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Hemoglobin C Disorder in Anemic Patients Referred to the National Center for Thalassemia and Genetic Counseling in Damascus

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ABSTRACT

Background: Hemoglobinopathies are common inherited blood disorders in our Mediterranean area. The main structural hemoglobin variants are hemoglobin S and hemoglobin C, due to their prevalence.
We conducted this retrospective study to investigate and characterize hemoglobin C patients referred to the National Center for Thalassemia and Genetic Counseling and the management of hemoglobin C disease in Damascus.

Materials and Methods: The study included patients referred to the National Center for Thalassemia and Genetic Counseling in Damascus between 2000 and 2022 for hemoglobin C detection. Gender, age, geographical origin, hemoglobin electrophoresis profile, and blood transfusion were considered for hemoglobin C patient classification. Blood transfusion in five consecutive years and linear regression with hemoglobin S and C values were determined.

Results: 30 (14 males and 16 females) out of 624 patients between 3 and 46 years old (mean ± SD: 17.3 ± 9.7 years) showed hemoglobin C disease. Only eight patients (one male and seven females) received blood transfusions, and the remaining patients (13 males and 9 females) did not receive any transfusion. Only one patient with 100% hemoglobin C was detected; 19 showed HbSC, and 10 had HbAC. There was a significant correlation between hemoglobin S and geographical origin (P-value=0).

Conclusion: A Homozygote hemoglobin C patient has mild hemolytic anemia, whereas the hemoglobin C 100% patient has only a one-time blood transfusion (he was 17 years old) in our study. The inherited combination of hemoglobin C and S is less severe than hemoglobin S alone. There is a significant relationship between hemoglobin S and geographical origin (p-value=0).

Keywords: Blood transfusion; Hemoglobin S (HbS); Hemolytic anemia; Sex

INTRODUCTION

Hemoglobin consists of two chains of proteins called globin and heme which is a complex of Protoporphyrin and Fe^{+2}, globin chains are **α, β, γ, δ, ε**[1]. **α**-chain contains 141 amino acids genetically encoded on chromosome 11**, β**-chain contains 146 amino acids genetically encoded on chromosome 16; this chromosome is responsible for coding **ε,δ,γ** as well[2,3]. Normal hemoglobins in adults are HbA_1 (2α2β) 95-98%, HbA2 (2α2δ) 1-2% and Fetal Hemoglobin (2α2γ) 0-1%[3,4].

Hemoglobinopathies are caused by mutations that lead to an imbalance in hemoglobin synthesis and were categorized as quantitative and qualitative[5,6].

Quantitative hemoglobinopathies are caused by abnormal production in α and β chains, such as α-thalassemia and β-thalassemia. On the other hand, qualitative hemoglobinopathies are the replacement of the positions of amino acids in α or β globin chains, such as HbS ($\beta 6^{Glu->Val}$), HbE ($\beta 26^{Glu->Lys}$), HbD ($\beta 212^{Glu->Gln}$) and HbC, which has Glutamic acid replaced by Lysine in sixth position in its β globin chain[1,3,5,4].

Our main study relates to HbC which is one of the most common abnormal hemoglobin found in the African ethnicity[1,7].

The presence of lysine gives it a positive charge, reducing its solubility and gives it crystallization form inside the Red Blood Cells[4].

HbC disease follows two genetic types heterozygous and homozygous; patients with the heterozygous type (HbCS, HbCA) do not show any signs, while patients with the homozygous type (HbCC) show more significant signs, such as hemolytic anemia, gallbladder stones and splenomegaly[1].

The prevalence of HbC reaches 40-50% in West Africa. The disease is also found in Togo and Benin, in individuals of African descent in the Caribbean, in the USA, and North Africa. HbC was present in major European cities and in the Middle East(Saudi, Iraq and Pakistan)[8,9] .

MTERIALS AND METHODS

The study was approved by the Research Ethics Committee at Al-Sham Private University.

This retrospective study was established between 2000 and 2022 at the National Center for Thalassemia and Genetic Counseling in Damascus/Syria. Thirteen patients with HbC disease were diagnosed, of whom eight patients received blood transfusion, while 22 patients were blood transfusion-free. The following information was taken and described as variables:

Blood transfusion: Frequency of blood transfusion for each patient in five consecutive years.

HbS: Sickle hemoglobin.

HbF: Fetal hemoglobin

Origin: Geographical origin of the patients. Syrian patients were coded as one and Palestinian patients as two.

Sex: The gender of the patients. Male patients were coded as one and female patients were coded as two.

HbC: Hemoglobin C.

Age: The patient's age.

Year4: Frequency of blood transfusion for each patient in the fourth year.

Year5: Frequency of blood transfusion for each patient in the fifth year.

The statistical study attempted to determine which variables are significantly correlated to one another, and the linear regression method was applied to estimate the linear relationship between the correlated variables if available. The Null hypothesis is the assumption that there is no correlation between the variables against the alternative hypothesis, which proves the significant correlation between these variables.

The Kendall τ_b is used because of the non-normality of the medical variables.

Data were analyzed using SPSS 22 for windows.

RESULTS

Complete data were available for only 624 out of 952 patients diagnosed with sickle cell at the National Center for Thalassemia and Genetic Counseling in Damascus between 2000 and 2022. Among the study participants, 30 patients have HbC. Demographic data of HbC, HbSC, and HbAC patients are illustrated in Table 1.

Table1: The patient's electrophoresis test and blood transfusion

Patient's number	Patient's gender	Patient's group	Electrophoresis%					Blood transfusion
			HbA1	HbA2	HbS	HbF	HbC	
1	Male	HbC	0.0	0.0	0.0	0.0	100.0	Yes
2	Female		3.8	0.0	53.0	0.0	43.2	Yes
3	Female		0.0	14.4	71.2	0.0	14.4	Yes
4	Female		0.0	1.3	46.2	10.3	42.2	Yes
5	Female		5.2	4.8	40.9	3.2	45.9	Yes
6	Male		5.7	0.0	38.0	15.7	40.6	No
7	Female		0.5	0.0	52.6	0.0	46.9	No
8	Male		2.5	6.2	35.5	7.5	48.3	No
9	Male		0.0	1.2	55.4	8.9	34.5	No
10	Male		0.0	0.0	52.4	0.0	47.6	No
11	Male		4.0	4.6	43.2	0.4	47.8	No
12	Male		3.9	3.6	38.6	7.5	46.4	No
13	Female	HbSC	3.8	4.6	43.2	1.6	46.8	No
14	Male		2.0	0.0	49.3	6.5	42.2	No
15	Female		3.0	1.2	51.3	0.0	44.5	No
16	Male		0.0	1.6	50.4	1.4	46.6	No
17	Female		0.0	0.0	49.3	0.0	50.7	No
18	Male		0.0	1.4	50.6	4.9	43.1	No
19	Male		0.0	1.7	52.4	1.8	44.1	No
20	Female		0.0	1.2	42.6	2.8	53.4	No
21	Male		0.0	3.6	0.0	1.3	95.1	No
22	Female		22.7	4.9	0.0	2.3	70.1	No
23	Male		48.3	5.9	0.0	1.1	44.7	No
24	Female		40.0	8.1	0.0	4.4	47.5	No
25	Male		0.0	6.0	0.0	9.9	84.1	No
26	Female	HbAC	61.7	0.0	0.0	0.0	38.3	No
27	Female		47.5	6.0	0.0	3.7	42.8	No
28	Female		0.0	2.8	0.0	2.6	94.6	Yes
29	Female		9.4	7.2	0.0	14.9	68.5	Yes
30	Female		63.8	0.0	0.0	0.0	36.2	Yes

First, histograms and the density estimation curves were plotted to examine the normality of the medical variables. Figure 1 shows the non-normality of these variables.

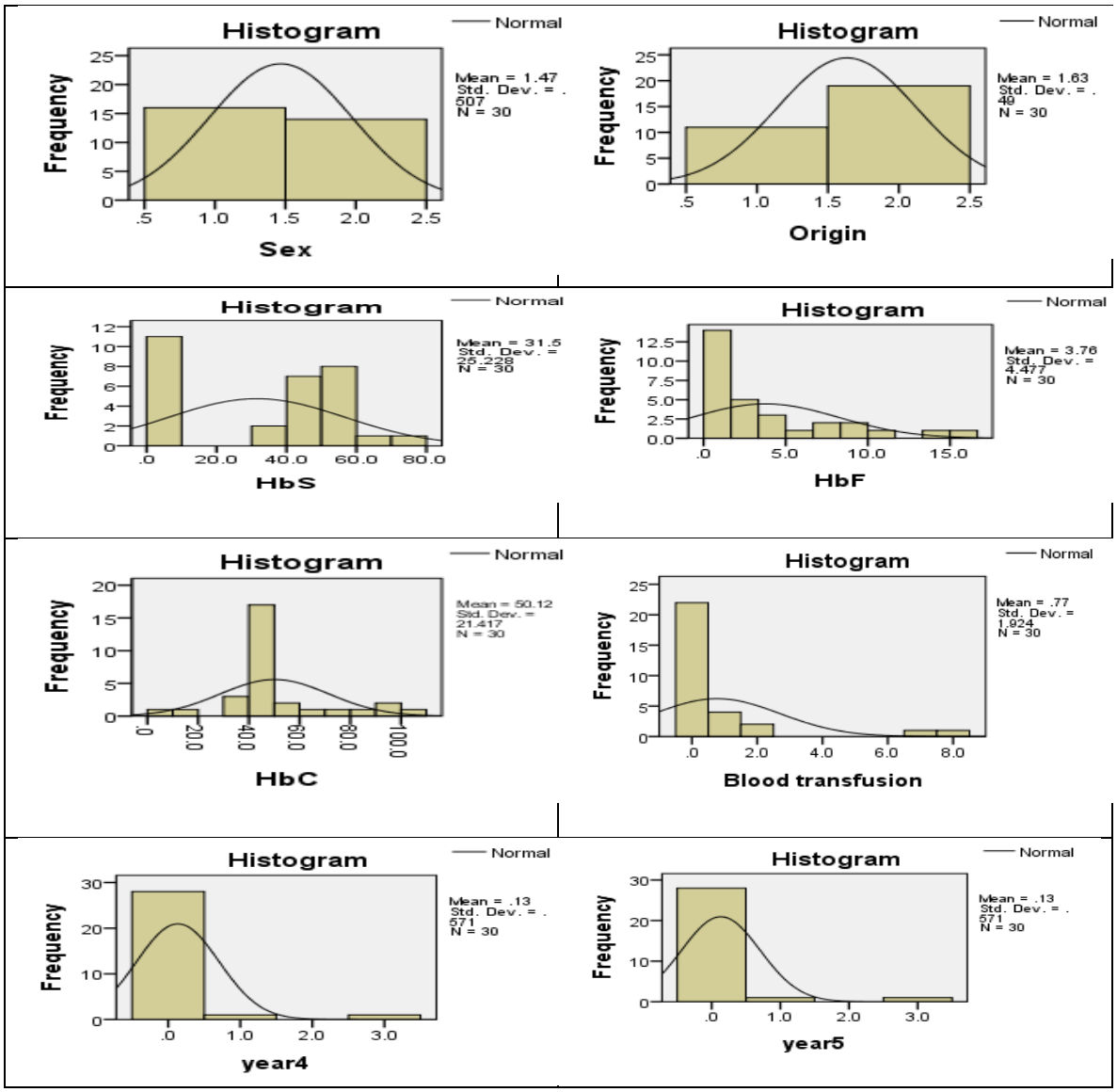

Figure 1. The non-normality of the variables' distribution

The findings showed that there is a direct relationship between HbS and the geographical origin of the patients. When we studied the correlation Kendall τ_b between the origin variable meaning that there is a significantly direct relationship between the origin and the HbS. Due to a significant correlation between the origin and the and HbS variable, we found τ_b=0.492 with a p-value =0 < α=0.05. Thus, we rejected the Null hypothesis H_0 and accepted the alternative hypothesis H_1,

HbS, we applied the least squares method to estimate the linear relationship (Figure 2).

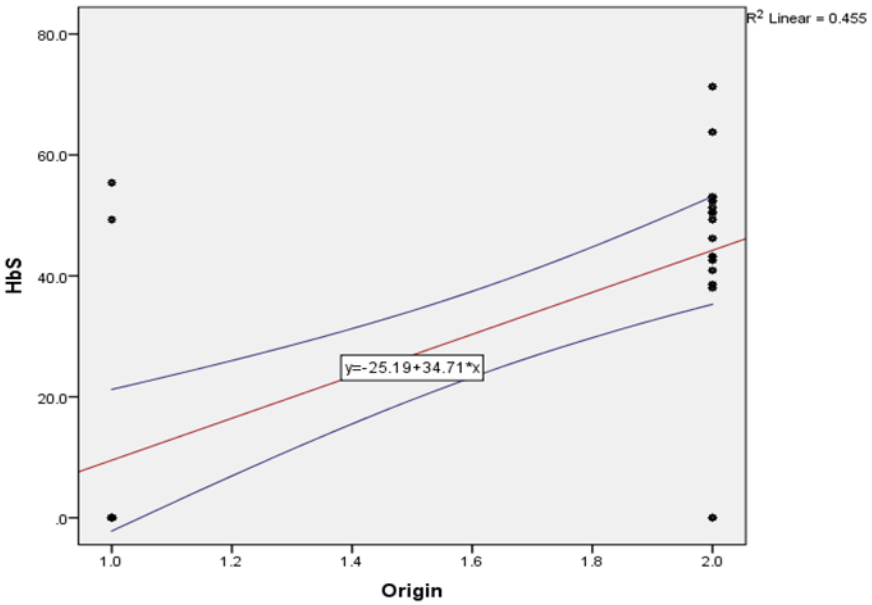

Figure 2. Linear relationship between HbS and geographical origin

The linear regression showed the significance of $\widehat{\beta_0}$ and $\widehat{\beta_1}$ estimated by this method. The intercept $\widehat{\beta_0}$ =-25.1 with p-value=0.049 < 0.05 indicates that $\widehat{\beta_0}$ is significant and not equal to zero. According to the same Table, the slope $\widehat{\beta_1}$ =34.7 with a p-value=0 < **α**=0.05 shows the significance of the slope, representing a significance relationship between the HbS and the origin as an independent variable.
HbS=-25.1+34.7*origin, with the 95% confidence interval is shown on the Figure applied on the mean.

Kendall τ_b correlation test showed an indirect relationship between blood transfusion and sex (τ_b=-0.4 with a p-value=0.02< 0.05). Thus, the Null hypothesis H_0 was rejected, and the alternative hypothesis H_1 was accepted, meaning that there is a significantly indirect relationship between the blood transfusion and the sex.
Due to a significant correlation between the blood transfusion and the sex, we applied the least squares method to estimate the linear relationship (Figure 3).

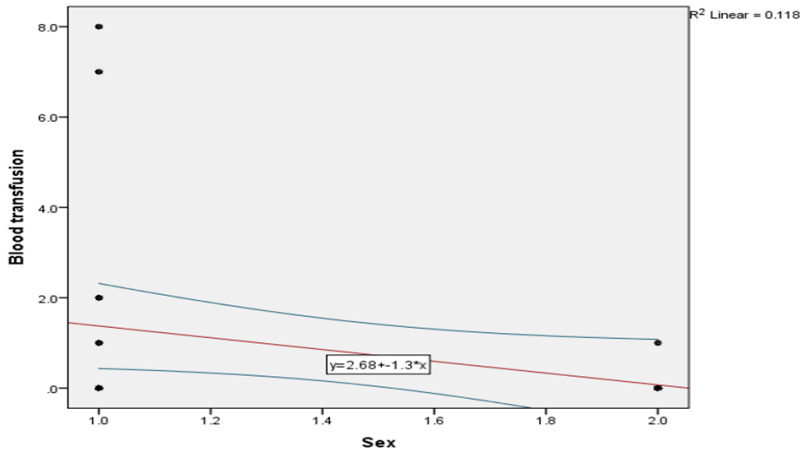

Figure 3: The linearity relationship between blood transfusion and sex

The linear regression showed the significance of $\widehat{\beta_0}$ and $\widehat{\beta_1}$ estimated by this method. The intercept $\widehat{\beta_0}$ =2.679 with p-value=0.016 < 0.05 indicates that $\widehat{\beta_0}$ is significant and not equal to zero. According to the same Table, the slope $\widehat{\beta_1}$ =-1.304 with a p-value=0.063 < α=0.07 shows the significance of the slope, representing a significant relationship

between the blood transfusion and the sex as an independent variable.

Blood transfusion=2.67-1.3*sex, with the 95% confidence interval is shown on the Figure applied on the mean.

Kendall τ_b correlation test showed that the HbC percentage is indirectly related to the fourth year of blood transfusion (τ_b=-0.324 with a p-value=0.043< 0.05). Thus, the Null hypothesis H_0 was rejected, and the alternative hypothesis H_1 was accepted, meaning that there is a significantly direct relationship between the HbC and the fourth year of blood transfusion.

Due to a significant correlation between the HbC and the fourth year of blood transfusion, we applied the least squares method to estimate the linear relationship (Figure 4).

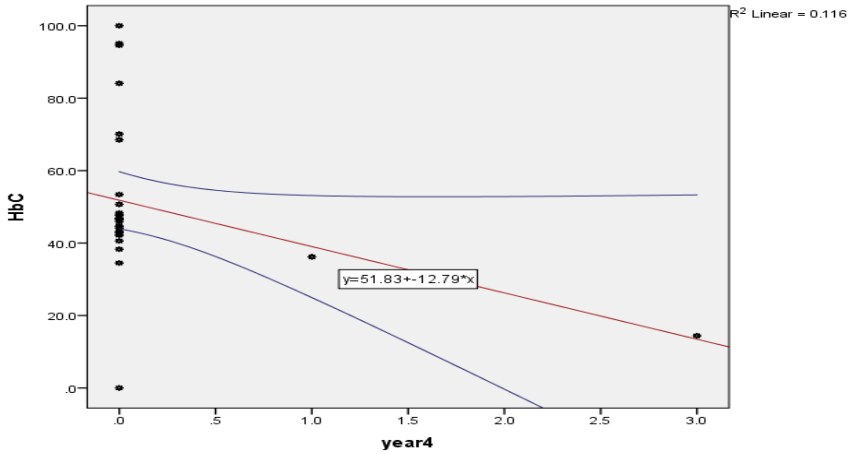

Figure 4: Linear relationship between HbC and the year4

The linear regression showed the significance of $\widehat{\beta_0}$ and $\widehat{\beta_1}$ estimated by this method. The intercept $\widehat{\beta_0}$=51.82 with p-value=0 < 0.05 indicates that $\widehat{\beta_0}$ is significant and not equal to zero. According to the same Table, the slope $\widehat{\beta_1}$=-12.79 with a p-value=0.065 > α=0.07 shows the significance of the slope, representing a significant relationship between the HbC and the fourth year of blood transfusion. HbC=51.8-12.79*year4, with the 95% confidence is shown on the Figure applied on the mean.

Kendall τ_b correlation test showed another indirect relationship between HbC percentage and the fifth year of blood transfusion, (τ_b=-0.324 with a p-value=0.043< 0.05). Thus, the Null hypothesis H_0 was rejected and the alternative hypothesis H_1 was accepted, showing that there is a significantly direct relationship between the HbC and the fifth year of blood transfusion.

Due to a significant correlation between the HbC and the fifth year of blood transfusion, the least squares method was used to estimate the linear relationship (Figure 5).

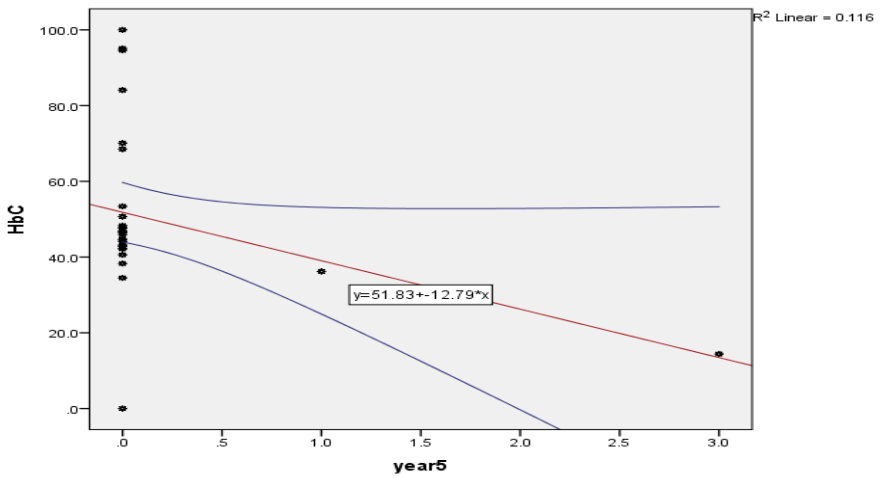

Figure 5: The linear relationship between HbC and year 5

The linear regression analysis indicated that the intercept β_0 with a value of 51.82 and a p-value=0 < 0.05 is significant. The slope $\widehat{\beta_1}$ was also significant, $\widehat{\beta_1}$=-12.79 and p-value=0.065 > α=0.07, demonstrating the significance of the relationship between HbC on the fifth year of blood transfusion, as shown in the equation HbC = 51.8 - 12.79 * year5. The analysis of other variables included in the study showed no significant relationships, indicating that not all variables are significantly related.

DISCUSSION

The study showed a direct correlation between HbS and origin and an indirect correlation between blood transfusion and sex, between HbC and the fourth year of blood transfusion, and between HbC and the fifth year of blood transfusion. Moreover, the linear relationship between variables was observed.

HbS is an abnormal hemoglobin arising from geographic areas in Africa, the Arab world, the Middle East, and the Mediterranean countries[10]**.** One study by Frédéric B. Piel et al. indicates that the mean HbC frequency is about 1.0% between West Africa and Egypt[11]. That means the immigration of African people was responsible for the presence of HbS and HbC in all regions of the world [12].

The study proved a direct correlation between HbS and the place of origin. In the present study, we found that HbS is more prevalent in Palestinian compared to Syrian patients.

Moreover, we found that female patients tend to need more blood transfusions compared to male patients because females have fewer hemoglobin values due to menstrual blood loss. In addition, Androgen stimulates the synthesis of red blood cells in males, which also applies to HbC patients[13].

The replacement of amino acids in β globin chains causes a reduction in hemoglobin solubility and an increase in oxidation exposure, resulting in unstable hemoglobin, which precipitates and forms bodies that harm the membrane of RBCs[3].

In HbC patients, the replacement of Glutamic acid by Lysine in position 6 of β globin chain induces an electrostatic interaction between positively charged β-6-lysyl group and negatively charged contiguous molecules, leading to solubility reduction, crystals formation, and water loss despite blood transfusion differs in these patients[1,14].

Unlike HbSS, the hemoglobin in HbCC does not polymerize, but it forms crystals with decreased RBC elasticity and life span and leads to mild hemolytic anemia. HbSC promotes the pathological characteristics of HbS, which leads to chronic anemia accompanied by milder symptoms than HbSS[7]. The study also showed that patients with high HbC values do not practically need a consecutive blood transfusion. Moreover, the need for blood transfusion rises four to five years after diagnosis regarding to HbC values. HbS and HbC combination will reduce the need for blood transfusion and is less serious than HbS disease.

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Abbreviation

HbC: Hemoglobin C, HbS: sickle Hemoglobin, HbF: Fetal Hemoglobin, RBCs: Red Blood Cells

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest in this research work.

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