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Original article

Molecular Characterization of Human Leukocyte Antigen Haplotypes in Coeliac Disease: Evidence from Libyan Patients

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Abstract
Coeliac disease (CD) is a complex condition characterized by chronic inflammation of the small intestine, triggered by gluten ingestion in genetically predisposed individuals. This study aimed to understand the genetic factors influencing CD and to evaluate the role of the Human Leukocyte Antigen (HLA) alleles in the different clinical manifestations of coeliac patients. The study was conducted between 2015 and 2019, involving 24 patients previously diagnosed serologically and histopathologically with CD, aged 1-45 years. Blood samples were collected, and extracted DNA was amplified using the AllSet+™ Gold SSP Kit. Screening for HLA class I (A, B, C) and class II (DQB, DRB) loci. The study showed the presence of multiple alleles at the DQB1 and DRB1 loci, which are well-established markers for CD susceptibility. Only three DQB1 alleles were detected among the tested coeliac patients, with 87.5% carrying the HLA-DQ2 (HLA-DQB1*02) haplotype. Whereas, for DRB1 loci 50% of the patients carried HLA-DRB1*03. The study also found that only three HLA-DRB3 alleles were detected, with HLA-DRB3*01 as the dominant allele (79.1%). The HLA-DQ2 is the most common HLA allele in Libya plays a significant role in CD. The HLA-DQ2 haplotype, a combination of HLA-DQB1*02, accompanied by HLA-DRB1*03 and HLA-DRB3*01, is the genetic risk factor for Libyan CD cases. The high prevalence of the HLA-DQB1*02 allele indicates a need for increased awareness and screening.
Keywords. Libya, Coeliac Disease, HLA-DQ2, HLA-DQ8.

Introduction

Coeliac disease (CD) is a complex condition characterized by chronic inflammation of the small intestine, primarily triggered by gluten ingestion in genetically predisposed individuals. It is a systemic autoimmune condition and is characterized by the presence of histological damage in the small-bowel mucosa and serum antibodies targeted against transglutaminase 2 (TG2-Abs) and endomysium (EmA) [1]. The symptoms may include abdominal pain, bloating, diarrhea, fatigue, and deficiencies in essential nutrients, such as iron, calcium, and various vitamins. The etiology of CD is regarded as multifactorial, in which Human Leukocyte Antigen (HLA) genes, the microbiome, gluten, and other environmental factors are involved in the development of the disease [2].

The development of CD is significantly influenced by genetic factors, particularly HLA alleles. The primary genetic risk factors associated with CD are HLA-DQ2 and HLA-DQ8, which account for a substantial portion of the disease's heritability [3]. A study published by Aboulaghras *et al.* (2023) showed that nearly 90% of individuals diagnosed with CD exhibit either DQ2 or DQ8 haplotypes, whereas only about 30% of the general population carries these risk alleles [2]. In addition to genetic risk factors, environmental factors also influence disease predisposition.

One of the most extensively studied environmental exposures is infections, which are often believed to trigger the onset of diseases. Furthermore, the commensal intestinal microbiota is known to play a crucial role in the maturation and functioning of the intestinal immune system [4]. The individuals who are diagnosed with CD are at increased risk for serious health complications, such as osteoporosis, infertility, neurological disorders, and certain cancers [5]. Therefore, advancements in molecular characterization techniques have significantly improved researchers' ability to identify and analyze genetic markers. This progress provides a solid foundation for understanding the genetic inheritance of CD [6]. A recent study suggests that CD may be more common than previously believed, particularly in non-Western populations. This underscores the urgent need for enhanced diagnostic protocols that accommodate diverse clinical presentations [6]. The purpose of this study was to determine the HLA allele structure associated with coeliac disease among selected Libyan patients.

Methods

Study design and setting

A genetic survey was conducted as part of a cross-sectional study from 2017 to 2019. The study involved 24 patients, aged 1–45 years, who had been previously diagnosed serologically and histopathologically with CD. This research was conducted at Al-Jala Hospital, Tripoli, Libya, and at the University of Tripoli Hospital (UTH), Tripoli, Libya. Laboratory testing was conducted in the Department of Medical Microbiology & Immunology, Faculty of Medicine-University of Tripoli.

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Samples and data collection
Blood samples were collected and immediately transported to the microbiology laboratories at the University of Tripoli. A volume of 5–10 mL of whole blood was drawn from participants and stored in a deep freezer at -60°C until further analysis. A questionnaire was used to collect demographic information (such as age, gender, duration of the disease, and family history) and clinical information (such as clinical symptoms of CD and the patients' serological and histological results).

DNA extraction
The extraction of DNA was performed according to the manufacturer's instructions using PureLink Quick PCR Purification kit (Invitrogen, Life Technologies, Ltd., Germany). After which, the extracted DNA was stored at -20ºC until it was required for PCR reactions.

SSP-PCR amplification for HLA typing
The AllSet+™ Gold SSP Kit (Invitrogen, Life Technologies, Ltd., Germany) was used to screen extracted DNA for HLA typing. This method employs formulations of allele- or group-specific primer sets to amplify genomic DNA using a 96-well thermal tray. In brief, the master mix was prepared by mixing 460 µl of PCR buffer (including dNTPs and gel loading buffer), 608 µl of water, 125 µl of DNA (50 ng/µl), and 7 µl of Taq DNA polymerase (5 units/µl) (Invitrogen, Life Technologies, Ltd., Paisley, UK). 10 µl of the master mix was added to each well. The tray was covered with a seal and closed tightly. The amplification of the targets was performed using an Eppendorf thermal cycler (Mastercycler; Helena Biosciences, Gateshead, UK) under the following conditions: an initial denaturation at 96°C for 1 minute, followed by five cycles consisting of denaturation at 96°C for 25 seconds, annealing at 70°C for 50 seconds, and extension at 72°C for 45 seconds. This was followed by 21 cycles of denaturation at 96°C for 25 seconds, annealing at 65°C for 50 seconds, and extension at 72°C for 45 seconds. Finally, four cycles of denaturation at 96°C for 25 seconds, annealing at 55°C for 60 seconds, and extension at 72°C for 2 minutes were performed, with a hold at 4°C.

Detection of PCR products
The PCR products were detected using agarose gel electrophoresis. The gels were prepared as follows: 2% (w/v) agarose gels were prepared by mixing 3g agarose powder (Invitrogen, Life Technologies, Ltd., German) with 150ml of 1x Tris-borate-EDTA (TBE) (Sigma Dorset, UK), and 15µl (0.5 µg/mL) of SYBRsafeTM DNA gel stain (Invitrogen, Germany). 5µl of loading buffer [70% (w/v) sterile injectable water (Phoenix Pharmaceuticals, Gloucester, UK), 29.5% (w/v) Glycerol (Sigma) and 0.5% (w/v) Bromophenol Blue (Bio-Rad, Hertfordshire, UK)] was added to 5µl of each PCR product and to 1.5µl of 2000bp molecular weight DNA marker (Invitrogen, life technologies, UK). Both the DNA molecular marker and each PCR product were loaded onto the 2% agarose gel and run at between 100 and 150 volts for about half an hour. Then, the gel was viewed under UV transillumination (Gel Doc 1000, Bio-Rad Laboratories). A photo of the gel was taken under UV illumination, and the results were documented by identifying the positive lanes.

Documentation and interpretation
After the completion of the electrophoresis, the gel was viewed under UV transillumination (Gel Doc 1000, Bio-Rad Laboratories), photographed, and the results were documented using the gel documentation form to identify the positive lanes. The analysis of allele distribution for HLA-DRB1 and HLA-DQ1 loci was performed in accordance with the guidelines provided by the manufacturer using Invitrogen AllSet+ HLA typing software (Invitrogen, Life Technologies, Ltd., Germany).

Ethical approval: The research proposal received ethical approval and authorization from the Faculty of Medicine, and Informed consent from adult participants and parents of children with CD was obtained, and they subsequently filled out a validated pre-test questionnaire.

Results
All 24 patients included in this study had a confirmed diagnosis of CD. They were randomly selected from those screened using the coeliac patient questionnaire and were between the ages of 1 and 45 years. For the HLA class I and II alleles characterized in the present study, the number of alleles for each targeted gene ranged between 3 to 6 alleles. Of the tested patients, a high percentage carried specific alleles: HLA-A*02 was found in 17 (71%) of patients, and HLA-C*12 was found in 14 (58%).
Regarding HLA class II (HLA-DQB1), interestingly, only three alleles (HLA-DQB1*02, HLA-DQB1*03, and HLA-DQB1*08) were detected among the tested coeliac patients; 21 (87.5%) carried the HLA-DQ2 (HLA-DQB1*02) alleles and 2 (8.3%) patients carried HLA-DQ8 (HLA-DQB1*03) alleles, and one patient (4.1%) had HLA-DQB1*08. Similarly, only three HLA-DRB3 (HLA-DRB3*01, HLA-DRB3*02, and HLA-DRB3*03) were detected, with HLADRB3*01 being the dominant allele and accounting for 19 (79%) of the tested patients (Table 1).

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Table 1. CD predisposing HLA-ABCDRDQ in 24 CD patients.

Sample no.	Low Resolution HLA -ABCDRDQ					
	A	B	C	DQB1	DRB1	DRB3
1	02	18	02	02	03	01
2	02	18	12	08	03	01
3	02	51	12	03	14	01
4	02	18	12	02	03	02
5	02	18	12	02	14	01
6	02	18	12	02	14	01
7	02	51	12	02	03	01
8	02	08	02	02	07	03
9	02	14	3	02	07	01
10	34	51	12	02	14	01
11	34	08	12	02	03	01
12	32	08	6	02	03	02
13	34	51	7	02	07	02
14	32	14	3	03	14	01
15	02	39	6	02	03	01
16	02	08	12	02	03	01
17	02	39	12	02	14	01
18	32	42	12	02	14	01
19	02	08	12	02	03	01
20	02	51	02	02	03	01
21	02	08	02	02	03	01
22	02	14	12	02	07	01
23	32	14	3	02	07	01
24	02	08	12	02	03	03

Discussion

Allelic profiles of 24 Libyan patients were analyzed, focusing on HLA class I (A, B, C) and class II (DQB1, DRB1, DRB3) loci to evaluate their genetic predisposition to coeliac disease. Key observations include the presence of multiple alleles at the DQB1 and DRB1 loci, which are well-established markers for CD susceptibility. The dataset showed allelic variability consistent with a heterogeneous genetic background. Notably, alleles corresponding to the DQB1 allelic group are associated with the formation of the HLA-DQ2 and HLA-DQ8 heterodimers. These heterodimers are critical in the pathogenesis of CD due to their role in presenting gluten peptides to T cells. The presence of these alleles aligns with global genetic profiles observed in CD patients. These genes were recognized with specific alleles encoding DQ2.5, DQ8 and DQ2.2 (HLA-DQA1*05, HLA-DQB1*02; HLADQA1*03, HLA-DQB1*03:02 and HLA-DQA1*02, HLA-DQB1*02), respectively [7]. The European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) in 2012 was proposed the above-described characteristics as diagnostic criteria for children and adolescents with coeliac patients [8]. Profiles detected in this study were compatible with other studies, which found 90-95% of CD patients carried the HLA-DQ2 (DQB1*02:01) haplotype, specifically the DQ2.5 (DQA1*05-DQB1*02:01) haplotype. Nearly all of the remaining 10% of patients have HLA-DQ8 (DQA1*03- DQB1*03:02) is permissive for CD [9].

In this cohort, 21/24 tested patients carried HLA-DQ2 or HLA-DQ8 haplotypes, the DQB1*02 (HLA-DQ2) allele was found in 21 patients (87.5%), the DQB1*03 (HLA-DQ8) allele was present in two patients (8.3%), and one patient carried the DQB1*08 allele (4.1%). A national study by Alarida *et al.* (2010) reported similar prevalence rates for HLA-DQ2 and HLA-DQ8 among coeliac patients, 80.6% and 12.9%, respectively [10]. Therefore, the HLA-DQ2 and HLA-DQ8 genes showed a significant genetic predisposition in Libyan coeliac patients. In North Africa, the prevalence of the HLA-DQ2 gene among coeliac patients was reported to be 67.4% in Morocco, 65.9% in Algeria, and 79.7% in Tunisia. Meanwhile, the rates of the HLA-DQ8 gene were 16.5% in Morocco, 22.73% in Algeria, and 8.1% in Tunisia [11–13]. Consistent with the previous research across North African populations, this study's results underscore a significant genetic link between the HLA-DQ2 and HLA-DQ8 alleles and an increased predisposition to CD in the Libyan population.

The study demonstrated the presence of the HLA-DRB3*01 allele in 19 (79.2%) coeliac patients. Of these, 16 (66.7%) possessed HLA-DQB1*02 in linkage with HLA-DRB3*01 alleles. Alshiekh *et al.* (2017) reported high risk of CD was associated with the presence of HLA-DQB1*02.5 and HLA-DRB3*01 among coeliac patients of Scandinavian ethnicity [14]. Therefore, suggesting that the HLA-DRB3*01 allele is strongly linked to CD susceptibility and may serve as a genetic risk factor among Libyan coeliac patients. Additionally, the current study found that 11 (45.8%) coeliac patients exhibited the co-occurrence of DQB1*02 and DRB1*03

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alleles, which is consistent with findings from several prior studies [14–18]. The high prevalence of DRB1*03 and DRB3*01 observed among our sampled patients agrees with the literature, suggesting these loci have a complementary, often secondary role in CD pathogenesis, possibly modulating immune responsiveness but not serving as primary susceptibility markers [19].

Conclusion

The study adds characterization data from Libyan patients, who have been underrepresented in genetic studies of CD, thereby expanding the understanding of its genetics globally [20].While HLA typing strongly predicts genetic risk, coeliac disease is multifactorial, and environmental triggers like gluten exposure and additional non-HLA genes influence disease manifestation [21]. Hence, the allelic profile provides insight into predisposition but must be integrated with clinical, environmental, and immunologic data for comprehensive management strategies. This study contributes to the growing evidence on HLA allele distribution and its pivotal role in coeliac disease in Libya. Future research in Libyan and broader North African populations with larger cohorts and molecular subtyping would enhance genetic and diagnostic precision.

Acknowledgments

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Conflicts of Interest

The authors declare no conflicts of interest.

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