Haemogiobin A_2 Levels - Reference Values in Healthy Pakistani Adults

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Abstract

Hemoglobin A_2 (HbA_2) was quantitated in blood from 212 healthy Pakistani adults with an age range of 16 to 60 years. HbA_2 level was observed in the range of 1.20 to 3.80% with mean ± SD as 2.67 ± 0.66% for both sexes. The mean level for males and females was 2.67 ± 0.66% and 2.66 ± 0.69% respectively. These values showed no significant difference ($P > 0.1$) among both sexes. The coefficient of variation (CV) was fairly high on a single sample within this range (i.e., 24.7% for combined sex; 24.62% for males and 25.93% for females). Other hematological parameters did not show significant variation within the groups. Predicted normal values of HbA_2 will be of great help in assessing conditions with abnormal HbA_2 levels (JPMA 34: 339, 1984).

Introduction

Hemoglobin A_2 (HbA_2) first demonstrated by Kunkel and Wallenius[1] is an adult hemoglobin component normally present in hemolysate made from human erythrocytes in variable concentrations. Two a chains and two chains are found in HbA_2. The a chains are identical to those of HbA, the normal adult hemoglobin most abundant in hemolysates (i.e.; > 95%). Delta chains are similar to but distinct from B chains of HbA, by a minimum of 10 of its 146 aminoacid residues. In alkaline buffers, HbA_2 has an electrophoretic mobility much slower than that of HbA but very close to the mobiities Hb's C, E and O[2]. The proportion of HbA_2 in the cord blood in-dicates an average value of 0.2%[3]. Following birth the proportion of HbA_2 increases steeply in the first six months and slowly thereafter, although the mean levels are still increasing at the end of third year[4]. Since HbA_2 is an adult hemoglobin, its concentration might be expected to rise in proportion to adult hemoglobin and hence inversely with the fall of HbF. The absolute values of HbA_2 show steady increase with age[5]. Therefore the values are low in infants and adults with chronic anemia. The normal values not only depend on the method used but also vary in different laboratories[6]. The value between 1.6 to 4.0% is considered as normal by various workers.[7,12] So far we have been refering to the values quoted by most of the western workers. The present study was conducted to estimate the values among our population and help the physicians in their clinical evaluation of cases having abnormal HbA_2 levels.

Material and Methods

Two hundred and twelve subjects (173 males and 39 females) were selectedin this study. The percentage of HbA_2 was quantitated using a method that involves elution of hemoglobin bands

following electrophoresis on cellulose acetate strips[13-15]. The details are as below:-

Collection of Blood Samples

5.0ml blood was collected in a vacutainer containing 0.1 ml of 10% solution of EDTA as an anticoagulant. The vial was gently rolled to ensure thorough mixing of the blood with the anticoagulant.

Preparation of Hemolysates

Anticoagulated blood was centrifuged at 2000 RPM for 10 minutes. The Plasma was removed and erythxocytes were washed three times in an equal volume of 0.85% NaCl solution. The supernatant was removed after the. final washing. Next, an equal volume of distilled water and 1/2 volume of toluene was added, the mixture was shaken vigorously for 10 minutes and then centrifuged at 2500 RPM for 10 minutes. The top layer of toluene and stroma was discarded. The red hemolysate was filtered through two layers of Whatman. I filter paper. It's hemoglobin concentration was checked by cyanmethemoglobin method and adjusted between 9 to 10 g/dl by adding distilled water. The hernolysate was then stored at 4°C ready for electrophoresis performed within 24 hours after preparing the hemolysates.

Reagents

A stock solution of Tris EDTA borate (TEB) buffer (pH 8.6 to 9.0) was prepared by mixing 55 g of THAM (Tris hydroxymethylaminornethane), 7 g of EDTA (ethylene diamine tetraacetic acid) and 9 g 'of boric acid in distilled water to a final volume of one litre. The working buffer was made by mixing 3 dl stock solution with 6 dl distilled water.

Electrophoresis

Cellulose acetate strips (Gelman), 2.54 x 15.24 cm were soaked in the working buffer for at least 30 minutes before use. A 3.5 dl volume of working buffer was poured into each side of a Gelman electrophoresis chamber. The cellulose acetate strips were removed from soaking and gently blotted with filter paper. Using Gelman applicator 4 ul hemolysate was applied to the centre of each strip. These strips were then placed in the electrophoresis chamber and kept in position with magnets. The centre of the strip was kept equidistant from the electrodes. Electrophoresis was run for 90 minutes at 300 volts and 1.0 to 1.5 milliamps per strip. The strips were cut with scissors midway between major hemoglobin component (HbA) and slower moving minor hemoglobin component (HbA2). The small band of HbA_2 and large band of Hb_2 were then eluted in 2.0 ml and 6.0 ml working buffer respectively for 20 minutes at 25°C. After the elution, the absorbance of these hemoglobin solutions were read at a wave length of 410 mn in a spectrophotometer.

Result

The values of HbA_2 in either sex ranged between 1.20 and 3.80% with a mean S.D. of 2.67 ± 0.66%.

Calculation:-

$$\%HbA_2 : \frac{\text{Absorbance of } HbA_2}{(\text{Absorbance of HbA} \times 3) + \text{Absorbance of } HbA_2}$$

The C.V. was 24.71% in a single subject and the mean values for males and females were 2.68 ± 0.66

with CV of 24.62% and 2.66 ± 0.69 with CV of 25.93% respectively (Table 1 and Fig 1).

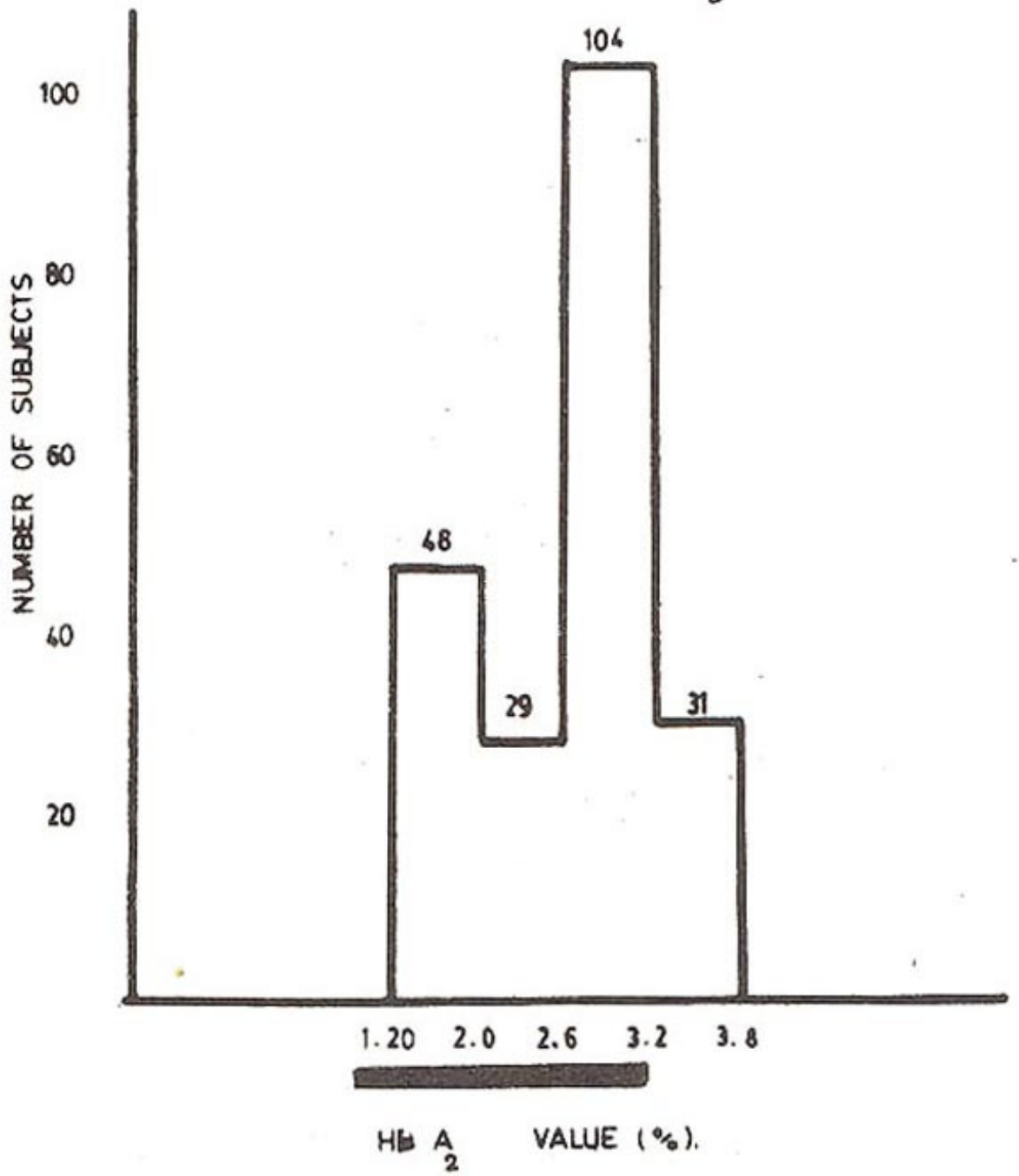
Table–I

Statistical Values of HbA_2 (Percentage).

	Sex Combined	Males	Females
n	212	173	39
$\bar{X}$	2.6723	2.6814	2.6602
SD	0.6614	0.6604	0.6901
SE	0.0454	0.0502	0.1105
CV	24.71%	24.62%	25.93%

n = Total number
$\bar{X}$ = Group mean
SD = Standard deviation
SE = Standard error
CV = Coefficient of Variation

FIG. 1. NUMBER OF SUBJECTS REPRESENTING Hb A_2 (%) IN MALES & FEMALES.

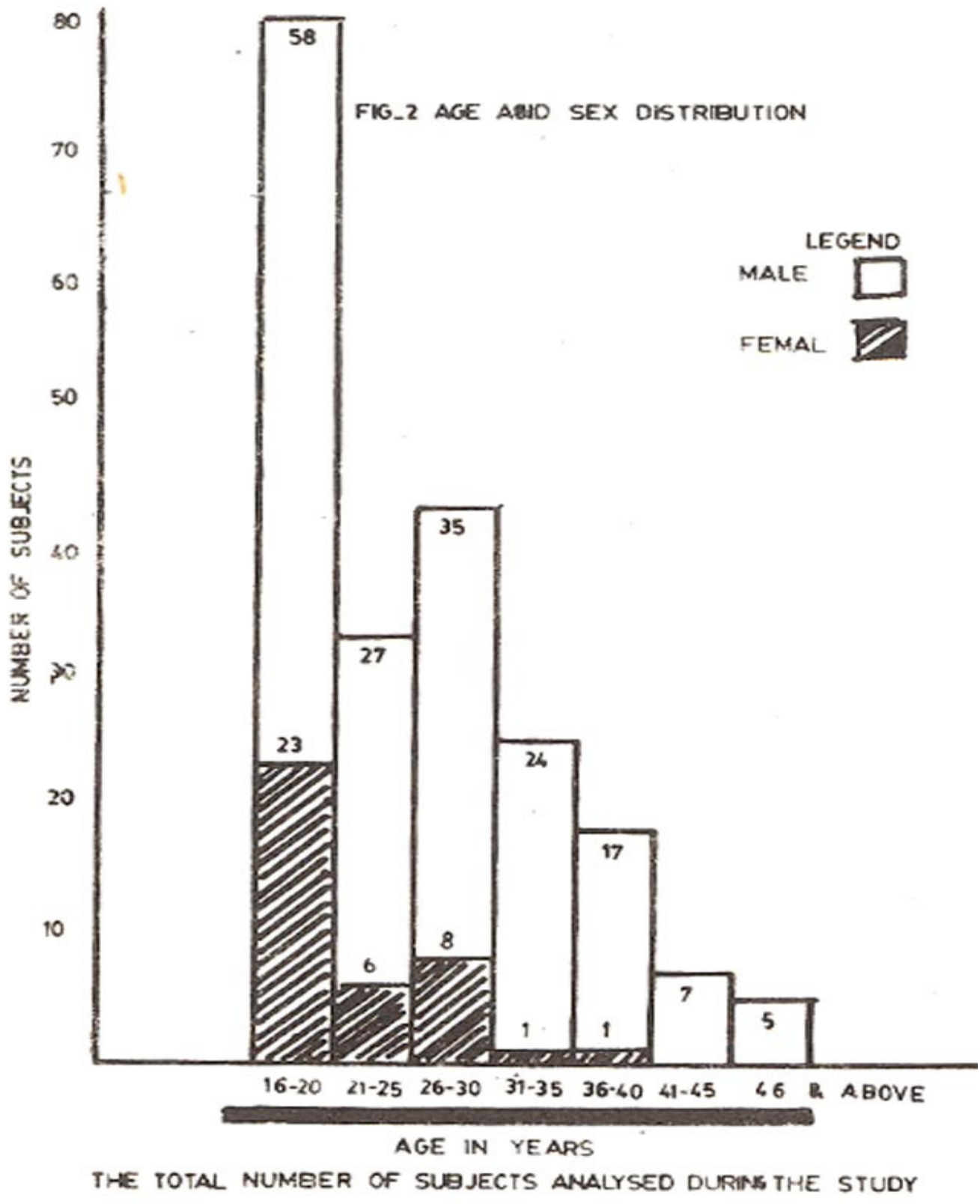


The mean values of HbA_2 in adult subjects. between the ages of 16 and 60 years in this study did not differ significantly ($P < 0.1$) (Table II and Fig 2).

Table–II Hematological Values for Both Males and Females (212) At Different Ages.

Age (Yrs)	No.	Hb (g/dl)		PCV (%)		RBC $10^6/\mu\ell$		MCV (fl)		MCH (pg)		MCHC (%)		HbA_2 (%)	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
16–20	81	14.8	1.6	46.0	3.9	5.4	0.7	85.1	7.9	27.4	3.3	32.1	2.1	2.64	0.63
21–25	33	14.8	1.7	46.2	4.1	5.5	0.9	84.0	10.9	26.9	3.6	32.0	2.1	2.61	0.64
26–30	43	14.6	1.7	45.8	4.2	5.4	0.9	84.8	9.2	27.0	2.6	31.8	2.5	2.67	0.67
31–35	25	14.8	1.6	45.8	3.2	5.3	0.5	86.4	8.0	27.9	2.9	32.3	2.6	2.77	0.82
36–40	18	14.7	1.7	45.7	4.3	5.2	0.7	87.8	8.5	28.2	3.2	32.1	2.4	2.81	0.65
41–45	7	13.8	2.2	45.7	5.6	5.2	0.7	87.8	8.7	26.5	2.8	30.1	2.5	2.70	0.64
46 +	5	15.6	1.2	47.8	2.8	5.7	0.9	83.8	8.3	27.3	5.5	32.6	3.3	2.78	0.58

X = Group Mean
SD = Standard Deviation.



Discussion

The values of HbA_2 obtained in this study co upare well with the results of other workers, who have

described a ranae between 1.40 to 3.80% with mean SD and 2.60 ± 0.51%[9] , 2.60 to 3.70% with mean SD 3.1 ± 0.4%[16] and a normal level of 2.80 ± 0.5% with a CV of 1 7.9%5

The mean values of HbA. in dulls in this study were sinutai to those reported by Ai pec in at al[17]

The similarity in the peresntage of HbA_2 between the male and femal subjects of this group (2.68 ± 0.69% and 2.66 0.69% respectively) corresponds with the observations inns of Aiperin et al[17] who reported a HbA^2 of 2.70 ± 0.38% in males and 2.64 ± 0.42% in termies in an age range of 2.89 years. However the age at which adult levels of HbA_2 are reached is of importance and is relevant for the differential diagnosis of conditions associated with abnormal HbA_2 levels.

The results obtained in this study are comparable to the other published data of the referred workers, The little disparity observed may be due to the smaller number of control subjects used by others. As a large number of subjects of both sexes have been studied , the values of Hb_2 A reported here can be taken as standard values for normal healthy adults in Pakistan (Table Ill and IV).

Table–III Hematological Values for Males (173) at Different Ages.

Age (Yrs)	No.	Hb (g/dl)		PCV (%)		RBC $10^6/\mu\ell$		MCV (fl)		MCH (pg)		MCHC (%)		HbA_2 (%)	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
16–20	58	15.2	1.6	47.1	3.8	5.6	0.7	84.0	7.9	27.1	3.5	32.2	2.3	2.61	0.70
21–25	27	15.1	1.5	47.0	3.5	5.6	0.9	83.9	11.9	26.9	3.8	32.1	1.9	2.70	0.54
26-30	35	14.6	1.6	47.2	3.1	5.6	0.7	84.2	9.2	26.0	2.5	30.9	2.2	2.75	0.83
31–35	24	14.8	1.6	45.8	3.2	5.3	0.5	86.4	6.7	27.9	2.8	32.3	2.5	2.73	0.83
36–40	17	14.8	1.7	45.8	4.3	5.2	0.7	88.0	8.3	28.4	3.3	32.3	32.3	2.79	0.67
41–45	7	13.8	2.2	45.7	5.5	5.2	0.6	87.8	8.6	26.5	2.8	30.1	2.4	2.70	0.64
46+	5	15.6	1.2	47.8	2.7	6.7	0.8	71.3	8.2	23.3	5.5	32.6	3.3	2.78	0.58

X = Group Mean
SD = Standard Deviation

Table–IV Hematological Values for Females (39) At Different Ages.

Age (Yrs)	No.	Hb (g/dl)		PCV (%)		RBC $10^6/\mu\ell$		MCV (fl)		MCH (pg)		MCHC (%)		HbA_2 (%)	
		$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD	$\bar{X}$	SD
16–20	23	13.9	0.8	43.0	2.5	4.9	0.5	87.7	6.8	28.3	7.9	32.3	1.5	2.72	0.46
21–25	6	12.9	1.6	42.0	4.7	5.0	0.5	84.0	4.8	25.8	1.7	30.7	2.0	2.2	0.93
26–30	8	12.3	1.6	39.8	3.2	4.4	0.5	90.4	8.0	27.9	3.2	30.9	2.2	2.70	0.99
31–35	1	13.25	–	45.0	–	4.10	–	109.7	–	32.3	–	29.4	–	3.2	–
36–40	1	13.25	–	44.0	–	4.50	–	97.7	–	29.4	–	30.1	–	3.20	–

X = Group Mean
SD = Standard Deviation.

References

1. Kunkel, H.G. and Wallenius, G. New hemoglobin in normal adult. Sciena, 1955; 122: 288.
2. Lehmann, H. and Huntsman, R.G. Man's hemoglobins : North Holland publishing company Amsterdam, Chapter 1,58-60 and chapter 14, 273-275.
3. Wilson, M.G., Schroeder, W.A., Graves, D.A. and Kach, V.D. Hemoglobin variation in D-Trisomy Syndrome. N. Engl. J. Med., 1967; 277: 953.
4. Scnrt. B., Mason, K. P. aM Serjeant, G.R. The development of hemoglobin A2 in nornrnl Negko infants and sicksl cell disease. Br. J.Hematol.1978;39: 259.
5. Angelova. Gatevs, P. and Marmova, M. Hemo globin A2 in eativ chUcznwc1, .. Pediat,, 1978; 92 944.
6. Sehmids R.M and Brosious EM. Quantitation of niiio:rInc,~n A2; an interiaboratory study. ton. J. CA.. t-c;tflOt.. 1979: 71: 534.
7. Mialt J.B. Laboratory medicine; hematology. 4th Saiatiouis Mosby 1972; pp. 833, 846.
8. Efremoy, G.D. and Huisman. T.H.J. The Lab wcn tory diagccn'sis of tat hemoglobinopathies. Clin. Hernia ol., 1974; 3 : 527.
9. Morin, L,G. Rapid estimation of hemoglobin A2 without chromatogtapcy or electrophoresis. Clin.chern.;1975;21: 1490.
10. Reiss, R.F., Morse, E.E. and Michini, L.J. An improved method for the quantitation of A2 hemoglobin utilizing cellulose acetate electrophorssis and densitometry. Am. 3. dIm. Pathol., 1975;63: 841.
11. Garver, F.A., Jones, C.S., Baker, MM., Altay, G., Barton, B.P., Gravely, M. and Huisman, T.H.J.Specific radio-immunochemical identitication and quantitation of hemoglobin A2 and F. Am. J. Hematal, 1976; 1: 459.
12. Gottfried, EL., Walk, B. and Robertson, N.A. Reliable Estimation of HbA2 concentration by electrophoresis with densitometry. A m.J. Clin. Pathol., 1979;72: 415.
13. Bartlett, R.C. Rapid cellulose acetate electrophoresis. II, Qualitaive and quantitative hemoglobin fractionation. Gin. Chem. 1 965; 9 : 325.
14. Marengo-Rowe, A.J.Rapid electrophoresis and quantitation of hemoglobins on cellulose acetate J.Clin pathol., 1965;18:790.
15. Schmidt, R.M., Rucknagel, D. L. and Necheles, T.F. Comparison of methodologies for thalassemia screening by HbA2 quantitation. J. Lab. Clin. Med., 1975; 86 : 873.
16. All, M.A.M. and Schwertner, E. Hemoglobin A2 level; a proposed test for confirming the diagnosis of iron deficiency. Am. J. Clin. Pathol, 1975; 63: 549.
17. Alperin, J.B., Dow, P.A. and Petteway, M.B. Hemoglobin A2 levels in health and various hematological disorders. Am. J. Clin. Pathol., 1977; 67 : 219.