Original Article

DETERMINATION OF FETAL HEMOGLOBIN LEVELS BY CORD BLOOD SAMPLING AND ITS ASSOCIATION WITH VARIOUS INFLUENCING PARAMETERS

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ABSTRACT

Objectives: To determine association between fetal hemoglobin levels and various maternal and infants' influencing parameters.

Material and Methods: This descriptive, cross-sectional study was conducted in department of hematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi from 30th March to 30th September 2017. Total 200 females (mothers) of age 20-45 years with gestational age of 25-45 weeks were included. Known parents with persistent fetal hemoglobin (Hb F) and both parents with beta thalassemia trait were excluded. The blood samples were taken under aseptic conditions from the umbilical cord of the fetus. The fetal hemoglobin levels were measured by HPLC (high performance liquid chromatography) (Bio Rad D10).

Results: Mean age of females was 29.26 ± 6.07 (±1 SD) years. Mean gestational age was 32.61 ± 4.09 (±1 SD) weeks. Out of these 200 infants, 129 (64.50 %) were male and 71 (35.50 %) were females, with male to female ratio of 1.8 : 1. Mean hemoglobin of mother was 11.01 ± 0.607 g/dl. Mean weight of infants was 3.32 ± 1.49 kg. Mean levels of fetal hemoglobin in infants from cord blood sample was 75.35 % ± 11.59.

Conclusions: This study showed no significant association between fetal haemoglobin levels in infants with age, haemoglobin level socioeconomic status of mother and also weight and gestational age of infant

Key Words: Fetal hemoglobin F, HPLC (high performance liquid chromatography), Infant, Cord blood.

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INTRODUCTION

Fetal hemoglobin (Hb F) is the main oxygen transport protein in fetus. After the first 10 to 12 weeks of gestation hemoglobin switches from embryonic hemoglobin to fetal hemoglobin. It persists in the newborn until roughly 6 months of age [1,2]. At birth Hb F comprises 50-80 % of the infant's haemoglobin [2]. Hb F has a slightly higher oxygen affinity, explained by low interaction of Hb F with 2,3-diphosphoglycerate (DPG) [3,4]. This characteristic makes the delivery of oxygen through placenta easier from mother's blood stream [4]. There are two physiological variations of fetal hemoglobin; Hb F 1 and acetylated HbF. Sum of both is calculated for total amount of HbF.

The percentage of fetal hemoglobin decreases with the increasing week of gestation. The higher percentage of Hb F is associated with male sex, low birth weight, non-Indo-European ethnic origin, twin delivery and cigarette smoking by the mother during pregnancy. Use of alcohol and drugs or vitamins by mother does not affect Hb F levels in fetus or new born. In a study conducted by Diana, *et al.*, (2001) the determinants showed HbF fraction percentages in males were 78.07 ± 7.01 and females were 76.62 ± 7.40 respectively [5].

In newborns, higher Hb F percentages were seen intra uterine growth retardation (IUGR), selected pregnancy complications and maternal weight gain of 9 kg or less. Higher percentages of Hb F were detected in newborns of mother with anemia, urinary tract infection (UTI), poor prenatal care, parity or multiple births [6,7].

The Hb F production rapidly decreases at birth and substituted by Hb A. The Hb F decreases to adult levels (less than 1%) within first two years of life [8,9]. The Hb F levels are increased in sickle cell anemia, sickle beta thalassemia and hereditary persistence of fetal hemoglobin (HPFH) in adults. Elevated levels of Hb F are also seen in leukemia, myeloproliferative disorders and embryonic tumors [11].

In a study conducted by Fagan DG, *et al*. in 1995 the data showed Hb F fraction percentages in preterm deliveries were found to be 80.5 ± 3.1, term

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deliveries 73.1 ± 5.8 and post term deliveries 69.9 ± 6.8 respectively [6].

MATERIAL AND METHODS

This was a descriptive cross-sectional study conducted in Department of Haematology, Armed Forces Institute of Pathology (AFIP), Rawalpindi. 30th March 2017 to 30th September 2017. The sample size was calculated by WHO calculator with a confidence level 95 %. The anticipated population mean was 76.62 ± 7.4. The sample size was 200, with absolute precision being 1.

The sampling was done by non-probability, consecutive technique. A total of 200 females (mothers) of age 20-45 years with Hb from <12.5 to >10 and parity from 1 to 5, and gestational age of infant being 25-45 weeks were included. Whereas, known parents with persistent Hb F and both parents with beta thalassemia trait were excluded.

Ethical committee approval was taken. The participants of the study included infants born to mothers at various gestational ages. Verbal and written informed consent was taken for usage of blood taken from umbilical cord of the infant/neonate was from mother. Age of mother was determined as per national identity card. The haemoglobin levels of mothers were determined by running the sample on Sysmyx K21 (automated blood analyzer). Parity was determined by answers given in questionnaire. Gestational age at the time of delivery was calculated by determining the first day of last menstrual cycle (LMP). The cord blood sample was run on the HPLC machine to determine the percentage of fetal hemoglobin (Hb) in it.

All the data was entered and analyzed by using SPSS version 21.0. The quantitative variables like gestational age, Hb F levels, Hb levels of mother, parity of female and weight of infant were presented as mean and standard deviation. The qualitative variables like gender, socioeconomic status and ethnicity were presented as frequency and percentage.

Effect modifiers like gender, gestational age, Hb of mother, weight of infant, socioeconomic status and ethnicity were controlled through stratification and post-stratification independent sample 't' test was applied to see their effect on outcome. P-value ≤ 0.05 was considered as significant.

RESULTS

Age range of females in this study was from 20 to 45 years with mean age of 29.26 ± 6.07 years. Majority of the females i.e. 117 (58.50 %) were aged between 20 to 30 years of age. Mean gestational age at time of delivery was 32.61 ± 4.09 weeks. Out of these 200 infants, 129 (64.50 %) were male and 71 (35.50 %) were females with male to female ratio of 1.8: 1was seen.

Mean hemoglobin of mothers was 11.01 ± 0.607 g/dl. Mean weight of infants was 3.32 ± 1.49 kg. Mean levels of fetal hemoglobin in infants from cord blood sample was 75.35 ± 11.59 as shown in Table-1.

Stratification of mean levels of fetal hemoglobin in infants from cord blood sample with respect to gestational age and gender of infant are shown in Table-2 & 3 respectively. Table-4 & 5 have shown the stratification of mean levels of fetal hemoglobin in infants from cord blood sample with respect to gestational age and Hb of mother. Stratification of mean levels of fetal hemoglobin in infants from cord blood sample with respect to weight of infant, socioeconomic status and ethnicity are shown in Table-6, 7 & 8 respectively.

Table-1: Mean levels of Fetal hemoglobin in infants from cord blood sample.

	N	Min	Max	Mean	Std. Deviation
Fetal hemoglobin	200	56.00	99.00	75.35	± 11.59

Table-2: Stratification of mean fetal haemoglobin with respect to age of mothers

Age groups	Fetal haemoglobin (%age of total Haemoglobin)		P-value
	Mean	SD	
20-30	74.80	± 11.38	
31-45	75.74	± 11.77	**0.578**

Table-3: Stratification of Mean Fetal Haemoglobin with respect to Gender

Gender	Fetal haemoglobin (% of total Haemoglobin)		P-value
	Mean	SD	
Male	74.31	10.72	
Female	77.24	12.90	**0.087**

Table-4: Stratification of mean fetal hemoglobin with respect to gestational age

Gestational age	Fetal hemoglobin		P-value
	Mean	SD	
25-32	75.44	± 11.29	
33-41	76.87	± 11.82	**0.383**

Table-5: Stratification of Mean Fetal Hemoglobin with respect to Hb of Mother

Hb of mother	Fetal Haemoglobin (% of total Haemoglobin)		P-value
	Mean	SD	
10.1-11.0	75.01	11.32	**0.735**
11.1-12.5	75.58	11.82	

Table-6: Stratification of Mean Fetal Haemoglobin with respect to Weight of Infant

Weight of infant (kg)	Fetal Haemoglobin (% of total Haemoglobin)		P-value
	Mean	SD	
≤3	76.68	12.01	
>3	75.92	10.93	**0.656**

Table-7: Stratification of mean fetal haemoglobin with respect to socioeconomic status.

SES	Fetal Haemoglobin		P-value
	Mean	SD	
Poor (< 20,000/ month)	73.92	11.76	**0.372**
Middle (20,000-100,000/ month)	75.62	12.00	
Upper (>100,000/ month)	77.26	10.33	

Table-8: Stratification of Mean Fetal Haemoglobin with respect to ethnicity.

Ethnicity	Fetal Haemoglobin (% of total Haemoglobin)		P-value
	Mean	SD	
Punjab	76.07	11.28	**0.558**
Sindh	71.04	10.82	
Kashmir	76.75	10.71	
Gilgit Baltistan	76.93	10.36	
KPK	75.79	13.38	
Baluchistan	73.89	12.30	

DISCUSSION

HbF is the primary protein for oxygen transport in the developing fetus. Hemoglobin production naturally shifts with advancing age from HbF to HbA such that HbF reserves are typically depleted by six months of age and while residual amounts of HbF continue to be synthesized in adult erythropoiesis, the majority of adults have < 1 % HbF [12]. It is known that HbF decreases gradually during the last trimester of fetal development, and premature babies have higher percentage of HbF than full-term neonates [13]. This study was conducted to determine the relationship of levels of fetal hemoglobin in infants with various parameters like age, haemoglobin level, socioeconomic status of mother and also weight, gestational age of the infant, in our population.

Age range in this study was from 20 to 45 years with mean age of 29.26 ± 6.07 years. Majority of the females i.e. 117 (58.50 %) were between 20 to 45 years of age. Mean gestational age was 32.61 ± 4.09 weeks. Out of these 200 infants, 129 (64.50 %) were males and 71 (35.50 %) were females with male to female ratio of 1.8:1. Mean levels of fetal hemoglobin in infants from cord blood sample was 75.35 ± 11.59. In a study conducted by Diana *et al.* in (2001), the determinants showed that males have 78.07% ± 7.01 and females have 76.62 % ± 7.40 of HbF from total Haemoglobin [5].

Studies have revealed that HbF is usually replaced by Hb A in red blood of infants at about 6 months [18]. However, the exact time of change of HbF to HbA may vary and the signal that determines the switch from fetal to adult hemoglobin is not known. Very small quantities of HbF < 0.5 %, have been detected in the blood of some adults. HbF may be raised in certain conditions such as childhood anemia, myeloid leukemia, hereditary persistence of fetal haemoglobin and sickle cell crisis [14,15]. It is not clear how HbF concentration will affect the severity of crisis in sickle cell individuals and in sickle cell variants.

Giulian *et al*. (1987), reported startlingly high values of percentage fetal hemoglobin (% HbF) in infants dying from cot death [16]. Fagan and Walker presented similar findings, showing raised values of % HbF in sudden infant death syndrome (SIDS) but only when an appropriate gestationally matched control population was used. Two significant differences between the postnatal decline in % HbF in preterm and full-term infants were also reported [17].

A study was devised to determine whether levels of fetal hemoglobin (HbF) synthesis are elevated in infants with bronchopulmonary dysplasia (BPD) when compared with the levels of HbF synthesis found in normal control infants. Twelve infants with BPD, whose postconceptional ages ranged from 40 to 62 weeks were studied. The mean (±SD) gestational age and birth weight was 29 ± 1.9 weeks and 1289 ± 376 g, respectively. The results demonstrated that the mean (±SD) level of HbF synthesis in infants with BPD was significantly higher than that in the control infants (42.6 ± 22.9 % vs 18.8 ± 12.8 %; P < .01) [18].

In a study hematological parameter in newborn umbilical cord blood samples (n = 476) collected at the Hospital Provincial del Centenario, Rosario, were studied [19]. They were divided into 3 groups: (I) full term newborns with weight according to gestational age; (II) low weight and normal gestational age; (III) preterm newborns. Decreased Hb concentration ($p < 0.05$) and increased MCV ($p < 0.01$) were observed in preterm newborns in comparison with normal ones, and a slight PCV increase and RBC values in low weight newborns compared to the control group ($p < 0.05$). Electrophoretic pattern was with the following Hb F values 66.3 +/- 6.8%, and Hb A2 0.45 +/- 0.3% in

group (I), with a significant increase of Hb F in 30-35 weeks preterm newborns [20].

CONCLUSION

This study concluded that mean levels of fetal hemoglobin in infants from cord blood sample was 75.35 % ± 11.59. These levels were influenced by gestational age of infant. The study provided the reference range of fetal haemoglobin according to geographic distribution of Pakistani population.

According to our study, there is no significant association found between fetal haemoglobin levels in infants with age, haemoglobin level, socioeconomic status of mother and also weight and gestational age of the infant.

AUTHORS CONTRIBUTION

Sarah Fatimah: Principal investigator
Asad Mahmood, Nadir Ali, Hamid Saeed Malik, Helen Mary Robert: Literature review, finalizing manuscript
Muhammad Tahir Khadim: Overall supervision

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