"COMPARISON OF RED BLOOD CELL MORPHOLOGY AND INDICES IN PATIENTS OF VEGETARIAN AND NON VEGETARIAN DIET, A ONE YEAR CROSS SECTIONAL STUDY IN KLES DR. PRABHAKAR KORE HOSPITAL AND MRC"

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ENDORSEMENT

This is to certify that the dissertation entitled **"COMPARISON OF RED BLOOD CELL MORPHOLOGY AND INDICES IN PATIENTS OF VEGETARIAN AND NON VEGETARIAN DIET, A ONE YEAR CROSS SECTIONAL STUDY IN KLES DR. PRABHAKAR KORE HOSPITAL AND MRC"** is a bonafide research work done by **REG NO. BG0118004.**

Dr. ARATHI DARSHAN MD, FICP
Professor and Head,
Department of Medicine,
J. N. Medical College,
Nehru Nagar, Belagavi – 10

Date:
Place: Belagavi

Dr. N.S. MAHANTSHETTI MD
Principal,
J. N. Medical College,
Nehru Nagar, Belagavi – 10

Date:
Place: Belagavi

KLE

JAWAHARLAL NEHRU MEDICAL COLLEGE

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Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)

Nehru Nagar, Belagavi- 590 010, Karnataka, INDIA

0831 - 2471350 0831 - 2470759 www.jnmc.edu principal@jnmc.edu

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Principal,
J. N. Medical College, Belagavi.

To,
Reg. No. BG0118004.
Postgraduate Student,
2018-19 Batch,
Department of General Medicine,
J. N. Medical College, Belagavi.

LIST OF ABBREVIATIONS USED

HTN	:	Hypertension
DM	:	Diabetes Mellitus
CLD	:	Chronic Liver disease
CKD	:	Chronic Kidney Disease
COPD	:	Chronic Obstructive Pulmonary Disease
BMI	:	Body mass index
Hb	:	Haemoglobin
MCV	:	Mean Corpuscular Volume
MCH	:	Mean Corpuscular Hemoglobin
MCHC	:	Mean Corpuscular Hemoglobin Concentration
AGEs	:	Advanced Glycated End products
TAC	:	Total Antioxidant Capacity
WHO	:	World Health Organisation
G6PD	:	Glucose-6-Phosphate dehydrogenase

ABSTRACT

Introduction:

The present study was conducted in department of Department of Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre in the study period from January 2019 to December 2019 was undertaken to study the RBC morphology and indices in patients of vegetarian and non-vegetarian diet.

Materials and Methods:

The study included inpatients of Dr. Prabhakar Kore Hospital and MRC, Belagavi. Each individual interested in participating the study was interviewed and asked to complete a questionnaire to determine if they met all the inclusion criteria. Vegetarians were broadly defined as those who did not consume meat or fish for approximately 6 months prior to and during the study.

Observations and Conclusion:

In our study, majority of patients of severe anemia were vegetarians. Most of the male patients were mildly anemic and females were moderate to severely anemic. The commonest type of anemia was microcytic hypochromic anemia followed by normocytic normochromic anemia in both the groups (vegetarians and non-vegetarians).

Hemoglobin was slightly more in non-vegetarian group. Amongst the different habits of patients, tobacco chewing had positive correlation with increased MCV values.

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INTRODUCTION

In the recent years, there is an increasing worldwide inclining towards a vegetarian diet because of its proposed health benefits, for example, higher content of fiber, vitamins A, C, E and K, folate, potassium, numerous phytochemicals, reduced blood pressure, BMI, and serum cholesterol levels.[1] However, the long-term omission of animal foods may lead to scarcity of certain nutrients which are not easily available through plant sources, and this in turn may affect the RBC morphology and hematological parameters.[2] For example, red meat poultry, and fish are good source of heme iron, and lack of consumption of these products may increase risk of iron-deficiency anemia. In addition, the presence of phytate, polyphenols and calcium in plant-based diets have been reported to inhibit the absorption of iron.[3]

Iron deficiency anemia can cause microcytic red blood cells and diminished hemoglobin synthesis. This in turn leads to inadequate oxygenation of cells, tissues, and organs, and the symptoms associated with this include fatigue, decreased cognition, compromised immune function, pregnancy related complications, and a higher risk of lead poisoning.[4]

A higher prevalence of vitamin B_{12} deficiency has been noted in vegetarians who exclude all animal foods from their diet.[5] Vitamin B_{12} is integral for the maturation of RBC and in the platelet life cycle. In addition, the bioavailability of other vital nutrients that are crucial for RBC and hemoglobin production, such as, vitamin A, riboflavin, and zinc are comparatively lesser through plant-based diet.[2]

The intake of vitamin B_{12} is primarily dependent on the inclusion of animal-source foods in the diet. Although, the inclusion of kelp, spirulina and algae in vegan has been suggested to be useful for getting the vitamin, cobalamins in these sources are not present in biologically active forms. Moreover, cobalamin analogs present in

these foods may inhibit cobalamin-dependent enzymes. Latest literature evidence has indicated that in pure vegetarian and in some lacto-ovo vegetarian diet consumers, there is actual depletion of vitamin B12, in contrast to non-vegetarian diet consumers. Increased folate deficiency has been linked to lactation and alcohol consumption.[6]

Need for the study

Review of literature shows that the risk of anemia is associated with dietary pattern and dietary diversity is linked to reduced anemia prevalence. However, there is a gap in evidence on how the vegan diet is linked to RBC morphology and dietary patterns. Evaluation of the morphology of RBCs and clinical features linked to dietary pattern may help in the early diagnosis of hematological abnormalities and developing effective management interventions.

OBJECTIVES

The objective of the present study was

- To compare the red blood cell morphology and indices in patients of vegetarian and non-vegetarian diet.

REVIEW OF LITERATURE

Anemia: Definition and cut-off levels

Anemia is defined as "a condition in which the number of red blood cells or the concentration of hemoglobin is lower than normal, which in turn decreases the capacity of the blood to carry oxygen to the body's tissues." This usually results in symptoms such as fatigue, generalized weakness, dizziness and shortness of breath.[7] The Hb distribution is dependent on the age, sex and physiological status of the subject (eg: pregnancy). In addition, physiological and statistical evidence corroborates that the smoking status also influence the Hb distributions.[8]

The levels of hemoglobin that are classified as healthy hemoglobin levels, according to the gender and age groups are given below: (WHO).[9]

- Children: 11-13 g/dL
- 12-15 years: ≤12 g/dL
- Adult male: 13.8 to 17.2 g/dL
- Adult female: 12.1 to 15.1 g/dL
- During pregnancy: At or above 11g/dL

The overall anemia cut-offs, recommended by WHO, have not been changed since 1968. However, the age group of children 5-14 years of age has been split, and a cut-off of 5 g/l lower was applied to children of 5-11 years of age based on the findings among non-iron deficient children in the US.

Global prevalence of anemia

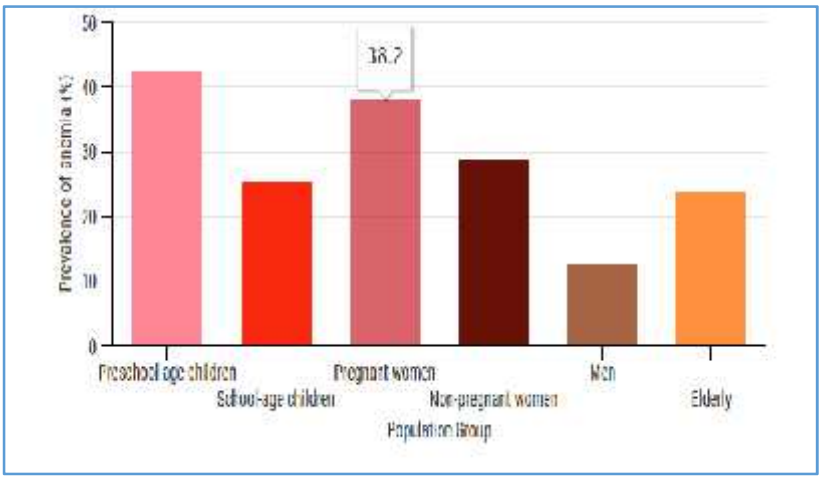
Anemia, one of the most common public health problems, affects approximately one- third of the global population. It is associated with substantial mortality and morbidity and they include impaired cognitive and

behavioraldevelopment in children, reduced work productivity and poor birth outcomes.[10] As per the 2000 reports of The Global Burden of Disease (GBD), around 2% of the years lived with disability and 1% of disability-adjusted life-years could be attributed anemia.[11]

WHO salient facts

- According to the 2011 WHO reports, the prevalence was highest among preschool-age children (42.6%) and lowest in men (12.7%). The estimation also reveals that the prevalence was also more among pregnant women (38.2%) (Fig. 1).
- Globally over 2 billion people are anemic, which could be mainly attributed to iron deficiency.
- In developing countries, every second pregnant women are estimated to be anemic.
- In many developing countries, worm infections, malaria and other infectious diseases such as HIV and TB aggravates the incidence of iron deficiency anemia
- As per WHO, 20% of all maternal deaths are attributed to Anemia (WHO).[12]

Fig. 1: Prevalence of anemia in different ethnic groups

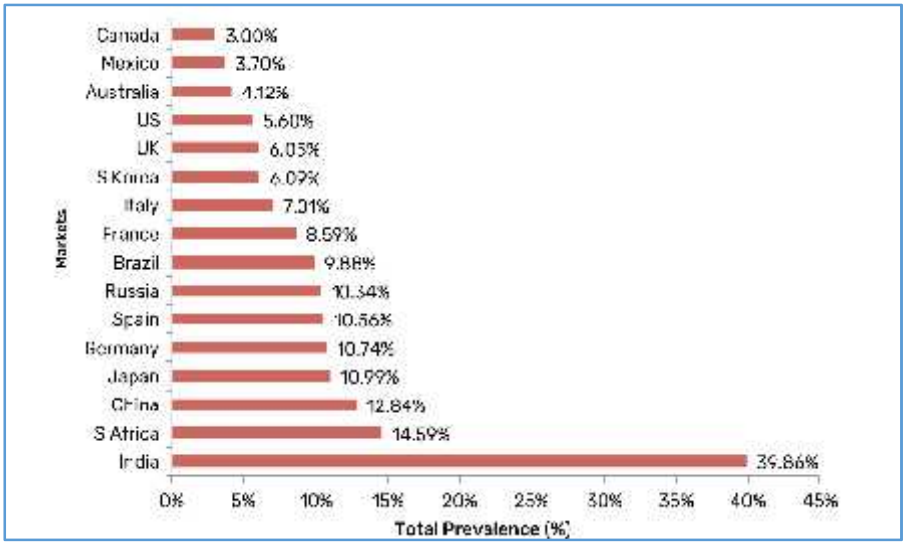


Source: WHO report, The global prevalence of anemia in 2011

Burden of anemia in India

According to the global epidemiological data, India has the highest prevalence of anemia (around 40%) when considering the 16 major pharmaceutical markets. The major causes noted by the survey are poor nutrition, lack of access to healthcare and anemia awareness and ineffective implementation of iron supplementation programs.The District Level Household Survey (DLHS) 2014 and Annual Health Survey (AHS) 2015 reports also corroborates the highest prevalence of anemia in the country. [13]

Fig. 2: Prevalence of anemia in 16 major pharma markets



Source: Global data's epidemiology data

In addition, the country has the highest prevalence of anemia in pregnancy and it leads in having largest number of anemic pregnant women globally. Anemia during pregnancy has been identified as a major public health problem in India, contributing to high maternal morbidity and mortality, high infant mortality and low birthweight. Low dietary intake of iron and folate have been identified as the major factors responsible for anemia during pregnancy.[14] As per the National Family Health Survey4 (NFHS4), the anemia prevalence noted in different groups namely children between the age group of 6 to 59 months, females aged 15 - 49 years, pregnant females aged 15 - 49 years and males aged 15 - 49 years were 53.1%, 58.6%, 22.7%, and 50.4% respectively.

Even among affluent population of the country, around 50% of children, adolescent girls and pregnant women are anemic. The major contributing factors include Inadequate dietary iron and folate intake due to reduced vegetable consumption, decreased B12 intake and poor bioavailability of dietary iron, and phytate-rich Indian diets. In India, an increase in 8- to 10-fold increase in maternal mortality ratio has been reported, and this could be attributed to fall in Hb<5 g/dl. Maternal anemia is a risk factor for poor intrauterine growth, increased risk of preterm births and low birth weight rates.[15]

A study by Kalaivani and Ramachandran has tabulated the reports of the National Family Health Survey (NFHS) 2, NFHS 3, Fact Sheets of NFHS 4 and District Level Household Survey (DLHS) 2 to estimate the anemia in pregnancy in India. The study also highlighted that around 50% of pregnant women in India were anemic, and in contrast to other South Asian countries, India had the highest burden of anemia in pregnancy.[13]

Table 1: Comparison of prevalence of anemia in India with neighboring countries

Countries	Percent with Hb <11 g%	Percent with Hb <7 g%
Afghanistan	44	1.2
Bangladesh	48	0.5
Bhutan	46	1.2
India	54	1.3
Maldives	39	0.6
Myanmar	33	0.7
Nepal	44	0.6
Pakistan	50	2.1
Sri Lanka	25	0.4
Thailand	30	0.6
Malaysia	27	0.4

Source:Kalaivani K, Ramachandran P. Prevalence of anemia in India and strategies for achieving Sustainable Development Goals (SDG) target. Proceedings of the Indian National Science Academy. 2018 Nov 13;84(4):899–912.

Morphological patterns of anemia

Evaluation of the RBC morphology and clinical features assist in diagnosis of anemia and management. The different morphological characteristics are listed below:

Based on RBC size

- If the RBC is of normal size, it is **Normocytic**
- If the RBC is smaller than normal, it is **Microcytic**
- If the RBC is larger than normal, it is **Macrocytic**

Based on degree of hemoglobinization

- If the RBC is of normal color, it is said to be **Normochromic**
- If the RBC has more of central pallor, it is said to be **Hypochromic**

Shape

- Abnormal Sickle shaped cells are seen in **Sickle cell anemia**

Based upon RBC morphology and blood cell indices, anemia is categorized into normocytic normochromic, microcytic hypochromic, and macrocytic. The details for classification are listed in table 2.

Table 2: Classification of anemia

Microcytic, hypochromic anemia	Normocytic, normochromic anemia	Macrocytic anemia
MCV <80 fL MCH <27 pg	MCV 80-95 fL MCH ≥27 pg	MCV >95 fL
Iron deficiency anemia Thalassemias, Lead poisoning Anemia of chronic disease, Sideroblastic anemia	Hemolytic anemias Renal disease Hemorrhage, Bone marrow suppression, Anemia of chronic disease	Megaloblastic type: vitamin B12 or folic acid deficiency. Non-megaloblastic type: alcohol induced, liver diseases, aplastic anemia, MDS, etc.

Existence of dimorphic pattern has also been characterized, which is marked by the presence of 2 cell populations, i.e. microcytic hypochromic, with other being either macrocytic or normocytic. Although, dimorphic pattern is common in India, it is usually an undetermined entity.[16]

A cross-sectional study conducted at the Department of Pathology, GMC, Jammu for a period of one year on 100 anemic subjects has noted that majority between the age group of 21-40 years were anemic. Normocytic normochromic anemia was the most common pattern of anemia seen (40%), followed by microcytic

hypochromic anemia (29%), and macrocytic anemia (10%). Dimorphic anemia was seen in 14 % of the subjects. [17]

A retrospective study conducted among Saudi anemic patients attending King Abdul-aziz Medical City, Riyadh has noted that microcytic hypochromic pattern of anemia was more prevalent in females as compared to males. The main causes identified were malnutrition, increase of blood loss during pregnancy and menstruation, and iron absorption defects.[18]

Classification of vegetarianism

Vegetarianism has become increasingly popular among people including adolescents in current years perhaps because this diet is believed to confer various health benefits. Nevertheless, there are still justified concerns about some critical nutrients whose lack may cause serious nutritional deficiencies, especially as diverse groups of women may have very different motivation to follow a vegetarian diet.[19]

The early studies on vegan diets in adults have concluded that daily intakes are nutritionally sufficient in protein and most vitamins except for vitamin B-12.[20] Since then, metabolic and neuropsychiatric abnormalities suggestive of vitamin B-12 deficiency, even in the absence of anemia, have been observed in vegans.[21,22] Also, the high-fiber and -phytate content of plant-based foods has prompted questions about the iron and zinc adequacy of the diet.[23,24] Adherence to largely vegan diets may compromise the immune status of individuals, including those living in developed countries.[25]

Vegetarianism can be classified into the following plant-based subgroups:

- **Lacto-ovo-vegetarian:** they consume animal products like eggs, milk and honey, excluding all flesh foodstuffs like meat, poultry, fish, etc.
- **Lacto-vegetarian:** they allow the consumption of dairy products, honey but exclude flesh foodstuffs and eggs etc.
- **Ovo-vegetarian:** the consume eggs along with vegetarian diet, excluding the consumption of all other animal products.
- **Vegan**: They exclude consumption of all animal products.
- **Vitarian**: They consume organic, raw and fresh foods, excluding tea and coffee.
- **Liquidarian:** They permit the consumption of vegetarian food like juices.
- **Fruitarian:** They exclude consumption of vegetables, flesh foodstuffs and animal products.
- **Sproutarian:** They consume sprouted plant seedling, like grains, vegetables & fruits.
- **Semi-vegetarian:** It is the transitional form between vegetarian and omnivorous diet.[26]

Influence of Indian diet on anemia

In India, the low-income population usually consume only two meals a day, comprising mainly wheat or millet chapatti or rice, with pickles, onion, salt and chillies, vegetables or pulses. Whereas, the middle- and high-income populations commonly consume three square meals per day. The general food consumption patterns followed by low-, middle- and high-income populations are briefed in table 3.

Table 3: General food consumption pattern of low-, middle- and high-income populations of India

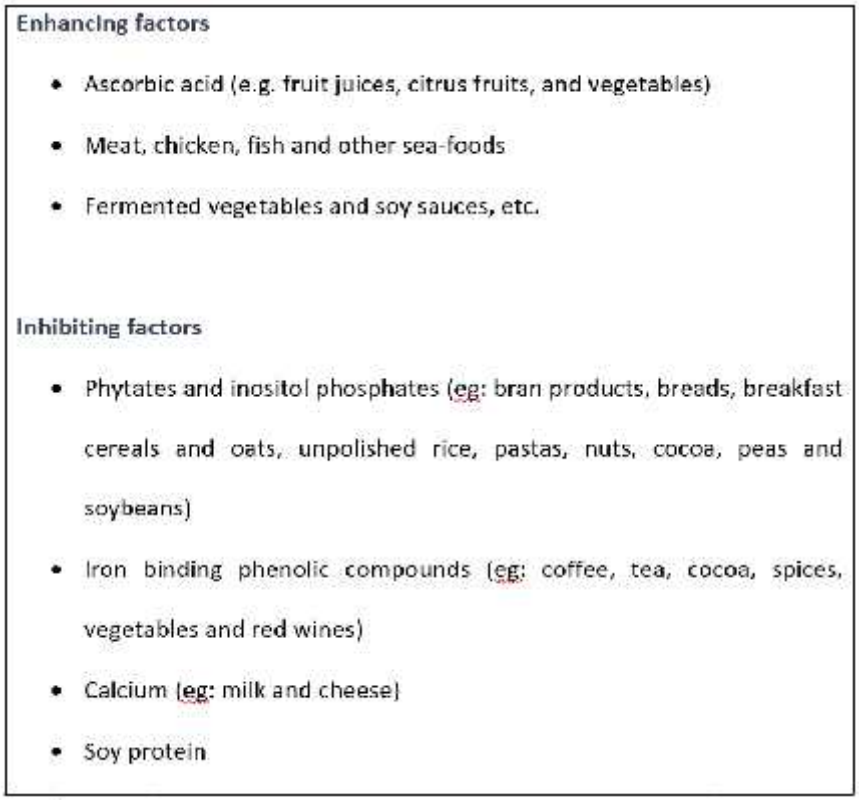
Meal	Food consumed	
	Low income population	**Middle or high income population**
Breakfast or brunch	Wheat or millet chapati or rice with tea; vegetables; pickle; onion + salt + chillies +/-jaggery	Wheat parathas and/or millet chapatti + milk or curd +/- tea; rice +vegetables +/- pulse, + bananas or breads with butter or jam + fruit
Mid morning	Tea	Coffee or tea with biscuits, samosa or pakora
Lunch	**----**	Wheat with millet chapati or rice with vegetable +/- pulse with salads or fruit
Mid afternoon	Tea + bun or biscuit	Coffee or Tea + biscuit, samosa or pakoras
Evening or dinner	Similar to brunch, usually including a vegetables or pulses	Similar to lunch

Source: Sharma KK. Improving bioavailability of iron in Indian diets through food-based approaches for the control of iron deficiency anemia.

In Indian diet, the iron absorption is inhibited by the presence of phytates in grains, nuts, vegetables, fruits, roots, and polyphenols in coffee, tea, vegetables, spices and herbs.[27] A study by Chiplonkar et al. has noted that cooking of pulses & cereals attributes to the losses of 22-24% of iron content.[28]

Jayanthi et al. have noted that in Indian family, the dietary pattern is mainly dependent on geographical location, religion, socio-economic status, and community and family practices.[37] In a chapter titled 'Geographic Aspects of Vegetarianism: Vegetarians in India', the author SutapaAgarwal has highlighted that the vegetarian dietary patterns in India are marked by reduced consumption of animal-source foods, in particular red meat, and inclusion of various vegetables, cereals, fruits, pulses, seasonings, spices, and cooking practices.[38]

Factors influencing dietary iron absorption



Source: Sharma KK. Improving bioavailability of iron in Indian diets through food-based approaches for the control of iron deficiency anaemia.

A 2012 survey by Ram Mohan et al. has found that 52% of Indian women of age between 15 and 49 years were anemic. Moreover, the study has highlighted that vegetarian women in India are significantly more prone to develop iron deficiency anemia than their non vegetarian counterparts. The researchers have recommended targeted iron supplementation, Mass food fortification with iron, control of diseases like hookworm and malaria, and effective public education about iron rich sources of food as effective strategies to reduce the prevalence of iron-deficiency anemia in Indian women.[29]

Influence of vegetarian diet on blood and morphology parameters

Despite the advantages of vegetarian diet, it is associated with a high risk of deficiency of certain nutrients, such as iron and vitamin B12. Low bioavailability of iron from plant sources could be due to the occurrence of absorption inhibitors such as phytate, polyphenols and oxalate.Phytate has been identified as one the dominant absorption inhibitors and is a key component of whole grains, nuts and legumes. Grain products are a source of more than 50% phytates in Indian diet. Due to these reasons, the Institute of Medicine has stated that vegetarians require 1.8 times more iron as opposed to non-vegetarians.[4]

A systematic review and meta-analysis done by Haider et al., which combined data of 24 cross-sectional studies, showed that adult non-vegetarians have significantly higher serum ferritin levels than their vegetarian counterparts. Their results showed that vegetarians are more prone to have lessened iron stores as opposed to non-vegetarians. And since higher iron stores are a risk factor type 2 diabetes, it is recommended that both vegetarians and non-vegetarians should regularly control their iron status and enhance their diet regarding the content and bioavailability of iron by consuming more of plants and less of meat. However, the

study was inadequate in the way that it did not consider other parameters like vitamin B12 or folic acid, which may too be affected by type of diet.[30] In line with these findings, a literature review by Pawlak et al. has noted that a higher percentage of vegetarian females, had ferritin below deficiency cutoff as opposed to the nonvegetarian subjects. Similar trend was showed by male vegetarians when compared to the nonvegetarians.[4]

Chaturvedi et al. evaluated 300 adolescent girls in age group of 10-19 years of two Government Schools in Ranchi from July 2015 to July 2016. The study noted the occurrence of anemia in 82% of the girls, despite majority (91.7%) of them having normal BMI. The incidence was more in vegetarians than in non-vegetarians, especially in those having rice-based diet. In addition, nearly 86% of the adolescent girls (200/233) who consumed junk foods frequently were found to be anemic. A gradual increase in anemia severity from early to late adolescent group was also noted.[49]A polish study by Nazarewicz has noted statistically significant reduction of white blood cells, neutrophils, and blood urea levels, and insignificantly lower levels of red blood cells, hemoglobin, hematocrit and platelet in vegetarian group, as opposed to omnivorous counterparts. Moreover, the study has associated these changes with protein deficiency linked to vegetarian diet.[31]

Obeid et al. have evaluated 29 vegans, 64 lacto & lacto-ovo-vegetarians, and 20 meat consumers to investigate the impact of hematological indices in relation to iron status and vitamin B12. The study noted an association between vitamin B12 and iron deficiencies with vegetarian diet. In addition, the researchers also noted reduced lymphocyte counts and an increased MCV in vegans when compared tolacto/ lacto-ovo-vegetarians.Vegans also displayed the highest methylmalonic acid and homocysteine levels.[32]

A comparative study by Mahajani and Bhatnagar evaluated the prevalence and severity of anemia in vegetarian and non-vegetarian women residing in Udaipur, Rajasthan. The authors have noted a highly significant difference in the BMI (P <0.01) and hemoglobin levels of vegetarian (12.07 ± 1.08 g/dl) and non-vegetarian group (10.09 ± 0.95 g/dl). Nearly 40% vegetarians were having moderate anemia and 60% were mild anemic, as opposed to 46.66% of non-vegetarians belonging to normal category.[33]

A study by Tong et al. evaluated the difference in hematological parameters and anemia prevalence in white and British Indian vegetarians and nonvegetarians. The researchers have found that, in contrast to white regular meat eaters, vegans, less meat eaters, poultry meat eaters and fish eaters had significantly lower platelet counts with higher platelet volume. Also, a Higher platelet count with lower volume was noted in vegans, as opposed to British Indian meat eaters. In line with these findings, a descriptive study conducted among Thai vegans has reported lower Hb and hematocrit in vegans, indicating the occurrence of anemia. Moreover, non-significantly increased mean corpuscular volume, RBC distribution width (RDW), and lower mean corpuscular Hb were observed in the group.[2]

A study conducted by Khanna et al in Indian female athletes found no significant difference for energy and carbohydrate intake on comparison of lacto vegetarians (27.2 +/- 4.2%) non- vegetarians (24.3 +/-4.0%) and ovo-lacto vegetarians. The study noted increased B-complex vitamins, iron intake, and hemoglobin levels (P<0.05) in non-vegetarians, and high calcium and fibre intake in lacto-vegetarians (P<0.05).[34]

Several literature studies have demonstrated that the variation in hematological parameters noted in vegetarians could be due to poor non-heme/ total

iron absorption, and reduced ferritin concentrations. Another probable reason could be the impairment of erythropoiesis due to vitamin B12 deficiency, and the abnormally developed RBCs might be too large to escape the bone marrow. This may subsequently manifest as decreased counts of reticulocytes and mature RBCs. In patients with severe iron deficiency or high folate intake, the simultaneous presence of microcytic anemia could conceal the occurrence of macrocytic anemia induced by vitamin B-12 deficiency.[2]

Lacunae in literature

Anemia had accounted for 8.8% of the total disability from all conditions in 2010 and the burden was highest in children <5 years and women. Although anemia is pathophysiologically diverse and often multifactorial, there is very limited literature evidence evaluating the influence of vegetarian diet on hematological parameters. Despite the belief that vegetarian diet confers various health benefits, there are still justified concerns that lack of certain critical nutrients in vegetarian diet may cause serious nutritional deficiencies. In addition, a large proportion of Indian population subsist on iron-poor vegetarian diets for religious, economic, and cultural reasons, Understanding the effect of diet on RBC morphology and indices is crucial to identify high-risk population, conduct surveillance of anemia and implement effective prevention strategies. The present study is intended to compare RBC morphology and indices in patients of vegetarian diet with non-vegetarian diet.

METHODOLOGY

The present study was conducted KLE Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi.

Studydesign

The study was a hospital-based cross-sectional study.

Study period

It was conducted over a period of 1 year from 1stJanuary 2019 to 31st December 2019.

Study site

The present study was carried out at KLE Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi. A tertiary care teaching hospital attached to Jawaharlal Nehru Medical College, Belagavi.

Study population

Patients admitted in the wards of General medicine at KLES Dr. Prabhakar Kore Hospital and MRC fulfilling the inclusion criteria.

Sample size

Sample size was calculated using the following formula:

$n=4\ pq/d^2$(n=sample size, p= prevalence of the disease, q= 100-p, d=absolute error).

Sample size= 96.

But for the convenience of the study, sample size has been taken as 100.

The prevalence of anemia in vegetarians was estimated to be around 40%.[33] Stratified sampling technique was used for the study.

Inclusion criteria

- Patients >18 years
- Non-vegetarian
- Lacto-ovo-vegetarian
- Lacto-vegetarian
- Ovo-vegetarian
- Vegan

Exclusion criteria

- Taking vitamin, mineral supplements or hematinics
- Hemoglobinopathies, hematological cancers
- Pregnant

Ethical clearance

Prior to the commencement, the study was cleared by the Institutional Ethics Committee, Jawaharlal Nehru Medical College, Belagavi.

Informed consent

Informed consent was obtained from all the study participants and only those participants who willingly signed the informed consent were included in the study. The risks and benefits involved in the study, and the voluntary nature of participation were explained to the participants before obtaining consent. Confidentiality of the study participants was maintained.

Data collection

All relevant parameters were documented in a structured Study Proforma.

Methodology

The study included inpatients of Dr. Prabhakar Kore Hospital and MRC, Belagavi. Each individual interested in participating the study was interviewed and asked to complete a questionnaire to determine if they met all the inclusion criteria. Vegetarians were broadly defined as those who did not consume meat or fish for approximately 6 months prior to and during the study. A written informed consent form was filled by each participant and blood samples were collected.

Venous blood samples were collected in dipotassium EDTA vial. The sample was run on hematologyautoanalyzer to calculate hemoglobin, RBC count, red blood cell indices including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW). Anemiacutoff was taken as hemoglobin (Hgb) <13 g/dL in males and <12 g/dL in females as per WHO recommendations. A peripheral blood smear (PBS) was prepared and stained with Leishman Stain in each case. PBS of each participant was examined under microscope to evaluate the type of anemia.

Investigations

- Hemoglobin
- RBC count
- MCV
- MCH
- MCHC
- RDW
- Peripheral smear

Severity Grading of Anemia

The WHO Grading for Anemia was employed in this study, categorising patients into mild, moderate and severe anemia based on the hemoglobin levels. The grading system is as below.

❖ **WHO Grading of Anemia**

Population	Non Anemia	Mild Anemia	Moderate Anemia	Severe Anemia
Non-Pregnant Women (15 years of age and older)	12 g/dl or higher	11-11.9 g/dl	8-10.9 g/dl	Below 8 g/dl
Men (15 years of age and older)	13 g/dl or higher	11-12.9 g/dl	8-10.9 g/dl	Below 8 g/dl

STATISTICAL METHODS

Data was analyzed using R i386 3.6.0 statistical software. Continuous variables are represented by mean ± SD form and categorical variables by a frequency table. Chi Square test was used to check the association between different categorical variables. ANOVA was used to analyze the relationship between severity of anemia and lab parameters. Independent t tests were used to compare diet and genders with lab parameters. In the tables below p-value <0.05 indicates statistical significance of variables.

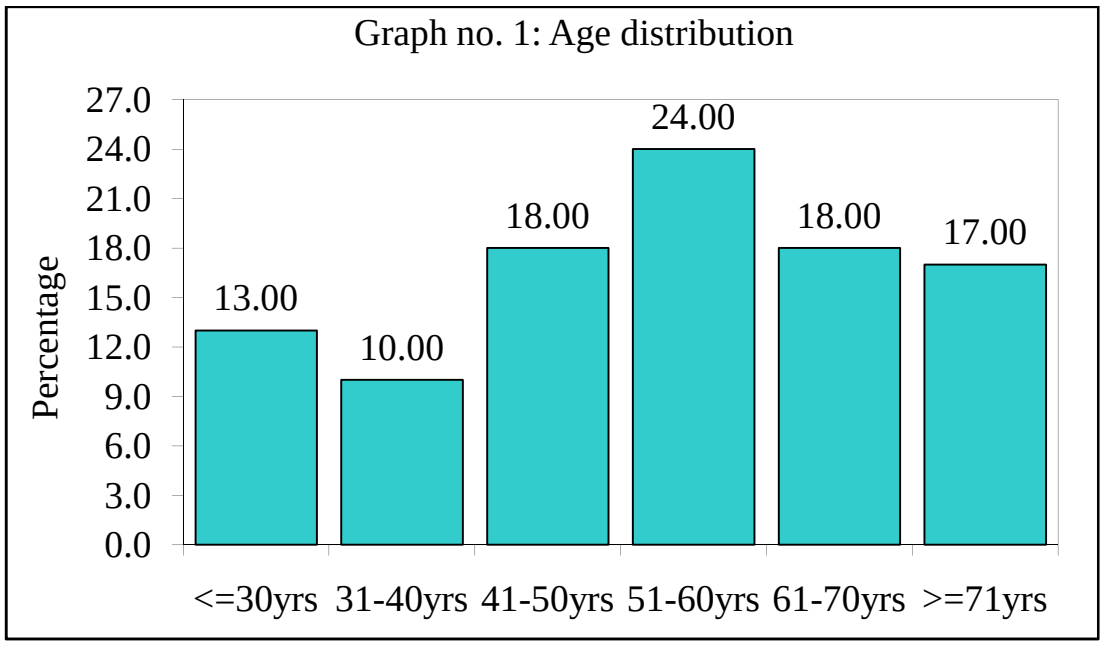
RESULTS

The present study was conducted in the department of General Medicine, KLES Dr. Prabhakar Kore Hospital and Medical Research Centre, Belgaum from January 2019 to December 2019.A total of 100 cases were studied and the findings obtained are tabulated as below.

Table no.1- Age distribution

Age groups	No of patients	% of patients
<=30yrs	13	13.00
31-40yrs	10	10.00
41-50yrs	18	18.00
51-60yrs	24	24.00
61-70yrs	18	18.00
>=71yrs	17	17.00
Total	100	100.00
Mean age	53.29	
SD age	17.39	

Graph No. 1- Age distribution



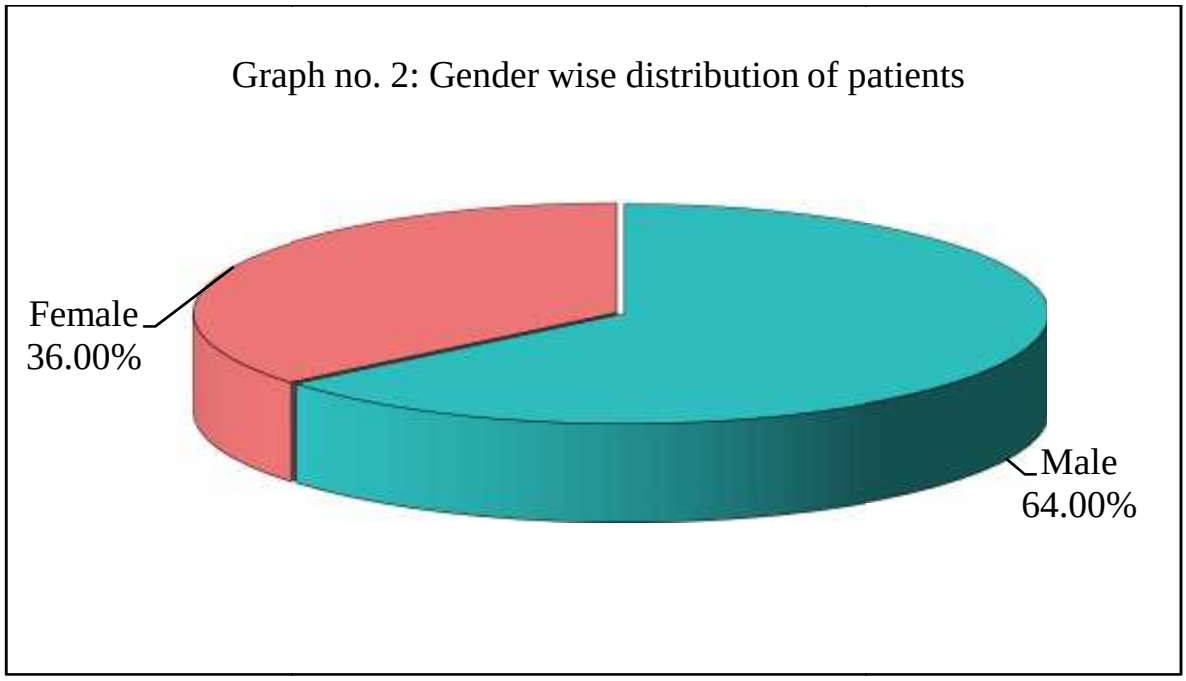
In the present study of 100 patients, the patients age ranged from 19-90 years. Maximum number of cases, that is, 24 were in the age group of 51-60 years (24%). Also observed was that more number of cases were above 40 years i.e. 18 between 41-50 years (18%), 24 between 51-60 years (24%), 18 between 61-70 years (18%) and 17 were more than 71 years (17%). Only 23 cases were seen in the age group below 40 years. The mean age at presentation was 53.29 ± 17.39 years.

Inference- Majority of patients were in the age group of 40 years and above.

Table No 2- sex wise distribution

Gender	No of patients	% of patients
Male	64	64.00
Female	36	36.00
Total	100	100.00

Graph No. 2- sex wise distribution



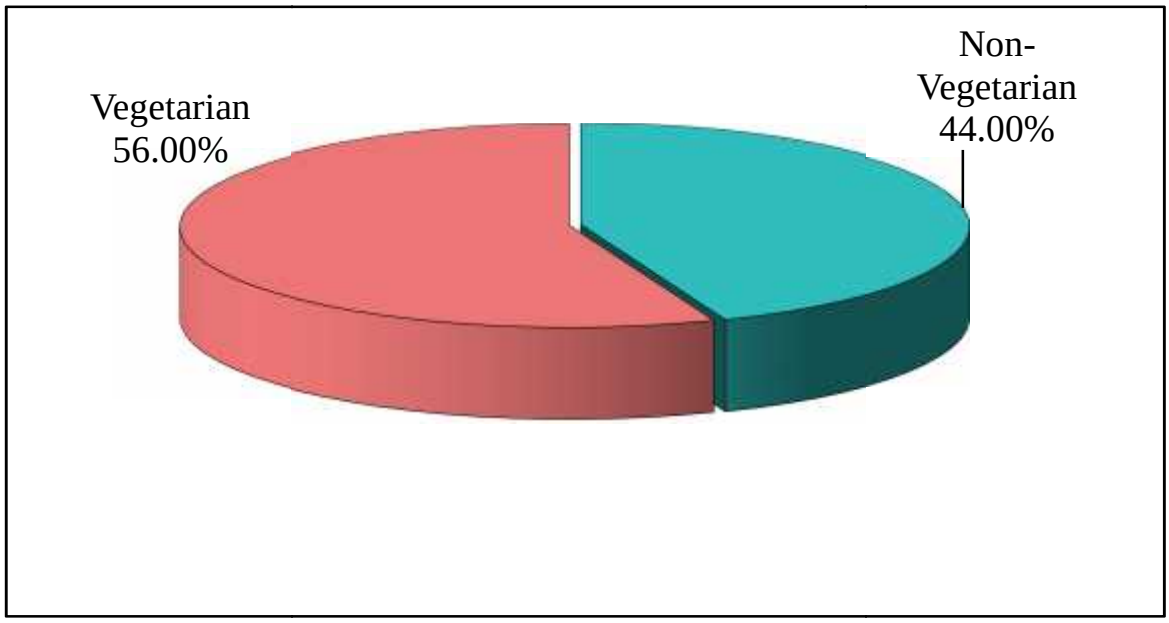
In the present study, males were 64 (64%) and females were 36 (36%), accounting for a male: female ratio of 1.78:1.

Inference: Male preponderance was observed.

Table 3: Dietetic habits of patients (vegetarianism/ non-vegetarianism)

Diet	No. of patients	% of patients
Non-vegetarian	44	44.00
Vegetarian	56	56.00
Total	100	100.00

Graph No. 3: Pie chart illustration of Dietetic habits of patients (vegetarianism/ non-vegetarianism)



In the present group of 100 patients, we observed that 56 patients were vegetarians (56%) and remaining 44 patients were non-vegetarians (44%)**.**

Table 4: Comparison of gender with their diet

Diet	Total (N=100)	Male (N=64)	Female (N=36)	P value
Non-vegetarian, N (%)	44(44.00)	28(43.74)	16(44.44)	0.9460 (Chi-square=0.0050)
Vegetarian, N (%)	56(56.00)	36(56.25)	20(55.56)	

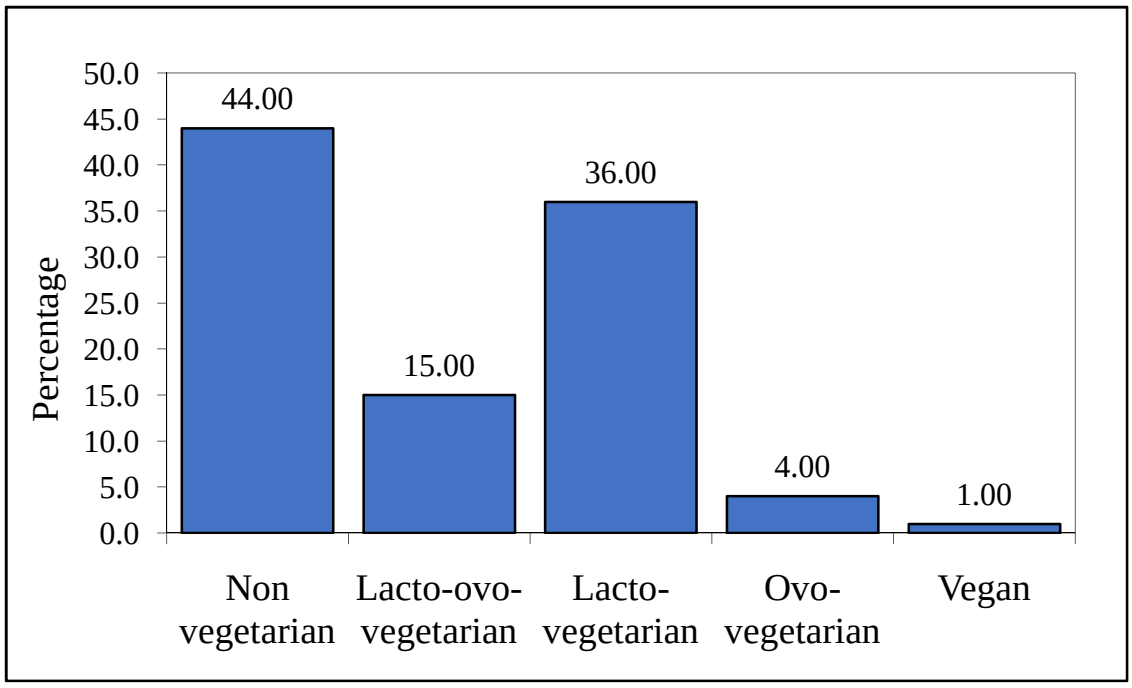
Similarly, when the dietetic habits of 100 patients was compared with gender, we found a total of 56 vegetarian patients (includes both males and females) and 44 non-vegetarians (includes both males and females).

The diet did not have any bearing on gender as shown in the above table. P value (0.9460) was statistically insignificant.

Table 5: Detailed different types of food habits

Groups	No. of patients	% of patients
Non vegetarian	44	44.00
Lacto-ovo-vegetarian	15	15.00
Lacto-vegetarian	36	36.00
Ovo-vegetarian	4	4.00
Vegan	1	1.00
Total	100	100.00

Graph no 4: Pictorial representation of detailed different types of food habits

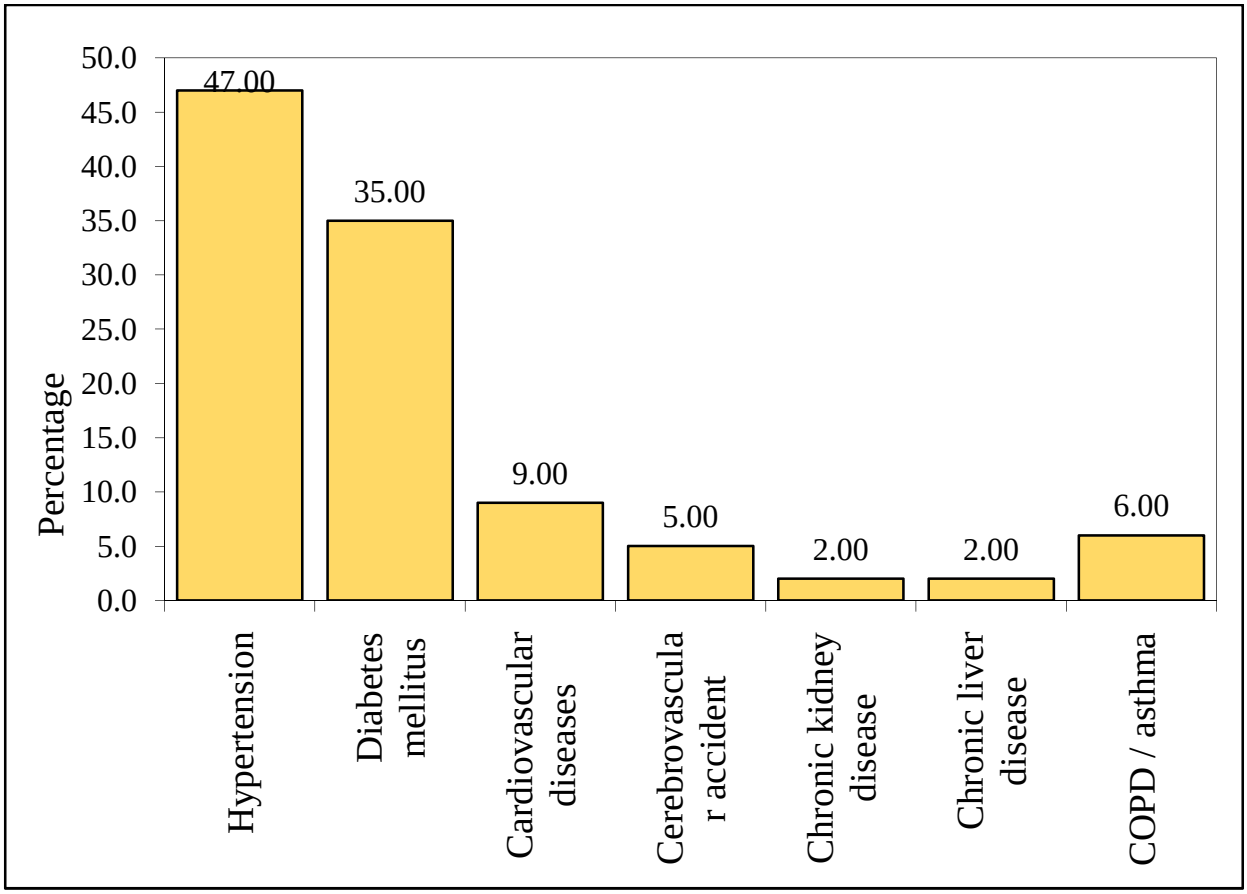


In our 100 patients, we observed the different types of food habits, and found to have 44 non-vegetarian patients (44%), 36 lacto-vegetarians (36%), 15 lacto-ovo-vegetarians (15%), and the other types of food habits are depicted in the above table.

Table 6: Distribution of patients with comorbidities

Co-morbidities	No. of patients	% of patients
Hypertension	47	47.00
Diabetes mellitus	35	35.00
Cardiovascular diseases	9	9.00
Cerebrovascular accident	5	5.00
Chronic kidney disease	2	2.00
Chronic liver disease	2	2.00
COPD / asthma	6	6.00

Graph no 5: Pictorial representation of distribution of patients with comorbidities



The following were the comorbidities associated with our study population. Majority of the patients were hypertensive (47 patients i.e. 47%), followed by 35 patients with diabetes mellitus (35%), 9 patients with cardiovascular diseases (9%), 6 patients with COPD/Asthma (6%), 5 patients had cerebrovascular accidents (5%), while out of the remaining 4, i.e. 2 patients each had chronic liver disease and chronic kidney disease (2% each).

Table 7: Comparison of diet with co-morbidities.

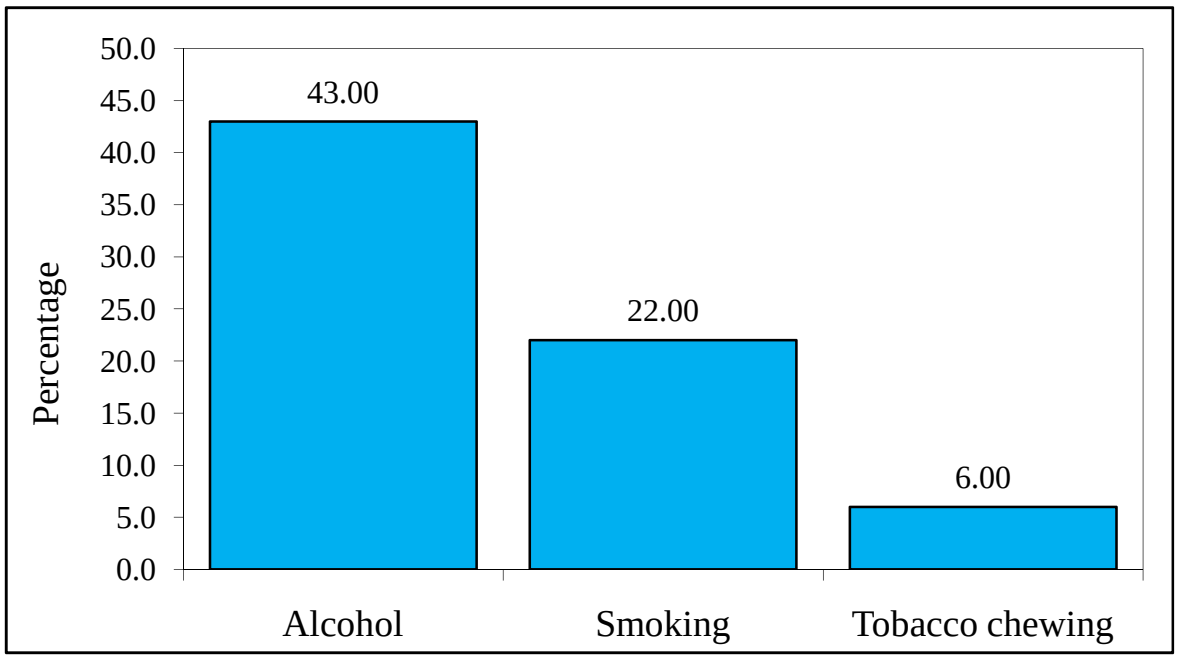
Co-morbidities	Total (N=100)	Non-vegetarian (N=44)	Vegetarian (N=56)	Chi-square	P-value
Hypertension, N (%)	47(47.00)	18(40.91)	29(51.79)	1.1700	0.2790
Diabetes mellitus, N (%)	35(35.00)	14(31.82)	21(37.50)	0.3500	0.5540
Cardiovascular diseases, N (%)	9(9.00)	3(6.82)	6(10.71)	0.4570	0.4990
Cerebrovascular accident, N (%)	5(5.00)	2(4.55)	3(5.36)	0.0340	0.8530
Chronic kidney disease, N (%)	2(2.00)	1(2.27)	1(1.79)	0.0300	0.8630
Chronic liver disease, N (%)	2(2.00)	0(0.00)	2(3.57)	1.6030	0.2050
COPD / asthma, N (%)	6(6.00)	5(11.36)	1(1.79)	4.0080	0.0450*

The above table depicts the dietetic habits of patients with their comorbidities.

Table 8: Habits of the patients

Habits	no. of patients	% of patients
Alcohol	43	43%
Smoking	22	22%
Tobacco chewing	6	6%

Graph no. 6: Pictorial depiction of distribution of patients based on their habits



In our present study of 100 patients, a total of 48 patients had 1 or the other habits (alcohol, smoking or tobacco chewing). Few had more than 1 habits (13 i.e. 13%) and very few had all the 3 habits (i.e. alcohol, smoking or tobacco chewing, i.e. 5 patients). 52 patients did not have any habits.

Table 9: Comparison of diet of patients with their habits.

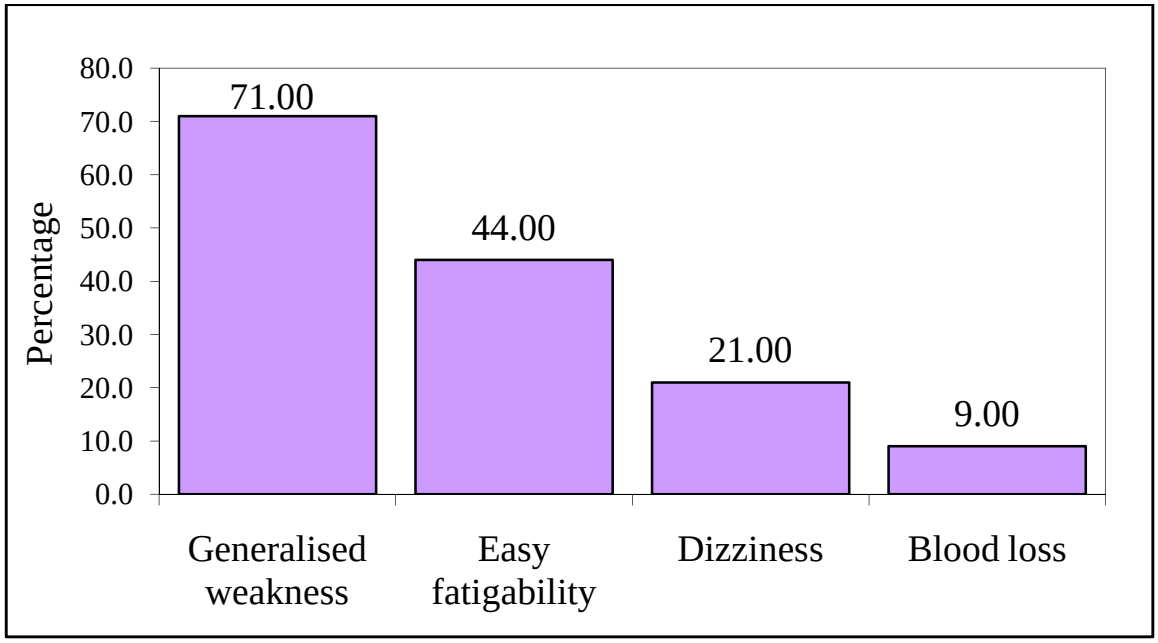
Habits		Total (n=100)	Non- vegetarian (N=44)	Vegetarian (N=66)	Chi-square	P-value
Smoking, N (%)	Yes	22	12(27.27)	10(17.86)	1.2730	0.2590
	No	78	32(72.73)	56(82.14)		
Alcohol, N (%)	Yes	43	19(43.18)	24(42.86)	0.0010	0.9740
	No	57	25(6.82)	42(57.14)		
Tobacco chewer, N (%)	Yes	6	3(6.82)	3(5.36)	0.0930	0.7600
	No	94	41(93.18)	63(94.64)		

The above table depicts that most of the patients who belonged to the non-vegetarian group had 1 or the other habits though the P value is statistically insignificant when compared to the diet and habits of patients.

Table 10: Clinical presentation of patients

Clinical presentation	No. of patients	% of patients
Generalized weakness	71	71.00
Easy fatigability	44	44.00
Dizziness	21	21.00
Chronic Blood loss	9	9.00

Graph no. 7: Graphical representation of clinical presentation of patients.



Out of 100 patients, majority had generalized weakness- 71 (71%) as the presenting complaint, followed by easy fatiguability in 44 (44%), dizziness in 21 (21%), and chronic blood loss (i.e. bleeding per rectum -4 , melaena -1, hematemesis -1, menorrhagia- 3) in 9 patients (9%). Some patients had overlapping clinical symptoms.

Table 11: Comparison of clinical presentation with dietetic habits of the patients

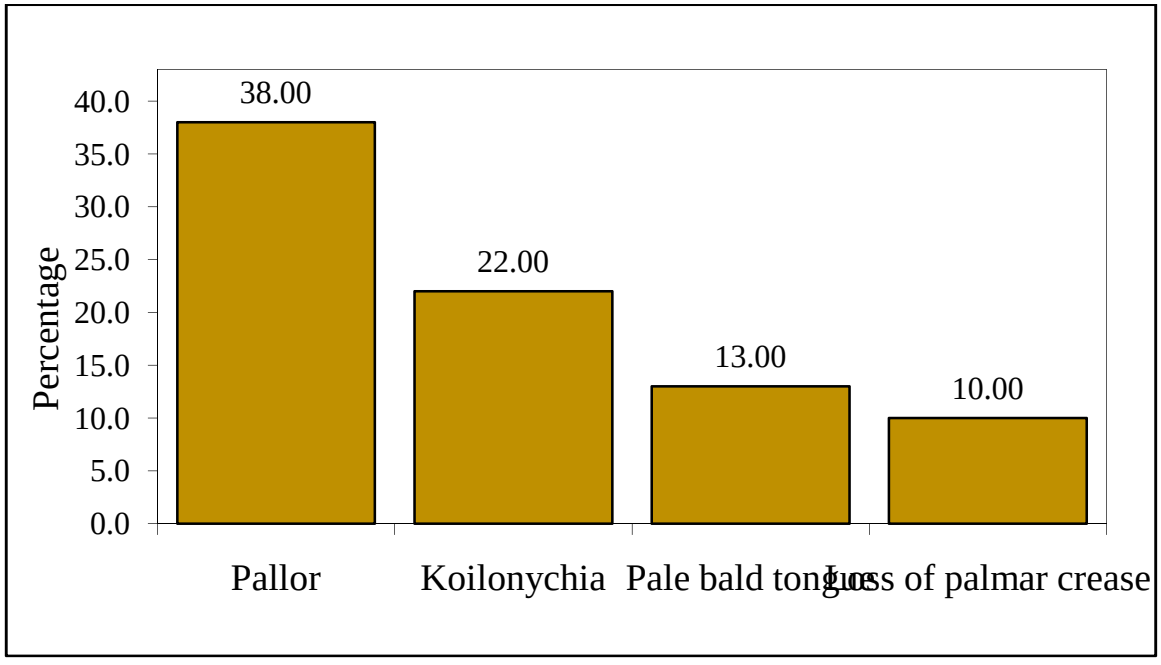
Clinical presentation		Non- vegetarian (N=44)	Vegetarian (N=66)	Chi-square	P value*
Generalised Weakness, N (%)	Yes	30(68.18)	41(73.21)	0.3030	0.5820
	No	14(31.82)	25(26.79)		
Easy fatigability, N (%)	Yes	16(36.36)	28(42.42)	0.404	0.2501
	No	28(63.64)	38(57.58)		
Dizziness, N (%)	Yes	3(6.82)	18(32.14)	9.5250	0.0020
	No	41(93.18)	36(67.86)		
Blood loss, N (%)	Yes	4(9.09)	5(7.56)	0.0010	0.9780
	No	40(91.91)	61(92.44)		

When we attempted to see clinical presentations of patients with their dietetic habits, the above observations were made, as shown in table no.11.

Table 12: Clinical signs

Clinical signs	No. of patients	% of patients
Pallor	38	38.00
Koilonychia	22	22.00
Pale bald tongue	13	13.00
Loss of palmar crease	10	10.00

Graph no. 8: Distribution of clinical signs



In our 100 patients, we observed various clinical signs of anemia, pallor in 38 (38%), koilonychia in 22 (22%), pale bald tongue in 13 (13%) and loss of palmar crease in 10 patients (10%). Some patients had overlapping clinical signs.

Table 13: Comparison of diet with clinical signs

Clinical signs		Non- Vegetarian (N=44)	Vegetarian (N=66)	Chi-square	p-value
Pallor, N (%)	Yes	13(29.55)	25(44.64)	2.3840	0.1230
	No	31(71.45)	41(55.36)		
Koilonychia, N (%)	Yes	7(15.91)	15(26.79)	1.6990	0.1920
	No	37(84.09)	41(73.21)		
Pale bald tongue, N (%)	Yes	2(4.55)	11(19.64)	4.9660	0.0260*
	No	42(95.45)	55(80.36)		
Loss of palmar crease, N (%)	Yes	1(2.27)	9(16.07)	5.2130	0.0220*
	No	43(97.73)	57(83.93)		

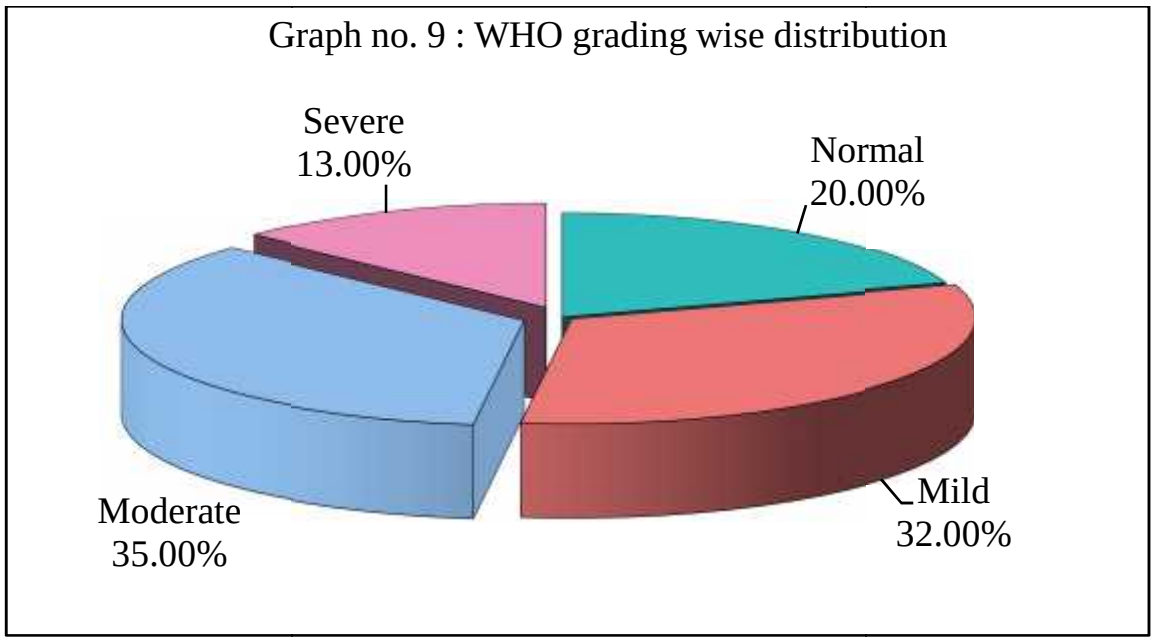
*$p<0.05$

The above table depicts the various clinical signs based on their dietetic habits. And we found statistically significant finding of pale bald tongue/ loss of palmar crease in vegetarian group.

Table 14: Grading of Anemia (WHO criteria)

WHO grading	No. of patients	% of patients
Normal (for males- 13 g/dl or higher. For females 12 g/dl or higher)	20	20.00
Mild (for males- 11-12.9 g/dl For females 11-11.9 g/dl)	32	32.00
Moderate (8-10.9 g/dl)	35	35.00
Severe (below 8g/dl)	13	13.00
Total	100	100.00

Graph no. 9: Distribution of grading of anemia (WHO criteria)



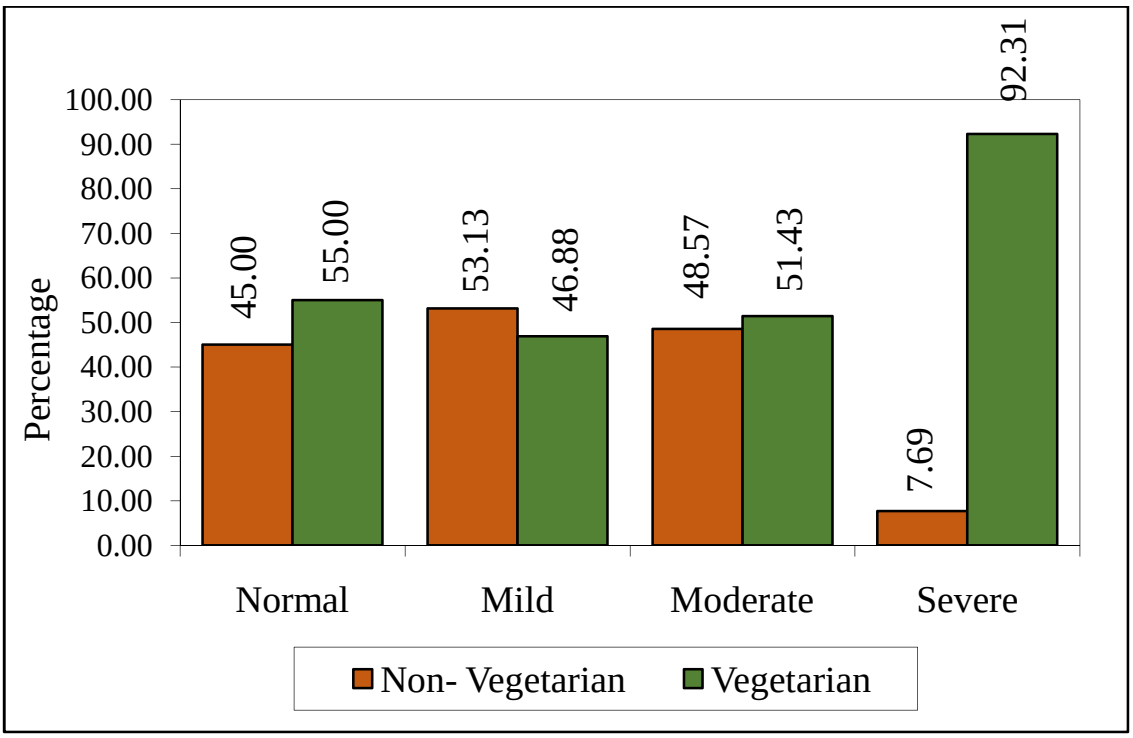
Majority of our patients i.e. 67 patients have either mild/moderate anaemia (mild- 32%, moderate- 35%), 13 had severe anaemia (13%) and remaining 20 (20%) did not have anaemia.

Table 15: Comparison of Anaemia with Diet

WHO grading	Non- Vegetarian (N=44)	Vegetarian (N=66)	Chi-square	P value*
Normal, N (%)	9(20.46)	11(16.67)	8.7142	0.0331
Mild, N (%)	17(38.64)	15(22.73)		
Moderate, N (%)	17(38.64)	18(27.27)		
Severe, N (%)	1(2.27)	12(18.18)		

*statistically significant if P<0.05

Graph no 10: Graphical representation of comparison of anemia with diet



In the present study of 100 patients, we observed different grades of anemia in 2 major dietetic groups (i.e. vegetarian and non-vegetarian groups), and we found statistically significant difference in both the groups, as far as anemia and severity were concerned (it was significant in vegetarian group)

Table 16 (A) Lab parameters

Lab parameters	Summary	Severity of anaemia					F-value	p-value
		Normal	Mild	Moderate	Severe	Total		
Haemoglobin	Mean	14.19	11.83	9.62	5.51	10.70	193.4892	0.0001*
	SD	1.59	0.59	0.77	1.65	2.82		
RBC count	Mean	9.66	4.39	4.06	2.24	5.05	2.1336	0.1010
	SD	21.27	0.78	0.73	1.22	9.65		
MCV	Mean	87.81	83.87	77.19	94.15	83.65	5.3863	0.0018*
	SD	2.58	11.52	11.37	29.77	15.08		
MCH	Mean	28.94	27.68	24.43	29.72	27.06	4.3783	0.0062*
	SD	1.35	4.34	4.59	11.95	5.92		
MCHC	Mean	32.95	32.89	31.45	30.64	32.10	8.1324	0.0001*
	SD	1.00	1.12	1.61	3.60	1.96		
RDW	Mean	13.84	15.61	18.51	19.88	16.83	11.7546	0.0001*
	SD	3.00	2.83	3.63	5.37	4.10		

*p<0.05

16 (B): Comparison of gender with severity of anaemia

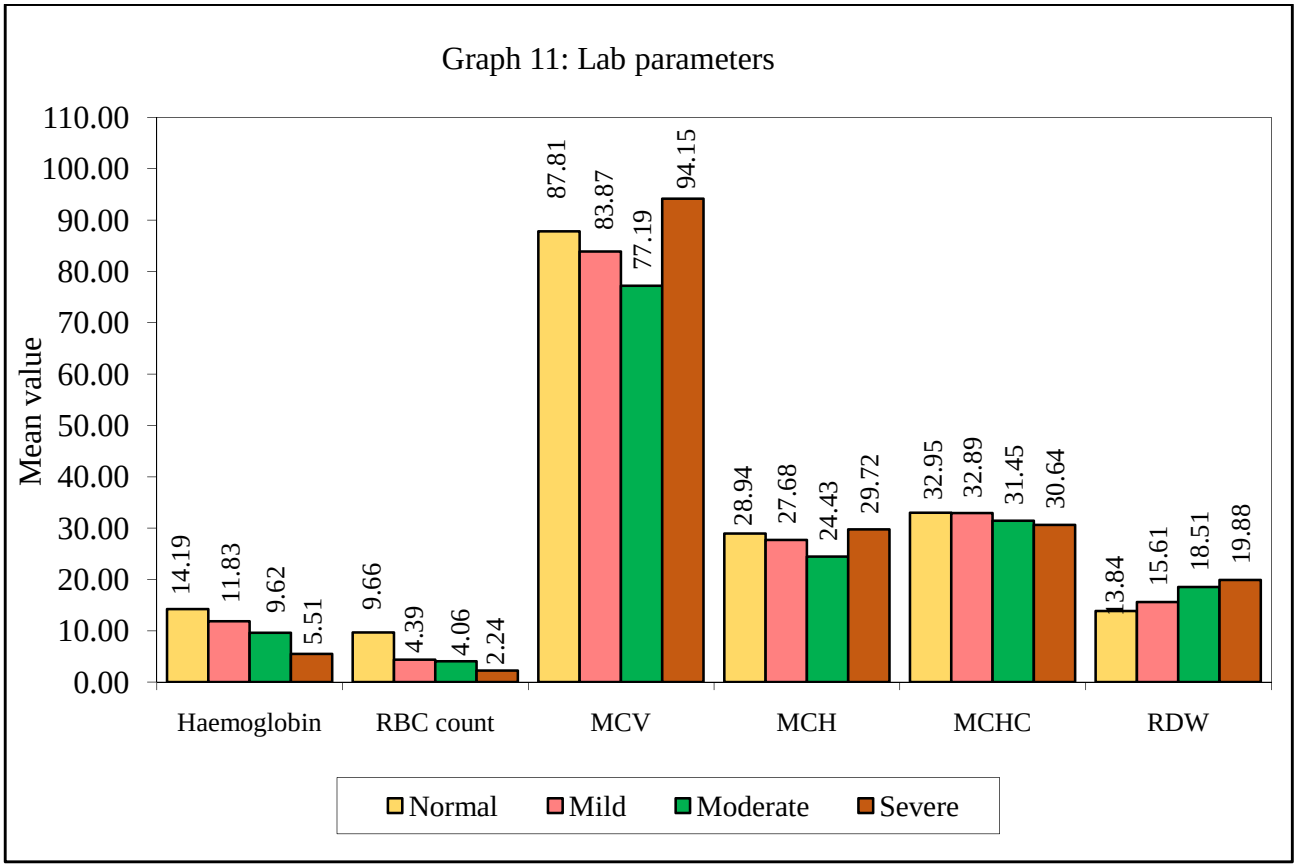
WHO grading	Male	%	Female	%	Total	Chi-square	p-value
Normal	15	75.00	5	25.00	20	7.9650	0.0470*
Mild	24	75.00	8	25.00	32		
Moderate	16	45.71	19	54.29	35		
Severe	9	69.23	4	30.77	13		
Total	64	64.00	36	36.00	100		

*p<0.05

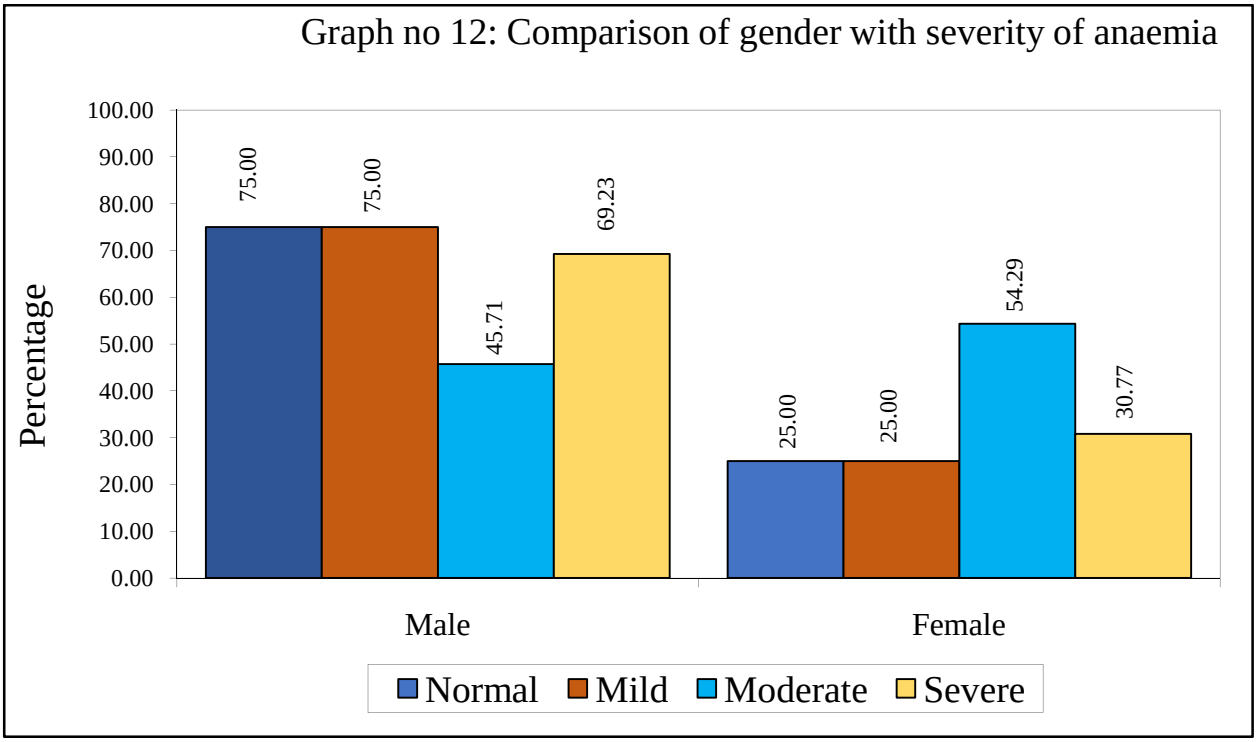
Table 16 (C): Mean values (Hb%, RBC count, MCV, MCH, MCHC, RDW)

	Haemoglobin	RBC count	MCV	MCH	MCHC	RDW
Mean	10.70	4.09	83.65	27.06	32.10	16.83
SD	2.82	1.10	15.08	5.92	1.96	4.10

Graph No 11- Lab parameters



Graph No 12- Comparison of gender with severity of anemia

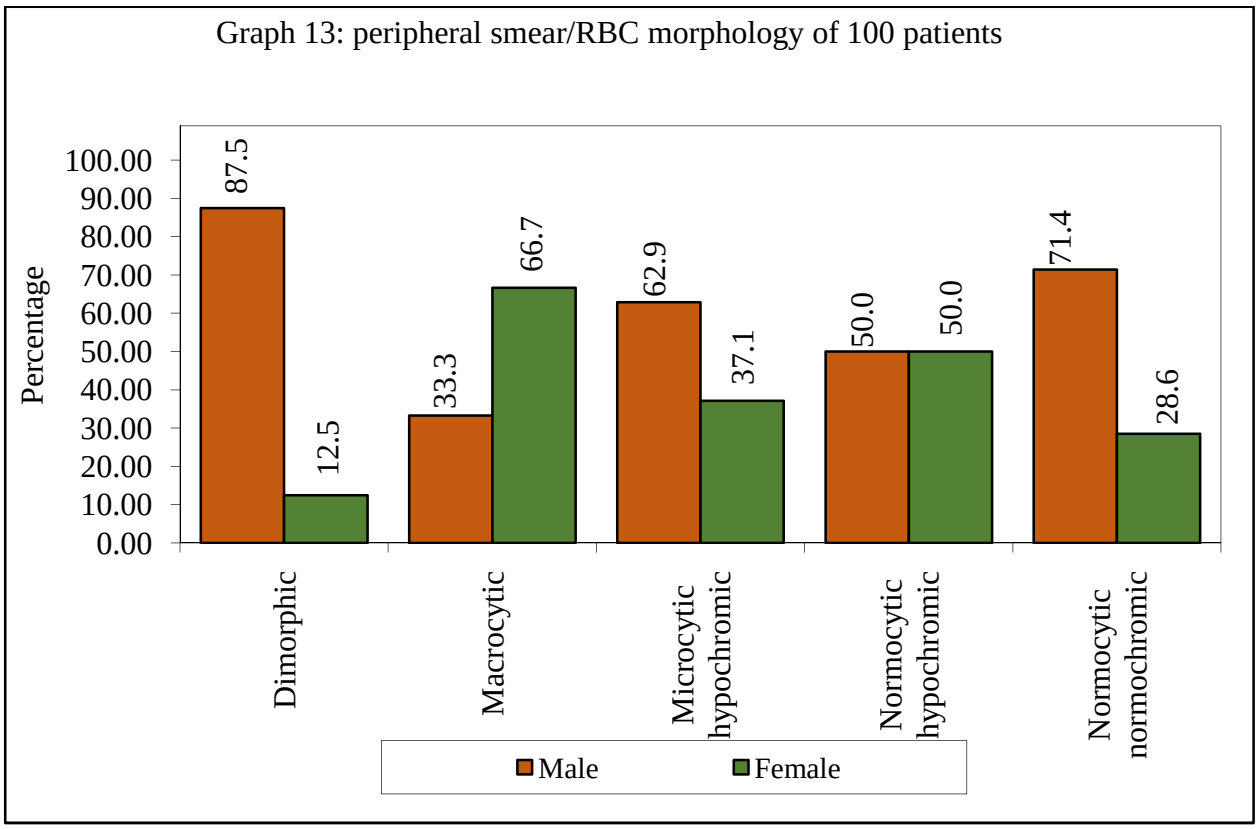


The above table depicts the patient's various blood indices like hemoglobin, Red Blood Cell count, MCV, MCH, MCHC, Red cell distribution width (RDW). There were 32 patients in the mild group of anemia, 35 in the moderate group and only 13 in the severe group.The mean values for hemoglobin was 10.70, RBC count was 4.09, MCV was 83.65, MCH was 27.06, MCHC was 32.10 and RDW was 16.83. Similarly, the Standard Deviation (SD) is depicted in the above table for all the parameters. P value (0.0470) was statistically significant in the severe group of anemia (males-9, females-4).

Table 17: Peripheral smear of 100 patients

Peripheral smear	Male	%	Female	%	Total	Chi-square	p-value
Dimorphic	7	87.5	1	12.5	8	7.345	0.2900
Macrocytic	2	33.33	4	66.67	5		
Microcytic hypochromic	22	62.86	13	37.14	35		
Normocytic hypochromic	8	50.00	8	50.00	16		
Normocytic normochromic	25	71.43	10	28.57	35		
Total	64	64.00	36	36.00	100		

Graph No 13- Peripheral smear/ RBC morphology of 100 patients

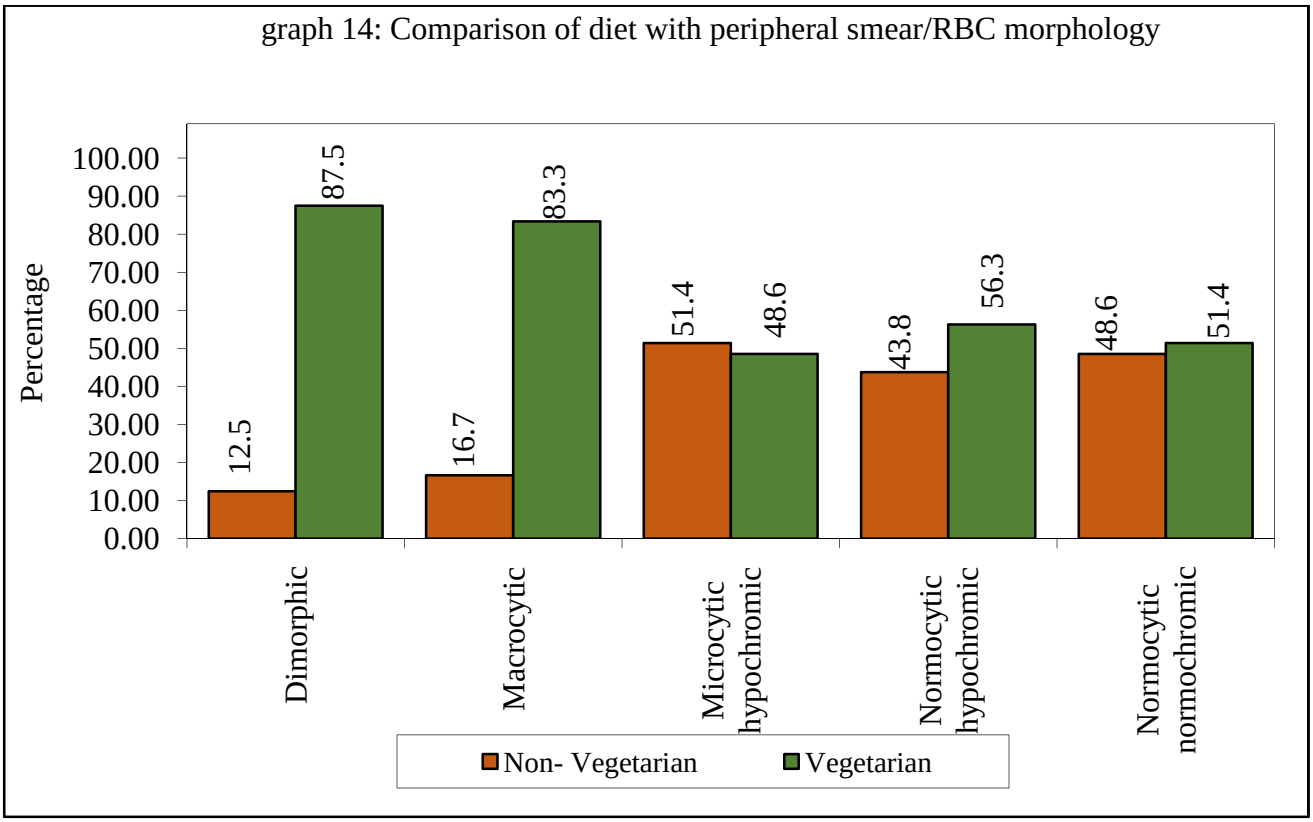


In the present study of 100 patients, the peripheral smears revealed findings which is depicted in table no. 17 gender wise. The p value was insignificant (p value = 0.2900).

Table 18: Comparison of diet with peripheral smear/RBC morphology

Peripheral smear	Non-Vegetarian	%	Vegetarian	%	Total	Chi-square	p-value
Dimorphic	1	12.50	7	87.5	8	7.7790	0.2550
Macrocytic	1	16.67	5	83.33	6		
Microcytic hypochromic	18	51.43	17	48.57	35		
Normocytic hypochromic	7	43.75	9	56.25	16		
Normocytic normochromic	17	48.57	18	51.43	35		
Total	44	44.00	56	56.00	100		

Graph No 14- Comparison of diet with peripheral smear/RBC morphology



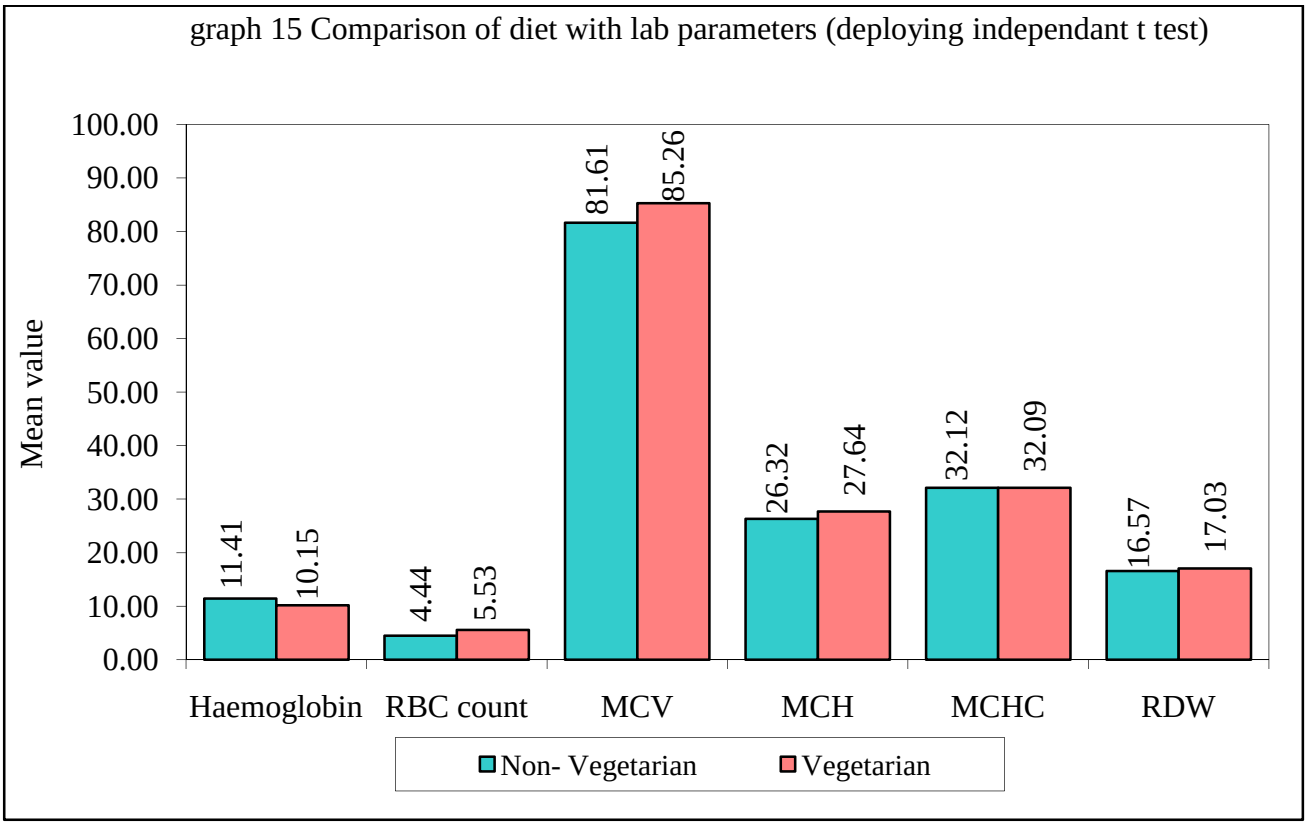
When we attempted to see if there was a correlation between diet and peripheral smear/RBC morphology, we found different pictures on peripheral smear. Again, the different types of anemia were more seen in vegetarian than in non-vegetarian patients, but the p value (0.2550) was statistically insignificant.

Table 19: Comparison of diet with lab parameters (deploying independent t test)

Clinical parameters	Non- Vegetarian		Vegetarian		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Haemoglobin	11.41	2.09	10.15	3.19	2.2771	0.0250*
RBC count	4.44	0.89	5.53	12.91	-0.5590	0.5774
MCV	81.61	12.40	85.26	16.83	-1.2028	0.2320
MCH	26.32	4.57	27.64	6.78	-1.1118	0.2689
MCHC	32.12	1.37	32.09	2.33	0.0890	0.9293
RDW	16.57	3.30	17.03	4.65	-0.5504	0.5833

*p<0.05

Graph No 15- Comparison of diet with lab parameters(deploying independent t test)

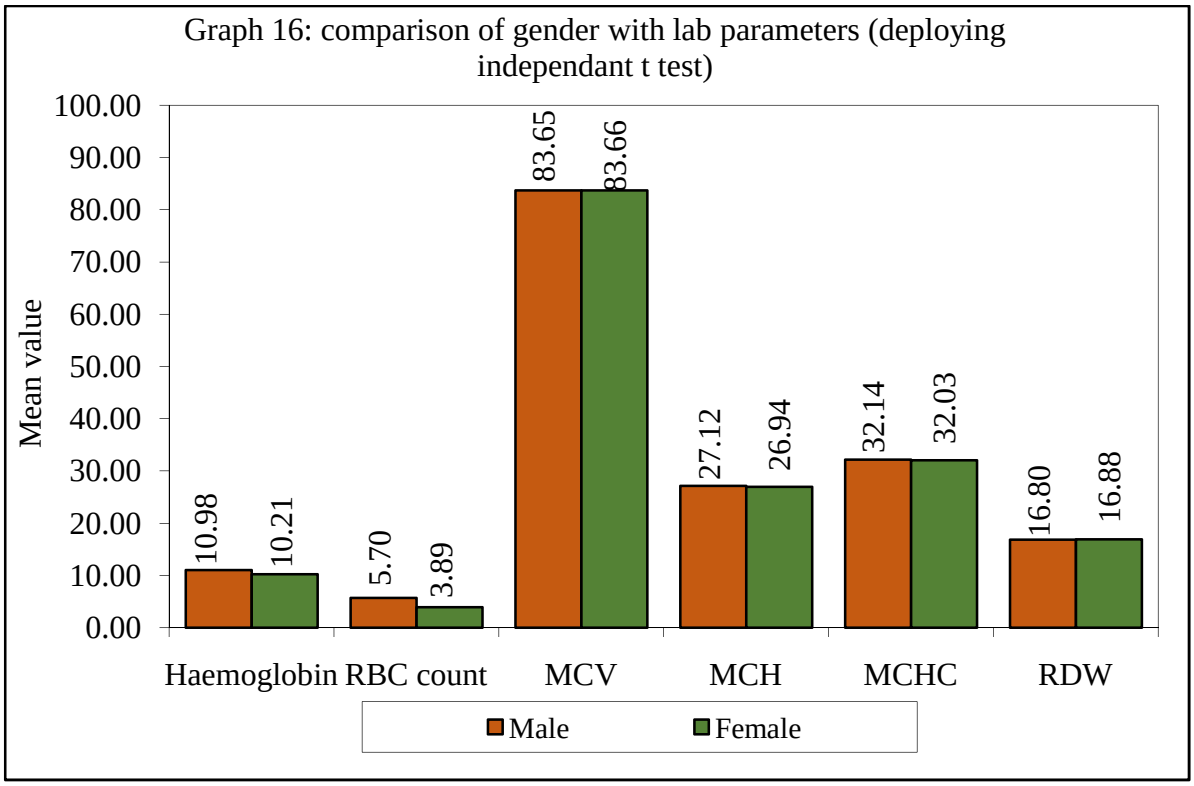


The table no. 19 depicts the t values and p values. Except for hemoglobin (Hb%), the rest of the RBC indices are statistically insignificant, as shown in the above table.

Table 20: Comparison of gender with lab parameters (deploying independent t test)

Clinical parameters	Male		Female		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Haemoglobin	10.98	3.16	10.21	2.02	1.3307	0.1864
RBC count	5.70	12.04	3.89	0.80	0.8987	0.3710
MCV	83.65	15.90	83.66	13.73	-0.0013	0.9990
MCH	27.12	6.22	26.94	5.42	0.1420	0.8874
MCHC	32.14	2.17	32.03	1.53	0.2764	0.7828
RDW	16.80	4.32	16.88	3.73	-0.0901	0.9284

Graph No 16- Comparison of gender with lab parameters (deploying independent t test)

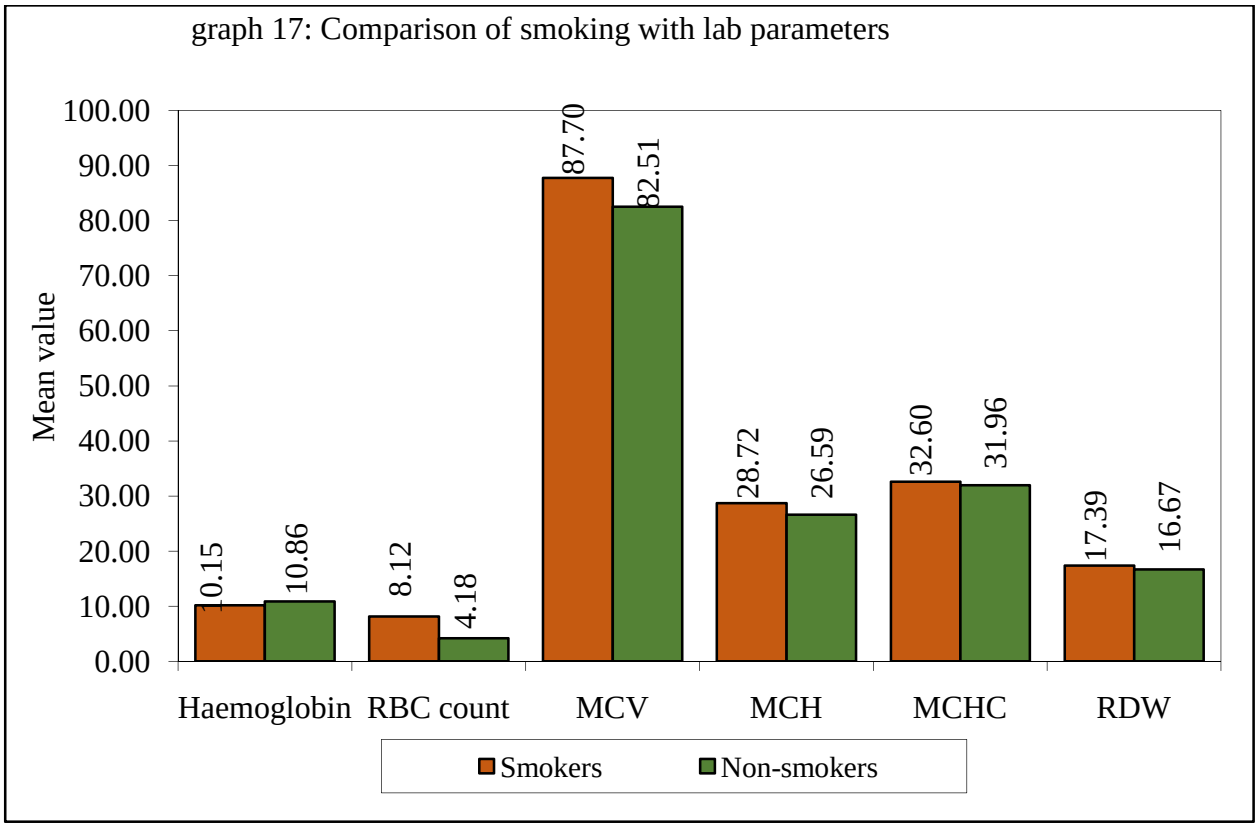


When both the genders were compared with different RBC indices deploying t test, p test, the p value was not significant.

Table 21: Comparison of smoking with lab parameters.

Clinical parameters	Smokers		Non-smokers		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Haemoglobin	10.15	3.10	10.86	2.74	-1.0351	0.3032
RBC count	8.12	20.57	4.18	0.98	1.7027	0.0918
MCV	87.70	17.23	82.51	14.33	1.4344	0.1546
MCH	28.72	6.28	26.59	5.76	1.5008	0.1366
MCHC	32.60	1.50	31.96	2.05	1.3678	0.1745
RDW	17.39	3.87	16.67	4.17	0.7307	0.4667

Graph No 17- Comparison of smoking with lab parameters



As shown in the above table, there was no comparison between smoking with RBC indices and the p value was statistically insignificant.

Table 22: Comparison of alcohol with lab parameters

Clinical parameters	With alcohol		Without alcohol		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Haemoglobin	11.03	2.91	10.45	2.75	1.0193	0.3106
RBC count	4.13	1.16	5.74	12.75	-0.8218	0.4132
MCV	84.86	15.42	82.74	14.89	0.6950	0.4887
MCH	27.69	5.88	26.58	5.95	0.9263	0.3565
MCHC	32.39	2.18	31.89	1.76	1.2606	0.2104
RDW	16.64	4.41	16.97	3.87	-0.3994	0.6905

Graph No 18- Comparison of alcohol with lab parameters

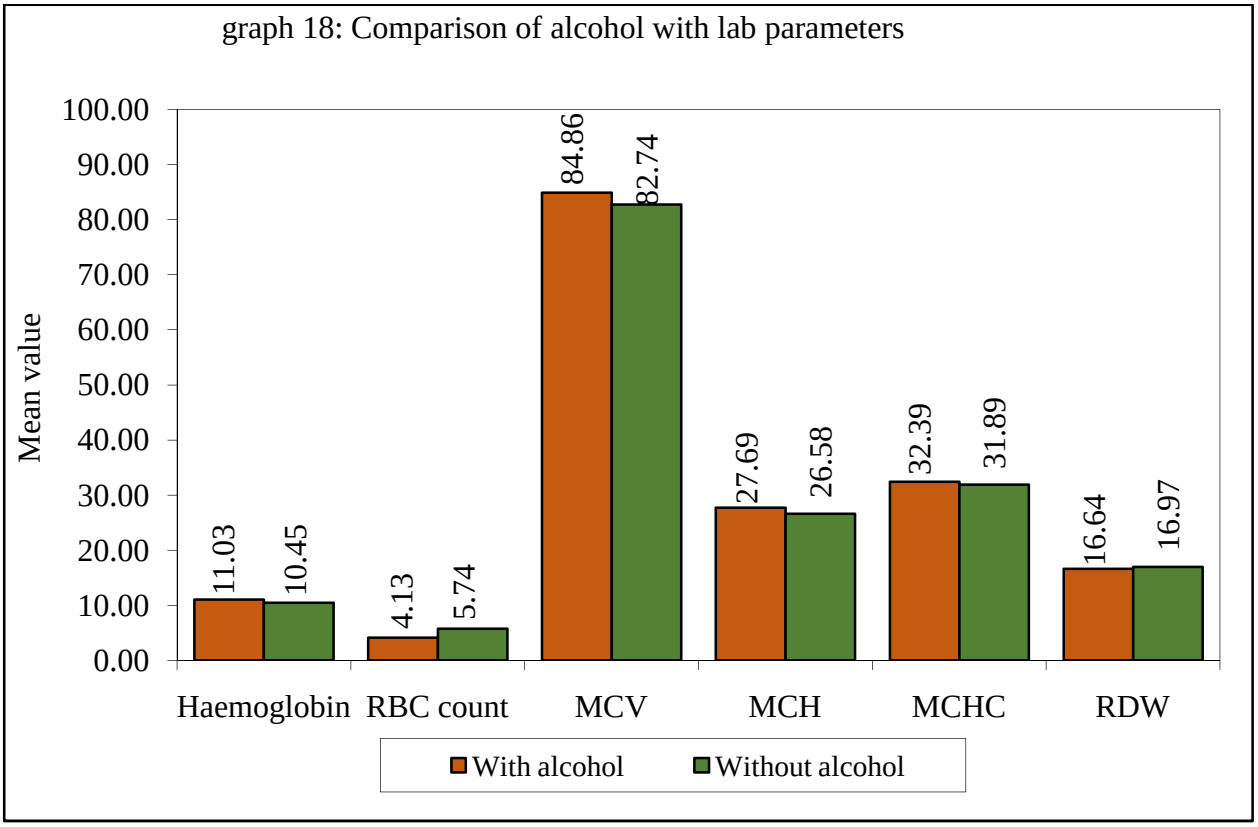


Table no. 22 shows that even with alcohol, there is no comparison between the different lab parameters.

Table 23: Comparison of tobacco chewing with lab parameters

Clinical parameters	With tobacco chewing		Without tobacco chewing		t-value	p-value
	Mean	Std.Dev.	Mean	Std.Dev.		
Haemoglobin	11.10	2.48	10.68	2.85	0.3532	0.7247
RBC count	3.72	1.15	5.13	9.95	-0.3464	0.7298
MCV	95.70	18.49	82.88	14.62	2.0507	0.0430
MCH	31.22	5.52	26.79	5.87	1.7964	0.0755
MCHC	32.67	0.75	32.07	2.01	0.7263	0.4694
RDW	15.92	3.73	16.88	4.13	-0.5590	0.5774

Graph No 19- Comparison of tobacco chewing with lab parameters

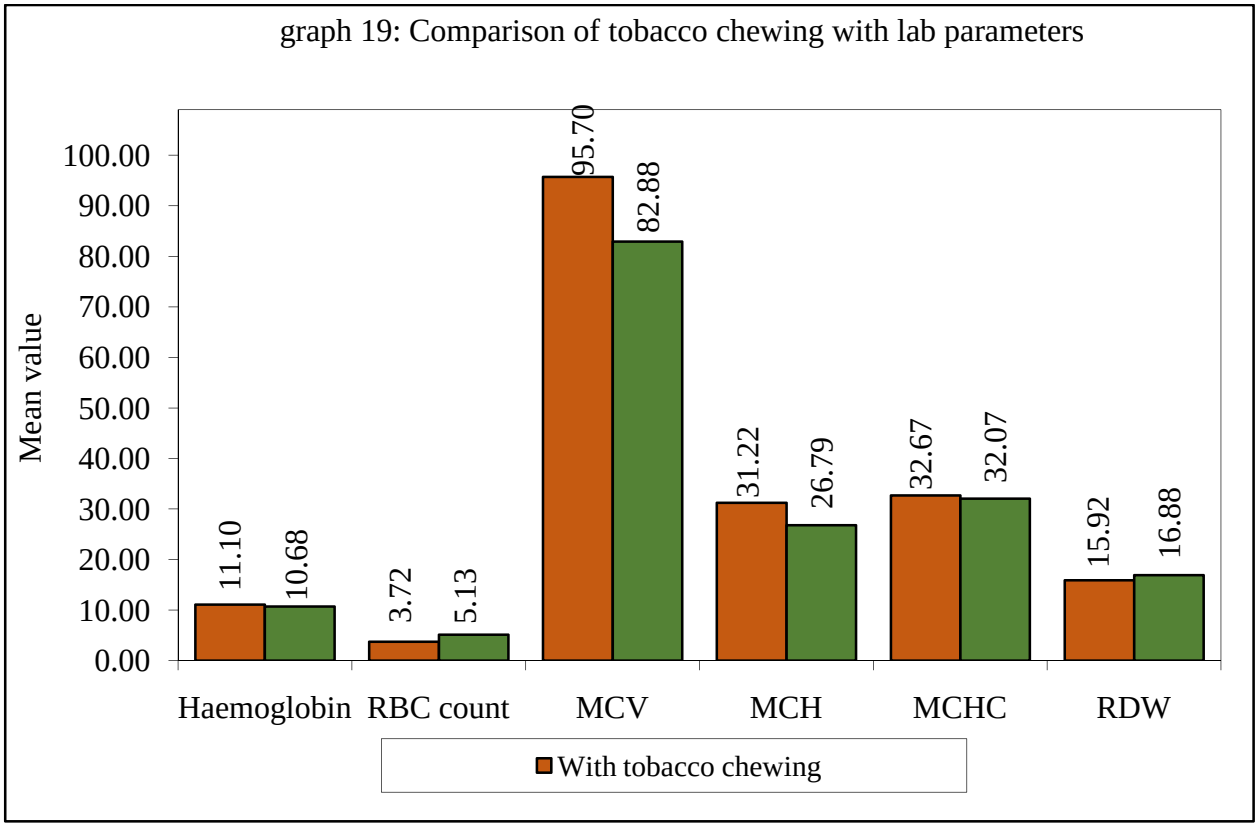


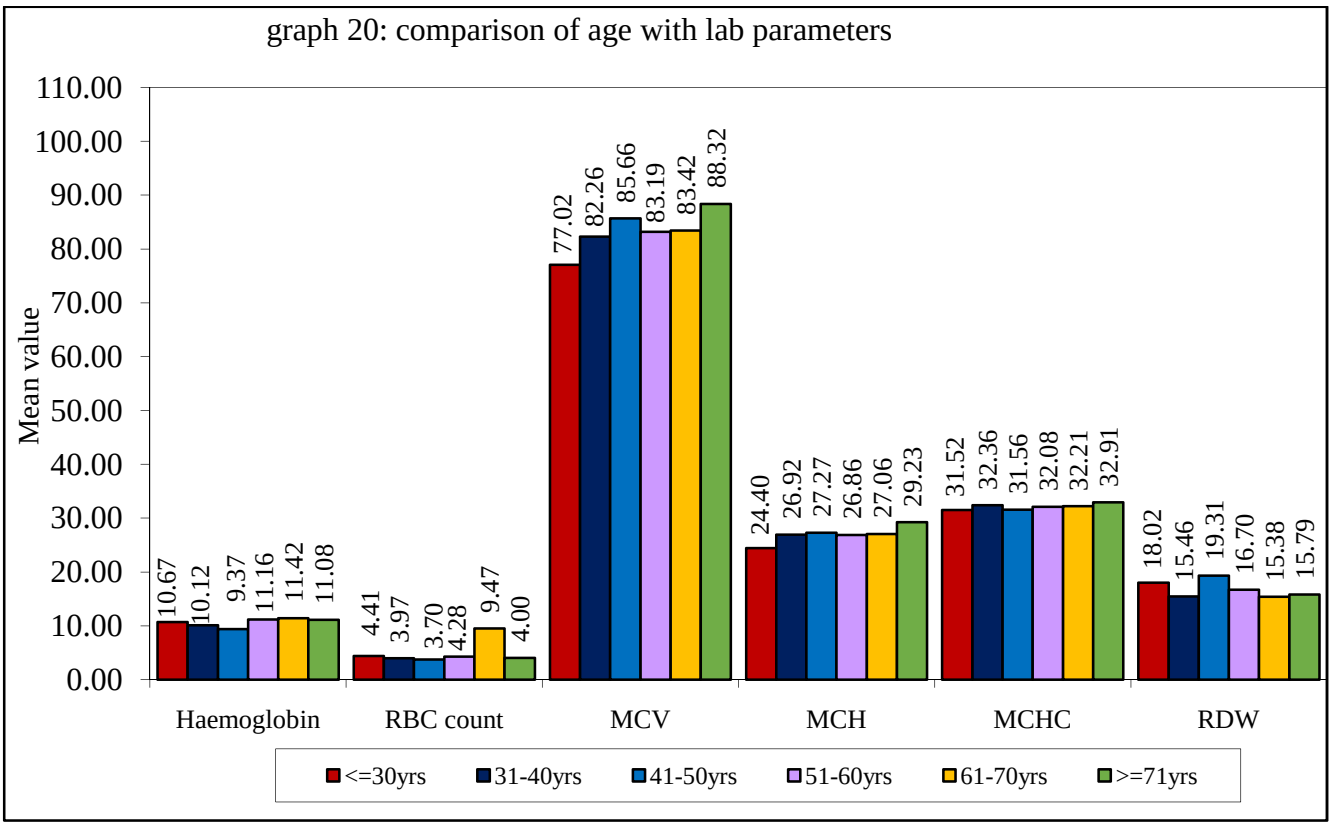
Table no. 23 shows that with tobacco chewing, except for MCV, there is no comparison between the different lab parameters.

Table no 24: Comparison of age with lab parameters

Clinical parameters	Summary	Age groups						F-value	p-value
		<=30 yrs	31-40yrs	41-50yrs	51-60yrs	61-70yrs	>=71 yrs		
Haemoglobin	Mean	10.67	10.12	9.37	11.16	11.42	11.08	1.3287	0.2588
	SD	1.99	3.16	2.84	2.34	3.48	2.83		
RBC count	Mean	4.41	3.97	3.70	4.28	9.47	4.00	0.9297	0.4655
	SD	0.54	1.24	1.33	0.97	22.62	1.30		
MCV	Mean	77.02	82.26	85.66	83.19	83.42	88.32	0.9100	0.4781
	SD	11.22	16.81	20.26	13.05	9.92	17.42		
MCH	Mean	24.40	26.92	27.27	26.86	27.06	29.23	0.9937	0.4259
	SD	4.51	7.09	7.65	4.98	4.45	6.57		
MCHC	Mean	31.52	32.36	31.56	32.08	32.21	32.91	1.1444	0.3426
	SD	1.63	2.30	2.08	1.99	2.27	1.25		
RDW	Mean	18.02	15.46	19.31	16.70	15.38	15.79	2.6360	0.0282*
	SD	3.99	3.38	4.84	3.58	4.22	3.24		

*p<0.05

Graph No 20- Comparison of age with lab parameters



The table no. 24 shows the comparison of age with lab parameters. Except for red cell distribution width (RDW), which was statistically significant, the other parameters, age did not influence.

DISCUSSION

In the present study of 100 patients with different dietetic habits, the effect of diet on morphology of RBC and various blood indices (RBC) was carried out. The same was compared with various factors like age, sex, severity of anemia and type of anemia, and following results were observed.

In our study of 100 patients, the patients age ranged from 19-90 years. Maximum number of cases, that is, 24 were in the age group of 51-60 years (24%). Also observed was that more number of cases were above 40 years i.e. 18 between 41-50 years (18%), 24 between 51-60 years (24%), 18 between 61-70 years (18%) and 17 were more than 71 years (17%). Only 23 cases were seen in the age group below 40 years. The mean age at presentation was 53.29 ± 17.39 years.

Similar observations were made by Obeid et al.[32] and Bhatnagar et al.[33] in their studies.

We observed 64 patients were males (64%) and remaining 36 were females (36%), accounting for a Male: Female ratio of 1.78:1 with a slight male preponderance in our study. A study by Kumar et al.[62] who observed a slight male preponderance in their study also. There is a sharp contrast in study by Haddadet al.[53]and Elorinne et al.[56], who observed a slight female preponderance in their study groups.

Further we studied the dietetic habits of our patients and there were slightly more vegetarian patients i.e. 56 (56%) and remaining patients were non-vegetarians (44 i.e. 44% - table no. 3). On further evaluation gender-wise (vegetarian versus non-vegetarian), we found gender-wise different diets of our study, depicted in table no. 4. A study by Bhatnagar et al. 2015.[33], who had an equal number of vegetarians and non-vegetarians in their study group, which included both genders. Another study by

Haddad et al.[53] had found slightly more vegetarians as compared to non-vegetarians, again which included both males and females.

We critically analysed all our 100 patients based on their dietetic habits and found to have majority were vegetarians (56 patients i.e. 56%). Remaining 44 patients were non-vegetarians (44%). On further analyses of different types of vegetarian dietetic habits, we found 36 patients were lacto-vegetarians (vegetarian/milk and milk products), 15 were lacto-ovo-vegetarians (vegetarian/milk and milk products/eggs), 4 were ovo-vegetarians (vegetarian/eggs. No milk or milk products) and only 1 was vegan (pure vegetarian, no eggs or milk and milk products). Similar observations were made by Obeid et al.[32] and Khanna et al. [34] in their study groups.

In our present study, 47 patients were hypertensive, 35 had diabetes mellitus, 9 had various cardiac aliments, and the other comorbidities of patients are depicted in table no.6. A study by Shridharet al.[57] and Okubo et al.[40]had different comorbidities in their study groups.

A study by Shridharet al.[57] did not find any correlation of diet with comorbidities like diabetes mellitus and hypertension. A study by Okubo et al.[40], observed that consumption of meat and meat products was associated with reduced lung functions in their study. A study by Miyamoto et al.[41]observed in their study of female patients, consumption of fish had lesser incidence of bronchial asthma in their study. Whereas in our study, we observed slight correlation of diet especially non-vegetarian diet associated with COPD/ bronchial asthma. P value was statistically significant (P value = 0.0450) The probable mechanism for reduced lung function, increased incidence of COPD/ bronchial asthma in non-vegetarian groups (meat and meat product eaters) could be because of high contents of nitrites. Nitrites being pro-oxidants can generate strong oxidative species like per-oxy-nitrite, which can induce

airways inflammation and pulmonary damage. Tobacco smoke could also be source of oxidants and nitrites, thus the interaction between meat consumption and tobacco use in declining lung function could be a biologically plausible mechanism. Another explanation for this could be that AGEs (advanced glycated end products) lead to inflammation and an increased oxidative stress by binding with their cell surface receptors which further stimulate nuclear factor (NF)-kB. Thus, the increased lung inflammation and hence reduced lung function could plausibly be explained by high consumption of foods rich in AGEs. The improvement in FEV1 by fruit and vegetable consumption and dietary TAC (total antioxidant capacity) was seen.

In our present study, majority of patients had 1 or the other habits (n=48). Few patients had more than 1 habits and very few had all the habits (alcohol, smoking and tobacco chewing). 52 patients did not have any habits. A study by Rizzo et al.[52] and Shridhar et al.[57] observed consumption of alcohol and tobacco use was more common in their non-vegetarian group of patients. In our study, we also observed one or the other habits seen more in non-vegetarian groups of patients, though p value was statistically insignificant.

In majority of our patients, generalised weakness (71%) was a major presenting symptom followed by easy fatiguability and dizziness. Some patients had overlapping of these symptoms. A study by Kumar et al.[62] and Dhanuka et al.[60] observed generalized weakness as commonest presenting symptom in their study population. Kumar et al.[62] further found that symptom of generalized weakness was more common in vegetarians in their study. Whereas in our study, the symptom of dizziness was more common in vegetarian groups. P value was statistically significant (P value= 0.0020).

In our study, the most commonly observed clinical signs were pallor (38), koilonychia (22), pale bald tongue (13) and loss of palmar crease (10). Some patients had overlapping of these physical signs. Studies byDhanukaet al.[60]and CH Manoj et al.[58] observed pallor as the most common physical sign in their study. In our study, we observed pale bald tongue and loss of palmar crease were significantly associated with vegetarian group of patients and p value was significant (P values for pale bald tongue- 0.0260, loss of palmar crease- 0.0220). Dhanukaet al.[60] did not observe at least in 15 patients of their study group any signs of anemia. 13 out of 15 had mild anemia and only 2 had moderate anemia. The current evidences suggest that conjunctival pallor may be more accurate clinical sign of anemia than pallor of nail beds and palms. Conjunctival pallor appears more frequently in patients of anemia, hence may be more sensitive as a sign of anemia as compared to other signs.

We attempted to categorize our patients based on WHO grading of anemia as mild, moderate, and severe and found to have majority (n=67) had mild to moderate anemia (mild-32 and moderate-35), 13 patients had severe anemia and in remaining 20 patients, the haemoglobin was normal. A study by Dhanukaet al.[60] have also categorized patients as per WHO grading, which is similar to our study.

We compared diet with different grades of anemia (mild, moderate and severe anemia- table no. 15). We found severe anemia was more common (n=12 pts) in vegetarian group. There was only 1 patient of severe anemia in non-vegetarian group. P value was significant (P value= 0.0331) for vegetarian diet associated with severe anemia. A study by Bhatnagaret al.[33], have found mild to moderate anemia more common in vegetarian group. Similarly, another study by Rammohanet al.[29] have also found similar findings in their study groups.

All 100 patients were subjected for Hb%. Based on Hemoglobin, we categorized them as mild, moderate, severe anemia and found to have 32 patients of mild anemia, 35 moderate anemia and 13 severe anemia. And remaining 20 patients had normal hemoglobin. This includes both male and female patients. Further we attempted to compare the gender with severity of anemia. The mean value of all 100 patients with Hb% and other RBC indices (Table 16 A, B, C). Study byDhanuka et al.[60] found in their groups evidence of mild to moderate anemia and did not have severe anemia in their group. Males were more in mild group of anemia whereas in moderate anemia, females were more in number. This is in sharp contrast with our study wherein we had all grades of anemia (mild, moderate and severe). Male patients were more in mild group of anemia, whereas in moderate and severe anemia, females were more. The reason for more anemic patients (moderate and severe anemia) could be overall poverty, health status of women in India reflects gender discrimination from birth, poor health resources, menstrual loss of blood. So high rates of anemia in India in women could be because of social and biological vulnerability, both within society and household. Again, the high incidence of anemia in female gender could be because of dietary patterns.

All patients peripheral smear revealed (table no. 17) different types of anemia. There was no much gender difference in type of anemia noted in our study, p value was statistically insignificant (p value = 0.2900). Whereas, a study by Elsayidet al.[18] found microcytic hypochromic anemia more common in female subjects. This could be because of menstrual loss of blood and loss of blood during delivery or malnutrition. They also found normocytic normochromic anemia more common in men. This was because of blood loss and chronic diseases. Another study by Deng et al.[65], male patients with omnivorous diet had less frequently microcytic hypochromic

and macrocytic anemia. The different types of anemia could be explained on the basis of deficiency of dietary factors like iron, vitamin B12 and folic acid. Apart from this, in female gender it could be because of chronic blood loss in menstruation or blood loss during delivery. It could be because of infections like malaria, worm infestations like hookworms. Normocytic normochromic anemia could be because of chronic infections. In rare instances, it could be because of genetic factors like hemoglobinopathies (thalassemia) or metabolic disorders like G6PD deficiency, sickle cell anemia, or it could be because of patients' habits (like alcohol, smoking or tobacco use).

Similarly, we compared peripheral smear with diet and we found diet has an influence on peripheral smear which was observed in our present study (table no.18). In patients with vegetarian diet, microcytic hypochromic anemia followed by normocytic normochromic anemia was also observed in our study groups of patients. Probably it was because of dietary factors, worm infestation or early stages of iron deficiency anemia in normocytic normochromic anemia or may be because of chronic infections. We also observed 18 patients of microcytic hypochromic picture and 17 of normocytic normochromic picture in non-vegetarian group. The probable mechanism could be as explained above. Deng et al.[65] observed higher incidence of macrocytic/ microcytic hypochromic anemia in patients of vegetarian diet (especially vegans).

Deploying independent t test, comparison was made with diet with hemoglobin and other RBC indices and found to have a significant correlation with hemoglobin, p value being statistically significant (p value= 0.025 - table no. 19). Hb% was higher in non-vegetarian group. Study by VirojWiwanitkit MD et al.[59] observed the hemoglobin was more in non-vegetarian group as compared to vegetarian group (vegans). He also observed in vegetarians, a non-significantly higher

RDW and MCV and a lower MCH. Similar observations were made byBhatnagar et al. [33], who found higher levels of hemoglobin in non-vegetarian group. Sheela Reddy et al.[54]observed higher MCV, MCH and RBC counts in vegetarian group. Deng et al.[65] has given a probable explanation for positive for RBC, Hemoglobin, hematocrit, and MCHC in non-vegetarian group (sea food) related to omega 3 fatty acid. As omega 3 fatty acid supplementation is safe and effective in patients of sickle cell anemia due to long chain polyunsaturated fatty acid, which is associated with lesser risk of iron deficiency. In our study, we have looked for all RBC indices except hematocrit. Sheela Reddy et al.[54] have offered an explanation for low levels of Hb concentration, low levels of MCHC, MCH in vegetarians. Also found RDW was higher in vegetarian groups, who are more susceptible to anemia as a result of maturation defect. All these factors are due to dietary deficiencies of essential factors required for hematopoiesis.

Similarly, gender was compared with different RBC indices and found no significant correlation. To best of our knowledge, many authors have not compared gender and RBC indices.

In our present study, a total of 48 had 1 or the other habits (alcohol, smoking and tobacco chewing), some had more than 1 and few had all 3 habits. Same was compared with different lab parameters (RBC indices). Comparison of smoking alone with Hb% and RBC counts and other indices did not have any correlation in our study, p value was insignificant (table no. 21). Similarly, alcohol alone also did not show any correlation, again p value was significant (table no. 22). But chewing of tobacco had some influence on MCV*, which was more in patients of tobacco chewing, observed in our study (table no 23). A study by Malenicaet al.[69] observed significant increase in Hb%, MCV and MCHC in smokers in their study. Study by

Aldosariet al.[68] found only RDW was significantly lower in smokers, other indices were not affected in their study. Latvalaet al.[70] observed higher MCV and MCH in alcoholic patients in their study. Study by Shukla et al. [71] observed tobacco chewing had influence on RBC indices, suggesting the direct tobacco effect on blood profile. Increase in RBC count and Hb% in smokers can be explained by tissue hypoxia leading to increased carboxy-hemoglobin causing increased production of erythropoietin and thus increasing erythropoiesis. Further, carbon monoxide from tobacco smoke may increase permeability of capillaries, which may decrease the volume of plasma, mimicking a condition like polycythemia. Same could be the explanation in tobacco chewing. The probable mechanism in alcoholics could be because of the deficiency of dietary factors in chronic alcohol consumption, causing alteration in RBC indices.

Finally, age was compared with different RBC indices. The age did not have any influence on RBC indices except for RDW which was increased in our study up to the age of 50 after which there was a progressive decline, with p value being statistically significant (p value = 0.0282). A study by Eun-Hee Nah et al.[66] observed the increase in Hb% and RBC counts in men upto the age of 45, thereafter decreased. Whereas in females, these indices reduced soon after puberty. He also observed increased RDW in older patients (>75 years). Alvarez-Uriaet al.[67] noted similar trend in their study group. The decrease in Hb% with advancing age could be because of either the age factor itself or because of underlying hidden diseases in old age which needs to be evaluated.

In our present study of 100 patients (vegetarians and non-vegetarians), their age ranged between 19-90 years. The number of male patients was slightly more. The most common symptoms of patients were generalized weakness, easy fatiguability

and dizziness. Some patients had overlapping of these symptoms. Most of our patients had physical signs of pallor, koilonychia and pale bald tongue. Most of our vegetarian patients had moderate to severe microcytic hypochromic anemia followed by normocytic normochromic anemia. We noticed in non-vegetarian group also, there were microcytic hypochromic anemia as well normocytic normochromic anemia. The probable explanation for this, we have already stated in our earlier discussion. We also observed severe anemia in the vegetarian group. The patients with non-vegetarian diet had high Hb% content. The reason for this, we have already given in the discussion. In our present study, most of the male patients had mild anemia, whereas in female patients, moderate to severe anemia was more common. As far as habits of patients was concerned, a total of 48 patients had 1 or the other habits, but the patients who had tobacco chewing habit, their MCV was more and same was statistically significant in our study. Age of patients did not have any influence on any of the factors (Hb% and other RBC indices), except for increased RDW up to the age of 50 followed by progressive decline, in our study.

We feel it is worth taking different variables like age, sex, Hb%, type of anemia, diet and habits to see whether these factors have effect on Hb%, peripheral smear studies and various RBC indices. Owing to a small sample size of 100 patients in our study, some of the facts, we could not ascertain. For example, normocytic normochromic anemia in both vegetarian and non-vegetarian groups. We assumed this could probably be because of deficiency of hemopoietic substance like iron (early stage of iron deficiency anemia) or could be due to chronic infection. Apart from a small sample size, we did not follow up our patients with change in the diet, stopping of habits and supplementation of nutrient factors which could affect these factors (Hb%, peripheral smear studies and various RBC indices).

CONCLUSION

In our present study of 100 patients, both vegetarians and non-vegetarians, we observed few factors correlating with Hb% and other RBC indices. Prominent features of our study are mentioned as follows:

- Among the patients of both vegetarian and non-vegetarian diet, there were 77 patients (including both males and females) in the age group of 40-90 years and only 23 patients (including both males and females) below the age of 40 years.
- Age did not have any influence on Hb% and other RBC indices except for increased RDW up to the age of 50 after which there was a progressive decline, in our study.
- Male patients were more in our study as compared to females.
- The common clinical presentations were generalized weakness followed by easy fatiguability and dizziness. Some patients had overlapping symptoms.
- The most common physical signs noted were pallor, koilonychia and pale bald tongue.
- Majority of patients of severe anemia were vegetarians.
- Most of the male patients were mildly anemic and females were moderate to severely anemic.
- The commonest type of anemia was microcytic hypochromic anemia followed by normocytic normochromic anemia in both the groups (vegetarians and non-vegetarians)
- Hemoglobin was slightly more in non-vegetarian group.
- Amongst the different habits of patients, tobacco chewing had positive correlation with increased MCV values.

Finally, we feel diet has got influence on Hemoglobin, type of anemia as well as other RBC indices. Whether or not factors like age, sex or habits have any effect on hemoglobin and other RBC indices has to be addressed with a large sample size. For normocytic normochromic anemia in our study group (both in vegetarians and non-vegetarians) the exact cause could not be ascertained. At the end, we conclude a large sample size taking these variables into account, with diet of patients has any effect, has to be studied.

SUMMARY

In the present study of 100 patients, including both of vegetarians and non-vegetarians, admitted in the department of General Medicine of KLES Dr. Prabhakar Kore hospital and MRC, Belgaum, with study period from January 2019 to December 2019, was conducted to know whether or not diet has any influence on various variables like age, sex, Hemoglobin, type of anemia and other RBC indices. The results observed were severe anemia in vegetarian group, mild anemia more in males, moderate to severe anemia in females, slightly more hemoglobin in non-vegetarians, tobacco chewers had higher MCV and that RDW increased up to the age of 50 after which it decreased.

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ANNEXURE I

CONSENT STATEMENT

TITLE OF RESEARCH AND STUDY:

Principal Investigator:-
REG NO. BG0118004
Post Graduate Student,
Department Of General Medicine,
JNMC, Belgaum.

Guide:-
Dr._____
Professor & Head of Unit,
Department of General Medicine,
JNMC, Belgaum.

Introduction and Purpose:-This research is intended to study effect of type of diet on red blood parameters . The principal investigator of the study is **REG NO. BG0118004**under the guidance of Dr. _____

Procedure:

If you agree to be part of the research study, you will be asked the relevant history and will be subjected to relevant clinical examination and investigations. You will also have to give blood for the necessary investigations.

Risk and Benefits:

The only risk and possible discomfort you might get is while blood is being drawn.You may/may not be benefitted by these investigations but you will be part of this study which is going to be useful to others in the future.

Alternatives:

Taking part in this study is voluntary. You may choose not to take part in this study.

If you decide to take part you can later change your mind and withdraw from the study. Your decision will not change the present or future health care or other services that you receive. The study doctor or sponsor may stop your participation in this study at any time. If you choose not to take part in the study, you will receive the standard treatment for patients with your condition.

Privacy and Confidentiality:

All information collected about you during the course of this study will be kept confidential to the extent permitted by law. The code numbers will identify you in this research record. Information from this study may be published but your identity will be confidential in any publication.

Institution / Sponsor's policy:

Does not apply to this research

Financial incentives for participation:

You will not be paid / offered any gifts /incentives for participating in the study.

Authorization to publish the results:

The results of the study would be forwarded to the KLE University, Belagavi as part of requirement towards the completion of MD degree, review and publishing.

In case of the queries during study or in future you may contact following persons,

1. REG NO. BG0118004
Investigator
PG In General Medicine
JNMC, Belagavi.

2. **Dr.**
Professor & Head of Unit,
Dept of General Medicine,
JNMC, Belgaum.

3. DR. ROOPA BELLAD MD
Professor of Paediatrics
Dept.of Paediatrics, J.N. Medical College, Belagavi 10

CONSENT FORM

I voluntarily agree to take part in this study by signing below. I may withdraw at any time. I am not giving up any of my legal rights by signing this form. My signature below indicates that I have read this consent form, or it has been read to me and has been explained to me in my vernacular language and all my questions have been answered. I will be given a copy of this consent form.

Signature / Left Thumb print of the Participant or legally authorized representative

Participant's name :……...

Signature / Left thumb impression :……...

of the participant

Name of the legally authorized :……...

representative / guardian

Signature / Left thumb impression :……...

Witness' name :……...

Signature / Left thumb impression :……...

Investigator's name and signature :……...

Date:

Place:

ANNEXURE II - PROFORMA

CASE NO:

NAME:

AGE/SEX:

IP NO.:

ADDRESS:

OCCUPATION:

HISTORY:

COMPLAINTS AT PRESENTATION:

Past history:

Family history

Personal history:

Treatment history

PHYSICAL EXAMINATION:

Temperature:

Pulse:

Blood pressure:

SYSTEMIC EXAMINATION:

R. S.:

C.V.S.:

P.A.:

C.N.S.:

Diagnosis:

QUESTIONNAIRE

1. DO YOU HAVE A VEGETARIAN OR NON VEGETARIAN DIET?

A.

2. IF VEGETARIAN, SINCE HOW LONG ARE YOU VEGETARIAN?

A.

3. IF VEGETARIAN, DO YOU EAT EGGS?

A.

4. IF VEGETARIAN, DO YOU EAT HONEY?

A.

5. IF VEGETARIAN, DO YOU EAT ONLY SPROUTS?

A.

6. IF VEGETARIAN, DO YOU EAT ONLY ORGANIC FOOD?

A.

7. IF VEGETARIAN, DO YOU HAVE DAIRY PRODUCTS?

A.

8. ARE YOU IN TRANSITION PERIOD OF VEGETARIAN TO NON VEGETARIAN DIET OR VICE VERSA?

A.

ANNEXURE III.ETHICAL CLEARANCE.

K.L.E. ACADEMY OF HIGHER EDUCATION AND RESEARCH
(Deemed – to- be- University)
Accredited 'A' Grade by NAAC (2nd Cycle) Placed in Category 'A' by MHRD (GoI)
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
Website: http//www.jnmc.edu
E-Mail : dome@jnmc.edu
Phone: (+ 91-(0)831 Office : 2472550
Principal: 2471701
Fax No. +91 (0)831 – 2470759

Ref: MDC/DOME/ 81 **Date: 24/11/2018**

To,

REG NO. BG0118004
PG student in Medicine,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"COMPARISON OF RED BLOOD CELL MORPHOLOGY AND INDICES IN PATIENTS OF VEGETARIAN AND NON VEGETARIAN DIET, A ONE YEAR CROSS SECTIONAL STUDY IN KLES DR. PRABHAKAR KORE HOSPITAL AND MRC"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Arathi Darshan)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Roopa M Bellad)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

Groups									Questionnaire							Co-morbidities								Habits			clinical presentation				clinical signs									
serial no.	ip no.	non vegetarian	lacto-ovo-vegetarian	lacto-vegetarian	ovo-vegetarian	vegan	Gender	Age	If vegetarian, since how long are you vegetarian?	If vegetarian, do you eat eggs?	If vegetarian, do you eat honey?	if vegetarian, do you eat only sprouts?	if vegetarian, do you eat only organic food?	if vegetarian, do you have dairy products?	are you in transition period of vegetarian to non vegetarian diet, or vice versa?	hypertension	diabetes mellitus	cardiovascular diseases	cerebrovascular accident	chronic kidney disease	chronic liver disease	copd/asthma	smoking	alcohol	tobacco chewer	generalised weakness	easy fatiguability	dizziness	blood loss	pallor	koilonychia	bald tongue	loss of palmar crease	hemoglobin	RBC count	mcv	mch	mchc	rdw	peripheral smear/RBC morphology	WHO GRADING
1	985698	1	0	0	0	0	male	77	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	12.8	6.42	63	20	31	18	microcytic hypochromic	mild
2	997903	0	0	1	0	0	male	66	birth	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	13.4	4..75	85	28	33	16	normocytic normochromic	0
3	946905	0	0	1	0	0	male	43	birth	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	1	1	1	1	1	0	1	5.4	2.7	71	20	28	21	microcytic hypochromic	severe
4	955337	0	0	1	0	0	male	67	birth	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	1	1	0	1	1	0	1	4.7	1.83	85	26	31	21	microcytic hypochromic	severe
5	979618	1	0	0	0	0	male	75	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	0	1	0	1	1	0	0	0	8.4	2.76	93	30	33	22	normocytic normochromic	moderate
6	984852	0	0	1	0	0	female	20	birth	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	10.2	4.11	78	25	32	18	microcytic hypochromic	moderate
7	993472	1	0	0	0	0	male	19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.1	4.51	87	29	33	16	normocytic normochromic	0
8	983833	1	0	0	0	0	male	85	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	12.4	4.72	79	26	33	15	microcytic hypochromic	mild
9	970833	0	0	1	0	0	male	66	birth	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0	1	1	1	0	1	1	0	3.8	2.92	53	13	25	23	microcytic hypochromic	severe
10	971323	0	1	0	0	0	female	28	birth	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	8.9	4.39	67	20	30	17	microcytic hypochromic	moderate
11	972048	0	0	1	0	0	male	54	birth	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	1	1	1	0	1	0	1	7.2	2.12	104	34	33	19	dimorphic	severe
12	963054	1	0	0	0	0	female	52	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.2	4.78	83	26	31	14	normocytic normochromic	0
13	984014	0	0	1	0	0	female	74	birth	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	11.1	3.51	95	32	33	12	normocytic normochromic	mild
14	989743	1	0	0	0	0	female	64	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.6	4.51	85	28	33	13	normocytic normochromic	0
15	974747	0	0	0	1	0	female	60	birth	1	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	9.8	3.93	79	25	31	24	normocytic hypochromic	moderate
16	983565	0	1	0	0	0	male	51	birth	1	1	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	12.4	4.5	84	28	33	18	normocytic normochromic	mild
17	983756	1	0	0	0	0	male	67	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	11	3.52	94	31	33	15	normocytic normochromic	mild
18	979046	0	0	1	0	0	female	50	birth	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	6.5	2.7	83	24	29	28	microcytic hypochromic	severe
19	1014131	0	0	1	0	0	female	57	birth	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	9.5	3.35	87	29	33	16	normocytic hypochromic	moderate
20	978351	0	0	1	0	0	female	63	birth	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	10.6	3.77	81	28	35	15	normocytic normochromic	moderate
21	978926	0	0	1	0	0	male	42	birth	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	1	0	1	8.6	2.57	100	34	34	27	dimorphic	moderate
22	978949	0	0	1	0	0	male	35	birth	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	8.9	5.01	59	18	30	19	microcytic hypochromic	moderate
23	1013440	1	0	0	0	0	male	60	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	16.4	5.79	87	28	33	13	normocytic normochromic	0
24	982571	0	0	1	0	0	female	61	birth	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	11.3	3.75	91	30	33	13	normocytic normochromic	mild
25	979686	0	0	1	0	0	male	45	birth	0	0	0	0	1	0	0	0	0	0	0	1	0	1	1	0	1	1	1	1	0	0	1	1	2.6	0.59	130	45	34	17	dimorphic	severe
26	982497	1	0	0	0	0	female	53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	1	1	0	8.9	3.75	76	24	31	16	microcytic hypochromic	moderate
27	981612	1	0	0	0	0	female	33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	10.5	3.97	86	26	31	15	normocytic hypochromic	moderate
28	980584	0	1	0	0	0	male	50	birth	1	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	12.6	4.24	88	30	34	15	normocytic normochromic	mild
29	949824	0	0	1	0	0	male	71	birth	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	12.2	3.25	110	38	34	13	dimorphic	mild
30	977562	0	0	1	0	0	male	36	birth	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	11.5	3.3	102	35	34	12	macrocytic	mild
31	975654	1	0	0	0	0	female	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	9.9	4.24	74	23	32	17	micocytic hypochromic	moderate
32	955962	1	0	0	0	0	male	55	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	10.9	3.83	85	29	33	16	normocytic hypochromic	moderate
33	973905	0	1	0	0	0	female	20	birth	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	1	0	0	0	10.2	3.58	89	28	32	20	normocytic hypochromic	moderate
34	931638	1	0	0	0	0	male	46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	11.3	4.88	71	23	32	17	micocytic hypochromic	mild
35	983862	0	1	0	0	0	female	66	birth	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	1	1	1	0	7.6	2.58	88	29	32	14	dimorphic	severe
36	787248	1	0	0	0	0	male	71	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	10.6	3.72	84	29	34	12	normocytic normochromic	moderate
37	970855	0	0	1	0	0	male	40	birth	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	1	1	1	0	1	0	1	3.3	0.87	107	37	35	11	dimorphic	severe
38	1014690	1	0	0	0	0	female	79	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14.5	4.97	89	29	33	13	normocytic normochromic	0
39	974408	1	0	0	0	0	female	42	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	11.2	3.17	104	35	34	15	macrocytic	mild
40	974765	0	0	1	0	0	male	67	birth	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	14	4.69	91	30	33	13	normocytic normochromic	no
41	971327	0	0	1	0	0	male	59	birth	0	0	0	0	1	0	1	1	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	0	12.2	4.77	80	26	32	14	normocytic hypochromic	mild
42	969876	0	0	1	0	0	male	60	birth	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	12.3	4.33	83	28	34	14	normocytic hypochromic	mild
43	954596	0	0	1	0	0	male	68	birth	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	10.5	4.57	73	23	31	19	microcytic hypochromic	moderate
44	956033	1	0	0	0	0	male	44	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	1	1	0	0	9.7	3.72	81	26	32	22	microcytic hypochromic	moderate
45	955651	0	1	0	0	0	male	60	birth	1	0	0	0	1	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	11.7	4.27	77	27	36	13	normocytic normochromic	mild
46	956294	1	0	0	0	0	male	61	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	12.7	5.49	72	23	32	16	normocytic hypochromic	mild
47	955643	1	0	0	0	0	male	55	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	11.7	5.94	66	20	30	17	microcytic hypochromic	mild
48	955816	0	1	0	0	0	female	18	birth	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	7.4	3.74	70	20	28	27	microcytic hypochromic	severe
49	957045	0	0	1	0	0	female	90	birth	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	11.1	4.34	77	26	33	16	normocytic hypochromic	mild
50	957286	1	0	0	0	0	male	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	11.7	4	91	29	32	25	normocytic normochromic	mild
51	957946	0	0	0	0	1	female	34	13 years	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	9.4	4.9	63	19	31	18	microcytic hypochromic	moderate
52	986418	1	0	0	0	0	male	19	birth	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	9.4	5.12	60	18	31	19	microcytic hypochromic	moderate

56	959113	1	0	0	0	0	female	74	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	11.9	4.02	89	30	33	14	normocytic normochromic	mild
57	980257	0	1	0	0	0	male	40	birth	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	15.2	4.9	89	31	35	15	normocytic normochromic	0
58	960983	1	0	0	0	0	male	36	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	10.3	3.47	89	30	33	22	normocytic hypochromic	moderate
59	988615	0	0	1	0	0	male	58	birth	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	1	1	1	0	1	0	1	1	6.6	1.76	114	37	33	21	macrocytic	severe
60	975730	0	1	0	0	0	male	55	birth	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	13.4	4.67	92	29	31	13	normocytic normochromic	0
61	1013574	1	0	0	0	0	male	65	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	4.54	88	29	33	15	normocytic normochromic	0
62	1013436	0	1	0	0	0	male	74	birth	1	0	0	0	1	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16.9	6.06	85	28	33	15	normocytic normochromic	0
63	960284	0	0	1	0	0	male	60	birth	0	0	0	0	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	12.2	5.36	72	23	32	16	microcytic hypochromic	mild
64	959107	0	1	0	0	0	male	60	birth	1	0	0	0	1	0	1	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	12	4.31	87	28	32	16	normocytic hypochromic	mild
65	961204	1	0	0	0	0	male	55	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	11.5	4.32	82	27	33	14	normocytic hypochromic	mild
66	978730	0	1	0	0	0	male	37	birth	1	0	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	13.1	4.3	89	30	34	15	normocytic normochromic	0
67	1014597	1	0	0	0	0	male	28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	10.1	5.01	67	20	30	18	microcytic hypochromic	moderate
68	960910	1	0	0	0	0	female	55	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	11.1	4.45	78	25	32	16	microcytic hypochromic	mild
69	960978	1	0	0	0	0	female	85	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	8.5	4.19	68	20	30	22	microcytic hypochromic	moderate
70	960960	1	0	0	0	0	female	65	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1	1	0	0	0	9.5	3.95	79	24	31	17	normocytic hypochromic	moderate
71	961631	0	0	1	0	0	male	72	birth	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	12.9	4.61	85	28	33	13	normocytic normochromic	mild
72	961661	0	0	1	0	0	female	54	birth	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	11.2	3.18	106	35	33	17	macrocytic	mild
73	960696	1	0	0	0	0	male	44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	1	1	0	0	0	9.6	5.2	66	19	28	22	microcytic hypochromic	moderate
74	989747	0	0	1	0	0	male	78	birth	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	1	0	1	1	1	0	1	0	1	0	8.8	2.62	99	33	33	17	dimorphic	moderate
75	990503	1	0	0	0	0	female	38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	10.9	4.3	78	25	33	13	microcytic hypochromic	moderate
76	962368	0	0	0	1	0	female	43	birth	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	9.6	3.25	93	30	32	16	normocytic hypochromic	moderate
77	978712	0	0	1	0	0	male	24	birth	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13	4.44	86	29	34	13	normocytic normochromic	0
78	962238	1	0	0	0	0	male	75	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	12.1	4.09	87	30	34	14	normocytic normochromic	mild
79	926185	1	0	0	0	0	male	67	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	18.5	5.86	93	32	34	12	normocytic normochromic	0
80	963030	1	0	0	0	0	male	62	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	12.8	5.18	79	25	31	14	normocytic hypochromic	mild
81	963044	0	0	1	0	0	female	87	birth	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	8.8	3.34	86	27	31	20	normocytic hypochromic	moderate
82	1010664	0	1	0	0	0	male	48	birth	1	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	8.9	4.29	69	21	30	29	microcytic hypochromic	moderate
83	976561	0	0	1	0	0	male	53	birth	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.8	4.92	88	28	32	16	normocytic normochromic	0
84	992215	1	0	0	0	0	female	43	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	10.1	3.63	85	28	33	16	normocytic normochromic	moderate
85	963249	0	0	0	1	0	female	45	birth	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	1	1	0	1	1	0	0	8.9	4.52	66	20	30	18	microcytic hypochromic	moderate
86	962194	1	0	0	0	0	male	48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	10.2	5.59	63	18	29	22	microcytic hypochromic	moderate
87	992714	1	0	0	0	0	male	41	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	1	0	1	1	6.5	1.56	132	42	32	15	dimorphic	severe
88	1014606	1	0	0	0	0	male	49	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	12.1	4.73	77	26	33	18	microcytic hypochromic	mild
89	993730	0	0	1	0	0	female	77	birth	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	0	1	1	4.2	0.85	140	49	35	16	macrocytic	severe
90	1011926	1	0	0	0	0	male	51	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	11.6	4.54	79	26	33	23	microcytic hypochromic	mild
91	1014757	0	0	1	0	0	male	58	birth	0	0	0	0	1	0	1	1	0	1	0	0	0	0	1	0	1	1	1	0	1	1	0	1	5.8	4.95	48	12	24	26	microcytic hypochromic	severe
92	976241	0	0	1	0	0	male	62	birth	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	13.3	4.79	86	28	32	4.8	normocytic normochromic	0
93	979208	0	0	0	1	0	male	47	birth	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	15	5.1	90	29	33	13	normocytic normochromic	0
94	924658	0	1	0	0	0	male	23	birth	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	0	0	9	4.77	64	19	30	17	microcytic hypochromic	moderate
95	975763	1	0	0	0	0	male	25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14.6	4.96	91	30	32	14	normocytic normochromic	0
96	1014296	1	0	0	0	0	female	23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	10	3.73	82	27	33	16	normocytic normochromic	moderate
97	615996	0	1	0	0	0	female	69	24 years	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	14.7	4.87	88	30	34	22	macrocytic	0
98	991178	0	0	1	0	0	male	40	10 years	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	8.1	4.7	61	17	28	16	microcytic hypochromic	moderate
99	991056	0	0	1	0	0	female	55	birth	0	1	0	0	1	0	0	0	0	0	0	0	0	1	1	0	1	1	0	0	0	0	0	0	10.4	4.2	75	25	33	16	microcytic hypochromic	moderate
100	991909	0	0	1	0	0	male	70	birth	0	1	0	0	1	0	1	1	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	11.5	3.7	93	31	34	14	normocytic normochromic	mild