Original Research Article

A case-control study to determine the relationship between lipid profile and different types of anaemia

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Received: 15-04-2021 / Revised: 12-06-2021 / Accepted: 22-07-2021

Abstract

Aim: To study the relationship between lipid profile and types of Anaemia.**Methods:** This case (N=154) control (N=154) study was carried out in the Department of General Medicine, Mahatma Gandhi Medical College, Jaipur from January 2019 to June 2020. This includes All proven cases of Anaemia of age >18yrs and Hb<12gm% irrespective of sex. A detailed history was obtained from the subjects of the study, with special emphasis on age, sex and occupation; non-specific symptoms of anaemia. Fasting venous blood sample (> 12 hours) was obtained for estimation of lipid profile. T3 and T4 levels, fasting and post prandial (two hours after an oral dose of 75gms of glucose) blood sugar levels, and bone marrow aspiration cytology was done in selected cases based on clinical assessment. **Results:** The cases and Controls are matched for Age, majority of the cases (35.1%) and Controls (33%) are in the age group of >50 years. 61.7% of cases are males and 38.3% are females, and 57.8% of Controls are Males and 42.2% are Females. Most common presenting symptom is Fatigue, which was present in 50% of the cases. The most common finding on general physical examination was pallor, which was present in 58.4% cases. Mean Total Cholesterol level is more in IDA (136.6±17.9 mg/dl) compared to other types of Anaemia, mean HDL levels is more in Vit B12 Deficiency Anaemia (31.67±5.4 mg/dl), mean LDL levels is more in Vit B12 deficiency Anaemia (84.7±14.9), mean VLDL levels is more in Dimorphic Anaemia (52.4±25.8 mg/dl), mean TG levels is more in Dimorphic Anaemia. **Conclusion:** Anaemia is associated with significant hypocholesterolaemia, with lowering in all lipid subfractions. The extent of hypocholesterolaemia is proportional to the severity of anaemia. The type of anaemia has no effect on the hypocholesterolaemia seen in anaemia.

Keywords: Anaemia, lipid profile, pallor, fatigue.

Introduction

Anaemia is a common blood disorder, an estimated 1.9 billion people, 27% of the world's population, hence anaemia is a major public health issue, and particularly so in low- income and middle-income countries where 93% of all cases of anaemia globally are thought to occur.[1] In India 23.2% of people aged 3-65 are suffering from anaemia, India is thought to account for approximately a quarter of all cases of anaemia globally.[2] Symptoms of moderate anaemia include fatigue, loss of energy, shortness of breath, and palpitations (especially during physical activity).[3-6] One of the most important types of anaemia is iron deficiency anaemia (IDA).[3] Iron is an essential element in the physiology of the body, such as the transport of oxygen and enzymatic reactions. Iron deficiency reduces the serum level of iron and ferritin and thereby causes impaired erythropoiesis[1,3], about 50% of anaemia cases in the 0 world can be attributed to iron deficiency which is responsible for about 841,000 deaths in the world every year.[7,8] Accordingly, about 23% of children under 2, 26% of children aged 6, 23% of male and female adolescents, and 43% of pregnant women suffer from iron deficiency.[2]

Elevation of serum lipid levels increases the risk of atherosclerosis and coronary heart disease4, because of its role in atherogenesis, its effects on health increase with age, thus great attention is paid to abnormal levels of lipids and its associated factors.[9] The results of some studies shows that anaemia symptoms are fewer in overweight or obese people than those with normal weight, on the other hand, the findings of another study indicate that anaemia is associated with increased risk of Cardiovascular complications and death, especially in obese patients.[10,11]

The exact mechanism by which anaemia causes a fall in serum lipids is not known. The simplest explanation is a dilution effect i.e., the increased volume of serum in anaemia carrying the same total load of cholesterol. Other possibilities are increased utilization of cholesterol by proliferating cells, decreased endogenous synthesis of cholesterol by the liver due to decreased liver oxygenation, elevated levels of

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granulocyte-macrophage colony stimulating factor and enhanced receptor mediated removal of LDL in the bone marrow.[10,11]

Only few epidemiological studies showed that there is positive correlation between high iron reserves in the body and coronary heart disease, hyperlipidaemia is the most important risk factor.[5,10,11] In this regard, although the relationship between iron intake and serum lipid level has been found in animal models, this relationship has not been studied extensively in humans.[5] Since studies have been conducted on the effect of anaemia on serum lipid profile, the present research aimed to study the relationship between lipid profile and different types of ananaemia.

Materials and Methods

This is a case control study was carried out in the Department of General Medicine, Mahatma Gandhi Medical College, Jaipur from January 2019 to June 2020

Inclusion Criteria

All proven cases of Anaemia of age >18yrs

Hb<12gm% irrespective of sex

Severity of Anaemia was graded:

Mild - Hb>9gm%

Moderate - Hb between 6-9gm%

Severe - Hb<6gm%

Exclusion Criteria

1. Cases below 18 years
2. Obesity/Overweight: BMI > 25 kg/m^2
3. Malnutrition: BMI < 19 kg/m2 or Serum Total Protein < 6 gm/dl or Serum Albumin <3.5/dl
4. Known case of Diabetes Mellitus or RBS > 200mg/dl or FBS > 126 mg/dl or PPBS > 200mg/dl
5. Known Hypertensives or Blood Pressure persistently more than 140/90 mm of Hg on three consecutive readings taken on different days.
6. Alcoholics
7. Smokers
8. Known case of AIDS.
9. Known case of Ischaemic Heart Disease/ Cerebrovascular Accident.
10. History of recent blood loss.
11. History of use of steroids, oral contraceptives, diuretics, beta-blockers.
12. Blood Urea > 40 mg% or Serum Creatinine> 1.4 mg%
13. SGOT > 40 U/L or SGPT > 40 U/L or Serum Alkaline Phosphatase > 250 U/L
14. TSH > 7.0 µU/ml or TSH < 0.3 µU/ml

Methodology

After taking approval from institutional research, review board and ethical committee, the present study was conducted in all the cases that satisfy inclusion and exclusion criteria using predesigned, pretested and validated questionnaire which consists of Basic Demographic characteristics and Clinical data.

Clinical evaluation

A detailed history was obtained from the subjects of the study, with special emphasison age, sex and occupation; non-specific symptoms of anaemia like fatigue ability, dyspnoea, giddiness, palpitations and angina; symptoms suggestive of a specific cause for anaemialikepica, dysphagia, abdominal pain, bony pain, fever, loss of appetite, weight loss, jaundice, bleeding, melaena, haemoglobinuria, menorrhagia, pregnancy and post-menopausal bleeding.

Past history of disorders associated with dyslipidaemia or anaemia was obtained, including diabetes mellitus, hypertension, ischemic heart disease, cerebrovascular accident, AIDS, recent blood loss and gall stones. Dietary habits and habits like alcoholism and tobacco smoking was ascertained. History of intake of drugs affecting lipid levels, such as oral contraceptives, beta blockers, diuretics, steroids and NSAIDs was obtained. Family history of anaemia, jaundice and gallstones was also obtained.

Each patient was subjected to a detailed general physical examination, with specialemphasis on pallor, koilonychias, icterus, pedal edema, lymphadenopathy, glossitis, angular stomatitis, petechiae, haemolyticfacies, ankle ulcers, perioral pigmentation and knuckle pigmentation.

Investigations

Venous blood was drawn for investigations like complete haemogram, random blood sugar, blood urea, serum creatinine, liver function tests, and thyroid stimulating hormone levels. A urine sample was obtained for urine analysis, including albumin, sugar and microscopy. Fasting venous blood sample (> 12 hours) was obtained for estimation of lipid profile. T3 and T4 levels, fasting and post prandial (two hours after an oral dose of 75gms of glucose) blood sugar levels, and bone marrow aspiration cytology was done in selected cases based on clinical assessment.

Complete haemogram was performed using the Sysmax automated analyzer. Haemoglobin levels were confirmed by the colorimetric method. Differential count and peripheral smear was done manually using Leishmann's stain by a qualified pathologist. Urine albumin and sugar was estimated by dipstick method. Urine microscopy was done manuallyby a qualified pathologist. Biochemical analyses were done using the fully automated Technicon RA-XT system by Bayer. TSH, T4 and T3 were estimated using the chemiluminescence method on the fully automated ADVIA Centaur system by Bayer. Estimation of total cholesterol, HDL and triglycerides was done with the commercially available Autopak cholesterol kit on Technicon RA-XT system. VLDL was calculated using the formula, VLDL = Triglyceride/5. LDL cholesterol was calculated using the Friedewald's equation. LDL = Total cholesterol – [(Triglycerides/5) + HDL] mg/dl.

Controls

Total 154 non anaemic age and sex matched subjects were selected and screened for compliance with the exclusion criteria. Complete hemogram, lipid profile and other investigations were performed on them.

Statistical Analysis

After collecting data, it is entered in MS Excel and Statistical analysis is done using SPSS 20.0 and Vassar stats, Student t test has been used to test the homogeneity of age between case and control. Chi-square test has been used to find the homogeneity of sex between case and control. Student t test has been used to find the significance of Lipid profiles between case and controls. Analysis of Variance has been used to find the significance of mean lipid profiles when there are more than 2 groups. Mann Whitney U test has been carried to find the significance between case and control for TC/HDL and LDL/HDL ratio. Kruskal Wallis test has been used to find significance of TC/HDL and LDL/HDL ratio when there are more than 2 groups.

Results

Table 1: Age and Gender distribution in cases and controls

Variables	Groups		p-value
	Case	Control	
Age (In Years)			0.91 (NS)
<20	13	16	
	8.4%	10%	
21-30	42	37	

	27.3%	24%	
31-40	23	26	
	14.9%	17%	
41-50	22	24	
	14.3%	16%	
>50	54	51	
	35.1%	33%	
Gender			
Female	59	65	
	38.3%	42.2%	
Male	95	89	0.56 (NS)
	61.7%	57.8%	
Total	154	154	
	100.0%	100.0%	

The cases and Controls are matched for Age, majority of the cases (35.1%) and Controls (33%) are in the age group of >50 years, there is no significant difference in the age group between Cases and Controls (p=0.91). 61.7% of cases are males and 38.3% are females, and 57.8% of Controls are Males and 42.2% are Females, there is no significant difference in the sex distribution of Cases and Controls (p=0.56).

Table 2: Comparison of Age and Sex with Type of Anaemia among cases

Variables	Cases			p-value
	Dimorphic	IDA	Vit. B12 Deficiency	
Age (In Years)				
<20	5	4	4	
	9.4%	6.8%	9.5%	
21-30	14	16	12	
	26.4%	27.1%	28.6%	
31-40	13	6	4	0.234 (NS)
	24.5%	10.2%	9.5%	
41-50	5	13	4	
	9.4%	22.0%	9.5%	
>50	16	20	18	
	30.2%	33.9%	42.9%	
Gender				
Female	25	16	18	
	47.2%	27.1%	42.9%	
Male	28	43	24	0.072 (NS)
	52.8%	72.9%	57.1%	
Total	53	59	42	
	100.0%	100.0%	100.0%	

Among 53 Dimorphic Anaemia, majority (30.2%) are in the age group of >50 Years, 33.9% of IDA and 42.9% of Vitamin B12 Deficiency Anaemia cases belongs to > 50 years age group, there is no significant difference in the age group compare to types of anaemia (P=0.234).Percentage of male population among dimorphic anaemia is 52.8%, 72.9% in IDA and 57.1% in Vitamin B12 Deficiency Anaemia. There is no significant difference in the sex distribution among cases (P=0.072).

Table 3: Comparison of Symptoms with Type of Anaemia

Symptoms	Cases			p-value
	Dimorphic	IDA	Vit. B12 Deficiency	
Fatigue	28	28	21	0.851 (NS)
	52.8%	47.5%	50.0%	
Dyspnoea	17	18	13	0.984 (NS)
	32.1%	30.5%	31.0%	
Giddiness	13	14	13	0.686 (NS)
	24.5%	23.7%	31.0%	
Palpitation	15	14	12	0.814 (NS)
	28.3%	23.7%	28.6%	
Angina	3	1	2	0.525 (NS)
	5.7%	1.7%	4.8%	
Pica	0	0	2	0.067 (NS)
	0.0%	0.0%	4.8%	
Dysphagia	1	0	2	0.233 (NS)
	1.9%	0.0%	4.8%	
Abdomen Pain	1	1	1	0.969 (NS)
	1.9%	1.7%	2.4%	
Bony Pain	2	3	4	0.470 (NS)
	3.8%	5.1%	9.5%	

Fever	4	4	2	0.855 (NS)
	7.5%	6.8%	4.8%	
Loss of appetite	5	4	5	0.673 (NS)
	9.4%	6.8%	11.9%	
Weight Loss	1	2	2	0.732 (NS)
	1.9%	3.4%	4.8%	
Jaundice	3	1	1	0.464 (NS)
	5.7%	1.7%	2.4%	
Bleeding	1	3	2	0.645 (NS)
	1.9%	5.1%	4.8%	
Melaena	0	1	0	0.445 (NS)
	0.0%	1.7%	0.0%	
Haemoglobinuria	0	1	0	0.445 (NS)
	0.0%	1.7%	0.0%	
Menorrhagia	1	1	1	0.969 (NS)
	1.9%	1.7%	2.4%	
Post Menstrual Bleed	2	1	1	0.784 (NS)
	3.8%	1.7%	2.4%	

Most common presenting symptom is Fatigue, which was present in 50% of the cases, followed by Dyspnoea (31.2%), palpitations (26.6%) and giddiness (26%), and few cases presented with other symptoms like Angina (3.9%), PICA (1.3%), Dysphagia (1.9%), Pain Abdomen (1.9%), Bony Pain (5.8%), Fever (6.5%), Loss of Appetite (9.1%), Weight loss (3.2%), Jaundice (3.2%), Bleeding (3.9%), Melaena(0.6%), Haemoglobinuria(0.6%), menorrhagia (1.9%) and Post menstrual bleed (2.6%).

Table 4: Comparison of General Physical Examination findings with Type of Anaemia

General Physical Examination	Cases			p-value
	Dimorphic	IDA	Vit. B12 Deficiency	
Pallor	31	35	24	0.976 (NS)
	58.5%	59.3%	57.1%	
Koilonychia	13	6	8	0.130 (NS)
	24.5%	10.2%	19.0%	
Icterus	3	2	3	0.692 (NS)
	5.7%	3.4%	7.1%	
Pedaloedema	3	1	1	0.464 (NS)
	5.7%	1.7%	2.4%	
Lymphadenopathy	3	2	2	0.845 (NS)
	5.7%	3.4%	4.8%	
Glossitis	10	9	10	0.556 (NS)
	18.9%	15.3%	23.8%	
Angular stomatitis	5	4	5	0.673 (NS)
	9.4%	6.8%	11.9%	
Petechiae	0	0	1	0.261 (NS)
	0.0%	0.0%	2.4%	
Haemolyticfacies	0	0	0	NA
	0.0%	0.0%	0.0%	
Ankleulcers	0	0	0	NA
	0.0%	0.0%	0.0%	
Perioral pigmentation	2	1	1	0.784 (NS)
	3.8%	1.7%	2.4%	
Knuckle pigmentation	3	2	3	0.692 (NS)
	5.7%	3.4%	7.1%	

The most common finding on general physical examination was pallor, which was present in 58.4% cases. Also seen were glossitis (29 cases), koilonychia (27 cases), angular stomatitis (14 cases), knuckle pigmentation (8 cases), pedal oedema (5 cases), icterus (8 cases), lymphadenopathy (7 case), perioral pigmentation (4 cases) and petechiae (1 case). None of the cases had haemolyticfacies or ankle ulcers.

Table 5: Comparison of Lipid Profile with Type of Anaemia

Lipid Profile		Cases			p-value
		Dimorphic	IDA	Vit. B12 Deficiency	
TC (mg/dl)	Mean	136.66	133.26	135.77	0.724
	SD	17.954	23.394	22.902	
HDL (mg/dl)	Mean	31.64	31.67	30.30	0.320
	SD	5.498	5.855	4.418	
LDL (mg/dl)	Mean	80.15	84.71	84.38	0.261
	SD	14.888	14.903	18.039	
VLDL (mg/dl)	Mean	47.02	50.60	52.43	0.527
	SD	25.603	25.729	25.836	

TG (mg/dl)	Mean	101.31	106.24	109.36	0.094
	SD	21.751	20.891	15.635	

Mean Total Cholesterol level is more in IDA(136.6±17.9 mg/dl) compared to other types of Anaemia, mean HDL levels is more in Vit B12 Deficiency Anaemia (31.67±5.4 mg/dl), mean LDL levels is more in Vit B12 deficiency Anaemia (84.7±14.9), mean VLDL levels is more in Dimorphic Anaemia (52.4±25.8 mg/dl), mean TG levels is more in Dimorphic Anaemia (105.4±19.7 mg/dl), even though there is slight difference in the mean TC, HDL, LDL, VLDL and TG levels between the groups, there is no statistically significantdifference.

Discussion

The observations made in 154 cases of anaemia and 154 non anaemic controls, who presented to Department of General Medicine, Mahatma Gandhi Medical College, Jaipur from Jan 2019 to June 2020 is discussed here and results have been compared with other similar studies.

All cases in this study samples were more than 18 years of age. Majority of the cases (35.1%) and Controls (33%) are in the age group of >50 years, ie., majority of the cases were old aged (>50 years). This is probably due to decreased nutritional intake. 61.7% of cases are males and 38.3% are females, and 57.8% of Controls are Males and 42.2% are Females, Percentage of male population among dimorphic anaemia is 52.8%, 72.9% in IDA and 57.1% in Vitamin B12 Deficiency Anaemia, there was no correlation between sex and Type of anaemia.

Among 154 cases, Iron Deficiency anaemia was the most commonly seen type of anaemia in this study. Dimorphic Anaemia was the second most common, followed by Vit B12 deficiency Anaemia. This is consistent with standard textbooks of medicine, which describe nutritional deficiencies, especially iron deficiency, to be the most common cause for anaemia.[12,13]

Most cases had mild to moderate anaemia, as defined by a haemoglobin level above 6 gm/dl.

Cases commonly presented with non-specific symptoms of anaemia, such as fatigue, dyspnoea, palpitations and giddiness. Symptoms suggestive of a specific cause for anaemia were rarely seen, most common presenting symptom is Fatigue, which was present in 50% ofthe cases, followed by Dyspnoea (31.2%), palpitations (26.6%) and giddiness (26%), and few cases presented with other symptoms like Angina (3.9%), PICA (1.3%), Dysphagia (1.9%), Pain Abdomen (1.9%), Bony Pain (5.8%), Fever (6.5%), Loss of Appetite (9.1%), Weight loss (3.2%), Jaundice (3.2%), Bleeding (3.9%), Melaena (0.6%), Haemoglobinuria (0.6%), menorrhagia (1.9%) and Post menstrual bleed (2.6%), Cases with more severe anaemia were found to be more likely to have symptoms. Cases with more severe anaemia were more likely to have symptoms and had a greater number of symptoms. Patients with haemoglobin>9 gm/dl were usually asymptomatic, and incidentally detected to have on anaemia on routine evaluation. This is consistent with standard textbooks of medicine which state that mild anaemias of insidious onset are usually asymptomatic.[12]

The most common finding on general physical examination was pallor, which was present in 58.4% cases. Also seen were glossitis (29 cases), koilonychia (27 cases), angular stomatitis (14 cases), knuckle pigmentation (8 cases), pedal oedema (5 cases), icterus (8 cases), lymphadenopathy (7 case), perioral pigmentation (4 cases) and petechiae (1 case). None of the cases had haemolyticfacies or ankle ulcers

Majority of the cases with Hb<6gm/dl have all the symptoms like Pallor (100%), Koilonychia (86.4%), Glossitis (72.7%), Pallor is present in 93.9% of cases with Hb level 6- 9gm/dl, Koilonychia (12.1%). >9gm/dl cases had clinical signs of only pallor (9.1%).There is statistically significant difference in presentation of all clinical signs between the groups (P<0.05)

Pallor was the most common finding on general physical examination. Cases with more severe anaemia were found to be more likely to have findings on general physical examination. Signs were usually not seen in cases with haemoglobin less than 10 gm/dl. Koilonychia, lymphadenopathy, glossitis and angular stomatitis were seen only in cases with dimorphic anaemia and microcytic hypochromic anaemia.

Knuckle pigmentation and perioral pigmentation was seen only in cases with megaloblasticanaemia and dimorphic anaemia. Similar study was done by Greer JP et al., showed similar results and also, it is consistent with descriptions given in standard textbooks of medicine.[12]

Mean Total Cholesterol level in cases is 135.4±21.2 mg/dl and 176.4±21.2 in controls, mean HDL levels in cases is 31.2±5.3 mg/dl and 39.19±5.2 mg/dl in Controls, mean LDL levels in cases is 82.8±16.1 and 115.8±16.08 mg/dl in Controls, mean VLDL levels in cases is 49.8±25.6 mg/dl and 53.8±25.7 in Controls, mean TG levels in cases is 105.4±19.7 mg/dl and 126.4±19.8mg/dl in controls, mean TC/HDL ratio is 3.97±0.8 in cases and 4.55±0.6 in controls, mean LDL/HDL ratio is 2.63±0.5 in cases and 2.99±0.47 in Controls.

Mean Total Cholesterol level is more in IDA (136.6±17.9 mg/dl), mean HDL levels is more in Vit B12 Deficiency Anaemia (31.67±5.4 mg/dl), mean LDL levels is more in Vit B12deficiency Anaemia (84.7±14.9), mean VLDL levels is more in Dimorphic Anaemia (52.4±25.8 mg/dl), mean TG levels is more in Dimorphic Anaemia (105.4±19.7 mg/dl),

Mean Total Cholesterol level is more in Hb>9 gm/dl (152.48±14.15 mg/dl) compared to other types of Anaemia, mean HDL levels is more in Hb>9 gm/dl (34.73±4.1 mg/dl), mean LDL levels is more in Hb>9 gm/dl (94.7±13.5), mean VLDL levels is less in >9gm/dl (24.52±4.2) mg/dl), mean TG levels is more in >9gm/dl (114.9±16.03 mg/dl).

The results of this study confirm the findings of previous investigators that the mean serum total cholesterol, HDL, LDL, VLDL and triglyceride levels are decreased in anaemia.

The mean total cholesterol was found to be lower in anaemic cases when compared to controls. The decrease in mean serum cholesterol was not due to a specific lowering of any of the serum lipoprotein families; hypocholesterolaemia was caused by a reduction in all the major lipoprotein families, including mean HDL, LDL, VLDL and triglycerides. There was a very large decrease in mean total cholesterol and LDL levels, and a large decrease in mean HDL levels, resulting in a mild fall in mean TC/HDL and LDL/HDL ratios. There was a mild decrease in mean VLDL and triglyceride levels.

Rifkind and Gale in 1967 showed that anaemia was associated with hypocholesterolaemia and the decrease in serum cholesterol was not due to a specific lowering of any of the serum lipoprotein families, and that hypocholesterolaemia was caused by a proportional reduction in all the major lipoprotein families.[3,4]

Elwood and Mahler, in 1970, conducted a study 4,070 women, and demonstrated a significant difference in cholesterol between women with haemoglobin levels above and below 10.5g/dL.[6]

A study conducted by Choi et al. in 2001 showed that lipid levels in patients with iron deficiency anaemia were directly related to the haemoglobin levels.[14]

A study by Westerman[7] in 1975 examined the relationship between hypocholesterolaemia and various types of anaemia, including megaloblasticanaemia, hereditary spherocytosis, homozygous sickle cell disease, aplastic anaemia, and liver associated anaemia. The study showed that the plasma cholesterol level is closely related to haematocrit levels, both initially and throughout the course of the anaemias associated with hypocholesterolaemia. This association was maintained regardless of the cause of changes in haematocrit

levels. The authors concluded that low haematocrit, not the type of anaemia, is the cause of low cholesterol levels. Seip and Skrede, in 1967, found an association between serum cholesterol and haemoglobin in all cases, regardless of cause of anaemia.[15]

Conclusion

Anaemia is associated with significant hypocholesterolaemia, with lowering in all lipid subfractions. The extent of hypocholeserolaemiais proportional to the severity of anaemia. The type of anaemia has no effect on the hypocholesterolaemia seen in anaemia.

Further studies are required to study the long-term effect of anaemia on the risk of developing atherosclerosis, and to study the long-term effect of treatment of anaemia on lipid levels and cardiovascular morbidity and mortality.

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Conflict of Interest: Nil
Source of support:Nil