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The percentage of hemoglobin Bart's among group of neonates in Al-Najaf City

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Abstract
Background: Alpha-thalassemia is caused by deletions of the α-globin genes on chromosome 16. The presence of hemoglobin Bart's on the newborn screen almost always indicates that one or more of the baby's α-globin genes are deleted.
Objectives: To identify the rate of occurrence of HbBart's among group of neonates by cord blood assay.
Materials and Methods: Total of 120 cord blood samples of newborn were examined by α-thalassemia short program utilizes the principles of cation exchange HPLC for the presence of Hb Bart's. Statistical analysis utilized SPSS 26.
Results: Hb Bart's was encountered in 93 cases (77.5%) neonates constituting 1 - 10.4% of their total Hb, of these 92 cases (76.67%) were of α-thalasse-2 (Silent alpha-thalassemia) and 1 (0.83%) of α-thalasse-1 (α-thalassemia trait). Our results found to be slightly higher than the prevalence of the disease in the Middle East and western Asia (12.55%) and Southeast Asia (6 -75%).
Conclusion: Our study clarifies the importance for further future study and follow up of neonates with high percentage of HbBart's.

Keywords: Alpha-thalassemia, Hb Bart's, HPLC

Introduction

Hemoglobin is a protein responsible for carrying oxygen and giving blood its red color. Worldwide, there are hundreds of different hemoglobin types. Each hemoglobin molecule contains two pairs of globin chains, one is called Alpha (α) and the other is called Beta (β).
Hemoglobin Bart's (HbBart's) is a relatively common hemoglobin variant detected by HPLC testing and is only seen during the newborn period. Infants with α-thalassemia syndrome usually show hemoglobin Bart's on the newborn screening test.
Alpha-thalassemia is caused by deletions of the α-globin genes on chromosome 16. The loss of one to four of these genes is possible. The presence of hemoglobin Bart's on the newborn screen almost always indicates that one or more of the baby's α-globin genes are deleted [1]. The severity of the α-thalassemia condition is directly related to the amount of gene deletions present [2].
The silent carrier: One gene deletion in the silent carrier, only three out of the four genes that regulate the production of alpha (α) globin chains are passed from the parent to the child. A very small amount of Hb Bart's (1-3%) is identified at birth, however it soon disappears. The child has no anemia and will require no medical treatment.
Alpha Thalassemia Trait: Two gene deletion only two genes are inherited for the production of α - globin chains, either (Cis) which common only in Asian or (Trans) which common in African-American. A small amount of Hb Bart's (3-10%) is identified at birth, however it soon disappears. A mild anemia may be present. Parents who have told their newborn had Hb Bart's at birth should their health care provider. The information could prevent unnecessary testing or treatment with iron. No medical treatment for α - thalassemia is necessary, even for the child with a two-gene deletion.
Hemoglobin H disease: Three gene deletion only one gene for the production of alpha chain production has been inherited. A large amount of Hb Bart's (15-30%) is usually identified at birth. Complications might include; severe, lifelong anemia, jaundice, enlarged spleen and gallstones. This complication is most common in people of Southeast Asian ancestry.
Fetal Hydrops Syndrome: Four gene deletion no genes for the production of α - chains have been inherited.

A very large amount of Hb Bart's (80-100%) is identified at birth ; the ϒ chains form the tetramer Bart's (ϒ4), the fetus stillborn or dies within the first few hours of birth. This condition is seen almost exclusively in people from south Asia [3].

The Prevalence of α-thalassemia

It is estimated that there are 270 million carriers of mutant globin genes that can potentially cause severe forms of thalassemia. Globally, more than 1.0% of couples are at risk of having children with severe hemoglobinopathy, with more than 330,000 affected babies born each year [4], more than 95% of which occur in Asia, India and Middle East [5]. Although the frequency of α-thalassemia carriers in Iran has not been well identified, a report from northern Iran has estimated its frequency to be around 15% [6].

Before the introduction of DNA analysis, population surveys for α-thalassemia were based entirely on the measurement of Hb Bart's levels in cord blood. However, single gene detection heterozygotes do not always have detectable Hb Bart's in the neonatal period. As a result, reliable data on population frequencies for various types of α-thalassemia are not always available [7]. The α-thalassemia is common throughout parts of the world where malaria is endemic. Multiple studies have suggested that the presence of both single and double globin gene deletions confer a protective effect from malaria [8]. Listed below are the approximate percentages of various populations with some forms of α-thalassemia:-

Europe (4-12%), Middle East and western Asia (6-75%), Southeast Asia (11-50%), Africa (11-50%) while South America and the Caribbean (7%).

Aim of the Study: To identify the rate of occurrence of **HbBart's** among group of neonates by cord blood assay.

Patient and Method: EDTA anticoagulant cord blood samples preserved for HPLC [9], obtained from 120 full-terms new born, all of them born by caesarean sections in obstetrics and gynecology department of Al-Kufa University in Al-Zahraa Teaching hospital. This obstetric service takes care of Al-Najaf population handling about 15100 deliveries per year.

The samples were taken from consecutives caesarian sections deliveries from the June to September 2023. This material is therefore representative sample of Al-Najaf population.

Specimen Collection

At least 0.5 ml of whole blood is required for this test. Whole blood specimens should be collected in a vacuum blood collection tube containing EDTA as an anticoagulant. Patient specimens are stable for the 2-3 days when stored at 2 – 8 ^{0}C.

Specimen Preparations

1. From each patient specimen, pipette 0.5 ml whole blood into a separate 2 ml sample vial.
2. Add 2 ml of α-thalassemia short hemolysis reagent to each sample vial.
3. Cover each sample vial and mix by inversion.
4. Allow to stand at room temperature for 30 minutes.
5. Place the sample vial into the variant for analysis.

The system will automatically enter into a 15-minute wash cycle during which a thorough flushing of the sample probe and line by removing any residual sample components and minimized cartridge or column is performed to prepare the system for shutdown. Following completion of the wash cycle, the system enters the idle mode, in which full power is supplied to all components; all actions that require interface with the display screen are done in the idle mode. At the end of each sample analysis, a copy of the chromatogram appearing on the analysis monitor screen is printed automatically along with report data for that analysis.

Excluded Cases

Prematurity, low birth weights, postdate newborn, newborn with congenital abnormality and birth asphyxia.

Statistical analysis

All values are expressed as mean ± standard deviation. Data were analyzed by SPSS version 26 and Microsoft Excel. Categorical variables were analyzed by Chi-square test. The relation between different variables was done by Pearson's correlation coefficient $p< 0.05$ was considered statistically significant.

Results

The different quantities of Hb Bart's were encountered by HPLC of 120 cord blood samples of newborn, Hb Bart's ≥ 1 was detected in 93 cases (77.5%), of these 92 cases (76.67%) were of silent carrier and 1 case (0.83%) of α-thalassemia trait (figure 1).

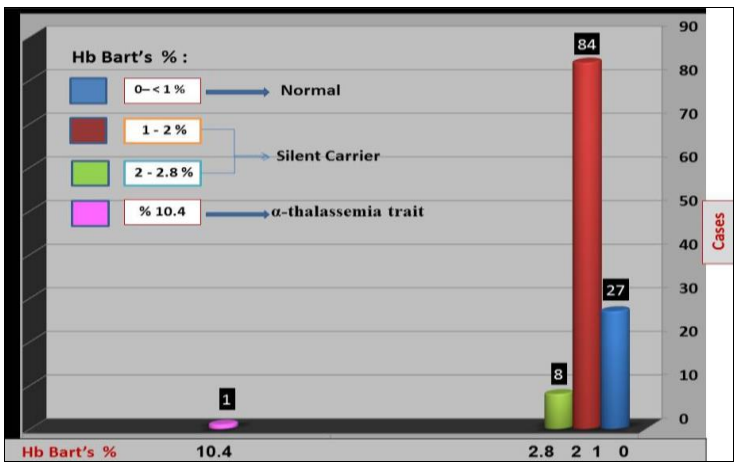

Fig 1: Show the HbBart's level in Al-Najaf and other cities of Iraq.

From the total of 120 cases , 65 cases (54.2%) where males, of whom 50 cases (76.9%) had Hb Bart's 1-3% and 15 cases (23.1%) had Hb Bart's <1% while 55 cases (45.8%) were female of whom 42 cases (76.36%) had Hb Bart's 1- 3%, 1 case (1.82%) had Hb Bart's > 3% and 12 cases (22.82%) had Hb Bart's < 1, there is no significant difference between 2 groups (p value = 0.16, more than 0.05), as shown in figure (2).

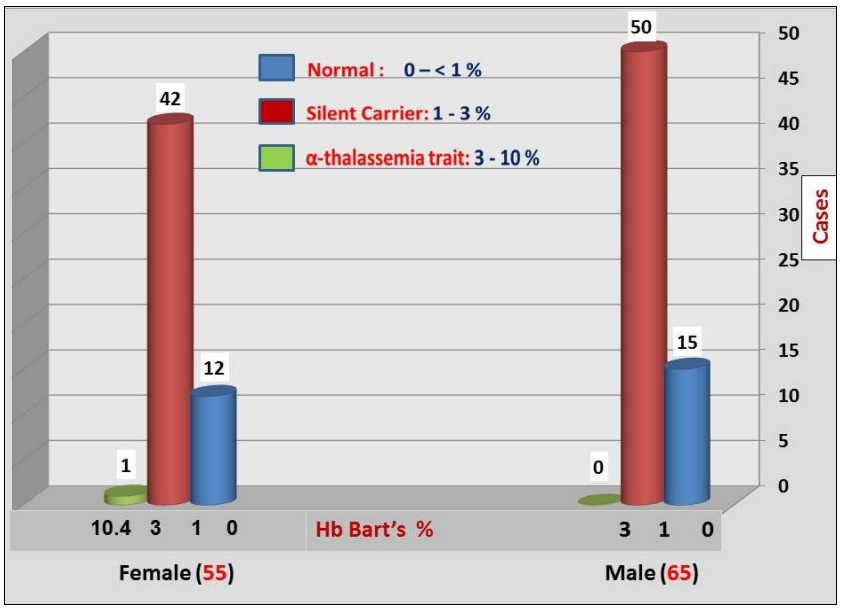

Fig 2: The difference of Hb Bart's quantities between male to female.

Figure (3) clarify that 61 cases (50.8%) demonstrate no consanguinity between parents, of whom 50 cases (82%) had Hb Bart's 1-3%, while 59 cases (49.2%) had consanguinity of parents of whom 43 cases (73%) had Hb Bart's 1-3%. There was high incidence of Hb Bart's > 1% in newborn of no consanguinity while the highest value of Hb Bart's (10.4) was in consanguineous group. There is a significant difference (p value = 0.02, less than 0.05) of no consanguinity group.

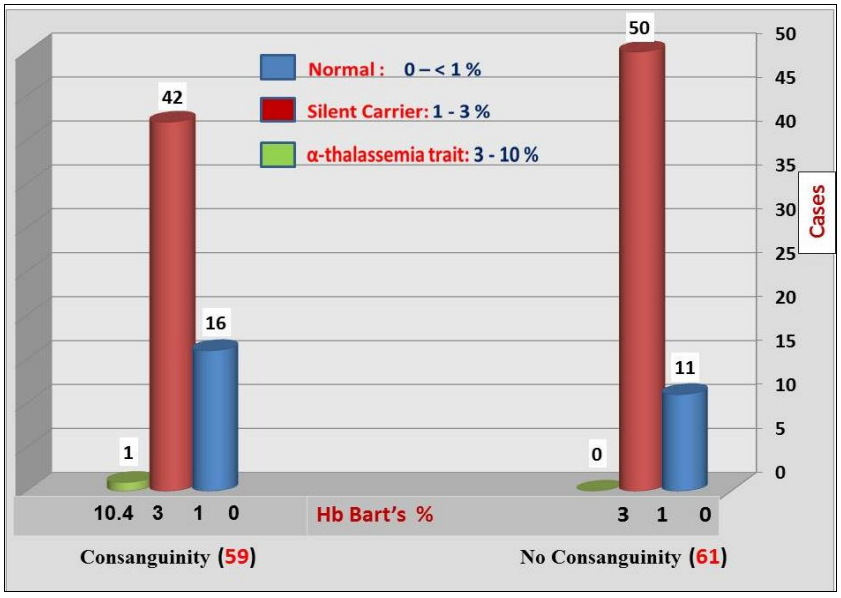

Fig 3: The percentage of Hb Bart's according to consanguinity of the parents.

Figure (4) demonstrate that 107 cases (89.2%) from Al-Najaf city, of whom 81 cases (75.7%) had Hb Bart's in range 1-3% and 1 case (0.9%) had Hb Bart's in range 3-10%, while 13 cases (84.6%) from other cities, of whom 11 cases (84.6%) had Hb Bart's in range 1-3%, there is no significant difference between 2 groups (p value = 0.070).

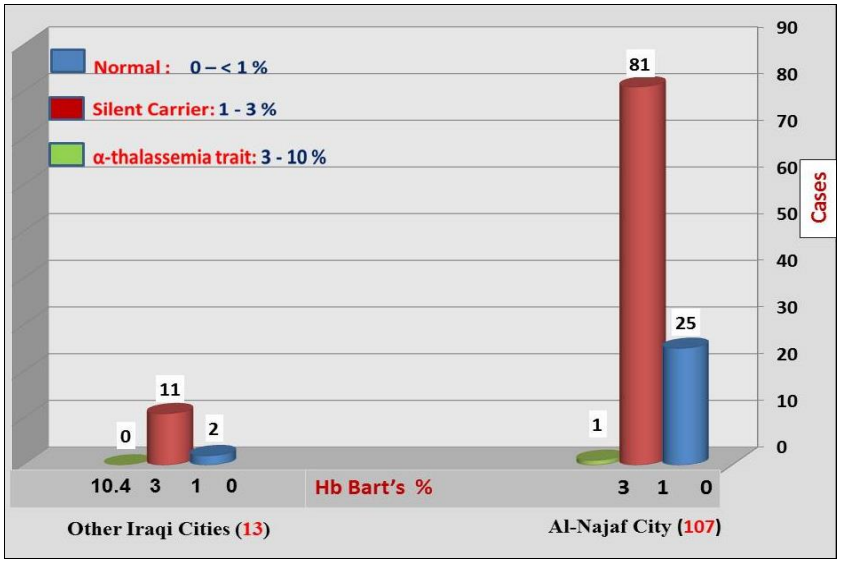

Fig 4: The percentage of Hb Bart's in the Al-Najaf city and other cities of Iraq.

Figure (5) clarify that 100 cases (83.3%) from urban area, of whom 78 cases (78%) had Hb Bart's >1%, on the other hand 20 cases (16.7%) from rural area of whom 15 cases (75%) had Hb Bart's >1%, there is a significant difference (p value = 0.03) of urban group.

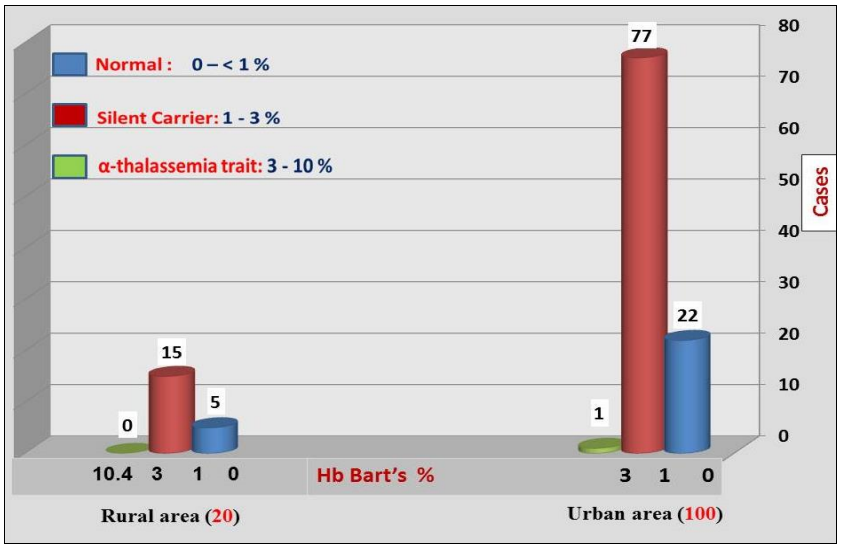

Fig 5: The percentage of Hb Bart's in the urban and rural areas.

From total of 120 cases, there was 75 cases (62.5%) of newborns had healthy mothers, of whom 56 cases (74.67%) had Hb Bart's > 1% while 45 cases (37.5%) of newborn had mothers with disease, of whom 37 cases (82.78%) had HbBart's >1%, Hypertensive mother were 21 cases, of whom 18 cases (85.7%) had newborn with Hb Bart's ≥ 1%, while only 3 cases (14.3%) had newborn with normal Hb Bart's < 1% as shown in table (1).

Table 1: Show the percentage of HbBart's according to mother's history of the disease.

	Mother and Disease	N (< 1%)	1 - 3%	3-10%	Total
1	No disease	19	55	1	75
2	Hypertension	0	9	0	9
3	UTI	0	2	0	2
4	Asthma	3	5	0	8
5	Anemia	0	3	0	3
6	Oligohydramnios	0	1	0	1
7	Hydatid cyst	0	1	0	1
8	DM	0	3	0	3
9	Urticaria	0	1	0	1
10	Hypertension + Asthma	3	4	0	7
11	Hypertension + D.M	0	2	0	2
12	Asthma + Urticaria	0	1	0	1
13	Goiter	0	1	0	1

14	Hypertension + Arthritis	0	1	0	1
15	Asthma + UTI	0	1	0	1
16	Disc Prolapse	1	0	0	1
17	Hypertension + anemia	0	1	0	1
18	DM + Asthma	1	0	0	1
19	DVT + Hypertension	0	1	0	1
20	Total	27	92	1	120

Discussions

Our study demonstrate that 92 cases (76.67%) of newborn infant have Hb Bart's 1-3% and 1 case (0.83%) have Hb Bart's 3-10%, this could indicate a genetically determined defect of α-chain synthesis, that is α-thalassemia prevalence is highest among Laotians and Cambodians and is also found among African [10], Chinese [11], Filipino, Vietnamese, and Thai persons [12], as well as among those with Middle Eastern ancestry [13]. The Mediterranean and Arab countries are considered high risk areas for thalassemia in general and for *α*-thalassemia in particular where alpha-thalassemia carrier frequency can vary among countries to reach the highest in UAE, Oman, and Saudi Arabia with a 50% carrier rate [14].

However, the presence of Hb Bart's in cord blood samples has been reported from different parts of India, and the prevalence of α-thalassemia has ranged from 1% to high frequency (18%) [15], the results have showed in certain communities by different electrophoretic techniques is less common as compared with neighboring in South East Asian Countries like China and Taiwan [16]. Hence, our result is higher than this range; this may indicate different sensitivity of technique using cation exchange HPLC performance, which is suggested to be more sensitive in detection of Hb Bart's [17]. Alpha-genotyping was done in India by southern blot hybridization in 24 cases who had shown variable levels of HbBart's at birth , where 7 of 24 cases (29.17%) showed no correlation between Hb Bart's level and α-genotypes [18].

Children with about 25% HbBart's at birth developed HbH disease and those with 3-10% HbBart's showed hematological finding such as microcytosis with α-thalassemia carrier state [19]. These results may indicate that the group of newborn with HbBart's ranging from 1-3% may include normal subject α-thalassemia carrier later on in childhood, although mild anemia can occur in some instances, like increased stress or pregnancy. These patients can pass on the mutation to their future progeny, which may or may not manifest as the thalassemia disease state [20].

Our finding may indicate that elevated level of HbBart's in group Al-Najaf population are due to the presence of α-thalassemia gene in order to clarify this issue more precisely, genetic study need to be done for those neonates, while the rarity of HbH disease and hydrops fetalis despite the intense in breeding in our population, points to an α-thalassemia genotype that is, in terms of phenotypic expression, intermediate between heterozygous state for α-thalassemia -1 and HbH disease, possible molecular basis for this genotype is suggested. Alternatively, our results can be explained by relative sensitivity by method used for detect of HbBart's and different methods of detection of HbBart's must be studied to clarify this issue [21].

In our study, we showed high incidence of Hb Bart's >1% in newborn of no consanguinity and urban area (p value= 0.02 and 0.03 respectively) that may go with most α-thalassemias are due to deletion mutations, our results supported by study of Chantal Farra *et al*. [22].

Our study showed the diseased mothers with hypertension especially in the presence of diabetes mellitus associated with of elevated Hb Bart's, this result is in accordance with Kwan WY *et al*. study [23], which observed Maternal complications are also common among women who carry a fetus with hydrops fetalis, Severe preeclampsia can occur in 30% of the mothers who are coexisting with hydrops fetalis.

Our study show no difference in expression of Hb Bart's percentage, in either female and male, which goes well with the recessive inheritance of the disease, these findings agreed with Chi Kong Li *et al*. study [24] which had been reported no difference in the prevalence of thalassemia between both sexes.

Conclusion

1. More study by different electrophoretic methods need to be clarifying the excat percentage of the carrier state of HbBart's.
2. Mother with hypertension during pregnancy are associated with higher rates of HbBart's, need to be carefully follow up and assess for expectation of higher rate of having children of Hb Bart's.

Conflict of Interest

Not available

Financial Support

Not available

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