{1A}2$	3	5	8	3.18	0.71 – 14.25	0.135
	O^1A^1	25	82	107	1.62	0.86 – 3.04	0.146
	O^2A^1	2	5	7	2.12	0.39 – 11.61	0.324
	O^1A^2	5	11	16	2.41	0.77 – 7.59	0.159
	O^2A^2	0	0	0	/	/	/
	A^2A^2	1	0	1	15.64	0.62 – 395.7	0.164
	O^1B	11	48	59	1.22	0.55 – 2.69	0.68
	O^2B	1	0	1	15.64	0.62 – 395.7	0.164
	BB	1	2	3	2.65	0.23 – 30.47	0.414
	A^1B	4	13	17	1.63	0.49 – 5.45	0.489
	A^2B	1	3	4	1.77	0.18 – 17.75	0.509
OO	$O^1O^1/O^1O^2/O^{2O}2$	23	122	145			

*Fisher's exact test
CRC colorectal cancer, *n* number, *OR* odds ratio, *CI* confidence interval

In the CRC patients group, the OR for non-O blood phenotypes did not show a statistically significant difference compared to the O blood phenotype (OR 0.6; 95% CI 0.35–1.02; $p = 0.061$). There was also no statistically significant difference for A phenotype compared to non-A phenotypes (OR 1.56; 95 % CI 0.95 – 2.55; $p = 0.081$); B compared to non-B (OR 0.98; 95 % CI 0.5 – 1.91; $p = 0.957$); AB compared to non-AB (OR 1.2; 95 % CI 0.42 – 3.37; $p = 0.735$).

There were statistically significantly more individuals with blood type A compared to O in CRC patients than in healthy blood donors (OR 1.79; 95 % CI 1.01 – 3.2; $p = 0.045$), Table 2.

There was no significant difference in the odds ratio for CRC in individuals with non-O genotypes compared to all genotypes constituting the O blood group (O1O1/O1O2/O2O2) (Table 3). When only individuals with A1 or A2 alleles were analyzed, compared to individuals with all geno-

types constituting the O blood group, significantly more A2 alleles were represented in the CRC group compared to the control group (p = 0.026, Fisher's exact test).

DISCUSSION

Since the first study of the multiple cancers association with ABO blood groups(4), numerous studies have proved higher risk for stomach cancer of type A carriers(19–22). Aird *et al*. also explored the connection between colon and rectal cancers and ABO blood groups and found no association(10). Thirty years later, no association of ABO blood groups with colon and rectal cancer was seen in a Norwegian retrospective study of 747 patients with CRC. Still, it was found that RhD-positive patients have a greater tendency to develop metastases in regional lymph nodes than RhD-negative patients(11).

Researchers are still looking for possible explanations of this correlation between ABO and cancers. Itzkowitz *et al*. studied the expression of ABO antigens in patients with CRC and identified three types of changes that contribute to carcinogenesis. Reexpression of ABH antigens in the distal colon (which usually occurs during fetal development) may occur, or expression in the proximal colon may be silenced, where ABO blood group antigens are commonly found in healthy individuals. Finally, the expression of carbohydrate antigens which are incompatible with the patient's ABO blood type may occur(23).

Case-control studies of CRC occurrence in relation to ABO blood groups show contradictory results in different studied populations. Urun *et al*. compared 1,620 patients with CRC in the Turkish population with the control group of voluntary blood donors. They showed that CRC is more often developed by carriers of non-O blood groups than by O blood group, with statistical significance for blood group A(8). On the other hand, a prospective study by Khalili H *et al*. conducted in the US on 1,025 patients with CRC did not confirm the association between ABO blood group phenotype and tendency to develop CRC. The authors considered the obtained statistically significant association of CRC with blood group B to be accidental and can not be explained by a clear biological mechanism(12).

A large study by Vasan *et al*. on 1.6 million voluntary blood donors from Denmark and Sweden, of whom 119,584 people have been diagnosed with cancer after more than 35 years, investigated the association of ABO blood group phenotypes with 45 specific anatomical sites. Previous studies of the association of blood group A with a higher risk of gastric and pancreatic cancer were confirmed, and a positive association of non-O blood groups was found for six more sites. For tumors of the colon and rectum, an association between the ABO blood group and the disease has not been detected(13).

A prospective cohort study in the male Chinese population concluded that individuals with the B phenotype have a lower risk of all cancers included in the study (lungs, colorectal, stomach, liver, bladder) compared to the A phenotype. Blood type B and AB had significantly lower risk of stomach cancer and CRC(5). Two studies conducted in China examined the effect of ABO blood types as a prognostic marker for patients with CRC. One study showed that the AB blood type was the best predictive factor(24), and the other resulted in the best survival of patients with O and B blood type and poor survival of patients carrying A blood type(25).

In a recent retrospective study conducted in Turkey, no relationship was found between blood type and stomach, CRC, thyroid, breast, and pancreatic cancers, but A RhD-positive blood group was more common in CRC patients(6). Kahramanca *et al*. also proved in the Turkish population that the A RhD-positive blood group is a risk factor in CRC. They found that the risk of liver metastasis was higher in blood group A(7). A recently published study from Saudi Arabia showed a higher risk for CRC in total non-O blood type carriers and AB blood type *versus* O blood type(9).

In Croatia, a study on the ABO phenotype and CRC was carried out in 2011 and showed no relationship between the ABO blood group and CRC(26). Our study, examining the association of the ABO blood group with CRC, included both phenotype and genotype of blood groups. Genotype consists of two alleles, one inherited from the father and one from the mother. Genotype provides more detailed information on a molecular basis than phenotype. For example, for the A phenotype, the genotype, in the majority of cases, consists of the combination of two of the following

alleles: A1, A2, O1, and O2. Our study showed statistically significant higher odds for blood group A (phenotype) compared to O in CRC patients (OR 1.79; 95 % CI 1.01 – 3.2; $p = 0.045$), which is in line with some previously conducted studies(5–8). Additionally, our study implies that allele A2 could be responsible for this finding. Still, we did not find any specific combination of the alleles (specific genotype) for blood group A that could be a risk factor.

A limitation of our study is the relatively small number of CRC patients included in the study. We found a significant age difference between the two groups. Still, since this study investigated the correlation of CRC with blood types, we consider the age difference irrelevant for this study because it does not influence blood groups.

CONCLUSION

Although ABO blood phenotypes and genotypes are not preventable causes of CRC, molecular findings of blood groups related to CRC could help in understanding the molecular background for developing CRC. The conclusions from our study that A blood group is a potential risk factor for developing CRC, as well as that the A2 allele could be responsible for this finding, need confirmation on a larger scale of patients. It should be considered that comparing the studies done on different ethnic populations can cause difficulties in drawing conclusions.

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Sažetak

POVEZANOST KRVNE GRUPE A S PREVALENCIJOM KOLOREKTALNOG KARCINOMA U REPUBLICI HRVATSKOJ

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Svrha: Cilj ovog istraživanja bio je ispitati povezanost fenotipa i genotipa ABO sustava krvnih grupa s prevalencijom kolorektalnog karcinoma u bolesnika u Hrvatskoj.

Metode: U studiju je uključeno 80 bolesnika (51 muškarac) s kolorektalnim karcinomom i 303 zdrava darivatelja krvi (180 muškaraca). Genotipizacija je izvršena na pet glavnih alela (O^1, O^2, A^1, A^2, B) koji rezultiraju s ukupno 15 mogućih ABO genotipova, koristeći PCR-SSP metodu.

Rezultati: Nije bilo razlike u prevalenciji kolorektalnog karcinoma između muškaraca i žena. Nije bilo razlike u fenotipu krvne grupe između bolesnika i kontrolne skupine. U grupi bolesnika pronađen je statistički značajno veći omjer izgleda za krvnu grupu A u odnosu na O. U genotipovima krvnih grupa nije bio kombinacija ne-O genotipova sa značajno većim omjerom izgleda za kolorektalni karcinom u odnosu na genotipove koji sačinjavaju fenotipsku krvnu grupu O.

Zaključak: U studiji je dokazan veći omjer izgleda za krvnu grupu A u odnosu na O u bolesnika s kolorektalnim karcinomom, što sugerira da bi krvna grupa A mogla biti potencijalni čimbenik rizika za kolorektalni karcinom. Ova povezanost je pokazana u nekoliko prethodno objavljenih studija na drugim populacijama. Potebne su opsežnije studije za potvrdu ovog opažanja.

KLJUČNE RIJEČI: *kolorektalne novotvorine; ABO sustav krvnih grupa; antigeni krvnih grupa*