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RESEARCH ARTICLE

"ESTIMATION OF SERUM FERRITIN LEVEL IN REGULAR BLOOD DONORS - A TERTIARY HOSPITAL BASED STUDY"

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Iron Deficency (ID), Sr. Ferritin, Hematological Indices, Iron Deficiency Anemia (IDA)

Abstract

Introduction: Nutritional Anemia is a worldwide problem with the highest prevalence in developing countries like India. Iron Deficiency Anemia (IDA) accounts for 8.41 lacs deaths annually worldwide. WHO/CDC in 2004 recommended Hemoglobin and Serum Ferritin level as the most sensitive markers for detection of Iron Deficiency (ID) and Iron Deficiency Anemia.

Aim: To assess and compare Serum Ferritin and the Hematological Indices between control (first time donor) and regular blood donors.

Materials and Methods: Donors were divided into Control (first time donors) and Case (donors who had donated atleast two times per year in the last five previous years). Following donation, blood samples from 80 donors were collected - 2 ml in EDTA tubes for CBC by using Automatic Hematolgy Analyzer machine and 4ml in plain tubes for Serum Ferritin estimation by ELISA method.

Results: In our Case Control study, we noted statistically significant association between difference of mean values and frequency of donation with respect to Age and Serum Ferritin level in control and case. We also noted statistically significant association of Serum Ferritin with respect to number of lifetime donation, donation per year, Hb and MCV ($p \leq 0.05$).

Conclusion: As a part of Donor Hemovigilance Program, awareness should be enhanced among blood bank professionals and regular donors about estimation of Serum Ferritin level to maintain adequate donor pool.

Introduction:-

Blood Transfusion Service plays a vital role in the modern health care system without which an efficient health care is not possible. The safety and availability of the national blood supply depends on individual who can provide repeated blood donation. Donor screening criteria are formulated so that both donors and the patients are safe.[1]

Despite all of the scientific and technical advancements, humans have yet to discover a reliable replacement for blood. Patients therefore have no other option except to accept blood from other people, which is why giving blood

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is referred to as a "life donation." All over world "World Blood Donor Day" is celebrated annually on 14th June. Whereas in India, National Voluntary Blood Donation Day is celebrated on 1st October every year.[2,3,4]

The WHO recommends the interval between donations can be as short as 8 weeks and a maximum of 3 litres of blood can be collected per year. [1]

In India the minimum interval from donation should be at least 3 months for males and 4 months for females.

At the time of donation, a donor usually donates maximum 450 ml of blood. In 450 ml of blood donation, men lose 242±17 mg and women lose 217±11 mg of iron. Adequate iron stores are very important in maintenance of the donor's health. It is not only important to give a safe blood donation to the recipient but it is also important that the donor is safe after donation.[5]

National Blood Donor Vigilance Programme (NBDVP) is an integral part of the Hemovigilance Programme of India (HvPI launched on 10th December 2017) and is a comprehensive, centralized, and well structured approach to collect, collate, and analyze data to continuously improve donor safety and satisfaction so that the blood donors have a feeling of being well-treated and well taken care of that may cause blood donors to continue as repeat donors and will have an positive impact. [6]

Chronic iron depletion and anemia due to Iron deficiency may result from the regular contributions. Rejection due to low Hb, increases the likelihood that the regular donor would withdraw from the donor pool.[1]

Deficiency of iron is the most common nutritional deficiency in the world and results in clinical signs and symptoms that are mostly related to anemia. About 10% of people living in developed countries and 25% to 50% of those in developing countries are anemic, and in both settings the most frequent cause is iron deficiency. IDA accounts for 8.41 lacs deaths annually worldwide. Africa and part of Asia bear 71% of the global mortality burden. [7]

In India, prevalence of Anemia is very high as compared to world and accounts for 51% of total populations.[8]

WHO/CDC (World Health Organization & Centers for Disease Control & Prevention) expert consultation in 2004 recommended Hemoglobin and Serum Ferritin as the most sensitive markers to detect Iron Deficiency (ID) as well as Iron Deficiency Anemia (IDA).

The most accurate way to assess iron store would be to measure Serum Ferritin levels in the blood as it identifies the individual most at risk for iron depletion. Hence, WHO has recommended estimation of Serum Ferritin as the laboratory test of choice for screening of iron deficiency.[1]

Hence the present study, aims to assess and compare Serum Ferritin and the Hematological Indices such as Hemoglobin (Hb), Hematocrit (Hct), Red Blood Cell (RBC) count, Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) and Red cell Distribution Width (RDW) between control (first time donor) and regular blood donors as per Operational Definition(donors who had donated atleast two times per year in the last five previous years).

Materials And Methods:-

The present study was a Case Control study conducted on blood donors presenting to the Model Blood Bank in the Department of Pathology and Dr. Bhim Rao Ambedkar Memorial Hospital Raipur, Chhattisgarh India, between 1st November 2021- 31st October 2022.

Inclusion Criteria:

As per norms by Drugs Controller of India, Directorate General Health Services (DGHS). Donors must be:-

1. Age- 18-65 years
2. Hemoglobin >12.5gm.
3. Weight more than 45 kg
4. The minimum interval from donation should be at least 3 months for males and 4 months for females.

Exclusion criteria:
1. Donors who are taking iron supplements.
2. All other Deferral criteria mentioned in guidelines issued by Drug Controller of India.

The total sample from 80 selected donors were collected and all relevant details were noted and were divided into 2 groups viz. control and case. Control consists of donors donating blood for the first time and case consisting of donors who had donated atleast two times per year in the last five previous years.

A six ml of whole blood was collected from each donor during process of blood donation. Out of six ml of the blood, two ml was collected in EDTA tubes for estimating Hb, PCV, MCV, MCH, MCHC and RDW while four ml collected in plain tubes for serum ferritin analysis.

Estimation of Blood Parameters
These tests were performed in MINDRAY- BC 6000® Automatic Hematology Analyzer.

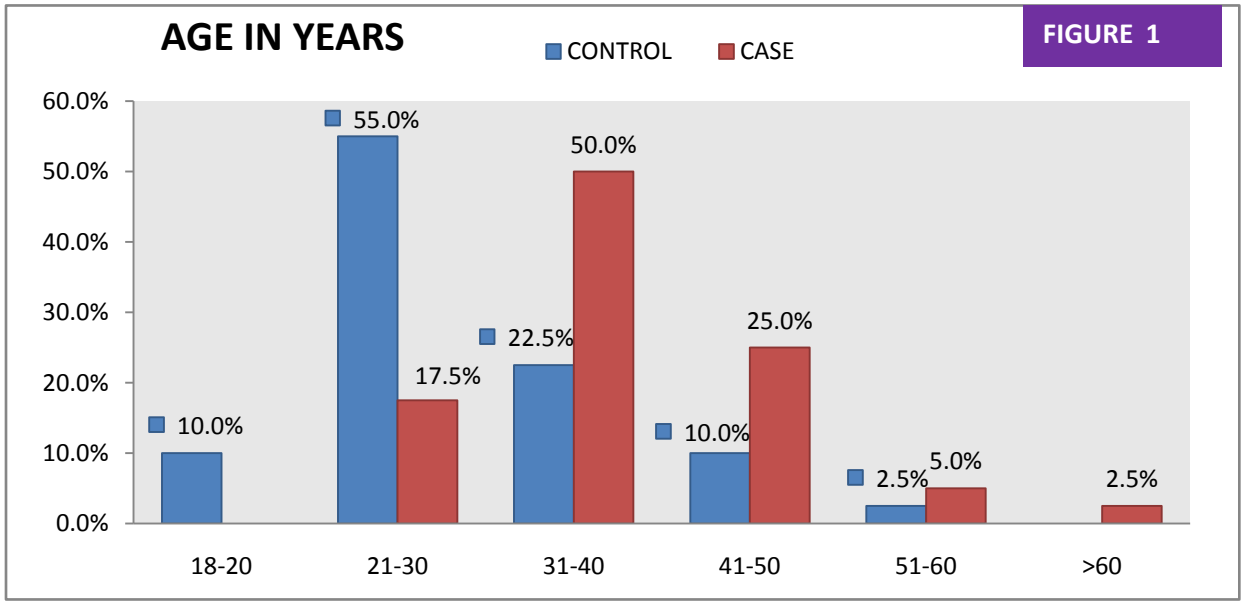
Serum Ferrtin Estimation:
Serum separated from the 4ml clotted blood from plain tubes from each donor was stored at -20ºC for estimation of Ferritin concentrations by DiaMetra ELISA® test kit and reading was obtained from HumaReader HS® ELISA Reader Machine.

During the analysis of results, the donors were grouped on the basis of Serum Ferritin values into those with < 15ng/ml and ≥15ng/ml.

This is based on the recommendation of the World Health Organization (WHO) that Serum Ferritin concentration <15ng/ml indicates depleted iron stores in those >5 years of age.[1]

Statistical Analysis:-
Statistical data was entered in Microsoft Excel and statistical analysis was performed in IBM SPSS software version 22. All the variables were analysed and compared between the two groups of study sample. Continuous data are reported as Mean±Standard Deviation (SD). ANOVA test was performed to find association between frequency of blood donation and blood parameters and $P \leq 0.05$ were considered as statistically significant.

Results:-
Out of 80 donors, Mean age of the study population was 29.87±8.68 years in control and 37.72±8.02years in cases. (Range: 18-65 years, with majority (96.25%) being males. [Fig. no.1]



Majority the study participants were taking a non-vegetarian diet and none gave the history of iron supplementation. Male: Female ratio in control group was 19:1, where as in case group ratio of Male: Female is 39:1 [Table no.1]

Table no.1:- Distribution of Donors based on Age, Gender and Diet.

GENDER	**Control (n=40)**		**Case (n=40)**	
	N	(%)	N	(%)
MALE	38	(95%)	39	(97.5%)
FEMALE	2	(5%)	1	(2.5%)
DIET				
NONVEGETERIAN	33	(82.5%)	31	(77.5%)
VEGETERIAN	7	(17.5%)	9	(22.5%)

Figure No. 2:- Distribution of Donors on Basis of Frequency of Lifetime Donation.

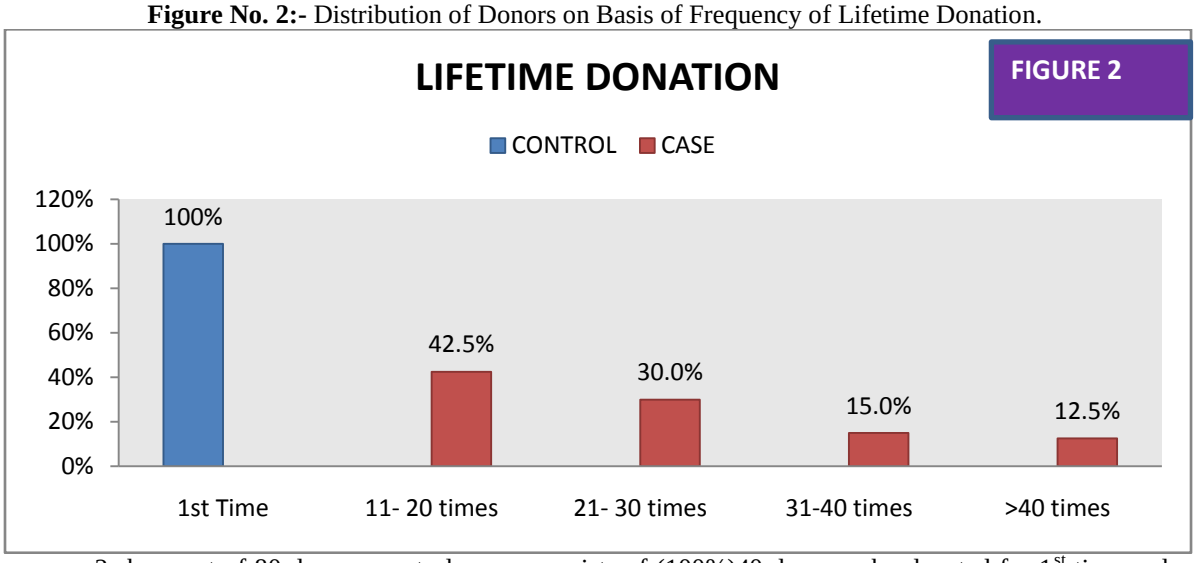


Figure no. 2 show out of 80 donors, control group consists of (100%)40 donors who donated for 1st time and case group- donors who donated for 11-20 times are 42.2%(17) were maximum.

Figure No. 3:- Distribution of Donors on Basis of Donation Per Year.

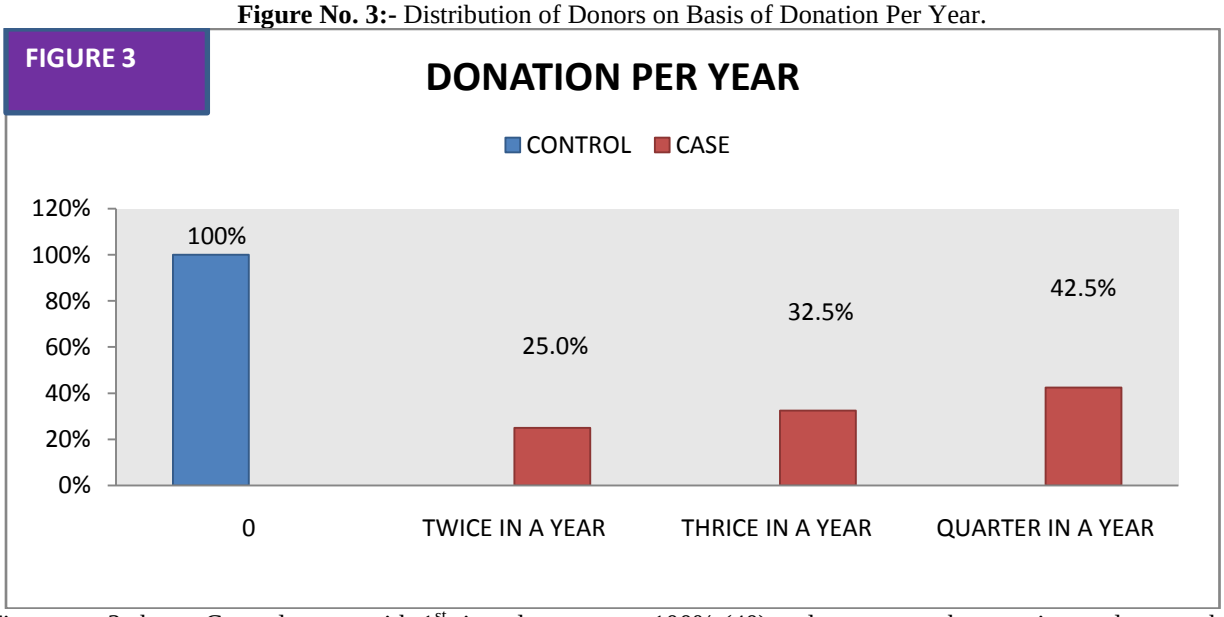


Figure no. 3 shows Control group with 1st time donors were 100% (40) and case group has maximum donors who donated 4 times per year was 42.5%(17).

Table No. 2:- Distribution of Donors on Basis of Serum Ferritin(ng/ml).

Serum Ferritin(ng/ml)		
Type Of Donor	CONTROL(n=40)	CASE(n=40)
	n %	n %
<15	1 (2.5%)	8 (20%)
≥15	39 (97.5%)	32 (80%)
Mean±SD	136.15±138.53	71.77±64.01

Table No. 2 show maximum donors with Sr. Ferritin ≥15 was 39 (97.5%) in Control donors followed by with Sr. Ferritin<15 was 1 (2.5%). Case donors had maximum donors with Sr. Ferritin ≥15 were 32 (80%) and Sr. Ferritin<15 were 8 (20%). Mean Sr. Ferritin ± Standard Deviation of control group and case group were 136.15±138.53 and 71.77 ± 64.01 respectively.

Table No. 3:- Comparision of Mean Differences Between Control And Case (According to Independent Samples Test).

Parameters	**t-test for Equality of Means**					
	T	**df**	**p-Value**	**Mean Difference**	**95% Confidence Interval of the Difference**	
					Lower	**Upper**
AGE (YRS)						
	-4.200	77.52	**0.000**	-7.8500	-11.57	-4.1284
SR. FERRITTIN(ng/ml)						
	2.668	54.926	**0.010**	64.3750	16.01	112.7346
HB(gm/dl)						
	1.597	71.809	0.115	.6250	-.155	1.4052
PCV(%)						
	1.471	70.17	0.146	2.6025	-.925	6.1308
RBC COUNT (million/mm3)						
	1.995	75.15	**0.050**	.330	.00053	.65997
MCV(fl)						
	0.627	76.19	0.533	1.4925	-3.2510	6.2360
MCH(pg)						
	-1.320	73.44	0.191	-1.0225	-2.5658	.5208
MCHC(gm/dl)						
	-1.619	69.68	0.110	-.5925	-1.3224	.1374
RDW(%)						
	0.608	74.34	0.545	.2000	-.4549	.8549

(p value<0.05 was considered as significant.)

There was a statistically significant mean difference in case and control group with respect to Age, Sr. ferritin and RBC Count. And other parameters were insignificant.

Table No.4:- Distribution Of Donors Based On Frequency Donation Per Year Among 1st Time Versus Regular Blood Donors.(According to ANOVA test).

Parameters	**Distribution Of Donors Based On Frequency Donation Per Year**								**P-Value**
	1st Time		**Atleast twice per year**		**Atleast thrice per year**		**Atleast quarter per year**		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
AGE(IN YEARS)	29.8	8.6	32.6	6.4	37.3	7.2	41.0	8.0	**0.00**
SR. FERRITTIN (ng/ml)	136.1	138.5	127.9	81.34	76.3	47.6	35.2	34.0	**0.01**
HEMOGLOBIN(gm/dl)	13.0	1.4	12.4	1.5	11.8	2.5	12.8	1.7	0.19
PCV(%)	42.8	9.1	41.0	6.1	37.9	7.2	41.6	5.8	0.28
RBC COUNT (million/mm3)	4.6	0.6	4.2	0.4	4.0	0.9	4.4	0.8	0.16
MCV(fl)	89.5	9.7	90.1	13.4	89.4	12.6	85.8	9.3	0.63

MCH(pg)	28.3	3.0	29.5	4.2	29.4	4.4	29.1	3.3	0.60
MCHC(gm/dl)	31.6	1.8	32.1	1.2	32.4	1.3	32.0	1.4	0.40
RDW(%)	14.7	1.6	14.3	1.1	14.3	1.4	14.7	1.3	0.79

(p value<0.05 was considered as significant.)

Table no 4.shows statistically significant association of frequency of donation per year with respect to Age and Sr. Ferritin ($p \leq 0.05$).

Table No.5:- Stages of Iron Deficiency in Both Case And Control[13,33] (n=80).

Stage	**S. ferritin (ng/ml)**	**Hb**	**Type of donor**	
1.Normal Iron Balance	Normal (15-300)	Normal	Control	Case
			25 (62.5%)	16 (40%)
2.Negative Iron Balance	< 20	Normal >12.5	-	2 (5%)
3.Iron Deficient Erythropoiesis	< 15	<12.5	1 (1.25%)	4 (10%)
4.Iron Deficiency Anemia	< 12	<12.5	-	2 (5%)

Table no 5 shows out of 80 donors; 62.5% (n=25) were in Normal Iron Balance and 1.25% (1) donors had Iron Deficient Erythropoiesis in Control donors. In Case donors 40%(16) were in Normal Iron Balance, 5%(2) had Negative Iron Balance, 10% (4) donors had Iron Deficient Erythropoiesis and 5%(2) donors had Iron Deficiency Anemia.

Table No. 6:- Stratification of Donors Based on Serum Ferritin.

PARAMETERS	**SR. FERRITIN(ng/ml)**				**P-VALUE (Chi- square test)**
	<15		**≥15**		
AGE (in Years)	**N**	**(%)**	**n**	**(%)**	
18-20	0	(0)	4	(5)	0.411
21-30	1	(1.25)	28	(35)	
31-40	5	(6.25)	24	(30)	
41-50	2	(2.5)	12	(15)	
51-60	1	(1.25)	2	(2.5)	
>60	0	(0)	1	(1.25)	
SEX					
Male	9	(11.25)	68	(85)	0.530
Female	0	(0)	3	(3.75)	
NUMBER OF LIFETIME DONATIONS					
1st Time	1	(1.25)	39	(48.75)	**0.001**
11-20 Times	1	(1.25)	16	(20)	
21-30 Times	2	(2.5)	10	(12.5)	
31-40 Times	2	(2.5)	4	(5)	
>40 Times	3	(3.75)	2	(2.5)	
PER YEAR DONATION					
1st Time	1	(1.25)	39	(48.75)	**0.00**
Twice	1	(1.25)	9	(11.25)	
Thrice	0	(0)	13	(16.25)	
Quarter	7	(8.75)	10	(12.5)	
DIET					
Nonvegeterian	7	(8.75)	57	(71.5)	0.86
Vegeterian	2	(2.5)	14	(17.5)	

PARAMETERS	SR. FERRITTIN(ng/ml)				P-VALUE
	<15		≥15		
HEMOGLOBIN(gm/dl)	**N**	**(%)**	**N**	**(%)**	
<12.5	7	(8.75)	30	(37.5)	**0.04**
≥12.5	2	(2.5)	41	(51.25)	
PCV(%)					
<40	6	(7.5)	26	(32.5)	0.34
>50	0	(0)	4	(5)	
38-45	0	(0)	3	(3.75)	
40-50	3	(3.75)	38	(47.5)	
RBC COUNT (million/mm3)					
<4.5	7	(8.75)	32	(40)	
>5.5	1	(1.25)	3	(3.75)	0.155
3.8-4.8	0	(0)	3	(3.75)	
4.5-5.5	1	(1.25)	33	(41.25)	
MCV(fl)					
<80	6	(7.5)	10	(12.5)	
>100	0	(0)	10	(12.5)	**0.001**
80-100	3	(3.75)	51	(63.5)	
MCH(pg)					
<27	3	(3.75)	16	(20)	
>32	0	(0)	9	(11.5)	0.46
27-32	6	(7.5)	46	(57.5)	
MCHC(gm/dl)					
<32	3	(3.75)	24	(30)	
32-36	6	(7.5)	47	(58.75)	0.64
RDW(%)					
>16	1	(1.25)	11	(13.75)	
12- 16	8	(10)	60	(75)	0.59

(P Value ≤0.05 was considered as significant.)

Table no. 6 shows statistically significant association of Sr. Ferritin with respect to number of lifetime donation, donation per year, Hb and MCV($p \leq 0.05$).Statistically insignificant association of Sr. Ferritin with respect to age, sex, diet, PCV, RBC count, MCH, MCHC and RDW ($p \geq 0.05$) were seen in our study.

Discussion:-

The World Health Organization (WHO) urges the establishment of a nationwide transfusion network under the Government's strong supervision. [1,9]

An issue for blood banks is the mismatch between the rising demand for blood transfusions and the difficulties in finding new blood donors and keeping the current blood donors. [1,10]

One gram of Hb contains 3.4 mg of iron. Thus, in a normal person with Hb of 15g/dl, 100ml of blood would contain approximately 50mg of iron. This accounts to removal of approximately 175 mgs of iron with 350 ml of blood donation and 225 mg of iron with 450 ml of donation.[1,11]

According to Rosvik A S et al, donors does not need additional iron if their Serum Ferritin levels are above 80µg/L (80ng/ml) and should only get iron if their levels are below 50 µg/L (50ng/ml).[1,12]

In this study, the Mean Age in control group was 29.87±8.68 and in case group was 37.72±8.02. Where as in Garg B et al, the mean age in control group was 27.26 ± 7.34 and in case group was 29.15 ± 7.55 (p=0.009). Also in Adediran A et al, the mean age in control and study group was 33 ± 9.15 and 35.19 ± 7.94 P=0.461). So our study correlated with study of Garg B et al but not with Adediran A et al in respect of mean age.[13,14].There were 38 males and 2 females in Control Group with Male:Female ratio of 19:1; whereas in Case Group Male:Female ratio was

39:1. This was similar to other studies where males were more as compared to female donors viz. Tailor H et al, Adediran A et al, Mahida VI. et al and Reddy K et al.[5,14,15,16]

In our study, the relationship between food history and serum ferritin levels was determined to be statistically insignificant. Similar findings were made in studies by Devi GD et al, Mittal R et al, Mahida V et al and Reddy KV et al which indicated no distinction between vegetarians and non-vegetarians in terms of their iron profiles among blood donors. However, research by Leggett et al. and Richard et al. had shown that serum ferritin levels were inversely correlated with diets high in meat, with vegetarians having lower levels.[1,17,15, 16, 18,19,20]

Table No. 7:- Comparision Between Present Study And Other Studies Based on P Values With Regard to Age And Hematoligical Indices.

Parameters	**References**						
	Yousefinejad V et al (2010) [21]	**Garg B et al (2012) [13]**	**Adediran A et al (2013) [14]**	**Datta S et al (2013) [22]**	**Tailor H.J. et al (2017)[5]**	**Waheed U et al (2018) [23]**	**Present Study**
AGE	-	0.09	0.461	>0.05	-	-	**0.000**
Sr. Ferritin	**0.000**	**0.01**	**0.001**	**<0.05**	**<0.001**	**0.001**	**0.010**
Hb	**0.05**	0.49	0.303	**<0.05**	**<0.005**	**0.004**	0.115
RBC Count	**-**	**0.05**	**-**	-	**<0.005**	**0.001**	**0.050**
PCV	**-**	0.98	0.119	-	**-**	**0.001**	0.146
MCV	**0.006**	**0.05**	**0.003**	-	**<0.001**	**0.001**	0.533
MCH	**-**	0.14	0.743	-	**<0.001**	**0.003**	0.191
MCHC	**-**	0.95	**0.036**	-	**-**	**0.002**	0.110
RDW	**-**	0.32	-	-	**-**	**-**	0.545

P Value ≤0.05 was considered as significant.

In the present study that Serum Ferritin level between Control & Case was significant (p=0.013).(Table no. 3) This was similar to studies of **Datta S et al, Adediran A et al, Yousefinejad V et al, Tailor H.J. et al, Waheed U et al** and **Garg B et al**.[22,14,21,5,23,13].

We noted statistically significant association between first time donor and regular blood donors with respect to RBC count. (Table no. 3) **Tailor H.J. et al, Waheed U et al** and **Garg B et al** found similar findings. [5,23,13].

Table No. 8:- Comparision of P Valvues Based on Frequency of Donation with Other Studies.

Reference	**Parameters**								
	Age	Serum Ferritin	Hb	RBC Count	PCV	MCV	MCH	MCHC	RDW
Mittal R et al (2006)[2]	-	**0.001**		**-**	-	**-**	**-**	**-**	**-**
Mahida V.I. et al (2008)[15]	-	**0.01**	**0.01**	**-**		**0.01**	**0.01**	**-**	**0.01**
Yousefinejad V et al (2010) [21]	-	**0.00**	**0.004**	-	-	0.08	-	-	-

Abdullah S.M. (2011)[24]	-	0**.016**	-	-	-	0.8	0.5	-	-
Devi G. D. et al (2017) [1]	**0.00**	**0.00**	0.494	-	0.16	0.286	-	0.169	0.841
Tailor H.J. et al (2017)[5]	-	**<0.001**	**<0.005**	-	-	-	-	-	-
Reddy K.V. et al (2019)[16]	**0.00**	**0.003**	**0.032**	-	**0.032**	-	-	-	-
Kumar A et al (2021)[3]	-	0.89	0.76		0.42	0.44	**0.039**	**0.007**	-
Present study	**0.00**	**0.01**	0.19	0.16	0.28	0.63	0.60	0.40	0.79

P Value ≤0.05 was considered as significant.

In our study, we found that p value (0.00) with regard to frequency of donation and age between control & case was significant and was similar with findings of **Devi G. D. et al** and **Reddy K.V. et al.**[1,16]

We found a statistically significant correlation with respect to frequency of donation and Serum Ferritin levels. This was in correlation with the findings of **Devi G. D. et al, Reddy K.V. et al, Abdullah S.M., Tailor H.J. et al, Mittal R. et al, Yousefinejad V et al, Mahida V.I. et al, Badar A et al, Finch et al, Norashikin et al, Rodalfo et al, Mozaheb Z et al, Boulahriss M et al, Badami K.G et al and Nadarajan et al.** [1,16,24,5,2,21,15,25,26,17,27,28-31]. But **Kumar A.S. et al** found insignificant correlation.[3]

Table No. 9:- Comparision of P Values Based on Serum Ferritin with Other Studies.

Reference	Parameters						
	AGE	Hb	PCV	MCV	MCH	MCHC	RDW
Adu P et al (2020) [32]	0.001	**0.00**	**-**	**0.001**	**0.00**	-	0.26
Kumar A et al(2021) [3]	-	**0.001**	**0.001**	**0.01**	**0.005**	0.156	-
Present study	0.41	**0.04**	0.34	**0.001**	0.46	0.97	0.79

(P Value ≤0.05 was considered as significant.)

In our study we found statistically significant correlation based on Serum Ferritin with regard to Hb and MCV. This was similar to studies of **Adu P et al** and **Kumar A et al**. [32,3]

Conclusion:-

The present study showed that there was a significant difference in Serum Ferritin level between Control and Case. The population we studied showed decreased Serum Ferritin with increasing frequency of donation and age. Hemoglobin estimation alone may not be enough to evaluate donor safety prior to phlebotomy. Therefore regular donors are at risk of leaving donor pool soon due to chronic iron deficiency as a consequences of regular donation and low body iron store, evident by Serum Ferritin levels and other laboratory tests.

We also found statistically significant correlation between Serum Ferritin level <15ng/ml, and Hb and MCV. It could be probably explained by different stages in development of iron deficiency anemia, in particular pre-latent and latent stages, and also due to their lack of dietary replenishment as per physiological demand required and low socioeconomic status.

We also noted statistically significant inverse correlation between Serum Ferritin and frequency of donation. Hence we concluded that estimation of Serum Ferritin level after five donations will help to identify iron deficient blood donors as chronic iron deficiency is one of the most frequent complication observed in long term blood donors.

As a part of Donor Haemovigilance Program of India (HvPI), our study suggests awareness should be enhanced among Blood Bank Professionals and Donors especially female donors and regular donors about estimation of

Serum Ferritin level, Iron supplementation and Donor Health Education on balanced nutritious diet to maintain adequate donor pool.

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None declared

Ethical approval:
The study was approved by the Institutional Ethics Committee Pt.JNM Medical College, Raipur, Chhattisgarh.

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