Original Article

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Prevalence of microcytosis in cord blood samples and admitted neonates in Tehran, Iran

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Abstract

Background: The study was established to define the prevalence of neonatal microcytosis and to estimate the incidence rate of alpha-thalassemia as its leading cause, in Tehran, Iran.

Materials and Methods: Overall, 800 neonates were selected from two populations of newborns and admitted neonates. Three hundred and sixty-one cord blood samples were obtained from deliveries in Mahdieh Hospital in April and May 2013. A second group of 439 neonates aged 1-5 days were subject to the study from admissions to the neonatal ward and neonatal intensive care units in Mahdieh Hospital between March 2011 and August 2014. All the included neonates were term, single, with normal birth weights. The admitted neonates suspected to have hemolytic anemia were excluded from the study. Microcytosis cut-off point was set at 95 fl.

Results: Prevalence of microcytosis was 2.5% in cord blood samples and 7.7% in admitted neonates. The admitted neonates showed a 3.28-fold higher risk of microcytosis compared to newborns. The average mean corpuscular volume was 104.6 ± 4.5 fl in newborns and 103.2 ± 6.0 fl in the admitted neonates. The admitted neonates had smaller and lower numbers of erythrocytes with higher mean corpuscular hemoglobin.

Conclusion: Prevalence of neonatal microcytosis was lower than expected in healthy newborns based on some previous studies, and therefore, alpha-thalassemia carriership as the main leading cause of neonatal microcytosis does not appear to be an urgent issue for mass screening to be considered. Selective screening should be taken into account as a more cost-effective option in neonatology wards.

Key words: Alpha-Thalassemia, Erythrocyte Indices, Neonatal Screening

Introduction

Alpha-thalassemia is an autosomal recessive disorder which is thought to be the most common monogenic gene disorder in the world(1). Mild forms of alpha-thalassemia are frequent across the tropical regions. However, severe forms are more prevalent in Southeast Asia and certain Mediterranean populations(2). In some of these populations, the carrier frequency of alpha-thalassemia even reaches 80-90%(1). High rates of carriership along with immigration and changes in demography is making this hemoglobin disorder a global concern. There is not sufficient data about the exact distribution of the disease in Iran. However, literature suggests a high prevalence of alpha-thalassemia and its gene deletions in neighboring countries especially around the Persian Gulf region(3, 4). Neonatal microcytosis is confirmed to have high sensitivity and specificity in high prevalent areas according to some published studies(5, 6). Different cut-off points have been introduced. This study was established to define the prevalence of neonatal microcytosis and to estimate the prevalence of alpha-thalassemia as the

main leading cause of neonatal microcytosis, in Tehran.

Materials and Methods

Participants

Two populations of newborns and admitted neonates were subject to the study. A group of newborns were selected as the first group from all the deliveries and cesarean sections in Mahdieh Hospital, located in Tehran, in April and May 2013. A second group of neonates aged 1-5 days were selected from admissions to the neonatal ward and neonatal intensive care units (NICUs) in Mahdieh Hospital between March 2011 and August 2014.

Neonates were allocated consecutively from routine clients of the hospital. All the included neonates were single, term, and with normal birth weights (2500-4000gr). Neonates suspected to have hemolytic anemia during their admission were excluded from the study.

Study design

A cross-sectional study was carried out on two groups of newborns and admitted neonates. From the first group, cord blood samples were obtained at delivery. Complete blood counts (CBC) were immediately determined by Sysmex KX-21N Hematology Analyzer (Sysmex®, Japan). Complete Blood Counts of the second group of admitted neonates were included in the study from hospital records.

The study design was approved by Ethics Committee of the Research Center in accordance with the Declaration of Helsinki, and parents were provided written informed consent to participate.

Statistical Analysis

A sample size of 811 was calculated to provide a microcytosis prevalence of 5% with a statistical power of 95%, and a Type I error of 0.05. Microcytosis cut-off point was set at 95 fl. Data were analyzed using SPSS software. A P-Value of less than 0.05 for a two-tailed interpretation was considered statistically significant. Blood indices as well as birth weight were described as mean ± standard deviation and frequencies were reported as percentages. Means of blood indices in different groups were compared using Independent-Samples T test, and Levene's test was used for comparing variances. Chi-Square test was used in analyzing nominal data and Odds Ratio was reported if possible. Normal distribution of variables was tested using One-Sample Kolmogorov-Smirnov Test.

Results

Eight hundred cases, including 343 females (43.1%) and 452 males (56.9%) were studied. Data for gender of 5 neonates were missing. Mean birth weight was 3159 ± 382 g. 361 neonates including 182 females (51%) and 175 males (49%) were studied as the first group. The second group included 439 admitted neonates including 161 females (36.8%) and 277 males (63.2%).

Diagnosis among the admitted neonates was mostly consisted of respiratory problems such as respiratory distress syndrome (41%), transient tachypnea of the newborn (17%), Meconium Aspiration Syndrome (4%) and Asphyxia (3%). Jaundice (15%), sepsis (9%) and seizure (2%) were other common diagnoses among neonates in the second group. However, neonates suspected to have hemolytic anemia during their admission were excluded from the study. Other nine percent had miscellaneous diagnoses during admittion.

The average mean corpuscular volume (MCV) was 104.6 ± 4.5 fl in newborns and 103.2 ± 6.0 fl in the admitted neonates. A significant difference was detected between MCV means of the two groups (P = 0.000).

Overall, 43 neonates were considered to have microcytosis including 9 neonates (2.5%) from the first group and 34 neonates (7.7%) from the second. The admitted neonates showed a 3.28-fold higher risk of microcytosis compared to

newborns in group I. Erythrocyte indices and Hematocrit (HCT) were significantly different between the two groups. The admitted neonates had smaller and lower numbers of erythrocytes with higher amount of mean corpuscular hemoglobin (MCH, Table I). The distribution of MCV is shown in each group in Figure 1. The Kolmogorov-Smirnov test was performed and the H0 hypothesis of normal distribution of MCV was accepted (P = 0.200). The distribution of MCV in each group was separately tested and proved to be normal. However, the second group displayed more kurtosis (Kg: 0.535>0.273).

Neonates with microcytosis appeared to have different RBC indices. A significant difference was found in RBCs, HCT, MCH and MCHC between the group with microcytosis and other neonates (Table I).

Cut-off for hypochromia was set at 30pg. The resulting prevalence was 2.2% (8 cases) in group I and 1.8% (8 cases) in group II. MCH distribution was normal in group I with a mean of 34.2 ± 1.9 pg (P = 0.200, Figure 2). However, the normality of MCH distribution in group II was rejected. The overlap between hypochromia and microcytosis occured in 2 cases (0.6%) in group I and 7 cases (1.6%) in group II (Table II). No correlation was observed between MCV levels and birth weights (P = 0.440). MCH and MCHC were different in male neonates and females (MCH P = 0.000, MCHC P = 0.011). No difference was detected in RBC, HGB, HCT, and MCV in male neonates and females.

Table I: Hematological characteristics of neonates in general, in group I (cord blood samples) compared to group II (admitted neonates), and in neonates with microcytosis compared to neonates with normal MCV (Mean ± Standard Deviation)

	Total (n=800)	Group I (n=361)	Group II (n=439)	*P-Value*	Neonates with microcytosis (n=43)	Neonates with normal MCV (n=757)	*P-Value*
RBC (10^6/µL)	4.2 ± 0.7	4.3 ± 0.5	4.2 ± 0.8	0.008	4.5 ± 0.6	4.2 ± 0.7	0.012
HGB (g/dL)	14.8 ± 2.5	15.0 ± 2.5	14.6 ± 2.5	0.060	14.3 ± 2.2	14.8 ± 2.5	0.224
HCT (%)	44.2 ± 6.1	45.4 ± 5.6	43.2 ± 6.3	0.000	42.1 ± 6.1	44.3 ± 6.1	0.024
MCV (fL)	103.9 ± 5.4	104.6 ± 4.5	103.2 ± 6.0	0.000	92.5 ± 2.6	104.5 ± 4.8	-
MCH (pg)	34.7 ± 2.1 33.3 ± 2.0	34.2 ± 1.9	35.1 ± 2.2	0.000	31.5 ± 1.7	34.9 ± 2.1	0.000
MCHC (g/dL)		32.6 ± 1.4	33.9 ± 2.2	0.000	34.0 ± 1.3	33.3 ± 2.0	0.024

RBC: Red Blood Cell, HCT: Hematocrit, MCV: Mean Corpuscular Volume, MCH: Mean Corpuscular Hemoglobin, and MCHC: Mean Corpuscular Hemoglobin Concentration.

Table III: Number of cases with microcytosis and hypochromia in group I (cord blood samples) compared to group II (admitted neonates)

	Group I (n=361)	Group II (n=439)	*P-Value*	Odds Ratio
Microcytosis	9 (2.5%)	34 (7.7%)	0.001	3.28
Hypochromia	8 (2.2%)	8 (1.8%)	0.407	-
Microcytic Hypochromia	2 (0.6%)	7 (1.6%)	-	

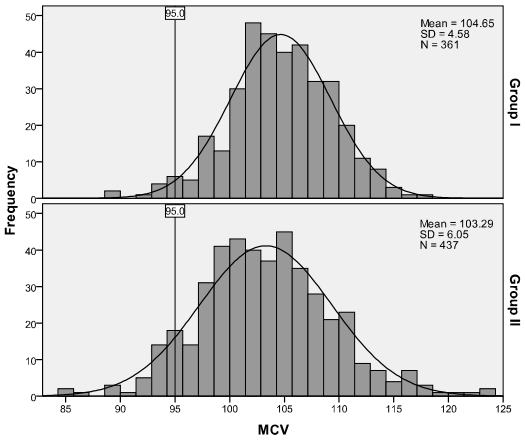

Figure 1. Distribution of MCV in group I (cord blood samples) compared to group II (admitted neonates)

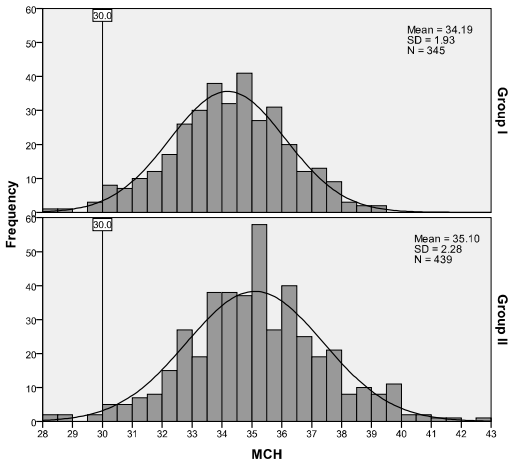

Figure 2. Distribution of MCH in group I (cord blood samples) compared to group II (admitted neonates)

Discussion

Two potential populations subjected to screening for alpha-thalassemia were studied. The mean MCV of newborns showed a statistically significant difference between the two groups. However, its clinical importance is under question. The admitted neonates showed a 3.28-fold higher risk of microcytosis compared to newborns. The higher risk is better explained by the distribution kurtosis than by the mean shift. Therefore, it brings a hypothesis of protective effects of medial MCVs against diseases and following admission in the early days.

Hypochromia is also thought to be benefitial for the screening of alpha-thalassemia. However, this criterion showed little overlap with microcytosis (0.6% of the total population), meaning that it targets a different population compared to microcytosis for further evaluation. MCV and MCH are calculated from HCT and HGB by the CBC counter used in the study rather than being measured. Therefore, technical and physiological factors should be examined as potential confounders of this difference.

Since many people from different ethnicities and social levels are referred to Mahdieh hospital, this obstetrics and gynecologic center can be considered as a reliable representative of the country. Reference ranges for erythrocyte indices was determined in the term newborns as well as the admitted neonates. The fifth percentile for cord blood samples of newborns were 96.95 fl for MCV and 30.73 pg for MCH. These values were 94.0 fl and 31.30 pg in CBC samples obtained from the admitted neonates of less than 3 days old.

Although admitted neonates in the second group were primarily decided to have a maximum age of 3 days, 23 of them were 4 and 5 days old to comply with the sample size requirements. The difference in MCV means between the two groups remained significant after omitting 4- and 5-day old neonates. To avoid higher MCV measures following the release of reticulocytes, neonates suspected to have hemolytic anemia during their admission were excluded from the study.

To control the possible effect of gestational age and intrauterine growth retardation on the erythrocyte volume, all selected neonates were term with normal birth weights (2500-4000g). Twins were not included in the study to prevent possible twin-to-twin hemorrhage leading to iron deficiency anemia.

Current studies suggest a high prevalence of alpha-thalassemia and its gene deletions in countries of the Persian Gulf region(3). A 33% prevalence of elevated Hb Bart's levels was reported in cord blood samples in southern regions of Iran. The study suggested a high prevalence of α+-thalassemia carriership (91%). However, no α0-thalassemia alleles in cis form (--/αα) were found among 78 samples with microcytosis(7).

Of cord blood samples studied in Shiraz (8), 2.3% were positive for Hb Bart's. A new screening criterion of MCV ≤ 96fl was introduced with a sensitivity of 78% and a specificity of 93% by means of ROC Analysis. The suggested cut-off point of MCH for the screening of alpha-thalassemia was 31pg. However definite diagnosis of alpha-thalassemia was based on electrophoresis in the analysis(8). In northern parts of Iran, Hb Bart's was detected in 10.1% of cord blood samples(9). It was suggested that 25% of the neonates with Hb Bart's had MCV>100(10). In another study of cord blood samples in the north of Iran, 15.29% of samples (CI 95%: 11.81-18.77) were reported to have at least one of five prevalent mutations in alpha-thalassemia(9).

Although many studies have been performed to discover deletional and nondeletional alpha-gene mutations in Iran, most of them have included suspected patients as their participants. Therefore, accurate data about the

prevalence of alpha-thalassemia in general population is not available. A 100% sensitivity of MCV≤95 is reported in eastern Asia for the diagnosis of alpha-thalassemia. However, its use for carriers of single α-globin gene defect is discouraged(5, 6).

Overally, it can be inferred from low rates of neonatal microcytosis that the dominant forms of defected alleles are in trans form (-α) or of non-deletional type (αTα) leading to more minor forms of alpha-thalassemia (-α/αα, αTα/αα). However, along with consanguineous marriage, higher prevalence of the disease is reported from southern areas with its genotype mostly in trans form (-α/-α) and is usually presented by borderline or low blood indices.

Conclusion

Since neonatal microcytosis has been suggested to have high sensitivity and specificity, this study was an attempt to estimate the current prevalence of the disease. It can be inferred from the present data that the current alpha-thalassemia gene frequency should be about 3-5%, which is near to half of the carrier rate and the emerging need for mass screening of alpha-thalassemia is therefore eased. Considering the higher risk of microcytosis among the admitted neonates, selective screening is a more cost-effective option in neonatal wards and NICUs which should be further studied.

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Conflict of interest

The authors have no conflict of interest to declare..

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