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Original article

Inbreeding in Al-Jabal Al-Akhdar, Libya: Evidence from the Fixation Index and Heterozygosity of Blood Groups

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Abstract
This study investigates levels of inbreeding among several towns in the Al-Jabal Al-Akhdar region of Libya using the ABO blood group system as a genetic marker. Analyses focused on observed heterozygosity (Ho), expected heterozygosity (He), and the fixation index (Fis), an estimate of the inbreeding coefficient. The Chi-Square Goodness-of-Fit Test was used to compare the proportions of the Fis across different towns. Results showed a large variation in genetic diversity across towns. The Ho ranged from 0.380 to 0.550, with Derna, Maraweh, and Shahat showing the highest diversity. In contrast, Al-Bayadah, Al-Haniya, Al-Qubah, and Qandula displayed high positive Fis values, indicating an excess of homozygotes and suggesting high inbreeding within these local populations. However, there is no statistically significant difference in the Fis of ABO blood types across towns in Al-Jabal Al-Akhdar. These findings highlight the need for further genetic and demographic studies to understand the factors that contribute to population inbreeding and substructure.

Keywords. ABO blood types, Allele frequencies, Fixation Index, Heterozygosity, Inbreeding.

Introduction

Inbreeding refers to the probability that two alleles at a given locus are identical by descent because of mating between related individuals, a concept formalized in quantitative genetics [1]. This phenomenon increases homozygosity and can expose deleterious recessive alleles, with important consequences for individual fitness and population viability. In population genetics, analyses of heterozygosity and fixation indices are central tools for characterizing mating patterns, genetic structure, and the overall genetic health of populations. Observed heterozygosity (Ho) quantifies the proportion of individuals that are heterozygous at a locus, while expected heterozygosity (He) represents the heterozygosity expected under Hardy–Weinberg equilibrium (HWE) given the allele frequencies; deviations between Ho and He therefore signal departures from random mating. The fixation index (Fis), often used as an inbreeding coefficient at the subpopulation level, captures this deviation by measuring the relative deficit or excess of heterozygotes: high Fis values indicate inbreeding, while low (or negative) values indicate excess heterozygosity [2]. Because Fis can be estimated directly from allele frequencies, it provides a practical and widely used metric for assessing inbreeding and nonrandom mating within populations. Nevertheless, commonly used heterozygosity measures have intrinsic limitations: their maximum value is bounded at regardless of the number of alleles, so they become progressively less sensitive to additional allelic variation as diversity increases [3]. Consequently, while Ho, He, and Fis are informative, they should be interpreted alongside other statistics and ecological or demographic data to obtain a comprehensive picture of genetic diversity and the impacts of inbreeding.

Some investigators believe that long-term inbreeding can benefit a population and its health by reducing deleterious, harmful genes through selection, provided ample time is given for selection to act on them [4]. However, inbreeding can cause negative health effects due to the expression of rare, recessive, deleterious genes inherited from common ancestors or from a single shared ancestor. In human populations, high levels of inbreeding can increase the prevalence of recessive genetic disorders [5]. It was found that, among adult Hutterite women in Canada and the Dakotas, recessive harmful alleles unmasked by inbreeding reduced female fertility [6]. But inbreeding can also yield completely healthy offspring [7]. In a study of the population in Dammam, Saudi Arabia, where first-cousin marriages represent the most frequent type of consanguinity, it was observed that rates of stillbirth, childhood mortality, and average birth weight did not differ significantly between children born to consanguineous versus non-consanguineous parents. This suggests that inbreeding does not always produce harmful outcomes and can result in normal offspring [8]. Some researchers argue that, over many generations, continued inbreeding could even improve population health by allowing selection to eliminate deleterious alleles, given sufficient time [4]. However, a study on the effects of inbreeding in Japan revealed that offspring of consanguineous marriages experienced higher rates of childhood mortality and morbidity, along with an increased incidence of congenital disabilities. Furthermore, these children exhibited developmental delays, specifically in the late onset of walking and talking [9].

The Al-Jabal Al-Akhdar region, like many areas in North Africa, may have a complex demographic history and social structure that influences its genetic makeup. These processes may leave detectable signatures in the genetic composition of present-day inhabitants, including deviations from Hardy–Weinberg expectations, elevated homozygosity, or pronounced differences in allele frequencies between localities.

In this study, we employ the classical ABO blood group locus as a genetic marker to investigate population structure within Al-Jabal Al-Akhdar. Although ABO is a single, autosomal locus with limited allelic diversity

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compared with genome-wide data, it remains informative for detecting modest levels of differentiation and for estimating parameters relevant to population genetics. Specifically, we estimate inbreeding coefficients within towns to quantify departures from panmixia. This paper aims to quantify the level of inbreeding and genetic differentiation among the towns of Al-Jabal Al-Akhdar using the ABO blood group locus to assess the extent of population substructure.

Methods

Study design and setting

The study utilized data from 3,363 individuals across several towns in the Al-Jabal Al-Akhdar region. The ABO blood group phenotypes were determined. The allele frequencies for the ABO blood group system (p, q, r) across various towns in the Al-Jabal Al-Akhdar region (Table 1) were retrieved from the study conducted by Aljebali & Sulaiman [10]. These frequencies served as the baseline for calculating the expected genotype counts and testing the Hardy-Weinberg equilibrium in the current population sample. The H_E is the heterozygosity predicted under Hardy-Weinberg Equilibrium (HWE) (Table 2). The H_O is the sum of the proportion of individuals in the town who are heterozygous at the ABO locus (Table 3).

The F_{IS} values were used to infer the degree of inbreeding within each sub-population. It was estimated as; $F_{IS} = H_E - H_O / H_E$ [4]

Table 1. Allele frequencies for the ABO blood group system (adapted from [10]).

Towns	**ABO blood type frequencies**		
	p	**q**	**r**
Al-Bayda	0.162	0.342	0.496
Shahat	0.236	0.258	0.506
Derna	0.275	0.249	0.476
Omer Al-mukhtar	0.321	0.17	0.509
Qandula	0.276	0.329	0.395
Maraweh	0.411	0.233	0.356
Al-Faydia	0.19	0.225	0.585
Al-Bayadah	0.161	0.342	0.497
Al-Haniya	0.229	0.233	0.538
Al-Qubah	0.3	0.253	0.447
Entire Population	0.280	0.242	0.478

Table 2. Applying the Hardy-Weinberg principle to the allele frequencies of the ABO blood group.

Genotypes	**Expected Heterozygosis (HWE)**
Ai	2pr
Bi	2qr
AB	2pq

Statistical analysis

The Chi-Square Goodness-of-Fit test was used to compare the proportions of the F_{IS} of ABO blood types across different towns in Al-Jabal Al-Akhdar to determine whether the observed distribution differed from an expected uniform distribution. The highest and lowest contributors to the chi-square statistic were identified. The collected data were analysed using Minitab Version 20.3, a statistical software package. To determine statistical significance, a p-value <0.05 was considered.

Table 3. Observed phenotypes and genotypes of ABO blood groups

Towns	**Phenotype**		**Genotype**		**Towns**	**Phenotype**		**Genotype**	
Al-Bayda	A	29.47	Ai AA	17.52 11.95	Maraweh	A	56.67	Ai AA	35.56 21.11
	B	23.66	Bi BB	16.27 7.39		B	25.56	Bi BB	8.89 16.67
	AB	5.00				AB	4.44		
	O	41.87				O	13.33		
Derna	A	34.19	Ai AA	22.68 11.50	Al-Faydia	A	25.35	Ai AA	14.08 11.27
	B	29.87	Bi BB	19.17 10.70		B	35.21	Bi BB	26.76 8.45
	AB	9.27				AB	1.41		
	O	26.68				O	38.03		

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Shahat	A	31.65	Ai AA	22.32 9.33	Al-Bayadah	A	18.42	Ai AA	10.52 7.89
	B	32.26	Bi BB	19.11 13.15		B	41.45	Bi BB	20.39 21.05
	AB	6.27				AB	5.92		
	O	29.82				O	34.21		
Omer Al-Mukhtar	A	37.61	Ai AA	15.39 22.22	Al-Haniya	A	29.31	Ai AA	17.93 11.38
	B	27.35	Bi BB	24.79 2.56		B	28.28	Bi BB	15.17 13.10
	AB	4.27				AB	5.17		
	O	30.77				O	37.24		
Qandula	A	37.14	Ai AA	23.81 13.33	Al-Qubah	A	35.88	Ai AA	18.21 17.68
	B	39.05	Bi BB	17.14 21.90		B	30.61	Bi BB	16.88 13.72
	AB	4.76				AB	6.33		
	O	19.05				O	27.18		

Results

In the current study, the Ho for the ABO gene ranged between 0.368 and 0.511 (Table 4). In the entire population, Ho was at 0.467. The highest level of heterozygosity was recorded in Derna at 0.511, followed by Maraweh at 0.488, Shahat at 0.47, and AL Bayda at 0.44. In contrast, the lowest Ho was observed in Al-Bayadah (0.368) and in Al-Haniya (0.380). The Fis for the ABO gene ranged from 0.19 to 0.39. In the entire population, Fis was low at 0.26. Between the towns, Fis was highest in Al-Bayadah (0.39), followed by Al-Haniya (0.36), Al-Qubah (0.35), and Qandul (0.30), suggesting elevated levels of inbreeding and, therefore, increased homozygosity within these populations. Conversely, Derna had the lowest Fis at 0.19, followed by Shahat (0.24), Maraweh (0.25), Al-Faydia (0.26), Al-Bayda (0.26), Omer Al-Mukhtar (0.26), and the entire population (0.26) (Fig. 1).

Table 4. Observed, Expected Heterozygosity and Fixation index of ABO blood types

Towns	Ho	He	Fis
Al-Bayda	0.44	0.60	0.26
Shahat	0.47	0.62	0.24
Derna	0.51	0.63	0.19
Omer Al-Mukhtar	0.44	0.60	0.26
Qandula	0.45	0.65	0.30
Maraweh	0.48	0.65	0.25
Al-Faydia	0.42	0.57	0.26
Al-Bayadah	0.36	0.61	0.39
Al-Haniya	0.38	0.60	0.36
Al-Qubah	0.41	0.64	0.35
Entire Population	0.46	0.63	0.26

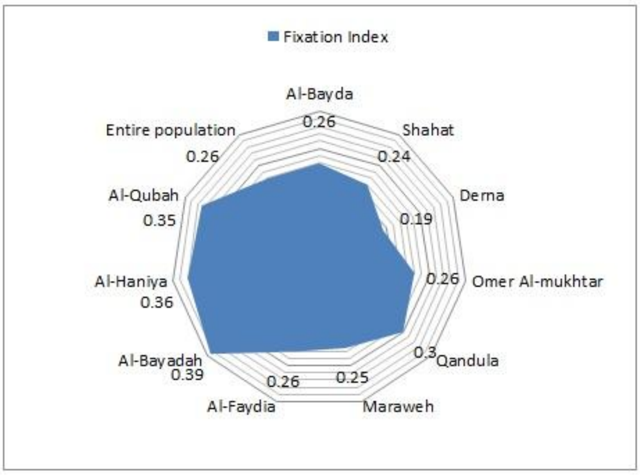

Figure 1. Fixation index of ABO blood type among different towns in Al-Jabal Al-Akhdar.

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The Chi-square goodness-of-fit test revealed no statistically significant difference in the FIS of ABO blood types across towns in Al-Jabal Al-Akhdar. The test reported a p-value of 1.000, indicating no evidence to reject the null hypothesis of equal proportions across towns (Table 5). The largest contributors to the chi-square statistic were Al-Bayada, Derna, Al-Haniya, and Al-Qubah, while the smallest contributors were Qandula, Al-Faydia, Omer Al-Mukhtar, and Al-Bayd (Table 6. fig 2).

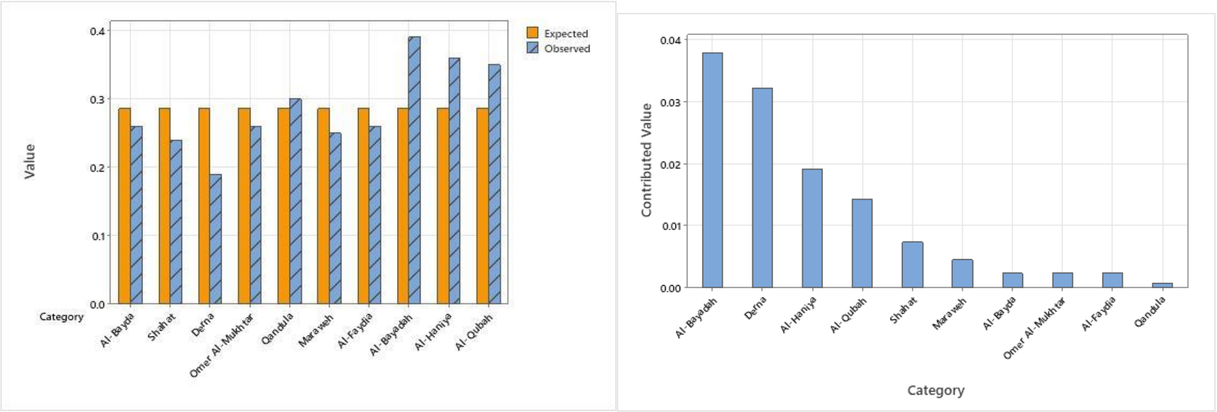
Table 5. Chi-Square Test of the fixation index of ABO blood types in the towns

DF	Chi-Sq	P-Value
9	0.123217	1.000

*P –Value *significant difference (p < 0.05). DF degrees of freedom. Chi-Sq is the calculated Chi-Square statistic*

Table 6. Observed and Expected Counts of Fixation index of ABO blood types in the towns

Towns	Observed	Expected	Contribution to Chi-Square
Al-Bayda	0.26	0.286	0.26
Shahat	0.24	0.286	0.24
Derna	0.19	0.286	0.19
Omer Al-Mukhtar	0.26	0.286	0.26
Qandula	0.3	0.286	0.30
Maraweh	0.25	0.286	0.25
Al-Faydia	0.26	0.286	0.26
Al-Bayadah	0.39	0.286	0.39
Al-Haniya	0.36	0.286	0.36
Al-Qubah	0.35	0.286	0.35



(a) **(b)**

Fig. 2: (a) Chart of Observed and Expected Values of Fixation of ABO blood type among different towns in Al-Jabal Al-Akhdar, (b) Contribution to the Chi-Square Value by Category of Fixation index of ABO blood type.

Discussion

Heterozygosity is commonly regarded as a measure of genetic health and adaptability within populations. Elevated heterozygosity levels indicate a strong gene pool, which is vital for species survival and their ability to adapt to changing environments [11]. In this investigation, the HO for the ABO gene was estimated between 0.368 and 0.511. The highest HO levels were found in Derna (0.511), Maraweh (0.488), Shahat (0.470), and Al-Bayda (0.440), reflecting significant genetic diversity among these populations. Factors contributing to this diversity may include larger population sizes, diverse mating patterns, or lower inbreeding rates [12]. However, from an evolutionary standpoint, outbreeding in human populations is generally preferred as it masks the expression of harmful recessive alleles in homozygous states and increases heterozygosity, which can enhance the adaptive capacity of future generations. However, this process simultaneously expands the population's genetic load by accumulating these deleterious recessive genes in their heterozygous form [13]. Conversely, the lowest HO was recorded in Al-Bayadah (0.368) and Al-Haniya (0.380), indicating a lack of genetic variation in these groups. This reduced diversity may stem from smaller population sizes or increased inbreeding.

A less diverse gene pool can heighten susceptibility to diseases, limiting the population's ability to adapt [14]. The positive and high FIS values observed in towns such as Al-Bayda and Al-Haniya strongly suggest the presence of significant inbreeding within these sub-populations. The effect of inbreeding is to increase

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the proportion of homozygotes and decrease the proportion of heterozygotes, which is precisely what the positive FIS values reflect.

The contrasting low FIS values in Derna, Maraweh, and Shahat suggest that these populations may be experiencing a higher degree of outbreeding or are acting as centers of gene flow, possibly due to their larger size or enhanced migratory activity. This variation in the FIS across the region points to a clear population substructure, where towns are not genetically homogeneous, but rather exist as distinct breeding units with varying degrees of isolation and mating practices. This genetic differentiation is a key finding for understanding the demographic history of the Al-Jabal Al-Akhdar region.In the towns of Al-Bayadah, Al-Haniya, Al-Qubah, and Qandul, there is a notable degree of inbreeding, which has resulted in higher levels of homozygosity within these populations, as reflected by their FIS values of 0.396, 0.360, 0.350, and 0.300, respectively. High FIS values indicate a greater likelihood that individuals are inheriting the same allele from both parents, leading to increased homozygosity [15]. This phenomenon can significantly impact the population's genetic diversity, adaptability, and overall fitness. Increased homozygosity is associated with various negative effects, such as inbreeding depression, where diminished genetic variation raises the risk of expressing harmful alleles.

Many studies have demonstrated that inbreeding depression can adversely affect reproductive success, survival rates, and general fitness across many species [16-17]. Factors contributing to this inbreeding include geographic isolation, cultural practices, and habitat fragmentation, which can all lead to a reduction in genetic diversity [12]. A decrease in genetic diversity can hinder a population's capacity to adapt to environmental changes, making it more vulnerable to diseases and less resilient to challenges [18].

Conclusion

The population genetic analysis of the ABO blood group system in the Al-Jabal Al-Akhdar region reveals a significant population substructure. The high positive FIS values in several towns provide strong evidence for substantial inbreeding, leading to an increased level of homozygosity. This finding is crucial for public health planning, particularly in the context of genetic counseling and the management of recessive genetic disorders. Further research utilizing an expanded panel of polymorphic genetic markers is recommended to confirm and elaborate on these findings.

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Conflicts of Interest

The authors declare they have no competing interests.

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