

Distribution of ABO Blood Groups in Iraqi Samples of Leukemia and Lymphomas

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Abstract :

The records of Hematological Diseases Unit at Baghdad Teaching Hospitals and National Center for Research and Treatment of Hematological Diseases (Al-Mustansiriyah University) were inspected for leukemia and lymphoma patients who were diagnosed and treated during the period 2008-2010, and their ABO blood groups were also obtained. The patients were distributed as 281 ALL, 128 CLL, 192 AML, 208 CML, 114 HDL (Hodgkin's lymphoma) and 197 NHL (non-Hodgkin's lymphoma). In addition, 595 blood donors were also included and considered as controls.

Testing the goodness of fit for ABO blood group allele and phenotype frequencies showed a good agreement with Hardy-Weinberg equilibrium (HWE) in controls and in ALL, CLL and HDL patients. In contrast, a significant corrected deviation was observed in CML ($P_c = 0.04$) and NHL ($P_c = 2.3 \times 10^{-6}$) patients. A further group of patients (CLL) also showed a significant deviation from HWE, but the difference was significant before correction ($P = 0.05$; $P_c = 0.20$). A further analysis of ABO blood group alleles revealed that their estimated numbers and frequencies varied between patients and controls, but a significant difference was recorded in CLL, CML and NHL patients.

The allele I*A was significantly decreased in CLL (32.0 vs. 41.5%) and CML (28.8 vs. 41.5%) patients as compared with controls, but a corrected significant level was only observed in CML patients ($P_c = 0.003$). In NHL patients, the allele I*B was significantly decreased and the difference remain significant after correction (25.9 vs. 34.1; $P_c = 0.03$). These findings suggest a role of blood group phenotypes and alleles in the etiology of hematological malignancies.

Keywords: ABO blood groups, Acute lymphoblastic leukemia, Chronic lymphocytic leukemia; Acute myeloid leukemia, Chronic myeloid leukemia, Hodgkin's lymphoma, Non-Hodgkin's lymphoma

Introduction:

The ABO blood group is the most important blood group system in transfusion medicine, and by employing classical serological agglutination test, it is possible to classify human populations into four main blood group phenotypes; A, B, AB and O, which are controlled by a single gene locus on chromosome 9 that has three alleles; I*A, I*B and i (1).

The distribution of these phenotypes and alleles is race-dependent, and the three main races (Caucasians, Orientals and Negros) have shown different frequencies in their populations (2).

Furthermore, associations between ABO blood groups and certain diseases have been described. These include the association between blood groups O and A individuals

with increased incidence of duodenal ulcers gastric carcinoma, respectively, as well as, aberrant expression of ABO antigens has been reported in pre-malignant and in malignant cells (3,4). The studies also suggested that ABO blood groups could be served as an epidemiological marker or a primary screening aid to identify populations at high-risk for certain hematological malignancies (5).

The etiology of leukemias and lymphomas is still largely unknown. The known risk factors (ionizing radiation and benzene exposure for leukemias; immunosuppression, HIV infection and pesticide exposure for non-Hodgkin's lymphomas) explain only a small proportion of the cases that occur in the world (6).

Therefore, studying ABO blood group distributions may be helpful in formulating new etiologic hypotheses, especially if such distribution is evaluated in the ground of Hardy-Weinberg equilibrium (HWE). Based on such theme, the HWE profile of ABO blood groups was examined in groups of leukemia (acute lymphoblastic leukemia; ALL, chronic lymphocytic leukemia; CLL, acute myeloid leukemia; AML and chronic myeloid leukemia; CML) and lymphoma (Hodgkin's; HDL and non-Hodgkin's; NHL

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lymphoma) Iraqi patients.

Material and Methods:

The records of Hematological Diseases Unit at Baghdad Teaching Hospitals and National Center for Research and Treatment of Hematological Diseases (Al-Mustansiriyah University) were inspected for leukemia and lymphoma patients who were diagnosed and treated during the period 2008-2010, and their ABO blood groups were obtained. The patients were distributed as 281 ALL (115 males and 166 females), 128 CLL (49 males and 79 females), 192 AML (82 males and 110 females), 208 CML (93 males and 115 females), 114 HDL (47 males and 67 females) and 197 NHL (102 males and 95 females). In addition, 595 blood donors (296 males and 299 females) were also included and considered as controls.

Blood groups for each sample of patients and controls were presented as observed and expected frequencies, as well as, allele frequencies of these blood groups were also estimated by the maximum likelihood method. After these estimations, the goodness of fit for allele and phenotype frequencies and agreement with HWE for each sample was inspected. A significant difference was assessed by Pearson's X² test, and the probability (P) was corrected (Bonferroni correction) for the number of blood group phenotypes (P_c).

These calculations were made using the computer package S2 ABOestimator. After that, the number of ABO al-

leles was estimated from the gene frequencies using the formula: Expected number = $2 \times N \times P \times (1-P) + (N \times P^2)$; in which P represents the allele frequency. Then, a comparison between patients and controls was made using the estimated number of ABO alleles. Significant differences were assessed by Fisher's exact test, and the P was also corrected for the number of alleles. In addition, relative risk (RR), preventive fraction (PF) and 95% confidence intervals (C.I.) were also estimated. These calculations were carried out using the computer package PEPI version 4 (7,8).

Results:

Testing the goodness of fit for ABO blood group allele and phenotype frequencies showed a good agreement with HWE in controls, as well as in ALL, CLL and HDL patients, in which no significant difference was observed between the observed and expected frequencies of ABO blood group phenotypes (Tables 1, 2, 3 and 6). In contrast, a significant corrected deviation was observed in CML (Pearson's X² = 6.320; D.F. = 1; P = 0.01; P_c = 0.04) and NHL (Pearson's X² = 25.020; D.F. = 1; P = 5.7 x 10⁻⁷; P_c = 2.3 x 10⁻⁶) patients (Tables 5 and 7); an observation that may suggest that HWE was disturbed in the two groups of patients.

A further group of patients (CLL) also showed a significant deviation from HWE, but the corrected P was not significant (Pearson's X² = 3.847; D.F. = 1; P = 0.05; P_c = 0.20) as shown in table 4.

Table 1: Distribution of ABO blood groups in healthy controls.

| Blood Group | Observed | | Expected | | Allele Frequency | |
|-------------|----------|-------|----------|-------|------------------|-------|
| | No. | % | No. | % | | |
| A | 186 | 31.3 | 194.1 | 32.6 | | |
| B | 142 | 23.8 | 150.2 | 25.2 | I*A | 0.235 |
| AB | 62 | 10.4 | 52.6 | 8.9 | I*B | 0.188 |
| O | 205 | 34.5 | 198.1 | 33.3 | i | 0.577 |
| Total | 595 | 100.0 | 595 | 100.0 | | 1.000 |

Pearson's X² = 2.717; D.F. = 1; P = 0.10 (Not significant)

Table 2: Distribution of ABO blood groups in acute lymphoblastic leukemia.

| Blood Group | Observed | | Expected | | Allele Frequency | |
|-------------|----------|-------|----------|-------|------------------|-------|
| | No. | % | No. | % | | |
| A | 83 | 29.5 | 81.5 | 29.0 | | |
| B | 74 | 26.3 | 72.5 | 25.8 | I*A | 0.203 |
| AB | 19 | 6.8 | 20.7 | 7.4 | I*B | 0.182 |
| O | 105 | 37.4 | 106.3 | 37.8 | i | 0.615 |
| Total | 281 | 100.0 | 281 | 100.0 | | 1.000 |

Pearson's X² = 0.227; D.F. = 1; P = 0.634 (Not significant)

Table 3: Distribution of ABO blood groups in chronic lymphocytic leukemia.

| Blood Group | Observed | | Expected | | Allele Frequency | |
|-------------|----------|-------|----------|-------|------------------|-------|
| | No. | % | No. | % | | |
| A | 34 | 26.6 | 35.5 | 27.7 | | |
| B | 35 | 27.3 | 36.4 | 28.4 | <i>I*A</i> | 0.199 |
| AB | 12 | 9.4 | 10.4 | 8.2 | <i>I*B</i> | 0.203 |
| O | 47 | 36.7 | 45.7 | 35.7 | <i>i</i> | 0.598 |
| Total | 128 | 100.0 | 128 | 100.0 | 1.000 | |

Pearson's $\chi^2 = 0.415$; D.F. = 1; $P = 0.52$ (Not significant)

Table 4: Distribution of ABO blood groups in acute myeloid leukemia.

| Blood Group | Observed | | Expected | | Allele Frequency | |
|-------------|----------|-------|----------|-------|------------------|-------|
| | No. | % | No. | % | | |
| A | 50 | 26.1 | 55.3 | 28.8 | | |
| B | 46 | 23.9 | 51.4 | 26.8 | <i>I*A</i> | 0.204 |
| AB | 21 | 10.9 | 14.9 | 7.8 | <i>I*B</i> | 0.191 |
| O | 75 | 39.1 | 70.4 | 36.6 | <i>i</i> | 0.605 |
| Total | 192 | 100.0 | 192 | 100.0 | 1.000 | |

Pearson's $\chi^2 = 3.847$; D.F. = 1; $P = 0.05$; $P_c = 0.20$ (Not significant)

Table 5: Distribution of ABO blood groups in chronic myeloid leukemia.

| Blood Group | Observed | | Expected | | Allele Frequency | |
|-------------|----------|-------|----------|-------|------------------|-------|
| | No. | % | No. | % | | |
| A | 39 | 18.8 | 45.9 | 22.1 | | |
| B | 60 | 28.8 | 66.7 | 32.0 | <i>I*A</i> | 0.157 |
| AB | 22 | 10.6 | 14.3 | 6.9 | <i>I*B</i> | 0.219 |
| O | 87 | 41.8 | 81.1 | 39.0 | <i>i</i> | 0.624 |
| Total | 208 | 100.0 | 208 | 100.0 | 1.000 | |

Pearson's $\chi^2 = 6.320$; D.F. = 1; $P = 0.01$; $P_c = 0.04$ (Significant)

Table 6: Distribution of ABO blood groups in Hodgkin's lymphoma.

| Blood Group | Observed | | Expected | | Allele Frequency | |
|-------------|----------|-------|----------|-------|------------------|-------|
| | No. | % | No. | % | | |
| A | 33 | 28.9 | 32.4 | 28.4 | | |
| B | 35 | 30.7 | 34.4 | 30.2 | <i>I*A</i> | 0.211 |
| AB | 10 | 8.8 | 10.7 | 9.4 | <i>I*B</i> | 0.223 |
| O | 36 | 31.6 | 36.5 | 32.0 | <i>i</i> | 0.566 |
| Total | 114 | 100.0 | 114 | 100.0 | 1.000 | |

Pearson's $\chi^2 = 0.080$; D.F. = 1; $P = 0.78$ (Not significant)

Table 7: Distribution of ABO blood groups in non-Hodgkin's lymphoma.

| Blood Group | Observed | | Expected | | Allele Frequency | |
|-------------|----------|-------|----------|-------|------------------|-------|
| | No. | % | No. | % | | |
| A | 49 | 24.9 | 61.2 | 31.1 | | |
| B | 27 | 13.7 | 39.6 | 20.1 | <i>I*A</i> | 0.205 |
| AB | 25 | 12.7 | 11.2 | 5.7 | <i>I*B</i> | 0.138 |
| O | 96 | 48.7 | 85.0 | 43.1 | <i>i</i> | 0.657 |
| Total | 197 | 100.0 | 197 | 100.0 | | 1.000 |

Pearson's $\chi^2 = 25.020$; D.F. = 1; $P = 5.7 \times 10^{-7}$; $P_c = 2.3 \times 10^{-6}$ (Significant)

A further analysis of ABO blood group alleles revealed that their estimated numbers and frequencies varied between patients (leukemia and lymphoma) and controls, but a significant difference was recorded in CLL, CML and NHL patients. The allele *I*A* was significantly decreased in CLL (32.0 vs. 41.5%) and CML (28.8 vs. 41.5%) patients as compared with controls, but a corrected significant

level was only observed in CML patients ($P_c = 0.003$). In NHL patients, the allele *I*B* was significantly decreased and the difference remain significant after correction (25.9 vs. 34.1; $P_c = 0.03$). The three variations were associated with PF values of 0.14, 0.18 and 0.11, respectively (Tables 8 and 9).

Table 8: Expected number of ABO blood group allele in leukemia and lymphoma patients and controls.

| Groups | No. | ABO Blood Group Alleles | | | | | |
|------------------------------------|-----|-------------------------|-------------|------------|-------------|----------|------|
| | | <i>I*A</i> | | <i>I*B</i> | | <i>i</i> | |
| | | No. | % | No. | % | No. | % |
| Controls | 595 | 247 | 41.5 | 203 | 34.1 | 489 | 82.2 |
| Acute lymphoblastic leukemia (ALL) | 281 | 103 | 36.7 | 93 | 33.1 | 239 | 85.1 |
| Chronic lymphocytic leukemia (CLL) | 128 | 41 | 32.0 | 47 | 36.7 | 107 | 83.6 |
| Acute myeloid leukemia (AML) | 192 | 70 | 36.5 | 66 | 34.4 | 162 | 84.4 |
| Chronic myeloid leukemia (CML) | 208 | 60 | 28.8 | 81 | 38.9 | 179 | 86.1 |
| Hodgkin's lymphoma (HDL) | 114 | 43 | 37.7 | 45 | 39.5 | 93 | 81.6 |
| Non-Hodgkin's lymphoma (NHL) | 197 | 73 | 37.1 | 51 | 25.9 | 174 | 88.3 |

Table 9: Estimated ABO blood group alleles showing significant variation between patients (leukemia and lymphoma) and controls.

| Type of Comparison | Allele | RR | PF | P | Pc | 95% C.I. |
|--------------------|------------|------|------|-------|-------|-------------|
| CLL vs. Controls | <i>I*A</i> | 0.66 | 0.14 | 0.029 | N.S. | 0.431-1.012 |
| CML vs. Controls | <i>I*A</i> | 0.57 | 0.18 | 0.001 | 0.003 | 0.399-0.813 |
| NHL vs. Controls | <i>I*B</i> | 0.67 | 0.11 | 0.01 | 0.03 | 0.460-0.979 |

Discussion:

In the present study, departures from HWE that can be created by sampling of individuals on the basis of the presence of a disease phenotype (leukemia and lymphoma) were examined, and they were present in two samples of leukemias (AML and CML) and one sample of lymphomas (NHL). These departures from HWE could be created because the selection criterion was based on presumed disease-susceptibility ABO blood group phenotypes, rather than on independently selected alleles, because alleles within phenotypes that confer greater susceptibilities or protection are represented in the sample at disproportionately high rates. Therefore, disequilibrium is expected to be greatest at the disease-susceptibility locus itself, since this is the factor that determines the selection criterion. Loci that are phenotypically neutral but are somehow associated with the disease-susceptibility locus, such as genetic markers in linkage disequilibrium with the disease-susceptibility locus, also experience disproportionate phenotype selection (9).

In testing gene-disease associations using a case-control design, the controls are expected to comply with HWE, because they are randomly selected from a general population, and it is assumed that the population is in equilibrium and it is not disturbed by external factors (10). In agreement with such concept, the distribution of blood group phenotypes and alleles in the present control sample showed no departure from HWE. In addition, other samples of patients (ALL, CLL and HDL) shared theme of controls; an observation that may suggest that either ABO blood group phenotypes had no etiological effect on ALL, CLL and HDL, or such phenotypes were not disturbed by the presented diseases. In contrast, AML, CML and NHL patients were significantly deviated from HWE, and ABO blood group phenotypes may serve as a risk epidemiological factor for these groups of leukemia and lymphoma, and such genetic risk prediction studies may represent the next step toward implementation of these genetic markers in clinical practice and, therefore, a higher level of alertness might be helpful (11,12).

For a further understanding of ABO blood groups role in the etiology of leukemias and lymphoma, the ABO alleles were estimated and comparisons between the six groups of patients and controls were made. Such comparisons

demonstrated that I*A allele was significantly decreased in CLL and CML patients, while NHL patients showed a significantly decreased frequency of I*B allele. Such findings may suggest the protective effect of these alleles against the development of these diseases. Reviewing the literature revealed that various studies have reported conflicting results in the distribution of blood groups among leukemias. Nagy and colleagues (13) showed an increase in the proportion of O blood group among female patients with acute leukemia and pointed to a similar finding from the previously published data by Mustacchi and colleagues (14). Steinberg reported in a study of 450 acute leukemia patients that the distribution was not significantly different from that of the general population (15). Shirley and Desai reviewed several previously published data and found no statistically significant difference in the distribution of A blood group with respect to O blood group in patients with acute leukemia when compared with the respective controls of each study reviewed (16). Jackson and colleagues reported a decrease in the proportion of O blood group among female patients with acute leukemias in the northeastern Malay Peninsula. Their results were not statistically significant when the data was analyzed after the reclassification of acute leukemia patients into two groups; ALL and AML (17). These conflicting findings can be explained in the ground of ethnic differences between the studied populations. Equally important, ABO alleles may represent immunogenetic predisposing factors for leukemias and lymphomas, and such predisposition require an environmental trigger, which may differ from population to other or may favors a specific ABO allele for interaction to precipitate the disease (18).

In conclusion, ABO blood groups are still fruitful research strategy in understanding the etiology of hematological malignancies, especially if they are evaluated in the ground of recent molecular typing of such genetic polymorphism. Such suggestion has been recently challenged by Novaretti et al. (19) who demonstrated that ABO molecular genotyping in leukemia patients reveals new ABO variant alleles, and elucidation of the diversity of these alleles in leukemia and in other diseases is important for the determination of the effect of changes in an amino acid residue on the specificity and activity of ABO glycosyltransferases and their function.

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توزيع مجاميع الدم (ABO) في عينات عراقية من ابيضاض الدم واللمفوما

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الخلاصة:

تم التحري من سجلات وحدة أمراض الدم في مستشفى بغداد التعليمي والمركز الوطني لبحوث وعلاج أمراض الدم (الجامعة المستنصرية) عن مرضى ابيضاض الدم واللمفوما والذين تم تشخيصهم وعلاجهم للفترة 2008-2010، كما تم الحصول على الطرز المظهرية لمجاميع الدم (ABO) لهؤلاء المرضى. توزع المرضى كالتالي: 281 ابيضاض الدم اللمفي الحاد (ALL) و128 ابيضاض الدم اللمفي المزمن (CLL) و192 ابيضاض الدم النقياي الحاد (AML) و208 ابيضاض الدم النقياي المزمن (CML) و114 هوجكن لمفوما (HDL) و197 لاهوجكن لمفوما (NHL)، فضلا عن ذلك فقد شملت الدراسة 595 شخصا من متبرعي الدم وعدوا كسيطرة. أظهر اختبار Goodness of Fit للطرز المظهرية وأليات مجاميع الدم تطابقا مع توازن هاردي واينبرغ في السيطرة ومرضى ALL وCLL وHDL، وعلى العكس من ذلك فقد لوحظ أن هنالك انحرافا معنويا مصححا في مرضى CML ($P_c = 0.04$) وNHL ($P_c = 2.3 \times 10^{-6}$). كما أظهر مرضى CLL انحرافا معنويا عن توازن هاردي واينبرغ ولكن كان الاختلاف معنويا قبل التصحيح ($P = 0.05$, $P_c = 0.20$). وفي تحليل آخر، أظهرت الأعداد والتكرارات المحسوبة لأليات الطرز المظهرية لمجاميع الدم (ABO) تغييرا بين المرضى والسيطرة ولكن سجل فرقا معنويا في مرضى CLL وCML وNHL. حيث أظهر الأليل I*A انخفاضا معنويا في مرضى CLL (مقابل 41.5%) ومرضى CML (مقابل 28.8%) بالمقارنة مع السيطرة، وعند تصحيح الاحتمالية كان الفرق معنويا فقط في مرضى CML ($P_c = 0.003$). أما بالنسبة للأليل I*B، فقد أظهر مرضى NHL انخفاضا معنويا وبقي كذلك حتى بعد تصحيحه (25.9% مقابل 43.1%; $P_c = 0.03$). تقترح هذه النتائج بأن للطرز المظهرية لمجاميع الدم وألياتها دورا في إحداث أمراض الدم الخبيثة.