Original article

Frequency of ABO and RhD Blood Groups in Patients with Celiac Disease

Khadija Alzagalie, Alaa Almabrouk, Mariam Elahjal∗ ⬤

Department of Medical Laboratory Sciences, Faculty of Medical Technology, University of Tripoli, Libya

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Corresponding Email. m.elahjal@uot.edu.ly

ABSTRACT

Celiac disease (CD) is a popular autoimmune disorder induced by gluten-containing foods and other environmental, and genetic factors. Every individual's red blood cell expresses over two million ABO blood-type antigens. Furthermore, the stomach and small intestine are major expression sites for the ABH antigens. This study was intended to assess the distribution of ABO and Rh blood types in Libyan patients with celiac disease as well as compare between CD and healthy control groups regarding complete blood count (CBC) parameters. Samples from 250 CD patients and 45 healthy control groups represented by 80 males (27.1%) and 215 females (72.9%) were enrolled in the study. The samples of CD were collected from patients who attended the gastroenterology outpatient clinic at Tripoli University Hospital (TUH) for follow-up. Two hundred and fifty blood samples were serologically screened for ABO, and Rh antigens using a tube agglutination test. Another group of healthy subjects (n = 45) and CD patients (n = 45) were analyzed for CBC test. The findings showed that the most observed ABO blood group among celiac patients was blood group O 129 (51.6%), followed by blood group A 80 (32%), and Rh-positive 222 (88.8%). Furthermore, the heritable proportion was 33%, with 21% classified as first-degree and 12% as second-degree hereditary. Additionally, the result of the independent Sample (T) test to compare RBCs, HGB, HCT, and NUT levels in blood between CD patients and healthy control showed that there were significant differences in the RBCs, HGB, and HCT counts with p-value = 0.034, <0.001, and <0.001 respectively. In contrast, Mann-Whitney U test results revealed significant differences in the PLT, MCV, MCH, and MCHC counts with p-value = <0.001, and for LYM with p-value = 0.003. Future studies on these simple inflammatory markers can guide us in predicting the diagnosis and prognosis.

INTRODUCTION

Celiac disease (CD) is a popular autoimmune disorder induced by gluten-containing foods and other environmental factors [1]. Chronic inflammation of the mucosa and submucosa of the small intestine is one of its most distinctive features [2,3]. Most research suggests that CD results from the interplay between genetic, environmental, and immune factors, or from the intestinal mucosal barrier, intestinal flora, and other factors [4].

The pathogenicity of CD seems to be substantially attributed to genetic vulnerability, particularly to class II genes correlated to the human leukocyte antigen (HLA) [5].

In recent years, the incidence rate of CD has increased globally; it has an estimated prevalence of approximately 1–2% of the population worldwide [3,6]. A systematic review exploring the epidemiology of celiac disease in Arab nations revealed that the highest incidence among the general population (3.2%) was reported in Saudi Arabia and the lowest (0.1%) was reported in Tunisia. The peak age at diagnosis fell between (1 –3) and (9 – 10) years old [5]. The disease can arise at any age, both during childhood and adolescence, and is also relatively common in adulthood [2].

 Clinical, immunological, and genetic research on CD show that despite small bowel villous atrophy, the disease may be subclinical or atypical without symptoms of malabsorption. Therefore, there are considerable delays in diagnosis, and many cases are not recognized [7,8]. The classic clinical presentation of celiac disease is characterized by abdominal symptoms, malnutrition, and impaired growth in childhood, and many patients exhibit only mild or no symptoms at all [3,9]. The only treatment for celiac disease is a life-long, strict gluten-free diet that leads to improvements in quality of life [10].

In addition to enteropathy, endomysium, and tissue transglutaminase antibodies are additional manifestations of CD. Patients with untreated CD almost constantly exhibit these antibodies. The targets of CD-specific antibodies, gliadin peptides, and tissue transglutaminase 2, have essential functions in CD and may contribute to disease progression [1]. Celiac disease tends to run in families, it does not follow a specific inheritance pattern. First-degree relatives of CD patients share genetic and environmental risk factors for CD. Therefore, they are at the most risk of developing the disease. The frequency of CD in these individuals is 10 to 20 times higher than in the general population [11]. Second-degree relatives also appear to have an increased prevalence [2].

 Every individual's red blood cell (RBC) expresses over two million ABO blood type antigens which is considered the predominant human blood group system [12]. The blood groups are defined by the presence of specific carbohydrate sugars on the surface of RBCs: N-acetylgalactosamine for the A antigen and D-galactose for the B antigen. The foundation of each of these sugars is the H antigen which is responsible for dividing blood into four main types: O, A, B, and AB [12]. the incidence of ABO groups varies markedly in different races, and, ethnic groups in different parts of the world [8]. Furthermore, the stomach and small intestine are major expression sites for the ABH antigens [4]. Currently, one of the major genetic variables affecting the gut flora is thought to be the *FUT2* gene. The *FUT2* gene encodes a key enzyme for the regulation of epithelial fucosylation and the production of secretory fucosylated ABO (H) histo-blood group antigens. Many research investigations have demonstrated an association between the probability of developing CD and the lack of *FUT2* [13].

The Rh blood system is more complicated than the ABO blood system and comprises up to 54 independent antigens [14]. Of all the Rhesus antigens (D, C, c, E, and e), the D antigen is the most immunogenic, major determinant, and clinically substantial of all the Rhesus antigens [15].

Research carried out in Libya to determine the association between ABO, Rh, and celiac disease in Libyan children showed that the most observed ABO blood group among coeliac patients was blood group O (54.8%), followed by blood group A (38.7%), and Rh-negative was 9.7% in celiac. Furthermore, it showed no firm relationship between ABO, Rh, and Coeliac disease [16]. The study's findings, which were intended to assess the distribution of ABO and Rh blood types in Iranian patients with celiac disease, demonstrated that blood group O is the most frequent among celiac patients [17].

On the contrary, many diseases outside the digestive system, such as autoimmune thyroid disease, type 1 diabetes mellitus, iron deficiency anemia, and several types of cancer, are closely associated with CD [9]. Type 1 diabetes mellitus (T1DM) and celiac disease (CD) are two of the most recognized related autoimmune disorders, as there are genetic and environmental factors that can increase the risk of developing diabetes, as well as certain drugs that trigger the specific destruction of beta cells. The condition is typically diagnosed in children or young adults [18]. Patients with type 1 diabetes mellitus (IDDM) have a prevalence of CD ranging from 3% to 8% [2].

Regarding autoimmune thyroiditis and CD. Several investigations proved that, like the Sweden study, the prevalence of thyroid disease among CD patients was 10.8%. Similarly, a Dutch study found that of 184 patients with CD, 39 (21%) were positive for thyroid antibodies [3]. Moreover, both Italian and Finnish data suggest an increased prevalence of hypothyroidism in individuals with CD [9]. However, this association remains controversial. This study aimed to assess the distribution of ABO and RhD blood types in Libyan patients with celiac disease as well as compare CD and healthy control groups regarding CBC parameters.

METHODS

Study area and design

This single-center study adopted an observational design. The present study was conducted at the Gastroenterology Department at TUH. This clinical study was conducted for four months; from the beginning of December 2023 to the end of April 2024.

A simple random sample of those patients with known celiac disease based on clinical manifestations, positive serological test (Anti tissue transglutaminase IgG, and IgA), or histopathology test and were on a gluten-free diet (GFD) included in this study to determine the ABO Blood groups and Rh factor. Patients with a medical history of chronic malignant tumors, chronic Inflammation, any infection or a history of cardiovascular disease were excluded. Participants were subjected to a brief interview by which data was collected, by the aid of A purpose -designed questionnaire which was used to collect some basic and clinical information. All aspects of the study protocol have been reviewed and authorized by the local ethics committee.

Sampling techniques and laboratory investigation

After verbal consent from celiac patients, 250 blood samples were collected from all candidates in tubes containing ethylenediaminetetraacetic acid (EDTA) tube and subjected to ABO, and RhD blood type testing by using direct Hemoagglutination tube techniques. Another group of healthy controls (n = 45) and CD patients (n = 45) were analyzed for CBC test. Blood samples were taken from the patients and then placed in the Sysmex Xp300 device to measure the CBC analysis, and it was serviced according to the manufacturer's instructions.

Data analysis

Data from the present study were gathered and statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software version 27 software (IBM SPSS Inc, Chicago, IL, USA). The Shapiro-Wilk test was used to evaluate the normal distribution of the data, and some data were found to be normally distributed while the other data were not. Proportions were created to establish the distribution of blood group analysis in the study participants. Descriptive statistics were used to describe the demographic characteristics of participants. Percentages (%) and frequencies (n) were used for categorical variables such as gender, while means and standard deviations were calculated for continuous variables such as age.

For the comparison between CBC count among CD patients and healthy control group, a statistical procedure was implemented to compare the means/means of two independent groups (CD patients and healthy control group) to determine if there was a significant difference between them. Independent T-test was employed for normal distributed data while the Mann-Whitney test was done for not normal distributed data. A p-value of 0.05 and below was considered statistically significant.

RESULTS

Demographic data

Two hundred and fifty samples were collected to analyze their ABO and RhD blood groups among CD patients; another group of healthy controls (n = 45) and a group of CD patients (n = 45) were analyzed for CBC tests. Both genders were included, with ages ranging from 1-63 years. the number of females was 215 (72.9%), and the number of males was 80 (27.1%). Of the 295 respondents in this study, 36.61% fell into the category of 20 to 39 years old (Adult), and 31.86% were middle-aged adults (40 to 59) the least one was infants (0-1 years old).

Data analysis and interpretation

Figure (1) shows that most of the patients were from blood group O 129 (51.6%) and blood group A 80 (32%), respectively. Additionally, figure 2 illustrated that 222 patients (88.8%) were RhD+.

Furthermore, out of 250 patients, 112 (44.8%) had blood group O+ and 74 (29.6%) had blood group A+, followed by B+ and O- blood groups with 25 (10%) and 17 (6.8%), respectively. While the less common blood group was AB- with 2 (0.8%). The prevalence of CD observed among first-degree relatives (FDRs) of patients with CD was 53 (21%) higher than that of second-degree relatives 31 (12%). Additionally, the current study observed that various autoimmune diseases associated with CD, more specifically, type-1 diabetes mellitus, were the most prevalent AD among celiac patients 32 (12.8%), followed by Hypothyroidism and Asthma with 7(2.8%) and 6 (2.4%) respectively. A Combination of Diabetic type 1 and hypothyroidism was observed in 2 (0.8%) of celiac patients.
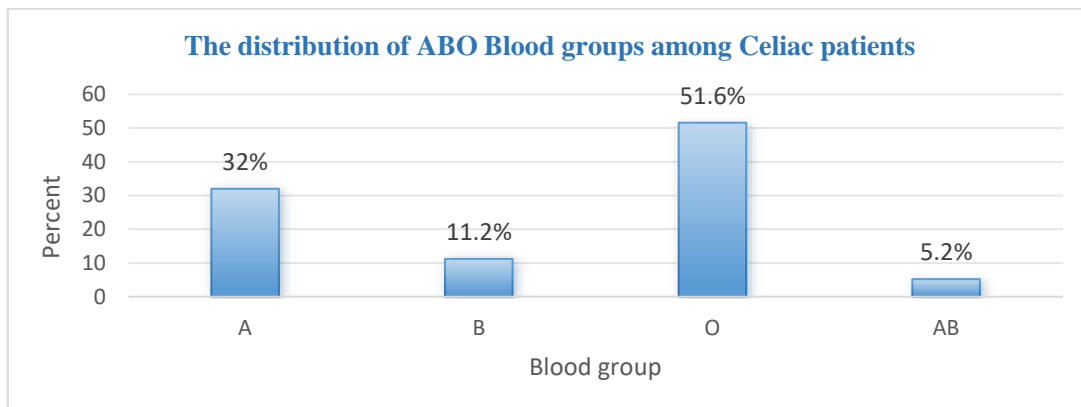
Figure 1. The distribution of ABO blood groups among CD patients.
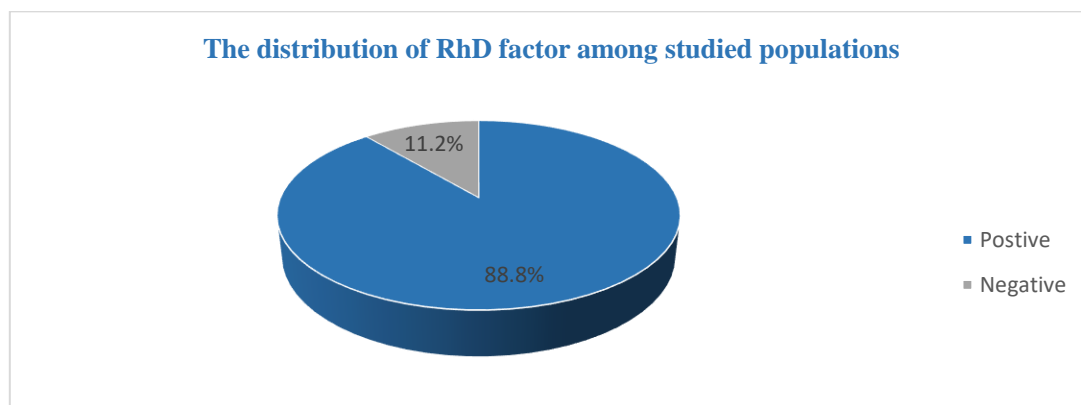
Figure 2. The distribution of RhD blood types among studied patients.

Comparison between Celiac patients and a healthy control group about CBC parameter levels
For study cases, table (1) shows the result of an independent sample (T) test that was performed to compare red blood cells (RBCs), hemoglobin level (HGB), hematocrit (HCT), neutrophil (NUT) cell levels in blood between CD patients and healthy controls. The data were expressed as Mean ± SD which revealed that there were significant differences in the RBCs, HGB, and HCT counts with p-value = 0.034, <0.001, and <0.001, respectively. On the other hand, there was no significant difference between the NUT count in blood among CD patients and controls (p- p-value = 0.963) (table 1).

Table 1. The average of some CBC parameters for study cases

CBC parameters	State	N	Mean SD	t	p-value
RBC	Control	45	4.58±0.68	2.154	0.034
	CD	45	4.31±0.50		
HGB	Control	45	12.90±1.86	4.779	<0.001
	CD	45	10.83±2.21		
HCT	Control	45	41.98±6.16	6.859	<0.001
	CD	45	33.83±5.05		
NUT	Control	45	52.18±10.28	0.046	0.963
	CD	45	52.05±14.56		

Mann-Whitney A U test was performed to compare white blood cells (WBC), platelets (PLT), The average size of RBCs (MCV), the average percentage of hemoglobin in RBCs (MCH), the amount of hemoglobin present in red blood cells (MCHC), and lymphocytes (LYM) cells in blood between CD patients and healthy controls. The data revealed that there were significant differences in the PLT, MCV, MCH, and MCHC counts with (p-value = <0.001) and for LYM (p-value

= 0.003). On the other hand, there were no significant differences between the WBC count in blood among CD patients and the control p-value = 0.787 (Table 2).

Table 2. The average of some CBC parameters for study cases.

CBC parameters	State	N	Mean Rank	Mann-Whitney U	P-value
WBC	Control	45	44.76	979	0.787
	CD	45	46.24		
PLT	Control	45	36.18	593	<0.001
	CD	45	54.82		
MCV	Control	45	61.87	276	<0.001
	CD	45	29.13		
MCH	Control	45	54.92	588.5	<0.001
	CD	45	36.08		
MCHC	Control	45	34.02	496	<0.001
	CD	45	56.98		
LYM	Control	45	37.39	647.5	0.003
	CD	45	53.61		

DISCUSSION

In our study, random samples were collected from cases registered in the celiac disease registry system at (TUH). Patients were categorized by gender, age, blood groups, and diagnostic techniques used in disease diagnosis. The relationship between blood groups and disease susceptibility., including many autoimmune disorders, malignancies, heart diseases, stomach ulcers, and diabetes, has been studied with various results [18]. The results of a recent study that focused on the distribution of ABO blood groups among 250 CD patients revealed that 129 patients had blood type O, accounting for 51.6%, followed by A blood group 80 (32%). Our findings are consistent with an Iranian study that showed a similar order: group O is the most common, followed by groups A with 71 (35.7%) and 69 (34.7%), respectively. Another agreement with a study conducted in Libya was that blood group O (54.8%) was the most observed, followed by blood group A (38.7%). Regarding RhD, the result obtained from this research was 88.8% of RhD positive, while in Iranian and Libyan studies, it was 58.9% and 85.2%, respectively [16,17]. Moreover, out of 250 patients, 112 (44.8%) had blood group O+ and 74 (29.6%) had blood group A+, followed by B+ and O- blood groups with 25 (10%) and 17 (6.8%), respectively. Furthermore, the least common blood group was AB- with 2 (0.8%).

According to many worldwide studies, CD tends to cluster in families: parents, siblings, or children (first-degree relatives) of people with CD have a higher risk of developing it. The current study, which was conducted to determine the hereditary nature of celiac disease, showed that the non-heritable proportion was 67% and the heritable proportion was 33%, with 21% classified as first-degree hereditary and 12% as second-degree hereditary. In contrast, a study conducted in North India and Brazil showed that the incidence of CD among first-degree relatives was 8.2% and 4.8%, respectively [19,20]. However, the inheritance pattern is unknown.

Celiac disease is more common in people with Type 1 diabetes and autoimmune thyroid disease. Many studies suggest that when the diagnosis of CD is delayed, the chance of developing other autoimmune disorders is increased. The findings of the current study observed that various autoimmune diseases (AD) associated with CD, more specifically, type-1 diabetes mellitus, were the most prevalent AD (12.8%), followed by hypothyroidism (7.2%). A combination of diabetes type 1 and hypothyroidism was observed in only (0.8%). Various research outlined the prevalence of CD was noted to be 1% to 19% in patients with type 1 diabetes mellitus and 2% to 5% in autoimmune thyroid disorders [21]. Numerous screening studies conducted around the world showed an increased prevalence of CD (2.4%–16.4%) in patients with DMT1 [22]. The CD has been found at an increased rate in patients with autoimmune thyroid disease (Grave's disease and Hashimoto's thyroiditis), with a prevalence ranging from 2% to 7% [23].

In the current study, we evaluated changes in hematologic indices, focusing on the rate of WBC, RBC, PLT, HGB, HCT, MCV, MCH, MCHC, NUT, and LYM in diagnosed CD patients and healthy individuals as controls. As previously shown in Table 1, our study revealed that there were significant differences in the RBC, HGB, and HCT counts between CD patients and controls, with p-values = 0.034, <0.001, and <0.001, respectively. The current results showed that there was a significant difference in RBC counts between CD patients and controls, with a p-value of 0.0001. Regarding hemoglobin level, our findings are consistent with study was conducted in Pakistan which also reported high statistical significance for the difference in HGB level between CD patients and the control group with p-values = 0.01 [22].

According to the findings of our study, the HTC comparison was identical to the Pakistani study, with a p-value <0.001 [22]. On the other hand, Neutrophils and lymphocytes are the cells that play a major role in inflammatory processes. Moreover, neutrophils and lymphocyte counts temporarily change in inflammation. Our study observed there was no significant difference between the NUT count in blood among CD patients and controls (p-value = 0.963).

Our data revealed that there were significant differences in the PLT, MCV, MCH, and MCHC counts between CD patients and controls (p-value = <0.001), which were in line with Pakistani research (MCV p-value = 0.009), MCH p-value (0.034), and MCHC p-value (0.056) [22]. Regarding PLT, our study results (p-value <0.001) are consistent with the findings reported by Purnak *et al*. There were significant differences in the PLT between CD patients and controls with a p-value = 0.001 in Ankara and another agreement with another Turkish study with a p-value = 0.001 [24,25]. Regarding LYM, our results showed that there was a significant difference between celiac patients and control groups, with a p-value = 0.003. Furthermore, our study results are consistent with the findings of the Ankara study, where the WBC (p-value = 0.245) was compared to our study, whose p-value = 0.787 (Table 4), indicating that there were no significant differences between the WBC count in blood among CD patients and the control group [24].

CONCLUSION
According to our findings, blood group O is the most frequent among celiac patients. Additionally, regarding CBC indices, results comparing CBC indices between CD patients and healthy controls showed that there were significant differences in most parameters with a p-value <0.05. Hematologic indices routinely measured by CBC, which is a simple, widely available, and cheap tool, could be useful in selecting patients with a high likelihood of CD. Future studies on these simple inflammatory markers can guide us in predicting the diagnosis and prognosis.

Conflicts of Interest
Declare conflicts of interest or state "The authors declare no conflicts of interest." Authors must identify and declare any personal circumstances or interests that may be perceived as inappropriately influencing the representation or interpretation of reported research results.

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توزيع فصائل الدم ABO و RhD في المرضى المصابون بحساسية القمح

خديجة الزقلعي ، الاء المبروك ، مريم الاحجل

قسم علوم المختبرات الطبية، جامعة طرابلس، طرابلس، ليبيا

المستخلص

مرض حساسية القمح هو واحد من اضطرابات المناعة الذاتية الشائعة الناجمة عن تناول الأطعمة التي تحتوي على الغلوتين وغيرها من العوامل البيئية والوراثية. تحتوي خلية الدم الحمراء لكل فرد أكثر من مليوني مستضد من مستضدات فصيلة الدم ABO. علاوة على ذلك، تعد المعدة والأمعاء الدقيقة مواقع رئيسية تحتوي على مستضدات ABH. تهدف هذه الدراسة إلى تقييم توزيع فصائل الدم ABOو RhD في المرضى الليبيين المصابين بمرض حساسية القمح وكذلك المقارنة بين مجموعة من المصابين بالمرض ومجموعة من الاصحاء فيما يتعلق بمعايير تعداد الدم الكاملCBC. شملت الدراسة عينات من 250 مصاباً بالمرض و45 مجموعة من الاصحاء ممثلة بــ 80 ذكراً (27.1%) و215 أنثى (72.9%). تم جمع عينات الدم من المرضى الذين حضروا العيادة الخارجية لأمراض الجهاز الهضمي في مستشفى طرابلس الجامعي (TUH) للمتابعة. تم فحص مائتين وخمسين عينة دم مصلياً بحثاً عن مستضدات ABO وRh باستخدام اختبار التراص الأنبوبي. تم تحليل مجموعة أخرى من الأصحاء (ن = 45) ومجموعة من المرضى (ن = 45) لاختبار CBC. النتائج: أظهرت النتائج أن فصيلة الدم ABOالأكثر شيوعا بين المرضى المصابين بحساسية القمح كانت فصيلة الدم ، (51.6%) 129 O، تليها فصيلة الدم (32%) A 80، وفيما يتعلق بالعامل الريزيسي الايجابيRhD كانت 222 (88.8%). علاوة على ذلك، بلغت نسبة الوراثة 33%، منها 21% مصنفة على أنها من الدرجة الأولى و12% على أنها وراثية من الدرجة الثانية. بالإضافة إلى ذلك، أظهرت نتيجة اختبار العينة المستقل (T) لمقارنة مستويات كرات الدم الحمراء و HGB و HCT و NUT في الدم بين مرضى حساسية القمح والاصحاء أن هناك اختلافات ذات دلالة إحصائية في عدد كرات الدم الحمراء و HGB و HCT مع قيمة p = 0.034 و <0.001 و <0.001 على التوالي. في المقابل، كشفت نتائج اختبار Mann-Whitney Uعن وجود اختلافات ذات دلالة إحصائية في تعداد PLTو MCV و MCHوMCHC بقيمة p = 0.001>،وبالنسبة لـــ LYM بقيمة p = 0.003. وفقا لنتائج الدراسة الحالية ، فإن فصيلة الدم O هي الأكثر شيوعا بين المرضى المصابين بحساسية القمح. فيما يتعلق بمؤشرات الدم التي تم قياسها بواسطة CBC ، كانت هناك اختلافات ذات دلالة إحصائية في معظم المؤشرات مع قيمة p> 0.05 يوصى بإجراء دراسات مستقبلية حول هذه العلامات الالتهابية البسيطة والتي يمكن أن ترشدنا في التنبؤ بالتشخيص والتشخيص.

الكلمات الدالة. مرض الاضطرابات الهضمية، عامل RhD، فصائل الدم ABO، تعداد الدم الكامل (CBC).