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Title: Association of carotid artery disease with ABO blood groups

Running Title: Carotid artery disease and ABO blood groups

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Association of carotid artery disease with ABO blood groups

Abstract

Objective: Carotid artery disease (CAD) is a cardiovascular disease leading to cause the formation of atherosclerotic plaques in carotid arteries. Diabetes mellitus, heredity, hyperlipidemia, and hypertension are the most effective major factors for the occurrence of CAD. There is strong evidence of a relationship between these major risk factors and ABO blood groups. Therefore, this study aims to detect whether ABO blood group is associated with CAD.

Material and Methods: The study was involved 230 consecutive patients diagnosed with CAD using carotid angiography between January 2012 and November 2019, and 136 consecutive subjects without CAD for the control group. The data of this retrospective study were collected from the patient files. Demographic characteristics, lipid profiles (total cholesterol, LDL, HDL, VLDL cholesterol, and triglyceride), hematological indices (leukocyte, platelet, hemoglobin, MPV, neutrophil, lymphocyte, monocyte) data of all the samples were analyzed.

Results: Chi-square test analysis indicated that there is a statistically significant difference in the distribution of non-O blood groups and O blood groups in the patient and control groups (p=0.017). A multiple logistic regression analysis demonstrated that the risk of developing CAD in the non-O blood group was 1.92 times higher than the O blood group (p=0.032).

Conclusion: It could be proposed that the non-O blood group may be evaluated as another risk factor for CAD. However, to understand the risk of developing CAD in individuals, blood groups and other major risk factors should be considered together.

Keywords: Carotid artery disease, O blood group, cardiovascular diseases, dyslipidemia, atherosclerosis.
INTRODUCTION

Atherosclerosis is a disease characterized by the formation of plaques called atheroma in the tunica intima layer. The main lesions in atherosclerosis are atherosclerotic plaques. Although the number, size, distribution, and content of these plaques vary from person to person, are also frequently occurred the abdominal aorta, coronary arteries, and internal carotid arteries (1). Due to the formation of atherosclerotic plaques in the inner part of the carotid arteries, and narrowing of the vessel lumen, carotid artery disease can cause a decrease in blood flow to the brain and leads to stroke. Carotid artery disease often doesn't produce symptoms until severe narrowing or obstruction occurs in the carotid arteries. Some diseases and habits can pose a risk for the development of CAD. These risk factors include age, obesity, familial history, insulin resistance, and diabetes mellitus, hyperlipidemia, hypertension, and smoking (2).

Blood group antigen proteins present on the cell membrane of human red blood cells. According to the presence or absence of two antigens and two antibodies determine a person’s blood type (3). It is known that ABO phenotypes are associated with cardiovascular risk factors. For example, some studies show that B blood types of persons have a higher risk of developing hypertension (4-6). In another study, it shows that individuals of the AB blood group have more sensitivity for hypertension. It has been recommended that the B blood group in Type 2 Diabetes patients carries a high risk compared to the O blood group and that the individuals in the B blood group should be closely followed for Type 2 Diabetes (7).

There is a significant increase in the risk of developing coronary heart disease (CHD) in people with a non-0 blood group (8). Besides, in another study that attempts to evaluate the relation between ABO blood groups and coronary plaque characteristics, they found that non-0 blood groups had more severe coronary artery stenosis than the 0 blood group (9). Diabetes, hypertension, dyslipidemia the commonest cause of etiology of atherosclerotic plaques and leads to CAD. It is recognized, there is strong evidence that these risk factors may be related to the type of blood group of individuals. Accordingly, the purpose of this study is to identify
whether the occurrence of carotid artery disease is related to the blood group owned by an individual.

MATERIALS AND METHODS

Subjects

The study was carried out by the department of cardiology and the department of physiology of the medical faculty. This study was designed retrospectively and was conducted by examining the files of patients diagnosed with carotid artery disease, who applied to the Cardiology Clinic of Faculty of Medical between January 2012 and November 2019 and who were performed carotid angiography. The patient group consists of 230 consecutive cases who underwent carotid angiography and detected 30% or more carotid artery stenosis with carotid artery duplex scanning. In our study, 136 consecutive individuals whose carotid arteries were found to be normal after angiography and who did not have any other heart disease such as coronary heart disease were included in control group. The sample size was calculated as 344 using power and sample size of software (Type I error $\alpha=0.05$, effects size medium, power= 80%). Following the approval of the ethics committee (Application number: 2019/2011-KAEK-2), data of patients and controls were obtained from the university's electronic database. Demographic information such as age and gender, and clinical backgrounds such as hypertension, hyperlipidemia, diabetes mellitus, smoking, cerebrovascular events, trans ischemic attack, and blood group were gathered from patient files. Hypertension was defined as systolic blood pressure $\geq 140$ mm-Hg and diastolic blood pressure $\geq 90$ mm-Hg, or as using antihypertensive drugs. Diabetes Mellitus was accepted as a fasting blood glucose of $\geq 126$ mg/dl or as being on oral antidiabetic or insulin treatment. Hyperlipidemia was described as having a total cholesterol value of $\geq 200$ mg/dl or receiving lipid-lowering therapy.

Statistical Analysis

The data were examined by using SPSS (version 22 for Windows). The distributions of numeric variables were considered as normal if $p>0.05$ for Kolmogorov–Smirnov test. For data meeting normality and variance homogeneity assumptions mean ± standard deviations were presented.

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and pairwise comparisons were conducted with Independent Samples t tests. For the other variables which did not meet normality and variance homogeneity assumptions (i.e. age, plasma levels of Glucose, HDL, VLDL, TG, leukocyte, platelets, neutrophils, monocytes) the medians ± interquartile ranges (IQR) were presented, and for pairwise comparisons, Mann-Whitney U tests were run. For the categorical variables, i.e. gender, blood type (O and non-O), history of HT, DM, SVO/TIA, and smoking, frequencies and percentages are presented and Pearson Chi-Square Tests were used for comparisons. A Multiple Logistic Regression was run to examine whether the occurrence of CAD can be predicted by blood type (O and non-O) and adjustment variables (i.e. gender, age, history of hypertension, diabetes and SVO/TIA, and smoking). All hypothesis tests were two-tailed, 95% CI was presented for odds ratios and p<0.05 was considered statistically significant.

RESULTS

There is no statistically significant difference between patients and controls in terms of age (respectively, 68 ± 13, 67.5 ± 17.75, p=0.574) and gender (p=0.092). In the study, 68.3% of the patients were male, 31.17% were female, 59.5% of the controls were male and 40.5% were female (Table 1.). As shown in Table 1, people with hypertension, diabetes, smoking, cerebrovascular history or temporary ischemic attack, coronary artery disease; compared to the control group, it is at a very high prevalence in the patient group. While fasting glucose, LDL, VLDL, triglyceride, and total cholesterol values were higher in the patient group, HDL value was lower. Differences in values other than total cholesterol showed a statistically significant difference. According to the results of complete blood count, platelet amount was lower in the patient group; the amount of hemoglobin and monocyte was higher and, these differences were statistically significant (p<0.05).

In the patient group, 50% of participants had blood type of A, 19.6% had B, 24.3% had O, and 6.1% had AB, while 44.9% had A, 13.2% had B, 36% had O, and 5.9% had AB in control. A Pearson Chi-Square test revealed that the distribution of O and non-O blood groups significantly differed between patients and controls (p=0.017, Table 2.), indicating that the risk of carotid artery disease in the non-O blood group was found to be 1.75 times higher than the
O blood group. Multiple logistic regression was run to test whether the association between CAD (i.e. patient or control) and blood type (i.e. O and non-O) was still significant even when adjustment variables (i.e. gender, age, history of hypertension, diabetes, and SVO/TIA, and smoking) were controlled. Findings revealed that predictors in the model explained the 48% of the variance (Nagelkerke R Square) and the model fit to the data (χ2Hosmer&Lemeshow (8)= 5.474, p=0.706). Beta coefficients indicated that all predictors, except age and history of smoking, were significantly related to the occurrence of CAD (Table 3). Odds ratios revealed that the risk of CAD for individuals with a history of hypertension, diabetes, SVO/TIA was 5.35, 3.98, and 4.53 times higher than individuals with no such histories, respectively. CAD risk for males was 2.02 times higher than for females. More importantly, the risk was 1.92 times higher among individuals with non-O blood type, compared to the ones with O blood type, indicating that blood type was still a significant predictor even after controlling the relations of other predictors with CAD.

Table 4 shows the expected and seen stenosis rates according to the blood groups of the patients diagnosed with the carotid artery. In the table prepared, firstly, the expected stenosis rates for blood groups were determined using the Chi-square test in line with the model that included other parameters included in the study, and the statistical program. Stenosis percentages are divided into 3 groups as 50 % and below, between 50-70 %, and above 70 %. When the number of cases seen and expected in stenosis percentages is compared in the O blood group, we would like to point on your attention to the fact that the number of cases seen is less than expected (p<0.05). The lipid profiles and hematological indexes were also compared between the O blood group and non-O blood group to determine the relationship between blood group and carotid artery disease (Table 5). There were no statistical differences in any parameters evaluated between the two groups.

DISCUSSION

Atherosclerosis is the most common cause of CAD. After the atherosclerotic process, the carotid arteries narrow and stenosis occurs. Cranial thromboembolisms are caused by these stenoses in the carotid artery leading to mortality and ischemic stroke. Various treatment
options have been developed to prevent or control such a situation, which can lead to such dramatic results. However, since this process caused ischemic strokes despite medical treatment, it directed the scientific world to alternative treatment methods (10). With the discovery of ABO blood groups in 1901, studies to explain the relation between blood groups and different diseases have continued until today without interruption (11). Many studies have been conducted showing that the ABO blood group type related the risk of developing in various diseases such as cardiovascular disease, hematologic disorders, metabolic diseases, cancer, cognitive disorders, and SARS-CoV-2 infection (12-14). In some population studies, non-O blood groups have been identified as potential risk factors for the occurrence of cardiovascular diseases (11). Among these risk factors, atherosclerosis, dyslipidemia, hypertension, and diabetes mellitus constitute the etiology of carotid artery disease (10). Although researchers have been working on evaluating the relationship between ABO blood groups and various diseases for years, no study to define the relationship between the ABO group and CAD could be found in the literature review. That's why the present study is original and valuable in terms of its contribution to the literature. That's why the present study is original and valuable with respect to data and context.

However, there are studies to explain the relation between CHD and ABO blood groups (15, 16, 17). and we know that coronary heart disease has a similar etiology with carotid artery disease. It was emphasized in one study that the individuals in the Non-O blood group had more severe coronary artery stenosis and that the Non-O blood group could pose a risk for CHD (9). Besides, coronary heart disease was found to be less common among people with an O blood group (15). Compared to the O blood group, A, B, or AB blood groups have an increased risk of coronary heart disease by 5%, 11%, and 23%, respectively (18). The findings obtained in our study appear to be compatible with the literature.

A, B, O blood group antigens as a potential independent risk factor for atherosclerotic plaque formation. At the same time thrombotic events, high systolic and diastolic blood pressure, and dyslipidemia could explain the relation between ABO blood groups and atherosclerosis (18). Some researchers have found that high plasma levels of Von Willebrand Factor (VWF) and
Factor VIII which are coagulation factors are interrelated with the clinic prevalence rate of thrombotic events (19). Additionally, various studies have shown that VWF is high in patients with AB blood group and this upward trend is associated with thrombus formation (20). Supporting the studies outlined above in similar studies, they reported that VWF levels were lower in those with O blood group than in non-O groups (21, 22). Another study, to support the studies summarized above, VWF levels were reported to be lower in those with O blood group than the non-O blood group. Researchers who aimed to answer a question as to why the non-O groups have high VWF plasma levels found that the plasma half-life of VWF was shorter in the O blood group than other blood groups (23).

Biswas et al. evaluated the distribution of the AB0 blood group in coronary artery patients in India in their study. According to the results of their studies, they have pointed out that individuals in the AB blood group have a higher risk of developing cardiovascular disease than other blood groups. They linked this finding to HDL cholesterol which was lower in 0 blood group, but the higher concentration in the AB blood group (24). Several studies have addressed the positive correlation between individuals with CHD with non-O blood groups and high serum cholesterol. In particular, they found that this positive correlation was stronger than individuals with an O blood group (25, 11). However, there wasn’t a statistically significant difference found to be between lipid profiles and the O blood group and non-O blood group in patients with carotid artery disease in our study.

There is also a contradiction in studies to associate blood groups with Type 2 Diabetes Mellitus is another important risk factor of CAD. Although it has been reported that the seen frequency of Type 2 Diabetes Mellitus is more common in some blood groups, an exact blood group has not been reached due to conflicting information in the literature (18). When evaluated our results, there was a significant accumulation in the group with carotid artery disease in terms of diabetes and hypertension compared to the controls. According to the results of our study, 0 blood group, which we think has a protective effect on CAD, reduces the percentage of stenosis in carotid artery patients to 70% and below. Huang et al. studied to evaluate the relation between ABO blood groups and coronary plaque characteristics. As a result of this study, they found
that non-0 blood groups showed more severe coronary artery stenosis than the 0 blood group (9). This finding supports the low percentage of stenosis in individuals in O blood group with CAD in the study.

CONCLUSION

According to the findings we obtained from our study, Non-O blood group increased the risk of developing CAD by 1.92 when the risk factors were controlled. Furthermore, in the Non-O blood group, the percentage of stenosis was higher in the carotid arteries. On the other hand, this result also strengthened the possibility of 0 blood group could be a protective factor in carotid artery disease. Racial and ethnic distribution of blood groups and sample size are important factors in predicting the risk of atherosclerosis. However, understanding the patient's risk, the type of blood groups should be considered in conjunction with other risk factors. The data from this study can be beneficial for health planners and it can help deal with future regional health problems. Briefly, building a database of blood groups and associated diseases not only provides data on the availability of human blood in case of regional disasters but also serves to learn about the possibilities of the future burden of disease.

Limitations of the study

Although the type of blood groups important factor in predicting the risk of atherosclerosis, to understand the patient's risk, the blood groups should be considered in conjunction with other risk factors. According to the power analysis, our sample size was sufficient, but accessing a larger sample would have enabled us to obtain healthier results. The fact that the study was conducted in a single-center constitutes the limitation of this study.

Ethics

Ethics Committee Approval: University Faculty of Medicine, Ethics Committee, Application number: 2019/2011-KAEK-2.
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**Table Legends:**

**Table 1.** Demographics and Clinical Features of the Patient and Control Groups

a Results were presented in the table as median ± interquartile range (IQR). Mann–Whitney U test was used for statistical analyses. p<0.05 was considered statistically significant.

b Results were presented in the table as n (%). Pearson Chi-Square Tests were used for statistical analyses. p <0.05 was considered statistically significant.

c Results were presented in the table as mean ± standard deviation. Independent Samples t test was used for statistical analyses. p <0.05 was considered statistically significant.

HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein, VLDL: Very Low-Density Lipoprotein, MPV: Mean Platelet Volume, SVO: Cerebrovascular Event, TIA: Transient Ischemic Attack.

**Table 2.** Distribution of O and non-O blood groups across patient and control groups

a Results were presented in the table as n (%). Pearson Chi-Square Tests was run for comparing patient and control groups in terms of their blood types (O vs. Non-O). p<0.05 was considered statistically significant.

**Table 3.** Multiple Logistic Regression of CAD on blood type and adjustment variables

**Table 4.** Distribution of percentage of stenosis according to blood groups

**Table 5.** Clinical variables between O and non-O blood groups in patients with carotid artery disease

a Results were presented in the table as median ± interquartile range (IQR). Mann–Whitney U test was used for statistical analyses. P<0.05 was considered statistically significant.
Results were presented in the table as mean ± standard deviation. Independent Samples t test was used for statistical analyses. P<0.05 was considered statistically significant.

HDL: High-Density Lipoprotein, LDL: Low-Density Lipoprotein, VLDL: Very Low-Density Lipoprotein, MPV: Mean Platelet Volume.

Table 1. Demographics and Clinical Features of the Patient and Control Groups

|                        | Patient (n=230) | Control (n=136) | p     |
|------------------------|----------------|-----------------|-------|
| Age (year)             | 68 ± 13        | 67.5±17.75      | 0.574 |
| Gender                 |                |                 |       |
| Female/male            | 73/157         | 55/81 (40.5/59.5)| 0.092 |
| History of             |                |                 |       |
| Hypertension           | 173 (76.5)     | 34 (25)         | <0.001 |
| Diabetes               | 101 (43.9)     | 15 (13.2)       | <0.001 |
| SVO/TIA                | 28 (12.1)      | 3 (2.2)         | <0.001 |
| Smokers                | 159 (69.1)     | 33 (24.2)       | 0.001 |
| Plasma                 |                |                 |       |
| Glucose (mg/dl)        | 109.9 ± 56.5   | 96.9 ± 18.9     | <0.001 |
| HDL (mg/dl)            | 36.2 ± 16.65   | 41.8 ± 17.15    | 0.005 |
| LDL (mg/dl)            | 110.16 ± 36.48 | 96.06 ± 25.34   | 0.002 |
| VLDL (mg/dl)           | 27.58 ± 16.78  | 22.3 ± 13.67    | 0.045 |
| Triglycerides (mg/dl)  | 135.25 ± 84.13 | 106.05 ± 61.05  | 0.009 |
Table 2. Distribution of O and non-O blood groups across patient and control groups

| Blood Group | Patient n (%) | Control n (%) | p       |
|-------------|---------------|---------------|---------|

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Results were presented in the table as n (%). Pearson Chi-Square Tests was run for comparing patient and control groups in terms of their blood types (O vs. Non-O). p<0.05 was considered statistically significant.

Table 3. Multiple Logistic Regression of CAD on blood type and adjustment variables

|                          | Beta | p     | Odds Ratio | 95% CI for Odds Ratio |
|--------------------------|------|-------|------------|-----------------------|
| Blood Type (O and non-O) | 0.654| 0.032 | 1.923      | 1.058                 |
| Gender                   | 0.704| 0.023 | 2.022      | 1.101                 |
| Age                      | 0.008| 0.536 | 1.008      | 0.983                 |
| Hypertension             | 1.677| 0.000 | 5.350      | 2.986                 |
| Diabetes                 | 1.381| 0.000 | 3.981      | 1.981                 |
| SVO/TIA                  | 1.511| 0.000 | 4.532      | 2.600                 |
| Smokers                  | 1.266| 0.065 | 3.547      | 0.924                 |
Table 4. Distribution of percentage of stenosis according to blood groups

| Blood Group | Lower than 50% | 50% -70% | Over 70% | Total |
|-------------|---------------|----------|----------|-------|
| O Count     | 20            | 26       | 10       | 56    |
| Expected count | 12.7        | 20.5     | 22.9     | 56    |
| A Count     | 8             | 41       | 66       | 115   |
| Expected count | 26         | 42       | 47       | 115   |
| B Count     | 16            | 14       | 15       | 45    |
| Expected count | 10.2       | 16.4     | 18.4     | 45    |
| AB Count    | 8             | 3        | 3        | 14    |
| Expected count | 3.2         | 5.1      | 5.7      | 14    |
Table 5. Clinical variables between O and non-O blood groups in patients with carotid artery disease

| Variable                  | O            | Non-O        | p      |
|---------------------------|--------------|--------------|--------|
| Age (year)                | 67 ± 14      | 69 ± 13      | 0.281<sup>a</sup> |
| Plasma                    |              |              |        |
| Glucose (mg/dl)           | 111.7 ± 93.85| 107 ± 48     | 0.323<sup>a</sup> |
| HDL (mg/dl)               | 33.2 ± 17.55 | 37.4 ± 16.8  | 0.359<sup>a</sup> |
| LDL (mg/dl)               | 110.75 ± 35.14 | 109.96 ± 37.04 | 0.891<sup>b</sup> |
| VLDL (mg/dl)              | 26.66 ± 18.82 | 26.92 ± 16.56 | 0.505<sup>a</sup> |
| Triglycerides (mg/dl)     | 133.3 ± 93.85 | 19.6 ± 82.65 | 0.371<sup>a</sup> |
| Total Cholesterol (mg/dl) | 165.73 ± 36.81 | 168.9 ± 42.1 | 0.625<sup>b</sup> |
| Leukocyte (10<sup>3</sup>/µl) | 7.85 ± 3.125 | 8.1 ± 3.28 | 0.568<sup>a</sup> |
Platelets ($10^3/\mu l$) & 229 ± 99 & 228 ± 80.5 & 0.907\textsuperscript{a}  \\
Hemoglobin (g/dl) & 13.1 ± 2.21 & 13.14 ± 1.89 & 0.907\textsuperscript{b}  \\
MPV (FL) & 9.65 ± 1.27 & 9.47 ± 1.44 & 0.411\textsuperscript{b}  \\
Neutrophils (%) & 5.43 ± 3.35 & 5.14 ± 2.97 & 0.845\textsuperscript{a}  \\
Lymphocytes (%) & 1.99 ± 0.97 & 1.87 ± 0.74 & 0.425\textsuperscript{b}  \\
Monocytes ($10^3/\mu l$) & 0.56 ± 0.33 & 0.6 ± 0.24 & 0.501\textsuperscript{a}  \\

\textsuperscript{a} Results were presented in the table as median ± interquartile range (IQR). Mann-Whitney U test was used for statistical analyses. P<0.05 was considered statistically significant.

\textsuperscript{b} Results were presented in the table as mean ± standard deviation. Independent Samples t test was used for statistical analyses. P<0.05 was considered statistically significant.

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