RESEARCH ARTICLE

PREVALENCE OF ANAEMIA AND HEMOGLOBINOPATHIES AMONG COLLEGE STUDENTS OF RAJKOT, GUJARAT.

Vachhani Nishith, Nandani Sanjeev and Vekariya Daya.
Indian Medical Scientific Research Foundation, Life Blood Centre, 24-Vijay Plot, Samir Dholakiya Marg, Malaviya Road, Rajkot – 360002, Gujarat, India.

Abstract

In India, major cause of anaemia is nutritional deficiencies which can be treated by medications. Haemoglobinopathies are the most common inherited red cell disorders world-wide. Most clinically significant haemoglobinopathies are inherited defects of the beta (β) globin chain of adult haemoglobin. Nutritional anaemia affects all age and sex groups in India. Iron deficiency anaemia is the most common micronutrient deficiency in the world affecting more than 2 billion persons. The present study is an attempt to assess the prevalence of anaemia and haemoglobinopathies among college students in order to sensitize the masses about its impacts. 650 college students in the age group of 16-28 years of Rajkot, Gujarat were the subjects of study. Haemoglobin estimation was performed by automated cell counter and observations were interpreted as per WHO criteria. The study showed that 17.69% were affected with various grades of anaemia condition. 11.69% subjects being mildly anaemic and 5.85% moderately anaemic while 0.15% suffered from severe anaemia. Various haemoglobinopathies were also studied by screening method – NESTROFT and confirmed by HPLC method. Prevalence of Beta thalassaemia carrier was 3.54%. Beta-thalassemia continues to be a cause of significant burden to the society particularly in the developing countries. Further studies with a large sample size are needed to draw out the exact proportion for prevalence of anaemia and haemoglobinopathies so that appropriate remedial measures can be taken.

Introduction:

Health, nutrition and population policies play a pivotal role in economic and human development and in poverty alleviation. Nutrition will remain a key element in ensuring security: adequate foods literally “vital” in keeping people alive as a basic need and human right. The prevalence of anaemia is an important health indicator for the country like India. Anaemia, defined as a low blood haemoglobin concentration (and consequently their oxygen-carrying capacity) is insufficient to meet the body’s physiologic needs and it has been shown to be a public health problem that affects low, middle and high-income countries and has significant adverse health consequences, as well as adverse impacts on social and economic development. Specific physiologic needs vary with a person's age,
gender, residential elevation above sea level (altitude), smoking behaviour, and different stages of pregnancy. Anaemia may result from a number of causes, with the most significant contributor being iron deficiency. The World Health Organization (WHO) global estimates of anaemia prevalence averaged 56%, with a range of 35–75% depending on the geographic location. Anaemia affects approximately 30–55% of young adults all over the world. South Asia has the highest prevalence of anaemia in the world, which is attributed to high rates of malnutrition. In South-East Asia region of WHO, mean haemoglobin concentration 10.7 gm% (10.4 to 11.2 gm%) and 53.8% (39.9 to 63.9%) of population is suffering with anaemia. Women of childbearing age are having an additional risk of developing anaemia because of their monthly menstrual blood loss and nearly 50 percent of females in this age group are anaemic. On average a healthy woman loses about 25–30 ml of blood monthly. Therefore, the body needs to produce blood in order to compensate for this loss and if the essential nutrients required for haemopoiesis are not supplied in their diet, anaemia will develop.

Correcting anaemia requires an integrated approach based on identifying and addressing the contributing factors. Low blood haemoglobin concentrations may be caused by genetic traits like sickle cell and thalassaemias; inadequate bioavailable dietary iron, folic acid and/or vitamin B12; malaria, schistosomiasis, hookworm or human immunodeficiency virus (HIV) infections; and some noncommunicable diseases.

Approximately 50% of cases of anaemia are considered to be due to iron deficiency, but the proportion probably varies among population groups and in different areas, according to the local conditions. Anaemia resulting from iron deficiency adversely affects cognitive and motor development, causes fatigue and low productivity and, when it occurs in pregnancy, may be associated with low birth weight and increased risk of maternal and perinatal mortality.

Iron deficiency anaemia (IDA) and beta thalassaemia trait (β-TT) are the commonest hematological abnormalities presenting with mild microcytic anaemia. Deficiency of elemental iron in diet accounts for the commonest hematological disorder in the form of IDA with approximately 30% of the world population (with majority in third world countries) suffering from it. In contrast, a common mimicker of microcytic anaemia which often confounds a diagnosis of IDA (nutritional case) in third world countries is thalassemia syndrome (trait) which is seen in 3% of the population. The main pathophysiology of these syndromes is absence / lack of either alpha or beta globin chains resulting in alpha or beta thalassaemia respectively.

The inherited hemoglobin (Hb) disorders are the most common single gene defect in man. The prevalence of haemoglobinopathies is on the rise worldwide. This is of special importance in developing countries where it increases the burden of health care delivery system. The abnormalities can either be quantitative (the thalassemia syndromes) or qualitative (the hemoglobin variants) or a combination of both.

Of these, the thalassemia syndromes particularly the beta thalassemia and some alpha thalassemia are the major cause of morbidity. It is estimated that 1.5% of the world’s population are carriers of beta thalassaemia - that is, at least there are 80 million to 90 million people with an estimated 60,000 new cases being born each year. The South-East Asia region (which includes India, Thailand and Indonesia) accounts for 50% of world carriers. In India, nearly 30 million people are carriers of beta thalassaemia and 7000 babies with beta thalassaemia major are born every year. The carrier rate varies between 0 to 17% in different ethnic groups.

Thalassemia is an autosomal recessive inherited group of disorders of hemoglobin synthesis characterized by the absence or reduction of one or more of the globin chains of the hemoglobin molecule. The structural variants result from substitution of one or more amino acids in the globin chains of the hemoglobin molecule. Being recessively inherited from the parents, the thalassemia and thalassemichemoglobinopathies pose serious health problem leading to severe morbidity and mortality in Indian population. With increasing global awareness and mass screening programs undertaken at various levels by health care system, the responsibility for laboratory personnel has greatly enhanced in detection and prevention of this problem. Awareness about the diagnostic problems as well as their solutions is very important so that one does not miss a single case.

Materials and methods:
A cross sectional survey of college going students was conducted to rule out the status of base line anaemia prevalence and to differentiate types of haemoglobinopathy at Rajkot, Gujarat, INDIA. A total of 650 students of
both the sex were enrolled in this study. All students’ ages ranged between 16 to 28 years. A consent form along with an information sheet giving details of the study (nature of the study, what will be expected from the participants, and expected risks and benefits) were provided to all students who were randomly selected to the sample. The details were also explained verbally to the potential participants.

A total volume of 2 ml of venous blood was obtained from each participant into EDTA (ethylenediaminetetraacetic acid) containers for CBC analysis, NESTROFT test and HPLC to classify the type of anaemia and haemoglobinopathies. Blood was drawn by skilled personal. Universal precautions were followed during blood collection, transportation, storage, and disposal to protect the participants as well as researchers. The blood samples were mixed one electric blood mixture for 8 to 10 minutes and thereafter analyzed on the Sysmex XP100, three part differential cell counter to obtain the hemoglobin values and indices as described in the instruction manual. Mentzer index (MCV/RBC) was also used for screening of thalassaemia carrier. All samples were analyzed by NESTROFT method as a primary screening test. All NESTROFT positive samples were reanalyzed on the Bio-Rad Variant II HPLC system (BioRad Laboratories, USA) as described in the instruction manual.

Naked-Eye-Single-Tube-RedCell-Osmotic-Fragility-Test as its name implies, is used to assess osmotic fragility of red cells at a single concentration of buffered saline (0.36% in single tube) visually without a spectrophotometer. A stock solution of 10% buffered saline (pH 7.4) is prepared by taking NaCl 90 gm, Na2HPO4 13.655 gm and NaH2PO4, 2H2O 2.4 gm and dissolving them in 1 litre of distilled water. From this, a litre of 1 buffered saline is prepared by 1:10 dilution with distilled water. 36 ml of 1% buffered saline with 64 ml distilled water to make 100 ml. 2 ml of 0.36% buffered saline is taken in one tube (10 cm x 1 cm diameter) and 2 ml distilled water is taken in another. A 20 µl of blood is added to each of the tubes, which are left undisturbed for half an hour at room temperature. After half an hour the contents of both the tubes are shaken and the tubes held against a white paper on which a thin black line is drawn\(^9\).

Interpretation of NESTROFT results: The line is clearly visible through the contents of the tube containing distilled water due to complete lysis. If the line is visible through the contents of the tube with buffered saline, the test is negative, whereas if the line is not visible, the test is positive. The tubes are then left undisturbed for a few hours. At the end of this period, contents of the tube with distilled water remain uniformly pink with no sediment at the bottom. In the case of a negative test, the tube containing buffered saline also presents a similar picture. With a positive test, the tube shows sediment of the red cells at the bottom and the top part of the saline is colourless. This is an additional confirmatory evidence of a test earlier interpreted as positive\(^9\).

A positive NESTROFT indicates that all red cells in the tested sample have not undergone lysis in 0.36% buffered saline. These unlysed red cells result in the hazy appearance of the contents of the tube and render the line on the paper indistinct. These red cells also sediment as a button at the bottom of the tube when it is left undisturbed for some time. Thus a positive NESTROFT indicates decreased red cell osmotic fragility and increased resistance to osmotic lysis. HbA2 was performed in all NESTROFT positive subjects to compute sensitivity of the NESTROFT.\(^9\) The Variant-II Beta Thalassaemia Short Program utilizes principles of ion-exchange high-performance liquid chromatography (HPLC). The samples are automatically mixed and diluted on the Variant-II Sampling Station (VSS) and injected to the analytical cartridge. The Variant-II Chromatographic Station (VCS) dual pumps deliver a programmed buffer gradient of increasing ionic strength to the cartridge, where HbA2/F are separated based on their interaction with the cartridge material. The separated HbA2/F then pass through the flow cell of the filter photometer where the changes in absorbance at 415 nm are measured. An additional filter at 690 nm corrects the background absorbance. The Variant-II CDM (CDM) Software performs reduction of raw data collected from each analysis. To aid in the interpretation of results, windows have been established for the most frequently occurring hemoglobins based on their characteristic retention time. For each sample, a sample report and a chromatogram are generated by CDM showing all hemoglobin fractions eluted, their retention times, the area of the peaks, and values of the fractions. Reports and chromatograms generated were studied and interpreted by observing HbA2 and F concentration for beta thalassemia and retention time and area percentage of other peaks and windows for structural variants. Each chromatogram shows peaks of HbA0, HbA2 and HbF along with C-window, D-window, S-window and two minor peaks, P2 and P3. Several hemoglobin variant selute same window; they were provisionally diagnosed by retention time and area percentage keeping in mind the ethnicity of the patients. Other relevant tests were done, for example, Sickling test (using sodium meta bisulphite) when D-window or/and S-window was eluted in the sample.\(^{18}\)
Results:-
The anaemia was observed in 115 (17.69%) students out of total 650 (396 male and 254 female) students selected to carry out study from college in Rajkot. The mean of haemoglobin among students was 13.79 gm% (14.71gm% in male and 12.34% in female). The prevalence of anaemia in male students was 6.82% and 34.65% in female students [Table-1]. As per haemoglobin concentrations for the diagnosis of anaemia and assessment of severity by World Health Organization, we observed there were mild anaemia among 76 students (11.69%), moderate anaemia among 38 students (5.85%) followed by severe anaemia in 1 female student (0.15%) [Table-2]. Among 650 study samples for NESTROFT, 75 (11.54%) were detected with positive findings [Table-3]. Total 77 samples (75 screening positive in NESTROFT + 2 abnormal indices in haemogram) were analyzed by high performance liquid chromatography (HPLC) on Variant-II (Bio-Rad Laboratories, USA) for haemoglobin fractionation study. Total 23 (3.54%) were diagnosed Beta thalassaemia trait and 8 (1.23%) were identified with other haemoglobinopathies [Table-4].

Table1:-Sex-wise distribution of anaemia

| Anaemia   | Male (%) | Female (%) | Total (%) |
|-----------|----------|------------|-----------|
| Present   | 27 (6.82)| 88 (34.65) | 115 (17.69)|
| Absent    | 369 (93.18) | 166 (65.35) | 535 (82.31) |
| Total     | 396 (100) | 254 (100) | 650 (17.69) |

Table 2:-Severity of anaemia among students

| Severity | Male (%) | Female (%) | Total (%) |
|----------|----------|------------|-----------|
| Mild     | 22 (5.56) | 54 (21.26) | 76 (11.69) |
| Moderate | 5 (1.26)  | 33 (12.99) | 38 (5.85)  |
| Severe   | 0         | 1 (0.39)   | 1 (0.15)   |

Table 3:-Screening test results of NESTROFT

| Result   | Male | Female | Total (%) |
|----------|------|--------|-----------|
| Positive | 36   | 39     | 75 (11.54) |
| Negative | 360  | 215    | 575 (88.46) |
| Total    | 396  | 254    | 650 (100)  |

Table 4:-Pattern of Haemoglobinopathies by HPLC method

| Result                                           | Number (%) |
|-------------------------------------------------|------------|
| Beta Thalassaemia Trait                          | 23 (3.54)  |
| Delta Beta Thalassaemia Trait                    | 1 (0.15)   |
| Hereditary Persistence of Fetal Hemoglobin heterozygous | 1 (0.15)   |
| Hereditary Persistence Fetal Hemoglobin/ Delta Beta Thalassaemia Trait | 1 (0.15)   |
| Homozygous for Hb D Punjab/ Double heterozygous for Hb D Punjab & Beta Thalassaemia Trait | 1 (0.15)   |
| Hb D (Punjab) Trait                             | 1 (0.15)   |
| Sickle Cell Trait                               | 1 (0.15)   |
| Borderline for Beta Thalassaemia Trait           | 2 (0.31)   |
| Total                                           | 32 (4.77)  |

Discussion:-
Globally, the mean blood haemoglobin concentration was 11.1 gm% (95% credibility interval [CI]: 11.0-11.3) in children, 12.6 gm% (95% CI: 12.4-12.8) in non-pregnant women, and 11.4 gm% (95% CI: 11.2-11.6) in pregnant women, indicating that, on average, all population groups were above the threshold formild anaemia (11.0 gm% for children and pregnant women and 12.0 gm% for non-pregnant women). In India, the mean blood haemoglobin concentration is11.9 gm% (95% CI: 11.3-12.5) in non-pregnant women. In present study the average haemoglobin concentration in non-pregnant women was 12.34 gm% which falls in WHO data. On the basis of the WHO cut-offs points, in this study, anaemia prevalence in this study population was 17.69%. The prevalence of anaemia was 34.65% for women, whereas only 6.82% of the men were anemic.
adolescent females (10–19 years old) and according their observations the prevalence of anaemia was found to be 35.1%.

Haemoglobinopathies are prevalent worldwide, but it is more prevalent in some geographical areas. The distribution of beta thalassemia is not uniform in the Indian subcontinent. Though certain communities are identified to have high prevalence, it has been detected in almost every Indian population. The haemoglobinopathies and thalassemia are the most common inherited single gene disorder in India. Therefore there is always a need for a screening method which can detect maximum variants. The prevalence of beta thalassemia trait varies from 1-17% in different populations of India. According to study done by Indian Red Cross society, Gujarat State Branch, prevalence of beta thal trait is 3.4% in Ahmedabad Gujarat. In our data, the prevalence of thalassaemia trait is 3.54%.

Conclusion:-
Reducing anaemia is recognized as an important component of the health of women and children, and the second global nutrition target for 2025 calls for a 50% reduction of anaemia in women of reproductive age by World Health Organization. Iron deficiency anaemia should ideally be addressed through dietary diversification and improved access to foods that have high levels of bioavailable iron, including animal products. Daily or intermittent iron supplementation, alone or together with folic acid and other micronutrients, can be used for high-risk groups (children, pregnant women and women of reproductive age), to improve iron intakes. Other food based approaches, such as fortification of staple foods and condiments can also be used to improve iron intake in the general population. Fortification of wheat flour with iron and other vitamins and minerals is currently mandated in 80 countries but the extent of coverage varies. In India, the prevalence of anaemia remains high and is an area of priority. If the current trends are maintained, there is a probability of less than 25% in all regions of reaching the global target of reducing the prevalence of anaemia by 50% in women of reproductive age. To make a significant impact, it is likely that a combination of key programmes that address the determinants of low blood haemoglobin concentrations will be required. These strategies should be tailored to local conditions, taking into account the specific etiology and prevalence of anaemia in a given setting and population group, and should be built into the primary health-care system and existing programmes.

NESTROFT is reliable, cost effective and better screening test for thalassaemia carrier detection. Positive cases can be confirmed by HPLC. NESTROFT can be used in mass haemoglobinopathies screening programmes.

Mass awareness programmes, anaemia screening programmes, integrated planning, multi-sectoral approach, community support along with anaemia prevention and treatment should be initiated for betterment of youth of India.

References:-
1. Al-Sayes et. al. "Prevalence of iron deficiency and iron deficiency anemia among females at university stage." Journal of Medical Laboratory and Diagnosis 2, no. 1 (2011): 5-11.
2. Bhalodia Jignasha et. al. "Study of hemoglobinopathies in patients of anemia using high performance liquid chromatography (HPLC) in Western India." Community Med 6, no. 1 (2015): 35-40.
3. Chakrabarti Indranil et. al. "Beta-Thalassemia Carrier Detection by NESTROFT: An Answer in Rural Scenario?" Iranian Journal of Pathology 7, no. 1 (2012): 19-26.
4. Chandrahas Prasad et. al. "Naked Eye Single Tube Red Cell Osmotic Fragility Test Screening For Detection Of B-Thalassemia Trait - An Evaluation Against HPLC Method At Rajendra Institute Of Medical Sciences, Ranchi" Research Journal of Pharmaceutical, Biological and Chemical Sciences July-Aug, 2014, 5(4) 1388-1396.
5. Chaudhary Sanjeev M.et. al. "A study of anemia among adolescent females in the urban area of Nagpur." Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine 33, no. 4 (2008): 243.
6. Colah, R. B. et. al. "HPLC studies in hemoglobinopathies." The Indian Journal of Pediatrics 74, no. 7 (2007): 657-662.
7. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. WHO - WHO/NMH/NHD/MNM/11.1
8. Hanafi et al. "Study of hemoglobin level and body mass index among preparatory year female students at Taibah University, Kingdom of Saudi Arabia." Journal of Taibah University Medical Sciences 8, no. 3 (2013): 160-166.

9. Himaxi Rathod et al. "NESTROFT AS A SCREENING TEST FOR BETA THALASSEMIA" Int. J. of Adv. Res. 2 (8).

10. Kabiret. et al. "A Screening Test for Iron Deficiency Anaemia and Thalassaemia Traits." Journal of Bangladesh College of Physicians & Surgeons 32, no. 4 (2014): 190.

11. Mehta, Kamal. "Prevalence of Nutritional Anaemia among College Students and its Correlation with their Body Mass Index." Journal of Bangladesh College of Physicians and Surgeons Vol. 32, No. 4, October 2014.

12. Mosca et al. "The role of haemoglobin A2 testing in the diagnosis of thalassaemias and related haemoglobinopathies." Journal of Clinical Pathology 62, no. 1 (2009): 13-17.

13. Nigam N. et al. "HPLC as a classical tool for screening of β-Thalassemia and Hemoglobinopathies in Kanpur" Rama Univ. J. Med Sci 2015;1(1):6-9.

14. Pandey Sachin et al. "A cross sectional study of nutritional anemia among medical students in a medical college, at Bilaspur, Chhattisgarh." National Journal of Medical Research 3, no. 2 (2013): 143-146.

15. Pant Leela et al. "Detection of abnormal hemoglobin variants by HPLC method: common problems with suggested solutions." International Scholarly Research Notices 2014 (2014).

16. Patel A. P. et al. "Prevalence of common hemoglobinopathies in Gujarat: An analysis of a large screening programme." Natl J Community Med 3 (2012): 112-6.

17. Piplani Sanjay et al. "NESTROFT-A Valuable, Cost Effective Screening Test for Beta Thalassemia Trait in North Indian Punjabi Population." Journal of clinical and diagnostic research: JCDR 7, no. 12 (2013): 2784.

18. Sachdev et al. "Detection of Hb variants and hemoglobinopathies in Indian population using HPLC: report of 2600 cases." Indian journal of pathology and microbiology 53, no. 1 (2010): 57.

19. Sharma Gopikishan et al. "Use of NESTROFT as a screening test for the carriers of thalassemia major." Pediatric Review: International Journal of Pediatric Research 3, no. 07 (2016).

20. Sharma Santanu K. et al. "Haemoglobinopathies-major associating determinants in prevalence of anaemia among adolescent girl students of Assam, India." (2012).WHO South-East Asia Journal of Public Health 2012;1(3):299-308.

21. The global prevalence of anaemia in 2011 - WHO 2015.

22. WHO Global Database on Anaemia - Worldwide prevalence of anaemia 1993-2005.

23. WHO Regional strategy on nutrition 2010-2019 and Plan of action. WHO-EM/NUT/256/E.