ORIGINAL RESEARCH

To evaluate the presence of hemoglobinopathies in patients of microcytic hypochromic anemia

[1]Dr. Neeta Kapoor, [2]Dr. Abhay Prakash

[1]Associate Professor, [2]Assistant Professor, Department of Pathology, NDMC Medical College, Hindu Rao Hospital, India

Corresponding Author
Dr. Neeta Kapoor
Associate Professor, Department of Pathology, NDMC Medical College, Hindu Rao Hospital, India

Received date: 10 March, 2024 Acceptance date: 01 April, 2024

ABSTRACT

Aim: To evaluate the presence of hemoglobinopathies in patients of microcytic hypochromic anemia. **Material and Methods:** This research included all patients who had microcytic hypochromic anemia and underwent HPLC owing to suspicion of having a hemoglobinopathy. The research eliminated those patients who had received blood transfusions during the last three months.There was a total of 956 individuals diagnosed with microcytic hypochromic anemia. Among them, 200 patients underwent HPLC and were included in the research group. **Results:** A total of 956 individuals were diagnosed with microcytic hypochromic anemia, of whom 200 were believed to have hemoglobinopathy and underwent HPLC testing. Out of a total of 124 instances, 62% were found to have an aberrant hemoglobin pattern, while the remaining cases showed a normal hemoglobin pattern. The results indicate that 30% of individuals are heterozygous for β Thalassemia, 2% are homozygous for β Thalassemia, 19.5% are heterozygous for HbS, 5.5% are homozygous for HbS, 2% are double heterozygous for HbS and β Thalassemia, 1% are heterozygous for HbE, 0.5% are homozygous for HbE, 0.5% are double heterozygous for HbE and β Thalassemia, 0.5% have HbJ Meerut, 0.5% are double heterozygous for HbS and HbE, and 38% have no genetic conditions (Normal).A typical chromatogram of a healthy adult typically displays mostly HbA0 (mean 84.21±3.75%), a modest proportion of HbA2 (mean 5.05±0.43%), and minimal amounts of HbF (mean 1.33±0.14%). The P2 and P3 windows were in a typical state.**Conclusion:** This area has a high occurrence of hemoglobinopathies, which suggests that giving iron supplements to all instances of microcytic hypochromic anemia without discrimination is pointless. Instead, there is a need for mass screening programs and genetic counseling.This work reaffirms that HPLC is a very effective and robust diagnostic method for directly identifying Hb variations.

Keywords: Hemoglobinopathies,Microcytic hypochromic anemia, HPLC

INTRODUCTION

Hemoglobinopathies refer to a collection of genetic illnesses that affect the formation of the hemoglobin molecule, either in terms of quantity or quality [1, 2]. The global prevalence of carriers with Hb diseases is estimated to be 269 million. Hemoglobin (Hb) anomalies are the most prevalent genetic disorders, impacting over 7 percent of the global population. Approximately 3% of the global population, which amounts to over 150 million individuals, has betathalassemia genes. Beta-thalassemia is the prevailing autosomal recessive monogenic condition in India, affecting almost 30 million individuals who possess the faulty gene. The carrier frequency of this disorder ranges from 3% to 17% [3].

It is essential for governments to spend substantial funds towards the diagnosis and treatment of people with hemoglobinopathies in order to significantly decrease illness and death rates. Prenatal screening for hemoglobinopathies and genetic counseling to prevent the occurrence of these conditions are now being implemented to a certain degree. India has a broad range of hemoglobinopathies [4,5]. Research conducted in India has shown that thalassemia major and minor are the most prevalent forms of hemoglobinopathies among affected individuals [6]. Minor hemoglobinopathies, including Hb Q India and Hb Lepore, are prevalent among the Indian population. Studies conducted in India have indicated a significant prevalence of homozygous and heterozygous variants of Hb S, as well as Hb S with β thalassemia.

The data from different regions of India align with the fact that β thalassemia is a significant hemoglobin disorder. High-performance liquid chromatography (HPLC) was used to identify mild hemoglobinopathy,

as indicated by previous findings [9-11].Sickling syndrome is a condition where red blood cells (RBCs) have a sickle shape due to the presence of HbS, which reduces their ability to deliver oxygen. The homozygous condition represents the most severe manifestation of the illness. These illnesses were mostly limited to certain geographic regions, religious groups, social classes, and ethnic communities, especially those with cultural practices that encourage marriage within their own group[2].

MATERIALAND METHODS

Cross-sectional research was carried out at the Pathology department, following the required consent from the Institutional Ethical Committee. This research included all patients who had microcytic hypochromic anemia (mean corpuscular volume < 80fL and mean corpuscular hemoglobin < 26pg for adults, and age and gender specific parameters for pediatric patients) and underwent HPLC owing to suspicion of having a hemoglobinopathy. The research eliminated those patients who had received blood transfusions during the last three months.

There were a total of 956 individuals diagnosed with microcytic hypochromic anemia. Among them, 200 patients underwent HPLC and were included in the research group. A comprehensive clinical history, examination results, and any pertinent investigation findings were documented. An Automated Hematology Analyzer (Sysmex XT 2000i) was used to conduct CBC. The Variant Hemoglobin Testing System (Variant II Beta Thalassemia Short Program, Bio-Rad Laboratories Inc., Hercules, CA, USA) was used to evaluate all samples according to the manufacturer's indicated experimental conditions. The peaks that were detected were matched with predefined "windows" established by the manufacturer based on certain retention time (RT) [12].

Statistical data analysis was conducted using Microsoft Excel 2012. The continuous variables were represented as the mean ± standard deviation (SD). The categorical variables were represented using frequencies and percentages. The data was tabulated using SPSS version 24.0, a statistical tool for social sciences.

RESULTS

A total of 956 individuals were diagnosed with microcytic hypochromic anemia, of whom 200 were believed to have hemoglobinopathy and underwent HPLC testing. Out of a total of 124 instances, 62% were found to have an aberrant hemoglobin pattern, while the remaining cases showed a normal hemoglobin pattern. Out of the 124 individuals exhibiting an aberrant hemoglobin pattern, 76 were male and 48 were female. All instances included individuals aged between 1 and 63 years, with an average age of 18.21 years. Table 1 displays the distribution of different types of genetic conditions in the population. The results indicate that 30% of individuals are heterozygous for β Thalassemia, 2% are homozygous for β Thalassemia, 19.5% are heterozygous for HbS, 5.5% are homozygous for HbS, 2% are double heterozygous for HbS and β Thalassemia, 1% are heterozygous for HbE, 0.5% are homozygous for HbE, 0.5% are double heterozygous for HbE and β Thalassemia, 0.5% have HbJ Meerut, 0.5% are double heterozygous for HbS and HbE, and 38% have no genetic conditions (Normal).

A typical chromatogram of a healthy adult typically displays mostly HbA0 (mean 84.21±3.75%), a modest proportion of HbA2 (mean 5.05±0.43%), and minimal amounts of HbF (mean 1.33±0.14%). The P2 and P3 windows were in a typical state.

The most often identified aberrant hemoglobin pattern was beta thalassemia trait, accounting for 60 (30%) cases. Hemoglobin A2 (HbA2) values ranging from 3.9% to 9% indicate the presence of beta thalassemia trait in a person who has no symptoms or just moderate anemia. The average Hb A2 in BTT patients in the current research was 5. Four instances (2%) were identified with beta thalassemia major. The average levels of HbF and HbA2 were 84% and 5% respectively.

In this research, sickle cell trait was the second most prevalent anomaly, accounting for 39 instances or 19.5% of the total. Hemoglobin S (HbS) is separated and collected in the S-window during the elution process, which occurs within a specific time range of 4.11-4.43 minutes. The average HbS level in individuals with sickle cell trait was 33.05±4.32%, while the average HbA0 level was 57.22±3.62% and the average HbF level was 3.06±0.33%. In individuals with sickle cell disease, the average level of fetal hemoglobin (HbF) was 17.91±2.21%, whereas the average level of sickle hemoglobin (HbS) was 75.43±3.38%.

Four instances (2%) of HbS - beta Thalassemia double heterozygous were observed. The mean levels of HbA2, HbF, and HbS were 4.57±0.54%, 14.06±1.11%, and 74.43±4.56%, respectively. One instance of HbS-HbE double heterozygous was identified, with an average HbA2 level of 27.87±0.33% and an average HbS level of 61.51±3.38%.

According to literature, sickle cell trait instances are identified when both HbA and HbS are detected, with HbA being greater than HbS. However, the normal range for HbS levels is between 37% and 46%. Nevertheless, the presence of alpha Thalassemia might result in decreased HbS levels. A comparative analysis of CBC parameters was conducted between individuals with sickle cell trait exhibiting HbS levels ranging from 37-46% and those with lower HbS percentages. Table 4 This data indicates that there were no significant differences in respect to hemoglobin and RDW. However, there were significant differences seen in terms of RBC count, MCV, and MCH.

Table 1: Spectrum of hemoglobinopathies

Hemoglobinopathies	Observed number of cases	%
$^{\beta}$Thalassemiaheterozygous	60	30
$^{\beta}$Thalassmiahomozygous	4	2
HbSheterozygous	39	19.5
HbS homozygous	11	5.5
HbS+$^{\beta}$Thalassemiadoubleheterozygous	4	2
HbE heterozygous	2	1
HbE homozygous	1	0.5
HbE-$^{\beta}$Thalassemiadoubleheterozygous	1	0.5
HbJ Meerut	1	0.5
HbS-HbEdoubleheterozygous	1	0.5
Normal	76	38
TOTAL	200	100

Table 2: RBC indices in various hemoglobinopathies

Hemoglobinvariants	HB (g/dL)	TRBC (million/µL)	MCV (fL)	MCH (pg)	RDW-CV (%)
$^{\beta}$Thalassemiaheterozygous	10.54±1.87	5.06±1.52	63.02±5.54	20.05±3.67	18.78±2.76
$^{\beta}$Thalassmiahomozygous	4.12±1.67	2.56±0.78	66.04±5.53	19.21±3.76	32.01±3.32
HbSheterozygous	10.56±1.79	4.95±0.87	67.24±5.78	22.03±2.32	18.43±3.23
HbS homozygous	7.98±1.14	3.52±1.05	70.36±5.45	23.32±1.98	20.22±2.54
HbS+$^{\beta}$Thalassemiadouble heterozygous	8.65±1.34	3.98±0.86	65.05±5.27	21.87±2.42	20.78±2.53
HbE heterozygous	9.44±2.46	4.66±0.94	63.12±5.89	19.12±2.86	19.21±2.42
HbE homozygous	7.56	3.07	60.22	22.05	19.04
HbE-$^{\beta}$Thalassemia	5.98±0.76	3.73±0.88	51.95±4.44	28.87±2.84	28.78±2.77
HbJ Meerut	8.65±0.33	3.94±0.12	66.77±3.39	22.96±2.21	17.89±2.53
HbS-HbEdoubleheterozygous	8.33±0.79	3.97±0.16	66.78±4.89	22.98±2.46	24.43±2.65
Normal	9.48±1.44	4.43±0.87	66.77±3.42	20.73±2.75	19.02±2.21

Table 3: Hemoglobin fractions in various hemoglobinopathies

Hemoglobinvariants	HBA0%	HBA2%	HBF%	HBS%	P3%
$^{\beta}$Thalassemiaheterozygous	84.21±3.75	5.05±0.43	1.33±0.14		
$^{\beta}$Thalassemiahomozygous	10.23±1.54	4.05±0.76	85.89±2.32		
HbSheterozygous	57.22±3.62	3.06±0.33	2.41±0.83	33.05±4.32	
HbS homozygous	1.94±0.78	2.87±0.39	17.91±2.21	75.43±3.38	
HbS+$^{\beta}$Thalassemiadoubleheterozygous	5.81±1.21	4.57±0.54	14.06±1.11	74.43±4.56	
HbE heterozygous	55.48±3.72	30.32±3.48	3.79±0.87		
HbE homozygous	3.11	83.01	2		
HbE-$^{\beta}$Thalassemia	8.39±1.21	63.99±3.37	16.96±1.22		
HbJ Meerut	65.38±3.63	2.54±0.89	5.41±0.52		23.32±2.55
HbS-HbEdoubleheterozygous	5.69±1.05	27.87±0.33	3.22±0.38	61.51±3.38	
Normal	86.98±4.48	2.61±0.58	0.91±0.11		

Table 4: Comparison of CBC parameters among sickle cell trait cases

HbS(%)	Hb	TRBC	MCV	MCH	RDW
20-39	10.27±1.46	4.97±0.46	70.04±4.43	22.65±3.83	18.77±2.11
37-46	10.87±2.67	4.63±0.76	71.89±3.39	24.31±3.69	17.21±1.98
P-VALUE	0.31 Notsignificant	0.01 Significant	0.02 Significant	0.01 Significant	0.12 Notsignificant

DISCUSSION

Anemia is a global public health issue that affects all regions of the globe. According to the World Health Organization (WHO), the worldwide impact of this condition is seen in 1.62 billion individuals [13]. Microcytic hypochromic anemia is the predominant kind of anemia seen in clinical settings. Multiple factors contribute to this condition, including a lack of iron and abnormalities in hemoglobin. Hemoglobinopathies refer to a collection of genetic illnesses that affect the formation of the hemoglobin molecule, resulting in either an abnormal quantity or quality of hemoglobin. Accurate laboratory tests are necessary to diagnose these illnesses, and HPLC has

been shown to be a fast, sensitive, specific, and reliable method [14,15]. A total of 956 individuals were diagnosed with microcytic hypochromic anemia, of whom 200 were believed to have hemoglobinopathy and underwent HPLC testing. Out of the total of 124 instances, which accounts for 62% of the sample, it was observed that they had an aberrant hemoglobin pattern. The remaining patients, on the other hand, exhibited a normal hemoglobin pattern. This conclusion is consistent with the research conducted by Balgir et al [16] in Odisha, which reported that around 65% of patients had hemoglobinopathies. Similarly, Baruah et al [17] also observed similar results.

Table 5: Incidence of various hemoglobinopathies

Hemoglobinopathies	Alam*etal*[18] (n=331)	Balgir*etal*[16] (n=1015)	Philip*etal*[19] (n=4335)	Raman*et al* [20] (n=788)	Bhagora*etal* [21](n=200)	Sarvaiya*et al*[22](n=2035)	Presentstudy (n=1260)
Sickle Heterozygous	15.4	29.8	1.24	15.1	3.5	4.71	19.5
Sickle homozygous	10.27	7.8	0.11	9.9	2.0	0.93	5.5
Betathalassemiaheterozygous	18.73	18.2	10.49	6.1	8.0	10.61	30
Betathalassemiahomozygous	2.11		0.55	0.89	2.0	-	2
Betathalassemiaintermedia	1.21	-	0.46	1.02	-	-	-
SickleBetathalassemia	23.00	1.7	0.48	2.79	0.5	0.68	2
Ehomozygous	1.51	0.9	0.46	0.76	-	0.04	0.5
Eheterozygous	0.91	0.3	0.85	0.12	-	-	1
E-B thalassemia	2.71	0.7	0.06	0.63	0.5	0.04	0.5
S-Edouble heterozygous	-	-	-	-	-	-	0.5
Dheterozygous	-	-	0.20	0.12	0.5	-	-
J Meerut	-	-	-	-	-	-	0.5

In Assam, a prevalence rate of 59.11% for hemoglobinopathies was recorded, however other studies reported lower rates of hemoglobinopathies. This may be attributed to the fact that the current research was conducted in a hospital setting and focused on a group of patients with anemia who were thought to have hemoglobinopathies. In contrast, previous studies with smaller sample sizes were conducted in the general community.

Out of the 124 individuals exhibiting an aberrant hemoglobin pattern, 76 were male and 48 were female.

The current investigation showed a clear dominance of males, with a ratio of 1.58 males for every female. An examination of existing literature demonstrates a diverse array of male to female ratios, indicating that there is no clear preference for either sex in hemoglobinopathies. The age range of all patients varied from 1 to 63 years, with an average of 18.21 years. Most instances of hemoglobinopathies manifest in early childhood, but in later years, their identification is mostly a result of chance discoveries or difficulties.

The current study found the following distribution of genetic conditions: β Thalassemia heterozygous 60 (30%), β Thalassemia homozygous 4 (2%), HbS heterozygous 39 (19.5%), HbS homozygous 11 (5.5%), HbS + β Thalassemia double heterozygous 4 (2%), HbE heterozygous 2 (1%), HbE homozygous 1 (0.5%), HbE- β Thalassemia double heterozygous 1 (0.5%), HbJ Meerut 1 (0.5%), HbS-HbE double heterozygous 1 (0.5%), and Normal 76 (38%).

Prior studies [21] on individuals with microcytic hypochromic anemia have shown that the most prevalent hemoglobin variations are beta thalassemia trait and sickle cell trait. Prior investigations conducted in Odisha [16,18,20] have shown that sickle cell syndromes are the predominant form of hemoglobinopathy. The increased occurrence of BTT in this research is likely due to the inclusion of only instances with microcytic hypochromic anemia in the study group.

The instances with BTT had red blood cells with the diagnostic features of microcytic hypochromia, with hemoglobin levels that were either normal or slightly decreased, and an elevated red blood cell count. The most crucial aberrant chromatogram result for diagnosis is an elevated A2 level. Nevertheless, cases characterized by borderline HbA2 levels need careful interpretation. The research used a range of 3.5-3.9% for borderline HbA2. HbA2 levels may be influenced by deficiency anemia, less severe types of Thalassemia, and the presence of delta thalassemia via co-inheritance. Genetic tests are essential and should be recommended in all situations when the diagnosis is uncertain [23].

Four instances (2%) had Beta Thalassemia significant. The majority of individuals had severe symptoms, including microcytic hypochromic red blood cells and a hemolytic blood picture, at a young age (under 10 years old). The hematological profile and HPLC results in patients with Thalassemia in this research showed a strong correlation with previous studies conducted in Odisha [20] and other locations in India

[24].

The prevalence of HbS is around 37-46% in individuals with the trait, 75-95% in those with homozygous HbS, and 60-90% in individuals with HbS-β thalassemias, depending on the existence of β0 or β+ [25]. The majority of individuals with the sickle cell trait were in the younger age range and had minor pallor or were mainly asymptomatic. The average hemoglobin level of these patients was 10.31 g/dl, and their mean corpuscular volume (MCV) was 67.12fL. The sickling test was positive in all instances, and the proportion of hemoglobin S (Hb S) was 33.12% with a standard deviation (SD) of 3.46.

Bhagora et al [21] and Sarvaiya et al [22] saw a microcytic hypochromic blood image in sickle cell patients, however other research [19,20,26] discovered normocytic normochromic red blood cells. The presence of a microcytic hypochromic blood picture in this geographic location is likely caused by either alpha thalassemia or iron shortage, both of which are common here.

A significant prevalence of iron shortage has been documented in Indian individuals with sickle cell disease [20]. Mohanty et al [27] used the ZPP/heme ratio (ZPP/heme) as a sole criterion to diagnose iron deficiency in Indian participants. Their findings revealed that 67.7% of patients with sickle cell disease (SCD) and 26.2% of those with sickle cell trait were identified as having iron insufficiency. Alpha thalassemia decreases the amount of HbS inside the cells, resulting in a decrease in cellular death caused by HbS. This ultimately leads to a reduction in the severity of the illness [28,29]. The 3.7 kb alpha-globin gene deletion has been seen in SCA patients in many countries, including as India (32%), Brazil (29%), and among African Britons in the UK (34%) [30]. Some instances with sickle cell trait had a HbS level of less than 37%, which may have been due to the concurrent presence of alpha thalassemia. However, molecular confirmation could not be conducted because to insufficient facilities. A comparative analysis of CBC parameters was conducted comparing sickle cell trait subjects exhibiting HbS levels within the predicted range of 38-45% and those cases with lower HbS percentages. The red blood cell (RBC) counts were elevated, but both mean MCV and mean MCH values were decreased ($p < 0.05$, statistically significant) in patients with lower HbS values. This suggests that these commonly accessible CBC characteristics might be potentially helpful in the preliminary diagnosis of alpha thalassemia. However, more research that includes molecular diagnosis of alpha thalassemia is necessary to validate these results.

CONCLUSION

This area has a high occurrence of hemoglobinopathies, which suggests that giving iron supplements to all instances of microcytic hypochromic anemia without discrimination is pointless. Instead, there is a need for mass screening programs and genetic counseling.

This work reaffirms that HPLC is a very effective and robust diagnostic method for directly identifying Hb variations. It also demonstrates that HPLC provides precise quantification of both major and minor Hb fractions, including normal and aberrant ones. However, it is crucial to note that the interpretation of chromatograms should only be conducted after considering the clinical history, family history, complete blood count, and the results of the blood smear investigation.

REFERENCES

1. PatelAP,NaikMR,ShahN M,SharmaNP,ParmarP H.PrevalenceofcommonhemoglobinopathiesinGujarat: Ananalysisofalargepopulationscreeningprogram. National journal of community medicine. Jan-March2012;3(1):112-116.
2. ParikhUR,GoswamiHM,MehtaRC,PatelPS,Gosai RN.IncidenceofhemoglobinopathiesinwomenattendingantenatalclinicsintheirfirsttrimesterNHLjournalofmedicalscience2014;3(1):63-67.
3. Patne SC, Shukla J. Hemoglobin E disorders in EasternUttarPradesh.IndianJPatholMicrobiol2009;52:110-2.
4. Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: report of 2600 cases. Sachdev R, Dam AR, Tyagi G. *Indian J PatholMicrobiol.* 2010;53:57–62.
5. Hemoglobin disorders in South India. Chandrashekar V, Soni M. *ISRN Hematol.* 2011;2011:748939.
6. Study of hemoglobinopathies and Hb variants in population of Western India using HPLC: A report of 7,000 cases. Shrivastav A, Patel U, Joshi JR, Kaur A, Agnihotri AS. *J. Appl. Hematol.* 2013;1:104.
7. Pattern of hemoglobinopathies and thalassemias in upper Assam region of North Eastern India: high performance liquid chromatography studies in 9000 patients. Baruah MK, Saikia M, Baruah A. *Indian J PatholMicrobiol.* 2014;57:236–243.
8. Hemoglobinopathy in India. Iyer S, Sakhare S, Sengupta C, Velumani A. *ClinicaChimicaActa.* 2015;15:229–233.
9. Study of hemoglobinopathies in patients of anemia using high performance liquid chromatography (HPLC) in Western India. Bhalodia JN, Oza HV, Modi PJ, Shah AM, Patel KA, Patel HB. *Indian J Community Med.* 2015;31:35–40.
10. Spectrum of hemoglobinopathies in Odisha--an institutional study by CE-HPLC. Alam S, Singh A, Chakrabarty S, Mohanty R. *Int J Med Sci Public Health.* 2016;1:208–212.
11. Incidence of hemoglobinopathies and hemoglobin variants using HPLC as a diagnostic tool in 6500 anemic patients in a tertiary care center in Western India. Biswas AK, Philip J. *Indian J Appl Res.* 2016;6:214–218.
12. Bio – Rad VARIANT thalassaemia short program. Instruction Manual 2003, 10.
13. Benoist B, McLean E, Cogswell M. Worldwide prevalence of anemia 1993– 2005: WHO Glob Database Anemia 2008; 440:516.
14. Archana Buch, et al, “Patterns of hemoglobinopathies diagnosed by high-performance liquid

chromatography in and around Pune (Western Maharashtra, India): A pilot study," Journal of Medical Society, vol. 30, no. 2, pp. 111, 2016.
15. Gupta PK, Kumar H, Kumar S, Jaiprakash M. Cation exchange high performance liquid chromatography for diagnosis of haemoglobinopathies. MJAFI 2009; 65:1.
16. Balgir RS. Spectrum of haemoglobinopathies in the state of Orissa, India: a ten years cohort study. J Assoc Physicians India 2005; 53:1021-6
17. 7. Baruah MK, Saikia M,Baruah A. Pattern of hemoglobinopathies &thalassemias in upper Assam region of NorthEasternIndia:Highperformance LiquidChromatographystudiesin9000 patients. Indian J PatholMicrobiol 2014; 57:236-43
18. Sadaf Alam et al; Spectrum of hemoglobinopathies in Odisha—an institutional study by CE-HPLC; Int JMedSciPublicHealth.2016;5(2):208-211.
19. MostafaA.S.Salama,MahaY.Kamal, Doren N. A. Younan, Gehad A. A. Henish. Hypochromic microcytic anaemia:a clinicopathological cross sectional study, Alexandria Journal of Pediatrics,2017,30:37-43.
20. RamanS,SahuN,Senapati U.Astudy of haemoglobinopathies and haemoglobin variants using high performance liquid chromatography (HPLC)inateachinghospitalofOdisha. J.Evolution Med. Dent. Sci. 2017;6(11):842-849
21. Bhagora S, Anand V, Goswami H. Evaluation of hemoglobinopathies in microcytic hypochromic anemia cases. International Journal of Clinical and DiagnosticPathology2021;4(1):17-19.
22. Ankur N. Sarvaiya, et al, Variant Hemoglobin Spectrum by Cation Exchange High Performance Liquid Chromatography: A Study of 2035 Subjects Published 2017
23. MaheshKumarU,DevisriY.Detection of hemoglobinopathies in patients of anaemiausinghighperformanceliquid chromatography - a hospital based prospective study. Trop J Path Micro 2019;5(2):51-57.
24. VaniChandrashekarandMamtaSoni, Hemoglobin Disorders in South India, ISRNHematology,Volume2011, Article ID 748939.
25. de Gruchy's Clinical Haematology in Medical Practice. Sixth adapted Edition.
26. Rao S, et al. Spectrum of haemoglobinopathies diagnosed by cation exchange-HPLC & modulating effects of nutritional deficiency anaemias from north India. Indian J Med Res 2010; 132:513-9.
27. MohantyD,MukharjeeMB,ColahRB, WadiaM,GhoshK,ChottrayGP,etalIron deficiency anemia in sickle cell disorders in India. Indian J Med Res 2008; 127:366–9.
28. Sheehan VA, Luo Z, Flanagan JM, Howard TA, Thompson BW, et al. Geneticmodifiersofsicklecellanemia intheBABYHUGcohort:infulenceon laboratory and clinical pehnotypes. American Journal of Hematology. 2013:88: 571– 576.
29. EmburySH.Age-dependentchangesin the membrane surface area: sickle red blood cell volume may account for differential clinical effects of coinheriteda-thalassemiaonsicklecell anemia. European Journal of Hematology. 2012: 88:363–4.
30. Rumaney MB, Ngo Bitoungui VJ, Vorster AA, Ramesar R, Kengne AP, Ngogang J, Wonkam A. The co- inheritance of alpha-thalassemia and sickle cell anemia is associated with better hematological indices and lower consultations rate in Cameroonian patients and could improve their survival. PloS one. 2014 Jun 30;9(6):e100516.