Iron Status of Blood Donors

BY

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LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF GRAPHS

LIST OF APPENDICES

Appendix

Introduction

I. INTRODUCTION

Man's desire for life is part of his nature. Of all things that he is constantly in persuit of is how to increase his life-span. During the past fifty years or more medical science has made starting progress in all branches of medicine. One of them is blood transfusion and storage of blood and its components (Indian Red Cross Society, 1987).

Human blood cannot be manufactured outside the body (Harold Shryock, 1986). A sudden loss or more than one fourth of blood volume will endanger a person's life and if blood replenishment is not made immediately, death is inevitable. For this, we used human blood as nothing can replace it and scientists have yet to evolve an ideal synthetic substitute for human blood. No wonder people consider blood to be synonymous with life (Tarkunde and Gupta, 1979).

According to Mavalankar (1987) the blood donation had now been accepted as a moral-social oblication. The concept of blood donation emerged with the success of blood transfusion operation in the early twentieth century. It is still to be developed and harnessed.

The amount of blood in each healthy persons body is five litres, 1/13 of the body weight is made up of blood. Blood donation is a painless process of extracting 300cc or five per cent of blood from a person for the purpose of transfusing it to a needy patient. This is absolutely harmless to the health (Henry, 1979). Blood is all the time being made in our system and this additional loss is made up in no time, this is made up in a few days on normal diet (Indian Red Cross Society, 1988).

Most of the big hospitals in India have their own blood banks, yet only a few have adequate stocks of blood of different groups (A, B, AB AND O) necessary to meet any emergency (Nair, 1986).

The blood donors can be classified as voluntary (no fee is paid) professional (paid according to the amount of blood donated), and relative (blood donation to the family members or friends) donors (Huestis et al., 1976). Proper selection of a blood donor is obviously a matter of importance. A great deal of individual variation will be found among prospective donors. Potential donors should be free of diseases, the donors and the patients must belong to the same blood group and Rh factor and their blood must be compatible by direct matching (Wintrobe et al., 1981).

According to Nutritional News (1986), the professional donors belonged to low socio-economic group. Most of them were under-nourished as judged by weight for height. In our country the professional class of donors is a problem. Shortage of any commodity leads to various evils and malpractices. Trading in human blood is however, fraught with grave dangers if not halted in time (Louis, 1979).

It is more convenient to use men as donors although women are more apt to volunteer. Female donors recover their cell volume and haemoglobin more slowly and are apt to develop a slight but definite anaemia. The females were found to have some slight haematological defects (Haemoglobin $<$11.8, Mean Corpuscular volume $<$ 50%, RBC count $<$ 3.8 MC) following blood donation (Feinblalt, 1976).

Nair (1986) stipulated that donors should be healthy not less than 45kg in body weight, age between 17 to 60 years and haemoglobin content of the blood should not below 12.5g/100ml.

To evaluate the iron nutritional status of blood donors, serum and blood iron, haemoglobin, red blood cell count, haematocrit, mean corpuscular cell volume, mean corpuscular haemoglobin concentration, mean corpuscular

haemoglobin, total iron binding capacity, ferritin, transferring saturation and ascorbic acid were used as indices. A few relevent studies on iron nutritional status of blood donors (Cook, 1982; Huebers et al.,1987; Nutritional News, 1986 and Arosio, 1981) reported in the literature are recalled in this section.

Keller et al. (1975), had reported that in all individuals after blood donation the haemoglobin content was found to be lower than before. There was no effect on haemoglobin value if the time between two donation was more than three months. The haemoglobin value was reported to be lower in the professional donors ($<$10.8g/100ml) than in the volunteer donors ($>$15.3g/100ml). This might be due to the fact that the low socio-economic status and malnutrition of the professional donors (Wintrobe et al., 1981).

The blood loss was found to be the formost important cause of iron deficiency anaemia among blood donors. Each unit of blood donated contained 250mg of iron, three to four such donations could exhaust the stores of normal man (Wintrobe et al., 1981).

Cook (1982) had found that routine blood cell count determination indicates haemoglobin, hematocrit results and also red cell indices such as mean corpuscular volume, mean corpuscular haemoglobin concentration. All these were found

to be in normal limits (Mean corpuscular volume 88.5cm) in early iron deficiency and decreased only in severe iron deficiency (Mean corpuscular volume < 80.2cm).

Total iron binding capacity measurement could be adopted as a measureof transferrin bound iron in the regular blood donors, female donors had high total iron binding capacity (3.4 per cent) than male donors (2.9 per cent). It was found to be low in volunteer donors and high in the professional donors (Huebers et al.,1987).

It was found out by Arosio (1981), and Poderson and Morhing (1978) in persons who had donated blood one or two times the ferritin value was less. It was < 110ng/ml in male donors and < 40ng/ml in female donors, indicating iron deficiency. The transferrin saturation (Nutritional News, 1986) was consistently below 0.5 per cent in the blood donors.

Thus the preliminary haematological survey is becoming important especially in professional donors, because the transfusion of their anaemic blood might lead to severe complications in the acceptors (Mahnovski et al., 1987).

In the present investigation sixty seven professional blood donors, fifty eight volunteer blood donors and thirty three relative blood donors were studied. Five c.c. of

blood samples were collected from each individuals. The samples were obtained from Coimbatore Medical College Hospital blood bank and two other private blood banks in Coimbatore City. These participants were evaluated for blood and serum iron, haemoglobin, Red Blood Cell count, packed cell volume, mean corpuscular cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, serum total iron binding capacity, ferritin, transferrin saturation and ascorbic acid and their levels were compared with twenty apparently healthy matching control invididuals. Blood was also analysed for blood grouping and cross matching. The most predominant sex and age of blood donors along with their nutritional status and body weight were recorded. A further attempt had been made to find out the frequency of blood donation, amount of blood donated by the individuals and also the category to which they belonged. Their socio-economic status was also studied to correlate their economic status and frequency of blood donation.

Review of Literature

II. REVIEW OF LITERATURE

The review of literature pertaining to this study on "Iron nutritional status of blood donors" are reviewed under the following headings:

1. Blood donation and blood bank
2. Type of blood donors
    - i. Volunteer Blood donors
    - ii. Professional blood donors
    - iii. Relative donors
3. Importance of blood donation
4. Profiles of blood donors
    - i. Age
    - ii. Sex
    - iii. Weight
    - iv. Blood pressure
    - v. Socio-economic status
    - vi. Nutritional status
    - vii. Frequency of donation
5. Biochemical findings in Blood donors
    - A. Blood
        - i. Iron
        - ii. Haemoglobin
        - iii. Red Blood cell count (RBC)
        - iv. Packed cell volume (PCV or Haematocrit)
        - v. Mean corpuscular volume, Mean corpuscular haemoglobin and Mean corpuscular haemoglobin concentration (M.C.V., M.C.H. and M.C.H.C.)

B. In serum and plasma

i. Serum iron
ii. Total iron binding capacity
iii. Transferrin saturation
iv. Ferritin
v. Ascorbic acid

6. Risks due to blood donation and transfusion

1. Blood donation and Blood Bank

Blood donation is a harmless process of giving 300cc or five per cent of blood by a person for the purpose of transfusing it to needy patient (Henry, 1979). According to Mavalankar (1987) blood donation has now been accepted as a social obligation and to enable easy access and success in all operations and emergencies. Today blood transfusion has become a common mode of therapy (Clinical Courier, 1983).

The Indian Red Cross Society (1988) had started two planes to honour the needs of blood donors: (1) Regular blood donors who have donated five times or more are given a separate card of identity assuming them that members of their immediate family will be provided blood in case of need by all the hospitals.

(2) For group donors, the blood donation camps can receive blood for members of their group directly from the hospitals on production of the coupons issued to them.

Blood Bank

Blood bank is a place where human blood is stored for use when needed in case of serious burns, accidents, operations and anaemic patients. A blood bank accepts blood donation from healthy people and maintains a list of donors who could be called upon to give blood whenever the need arises. Depletion of stocks without replacement could cause a blood bank to cease functioning. It therefore becomes absolutely essential for a blood bank to maintain a steady supply of blood, so that needy patients can bank upon it (Tarkunda and Gupta, 1979).

Henry (1979) quoted that the phlebotomists should be well-trained in aseptic techniques materials used should be sterile (2 hours at 170°C) donors blood bag, samples tube, and donor record should be properly identified and labelled before drawing. The vein puncture site should be free of skin lesions of an infectious nature. Thorough mixing of the blood and anticoagulants (Wintrobe et al., 1981), in the bag and tubing is essential, stripping the tubing several times before sealing is important.

According to Nair (1979), the blood can be stored in a blood bank for three weeks at a temperature of 4° to 6°C. Large blood banks have refrigerated rooms to store blood bottles. The blood bank are not only storage

places, they also coordinate supply to needy peoples. In the event that the stored blood is not used within the stipulated period, it is split into various components which then can be stored for longer period.

2. Types of Blood Donors

Proper selection of a blood donor was obviously a matter of importance (Feinblalt ($_1$976). The blood donors are custamarily divided into three groups (Indian Red Cross Society, 1979), volunteer donors, professional donors, and relative donors.

i. Volunteer Blood Donors

In voluntary donation a donor comes forward to give blood on his own, with the sole purpose of saving a life in danger and expects nothing in return for his gift of blood. His is only a humanitarian gesture.

ii. Professional Blood Donors

In the professional category the donors primarily concerned with monetary gain and not at all bothered about the patients health. He tries to sell his blood a number of time with in the restricted period and also conceals his communicable disease, if any (Huestis et al., 1976).

iii. Relative Blood donors

The third group of blood donors are the relative donors, donate their blood to only their relatives or friends. The patients and occasionally their physician think that direct donations (the patient directly solicits donations from family or friends) must be safer than regular blood bank donors blood (Moore, 1986).

The professional class of donors are the problem in our country. Shortage of any commodity leads to various evils and malpractices (Louis, 1979).

4. Importance of Blood donation

It might be thought often donation of blood by the professional and volunteer donors will leads to the anaemia, but such does not appears to be the rule. Following loss of 500cc or even more of blood, the average individual practically experiences, no discomfort. As a matter of fact, the majority of donors seems to feel somewhat better a few days after the donation and there seems to be tendency towards a slight gain in body weight (Feinblalt, 1979).

According to Tarkunde and Gupta (1979), the blood donation is a social obligation on all healthy people between the age group of 17 and 60 to donate their blood.

The blood transfusion provides a vital therapeutic modality (Walkar, 1987). The blood banks and transfusion services make available atleast twenty different blood products. Whole blood is used in large quantity in massive trauma and in open heart surgery. Where as the packed red cell with a smaller total volume as whole blood are effective for treatment of anaemia. The random donor platelet are used for patients with bleeding secondary thrombocytophenia or abnormal platelet function (Desiree et al.,1985).

5. Profiles of blood donors

i. Age

The vigorous young adults make the best donors, the military age that is from eighteen to forty five years. If the donor is too young, his psychologic reaction may render him undesirable, if he is too old, there is always a danger of inadequate blood regenaration (Feinblalt, 1976).

Henry (1979), suggested that the blood drawn from a donor of 45kg should be 450 ± 45ml. The professional donors belonged to ages ranged from 19-45 years (Nutritional News, 1986). The healthy volunteer male donors should belong to 19 to 46 years old and female donors from 22 to 46 years.

ii. Sex

According to Mollison (1972), in many transfusion services blood donation should not be more than twice a

year and they do not bleed the women. The major object of that policy was to protect the donors from iron deficiency. The donation of one unit (300cc) per year by a women is equivalent to an increase in daily iron requirement of 0.58mg.

iii. Body Weight

The donor should be healthy not weighing less than 45kgs in body weight (Indian Red Cross Society, 1988), the normal weight of the blood donors should be 45kg and amount of blood collected should not exceed 450ml.

iv. Blood pressure

Feinblalt (1976), reported that as a rule, there was rather a sharp fall in the systolic and diastolic pressure (120/80) immediately after the bleeding, all of these figures rapidly returned to approximately normal readings. The blood pressure of blood donors should be 150/90 (Indian Red Cross Society, 1988). High blood pressure and low blood pressure patients are not eligible to be blood donors.

v. Socio economic status

Huestis et al.(1976) reported that the volunteer donors were well nourished and the professional donors

belonged to low socio-economic group. Most of them were under nourished as judged by weight for height.

vi. Nutritional status

The intake of meat, dairy products and coffee had a significant effect on the iron status of the subjects (Lewiston, 1986). Most of the professional donors were under nourished (Nutritional News, 1986).

vii. Frequency of donation

According to Nair (1986), a donor can donate blood safely once in every three months. A study on the 803 men and 812 women blood donors who gave blood for the first time and 4 to 8 times during 4 years revealed that not only females iron stores depleted but also that of males (Finch et al., 1977).

5. Biochemical findings with respect to iron status in Humans

Iron deficiency anaemia is an important public health problem in developing countries. The prevalence of iron deficiency had been reported to range from 10 per cent in adult men and 35 per cent in women (Nutritional News, 1980).

In developing countries the incidence of iron deficiency anaemia was often extremely high compared with that in the industrialized part of the world.

a. Blood

i. Iron

The body contains 4 to 5g of iron of which roughly two thirds is storage iron. Except for the plasma iron which is bound to the globulin transferrin also known as siderophilin, iron is present in haem compounds, over 90 per cent as Haemoglobin and 5 per cent as myoglobin and under 1 per cent as the haem enzymes (Varley et al., 1980).

According to Wintrobe et al. (1981) the body iron content of normal adult male was approximately 50mg/kg body weight were as that of adult women was about 35mg/kg.

The blood was sampled from 196 professional and 27 voluntary donors, the iron status was studied in terms of Haemoglobin and serum ferritin (Nutritional News, 1986). The blood loss was the foremost important cause of iron deficiency anaemia. The mobilization of iron stores in normal men averages 750mg with a range from 180 to 350mg. The blood donors had significantly lower level with a mean of 110mg and a range from zero to 250mg (Ocsson, 1972). This iron deficiency result in a consequences of defect in the immune system (Dallman et al., 1978).

ii. Haemoglobin

Normal Haemoglobin was significantly higher in men than in women (Mattila et al., 1986). The mean Haemoglobin

value for men was 14.8 ± 1.1 and for women it was 13.6 ± 1.0g/100ml (Zauber and Zauber, 1987). The haemoglobin concentration required for the male blood donors is 13.5g/dl and 12.5g/dl for female blood donors (Henry, 1979).

According to Nutritional News (1986) mean haemoglobin level was significantly lowered in the professional donors than in voluntary donors. All the professional donors were with a haemoglobin content below 85 per cent. It was considered that substitutions were necessary only in donors with low initial Haemoglobin values with short intervals between donation.

The haemoglobin values in donors were not significantly lower than in controls. Haemoglobin regeneration was notably depressed until five weeks after blood letting, but reaches predonation value before the next donation. There was no significant difference between the groups, the haemoglobin value for all groups were between 15.5 and 16.1g per 100ml (Lieden, 1973, Deing and Sachs, 1973).

iii. Red Blood cell count

The reference values for haemotological variables were derived by Bachus et al.,(1986) for males Red blood cell count was 5.0 million/cumm and for females 4.8 million/cumm.

Indians had smaller, more red blood cells than the other groups of (144 black and 237 white) blood donors. They also had found that the alteration of RBC count between the two groups of donors (Brain, 1982). Red blood cell indices are average value and with in the normal limits in early iron deficiency, it falls only in severe iron deficiency. And it was abnormal during the development of iron deficiency (Kaplan and Pesce, 1984, McClure et al., 1985).

iv. Packed cell volume (PCV or Haematocrit)

The normal reference value for male was 60 per cent and for female it was 40 per cent. The blood donation patterns alter the normal haematocrit values (Blum et al., 1986). According to Huestis et al. (1976) the lower limit of PCV for men was 41 per cent for women it was 37 per cent. Haemocrit was significantly lower in professional donors than volunteer donors.

v. Mean corpuscular volume, Mean corpuscular Haemoglobin and Mean corpuscular haemoglobin concentration (M.C.V., M.C.H., M.C.H.C.)

The third state of iron deficiency associated with reduced red cell indices, Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration all with in normal limits in early iron

deficiency, falls only in severe iron deficiency and in other comparably severe defect of haemoglobin synthesis. MCV was less than 80 cubic microns (Cook, 1982).

B. Serum and Plasma

i. Iron

In iron deficiency the iron stores become depleted, the serum iron falls and lastely anaemia becomes evident with reduction in Haemoglobin concentration. The serum iron level was reduced in professional donors (70µg/dl) than the volunteer donors (80-130.8 µg/dl) (Neumann and Wequer, 1984).

ii. Total Iron binding capacity

The total iron binding capacity was higher in men in all age groups than in women (Davilanky et al.,1979). Determination of total iron binding capacity of the serum gives a measure of transferrin and it was low in the voluntary donors and high in professional donors (Huebers et al., 1987).

The total iron binding capacity was estimated for 117 females and 159 male blood donors from 18 to about 70 years old, 113 and 136 of them were regular blood donors. The number of donation ranged from one to about 180. Total serum iron and total iron binding capacity were measured, 23.9 per cent of women and 19.1 per cent of men had low serum iron, and high total iron binding capacity

were found in 2.4 and 2.9 per cent of the women and men (Neumann and Weguer, 1973; Brain, 1982).

iii. Transferrin Saturation

The transferring saturation decreased with age in females and it showed normal values in males (Ajayi, 1985). The iron status was defined as a function of percentage transferrin saturation. The healthy volunteers 12 men from 19 to 46 years old and 10 women from 22 to 40 years had serial phlebotomines of about 400ml at weekly intervals which were continued until haemoglobin concentration remained below 11g/100ml without further bleeding for 14 days and transferrin saturation was consistently below 15 per cent of the men (Walters et al.,1973).

iv. Ferritin

The values of serum ferritin obtained in normal female and male were 58.9ng/ml and 31.6ng/ml respectively (Bhargava et al.,1987). A study conducted by Bailey et al. (1982) states that the serum ferritin concentration was a valid measure of iron status.

Serum ferritin was measured by Finch et al. (1977), in 803 men and 812 women who gave blood for the first time and 732 men and 630 women who had given 4 to 8 units during 4 years. In the former groups, the men had a geometric

mean of 127 and the women 40µg/litre. It seemed that male donors could give 2 to 3 units per year without an appreciably incidence of iron deficiency. Women could give about half the amount that men could without considerable risk of iron deficiency. According to Birgegard et al. (1978), the mean ferritin value significantly lower in the blood donors than in non-donors after 6 to 8 phlebotomies.

v. Ascorbic Acid

The content of the ascorbic acid was closely correlated with iron absorption (Hallberg et al., 1982). Ascorbic acid was found to be the most potent factor in the diet, enhancing the absorption of iron (Hallber et al.1986; Krause, 1979). During the iron deficiency ascorbic acid level was reduced (below the normal) supplementation of ascorbic acid improves the haemotologic status of the anaemic patients (Seshadri et al. 1985).

6. Risks due to blood donation and Transfusion

The modern technique reduces the serious accidents to the donors some donors faint but the symptom was due to the sight of the blood, not due to the loss of blood from the body. After a large donation, there might be a certain amount of giddiness but that symptom was only transient.

According to Natarajan (1983), the Hepatitis-B surface antigen incidence in the paid donors was 10 to 14 per cent. And the iron deficiency due to blood donation some times leads to inflammation (Herberg et al., 1986). A study in a total of 115 professional and 212 voluntary blood donors for screening of hepatitis-B surface antigen and Anti-HBs, it was found, in 4.3 and 125 per cent professional donors and 2.8 and 7.0 per cent voluntary donors. The higher carrier rate found in the professional donors, because of low socio economic status (Nayana Joshi et al., 1986).

The donors blood cannot be assumed to be free of drugs, out of 104 volunteer donors 16 donors had one or more drugs in their urine (Mahnovshi et al., 1987). Hossely (1985), reported that the acquired immuno deficiency syndrome has resulted in major chance in blood transfusion practices.

Experimental Procedure

III. EXPERIMENTAL PROCEDURE

The aim of this study was to find out the iron nutritional status of blood donors. One fifty eight blood donors were studied. Among them sixty seven were professional donors (fee paid according to the amount of blood donated), fifty eight volunteer donors (no fee is paid), and thirty three relative donors (blood donation to the family members or friends). Estimation of Haemoglobin, blood and serum iron, Red blood cell count, Haematocrit, Mean Corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, serum estimation of total iron binding capacity, Ferritin, transferrin saturation and plasma ascorbic acid were carried out. Twenty normal non-donors, who were of similar age groups were studied for the same blood and serum parameters. The experimental procedures related to this study are presented in the following sequence in this chapter.

1. The target participants and their profiles
2. Collection of blood samples
3. Separation of serum
4. Estimation of Haemoglobin
5. Estimation of blood iron and Haemoglobin
6. Estimation of Red blood cell count
7. Estimation of packed cell volume (Hematocrit)

8. Estimation of M.C.V.,M.C.H. and M.C.H.C.
9. Estimation of serum iron
10. Estimation of serum total iron binding capacity (TIBC)
11. Estimation of Transferrin Saturation
12. Estimation of Serum Ferritin
13. Estimation of plasma ascorbic acid

1. The Target Participants and Their Profiles

One hundred and fifty eight blood donors were selected for the present study from Coimbatore Medical College Hospital and from two other private blood banks. Among them sixty seven were professional donors (fee paid according to the amount of blood donated), fifty eight volunteer donors (no fee is paid) and thirty three relative donors (blood donation to the family members or friends). In the case of the professional group, the female donors were not preferred in all the blood banks, the voluntary group included the female donors much larger number than the relative donors.

The normal apparently healthy non-donors of the same age group were selected as matched controls for comparison with all three groups.

The age group, sex, occupation and income pattern were enquired and recorded in the questionnaire.

The type of blood donation pattern and duration between the donations were also enquired. Dietary survey of the target participants were also carried out. All the foods consumed by the participants were quantified. Equivalent raw food weights were calculated, iron and vitamine C intakes by the participants were arrived at. Before the blood donation, donors weight were checked, it should not be less than 45kg in body weight. The subjects were asked to made light clothings. The weight of the target participants were measured using a weighing machine.

Blood pressure: Blood pressure of the target participants were measured by the Ausculatory method. The instrument was kept at the level of the heart and the cuff was tied round the upper arm. Pressure was raised to 200mm of mercury and then gradually released. Variations of sounds were heard with a stethoscope placing its chest piece on the brachialartercy, a little below the cuff. The sounds are heard due to occurrence of turbulence in the flow of blood through the narrowed blood vessels when the manometric pressure just coincides with the systolic blood pressure.

Due to giving air pressure in the cuff the vessels is pressed and blood flow was obliterated. But while releasing the air pressure gradually, blood just begin to flow through the narrowed blood vessels and the pattern of

flow was changed from streamline flow (silent) to turbulent flow (noisy). When the pressure was further released, normal streamline flow sets in and the sound was no longer heard. At this point manometric pressure coincides with the diastolic pressure. So, as the pressure was released the following variations of sounds are heard. First phase- sudden appearence of a clear tapping sound. This indicates systolic pressure. It persists while the pressure falls through 15mm of mercury. Second phase - the tap sound was replaced by a murmur persisting for another 15mm of mercury. Third phase - the murmur was replaced by a clear loud gong sound lasting for the next 20mm of mercury. Fourth phase - the loud sound suddently becomes muffled and rapidly begins to fade. This point indicates diastolic pressure. Fifth phase - absence of all sounds.

Blood grouping and cross matching

The plasma of a patient may agglutinate or haemolyse the corpuscles of donor or the plasma of a donor. Therefore for the purpose of the blood transfusion it is important to do grouping and cross matching to confirm that no agglutination has taken place between the donors blood and recipients blood. There are four major groups such as

1. Blood group 'A' (Anti-B, agglutinin in serum or plasma)
2. Blood group 'B' (Anti-A, agglutinin in serum or plasma)
3. Blood group 'AB' (Anti-A and Anti-B, agglutinin in serum or plasma)
4. Blood group 'O' (Anti-A and Anti-B, no agglutinin in serum or plasma)

Procedure

Slide method: Punctured a finger and collected two drops of blood in a small test tube containing about 2ml of normal saline. Mixed gently. Took a glass slide and divided into two with the help of a wax pencil and marked A and B. A drop of blood suspension and a drop of group A sera (Anti-B) on one side of the glass slide and on the other side a drop of suspension of blood and a drop of group B sera (Anti-A). Mixed gently, by rocking the slide. Allowed to stand for 5 minutes, occassionally rolling or tilting slide to ensure thorough mixing. The cells then nearly always settled out conspicuously and completely clumped. When no agglutination occured the cells will remain evenly distributed for hours.

No agglutination by either group 'A' or 'B'

--Blood group 'O'

No Agglutination in 'A' and there was agglutination in 'B'

--Blood group 'A'

No agglutination in 'B' and there was agglutination in 'A'

--Blood Group B

There was agglutinationin both Sera 'A' and 'B'

--Blood Group AB

The third test with group 'O' sera was not essential but it serves as a valuable check on the accuracy of the result of the first two.

Anti-D (Rho) test: Oxalated blood was used. Added one drop of the cell suspension to a prewarmed slide (37^{o}-40^{o}C) surface. Added one drop of Anti-Rho (Anti-D) slide test serum. Mixed well. Tilted the slide back and forth for two minutes. Agglutination means Rho positive, and No agglutination means Rho negative (Samuel, 1986).

2. Collection of Blood

Blood was collected as follows (Oser, 1976). Tied a tourniquet (of soft rubber tubing or a strip of bandage) tightly around the arm of the patient, a couple of inches above the elbow. Washed the skin surface about the prominant vein on the inner surface of the elbow with 70 per cent of alcohol, allowed to dry, held the vein

immobile by pressing in it with the thumb below the elbow and into the vein inserted a sharp sterile hypodermic needle (No.22), an inch and a 1/2 long which was attached to a dry, sterile syringe of 10ml capacity. The needle should penetrate the vein from the side and at an angle of 50°, with the needle being kept upward or to the side. As soon as the blood was seen to enter the syringe, retracted the plunger slowly until 5.0ml of blood had entered the syringe before removing the needle from the vein, loosened the tourinquet, had the patient unclench his fist and on the skin, at the point of entrance of the needle, held in place a small pad of folded quaze moistened with 70 per cent alcohol, withdraw the needle detached it from the syringe (not too vigorously which might cause hemolysis) and then transferred 4.0ml of blood to a centrifuge tube to separate serum and rest to the small bottle with potassium oxalate as anticoagulant to store the blood and for plasma separation. The blood collection and method for Red Blood Cell Count and packed cell volume were given in Appendix IV and Appendix V.

3. Separation of Serum

The blood after being transferred to a centrifuge tube was allowed to clot. The clot was removed and centrifuged after which the supernatant was separated. The separated serum was frozen until used for analysis.

4. Estimation of Haemoglobin

Blood haemoglobin was estimated by the method of samuel ($_1$986). The details of the method is given in appendix II.

5. Estimation of Blood Iron and Haemoglobin

Blood trace element iron was estimated by the method of Wong's (Varley, 1980).

The details of the method is given in Appendix III.

6. Estimation of Red Blood Cell Count

Total count of Red Blood Cells are estimated by the method of Henry($_1$985). The details of the method are is given in Appendix IV.

7. Estimation of Packed Cell Volume (Haematocrit)

Haemotocrit was estimated by the Wintrobe Macro Method (Henry, 1986). The details of the method is given in Appendix V.

8. Estimation of M.C.V., M.C.H. and M.C.H.C.

The blood mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration were estimated by the method of Samuel (1986).

The details of the method is given in Appendix VI.

9. Estimation of Serum Iron

Serum iron was estimated by the method of Dipyridyl (Varley, 1969). The details of the method is given in Appendix VII.

10. Estimation of Total Iron Binding Capacity (TIBC)

Total iron binding capacity was estimated by the method of Ramsay (Varley, 1969). The details of the method is given in Appendix VIII.

11. Estimation of Transferrin Saturation

The transferrin saturation was estimated by the method of Wintrobe (1981).

The transferrin saturation was calculated directly from the values of serum iron and serum TIBC by using the following formula:

$$\frac{\text{Serum iron } (\mu g/dl)}{\text{TIBC } (\mu g/dl)} \times 100 = \text{Transferrin saturation (percentage)}$$

12. Estimation of Ferritin

Serum ferritin was estimated by the method of double antibody radio immuno assay (Arosio, 1981). The details of the method is given in the Appendix IX.

13. Estimation of Ascorbic Acid

Plasma ascorbic acid was estimated by the method of Tietz (1976). The details of the method is given in Appendix X.

14. Statistical Analysis

'z' and 't' tests were conducted for the large and small samples respectively wherever necessary to check if the results were significant using the formulas:

$$z \text{ (C.D)} = \text{Test criterion} = \frac{(\bar{x}_1) - (\bar{x}_2)}{\text{SE of the difference } (\bar{x}_1 - \bar{x}_2)}$$

C.D. = Critical difference

$\bar{x}_1$ = Mean of the first sample

$\bar{x}_2$ = Mean of the second sample

SE = Standard error

$$\text{Se of the difference } (\bar{x}_1 - \bar{x}_2) = \sqrt{SE_1^2 + SE_2^2}$$

$$= \sqrt{(SD_1^2/n_1) + (SD_2^2/n_2)}$$ (Raghuramulu, et al., 1983)

$$t = \frac{\bar{x}_1 - \bar{x}_2}{S} \times \sqrt{n_1 n_2 / n_1 + n_2}$$

n_1 = Size of the first sample

n_2 = Size of the second sample

S = Combined standard deviation

$$S = \sqrt{\frac{(x - \bar{x}_1)^2 + (x_2 - \bar{x}_2)^2}{n_1 + n_2 - 2}}$$

Regression Analysis

Regression analysis was carried out to establish the universe relationship between the haemoglobin concentrations and the frequency of blood donation (For the period of three months).

Regression Equation of x on y

$$X_c = a + by$$

values of a and b were determined by using the Equations:

$$\Sigma x = Na + b\Sigma y$$
$$\Sigma xy = a\Sigma y + b\Sigma y^2$$

Regression Equation of Y on X

$$Y_c = a + bx$$

values of a and b were determined by using the Equations:

$$\Sigma y = Na + b\Sigma x$$
$$\Sigma xy = a\Sigma x + b\Sigma x^2$$

Correlation Analysis

Correlation analysis was done to find out the correlation between serum iron and serum ferritin levels using the formula:

$$r = \frac{\Sigma xy}{\sqrt{\Sigma x^2 \; x \; \Sigma y^2}}$$

where $x = x - \bar{x}$

$y = y - \bar{y}$

$\bar{x}$, $\bar{y}$ = means of x and y respectively

Results and Discussion

IV. RESULTS AND DISCUSSION

The results pertaining to the study 'Iron Nutritional status of blood donors' are discussed in this chapter.

The serum andplasma of volunteer blood donors, relative donors and professional donors were analysed for haemoglobin, blood iron, RBC, PCV, M.C.V., M.C.H., M.C.H.C. Serum iron, TIBC, Transferrin saturation, ferritin and plasma ascorbic acid. The values obtained were compared with those of normal individuals, who served as controls (non donors). This study consisted of evaluating the biochemical and haemotological parameters in blood, serum and plasma of three different types of blood donors (Volunteer, Relative and professional donors). It was also aimed to establish the iron status of blood donors.

Universally known fact is that blood loss would lead to iron deficiency. The haemoglobin levels of the participants could be used as a rough estimation of iron status, serum iron, ferritin and transferrin saturation could give the exact amount of body iron and losses due to blood donation. Other haemotological parameters would be used as an index to assess iron deficiency in the blood donors. Blood was also analysed for blood grouping and cross matching, whose identification was found to be an important factor for the transfusion practices. The sex and age of

blood donors were assessed along with their blood donation pattern. Nutritional status and body weight was recorded simultaneously. The socio economic status of the target, Participants were also studied to correlate their economic status with that of frequency of donation.

The results of the above study are presented as follows:

1. The target participants: Blood donors, their classification as volunteer, Relative, professional donors and non donors.

2. Distribution of blood groups and Rh factor of the blood donors and non donors selected for the study.

3. Mean body weight, Blood pressure and frequency of donation of the participants.

4. Haemoglobin levels of selected volunteer, Relative, professional blood donors and non-donors.

5. Blood iron level of selected Volunteer, Relative, professional blood donors and non-donors.

6. Red blood cell count of selected Volunteer, Relative professional blood donors and non-donors.

7. Packed cell volume level of selected Volunteer, Relative, professional blood donors and non-donors.

8. M.C.V., M.C.H., M.C.H.C. levels of selected Volunteer, relative, professional blood donors and non-donors.

9. Serum iron level of selected volunteer, Relative, professional blood donors and non-donors.

10. Serum TIBC level of selected volunteer, Relative, professional blood donors and non-donors.

11. Percentage transferrin saturation of selected Volunteer, Relative, Professional blood donors and non-donors.

12. Serum ferritin level of selected Volunteer, Relative, professional blood donors and non-donors.

13. Plasma ascorbic acid level of selected Volunteer, Relative, professiom l blood donors and non-donors.

14. Dietary intake of selected Volunteer, Relative, professional blood donors and non-donors.

1. The target participants: Blood donors, their classification as volunteer, Relative, Professional donors and non-donors.

TABLE I

THE TARGET PARTICIPANTS AND THEIR CLASSIFICATION

Blood donors	Types of blood donors			Total
	Volunteer	Relative	Professional	
Men	50	31	67	148
Women	8	2	-	10
Non-donors (Men)	-	-	-	20
Age in years	19-58	21-54	21-55	19-58

Table I presents the classification of participants. The blood donors were grouped as volunteers (No fee is paid). Relatives (blood donated to only relatives or friends), and professional donors (paid according to the amount of blood donated), this pertains to the classification given by Indian Red Cross Society (1987).

Of 158 blood donors selected for the study 148 were men of whomwere 50 volunteers, 31 relatives and 67 professional donors and 10 were women of whom eight were volunteer donors and two were relative donors. The age groups of these blood donors ranged between 19 and 58. Twenty apparently healthy subjects of the same age group who had not donated their blood until the study was conducted served as controls.

Women were not preferred as professional donors by the blood bank authorities and hence no samples were available. This fact might be due to the menstrual loss of iron in women (Umoren and Kies, 1982) and delayed degeneration of the blood cells and haemoglobin levels after blood donation. In fact the present study indicated that women volunteer blood donors were more (8 members) than the relative blood donors (2 members).

2. Distribution of blood groups and Rh factor of the blood donors and non donors selected for the study:

TABLE II

DISTRIBUTION OF BLOOD GROUPS AND Rh FACTOR OF THE PARTICIPANTS

Blood donors and non donors	Blood groups and Rh factors								Total
	'A'		'B'		'AB'		'O'		
	Rh+Ve	-Ve	Rh+Ve	-Ve	Rh+Ve	-Ve	Rh+Ve	-Ve	
Non donors	2	1	4	1	4	-	6	2	20
Volunteer donors	12	1	15	2	13	2	12	1	58
Relative donors	4	2	6	1	8	3	8	1	33
Professional donors	12	2	23	1	8	3	17	1	67

Table II presents the distribution of blood groups and Rh factors of the volunteers, relative and professional blood donors and non donors. Fig.1 indicates the same.

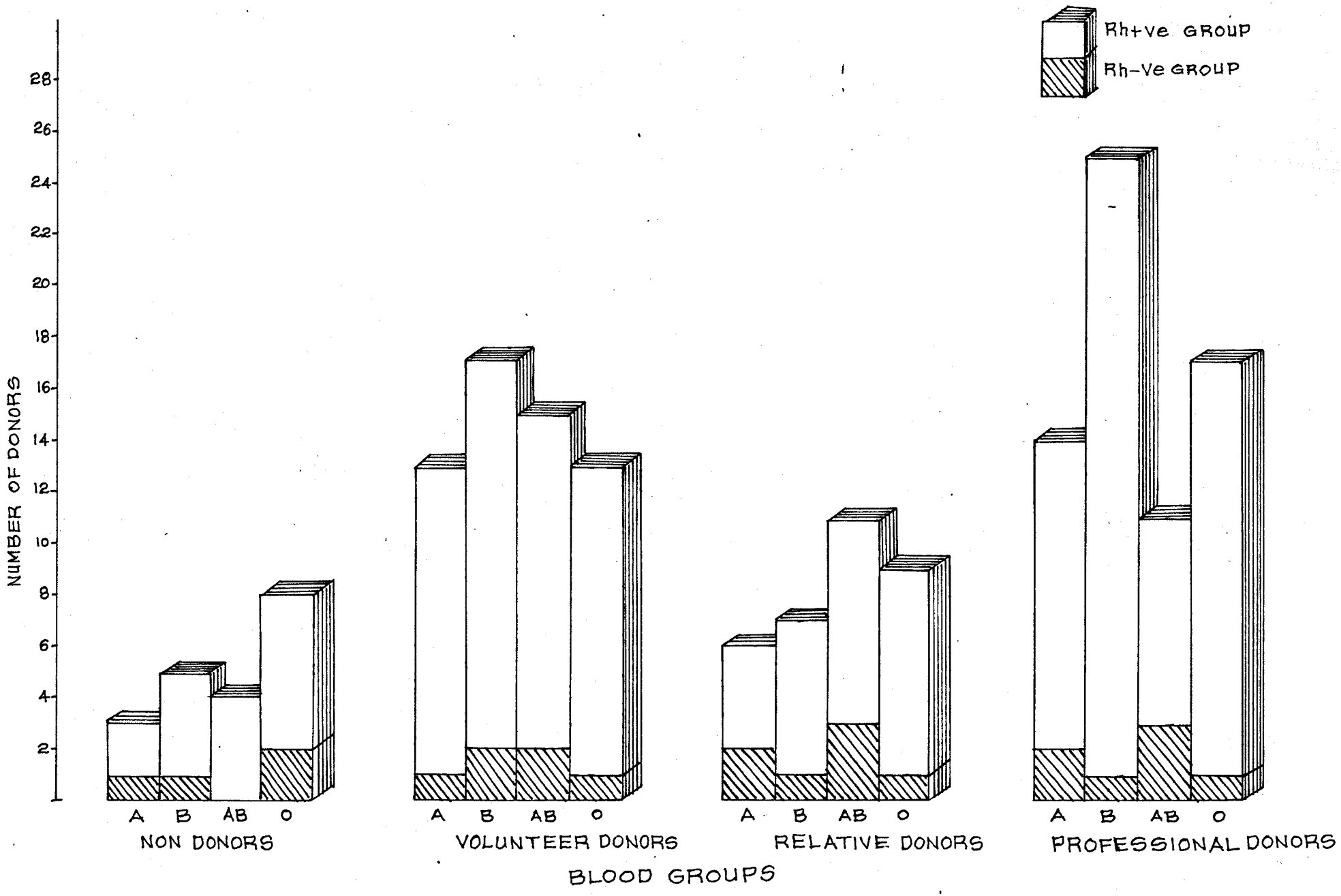


Figure. 1 GROUP WISE DISTRIBUTION OF NON DONORS AND VOLUNTEER, RELATIVE AND PROFESSIONAL BLOOD DONORS

Of 178 participants, 158 were blood donors of whom 58 were volunteer donors, among them 13 had blood group 'A'. 17 had group 'B', 15 had blood groups 'AB' and 13 had blood group 'O'; Relative donors were found to be 33 among them six belonged to blood group 'A', seven to 'B' group, 11 to group 'AB' and nine of them had group 'O'. Among 67 professional donors 14 had blood group 'A', 25 had blood group 'B', 11 had blood group 'AB' and 18 had blood group 'O'. Non donors (20) constituted group A, B, AB and O of three five, four and eight respectively.

It was found that in professional and volunteer groups, blood group B was predominant followed by blood group 'AB, 'O' and 'A'. With in the relative donors and non donors the blood groups 'AB' and 'O' were predominant than 'B' and 'A' groups. These blood grouping and classifications were very much essential for the use of donated blood other wise the blood groups and Rh factors do not have any relevance in this project.

3. Mean body weight, blood pressure and frequency of donation of the participants:

TABLE III

MEAN BODY WEIGHT, BLOOD PRESSURE AND PREQUENCY OF BLOOD DONATION OF THE PARTICIPANTS

Participants selected for the study	Weight (kg) Mean ± S.D.	Blood pressure (mmHg) mean	Fequency* of donation Mean± S.D.
Non donors	64.7 ± 10.1	116/75	--
Volunteer donors	62.9 ± 5.8	118/85	1.4 ± 0.64
Relative donors	58.5 ± 11.0	117/75	2.0 ± 1.3
Professional donor	54.2 ± 5.7	117/80	5.0 ± 1.10

*For the period of three months

Table III presents the mean body weights,blood pressure data of the volunteers, relative and professional blood donors and non donors. The frequency of blood donation is also presented.

The mean body weight of non-donors, volunteer relative and professional donors were 64.7 ± 10.1 kg 62.9 ± 5.8kg, 58.5 ± 11.0kg and 54.2 ± 5.7kg respectively. Volunteer relative, professional blood donors and non donors had mean blood pressure, of 116/75 mmHg 118/85 mmHg 117/75 mmHg and 117/80 mmHg respectively. The mean frequency of blood donated by donors withinaperiod ofthree months found to be

1.4 ± 0.64 for volunteer, for relative donors it was 2.0 ± 1.3 and for professional donors it was found to be 5.0 ± 1.1 respectively.

The mean body weight of non donors were found to be greater (64.7kg) when compared tothree other groups (volunteer blood donors relative and professional blood donors). The mean body weight of professional donors was low when compared against the volunteer and relative blood donors. The normal weight of the blood donors should be 45kg (Indian Red Cross Society, 1988). The mean body weight of three group of blood donors and non donors were found to be greater than the recommended body weight. The body weight of participant were also greater than the ICMR reference body weight (adult man 55kg;women 45kg) except the professional donors (54.2kg). Mean blood pressure of the volunteer, relative and professional donors and non donors were found to be normal.

The frequency of blood donated by the professional donors were found to be greater (5.0 times) when compared to volunteer and relative blood donors. The data was collected with in the period of three months. The poverty and the need of money made the professionals to donate the blood often. For the selection of the blood donors, body weight and blood pressure were the prime considerations. The professional

donors due to their low socio economic status, and to satisfy their hunger needs they were in need of frequent blood donation but due to their low body weight, the officials refused to take blood from them. So in the present study it was found to surprise that some of the professional donors in order to make themselves to weigh above 45kg, they hide some heavy objects inside their dresses.

Nair (1986) have stated that a donor could donate blood safely once in every three months. But the professional donors in order to get money in the easiest way donated their blood even 5 times within the period of three months to different blood banks in different places and also followed the habit of giving wrong addresses and names.

4. Haemoglobin levels of selected volunteer, relative and professional blood donors and non-donors:

TABLE IV

LEVELS OF HAEMOGLOBIN IN VOLUNTEER, RELATIVE, PROFESSIONAL BLOOD DONORS AND NON DONORS

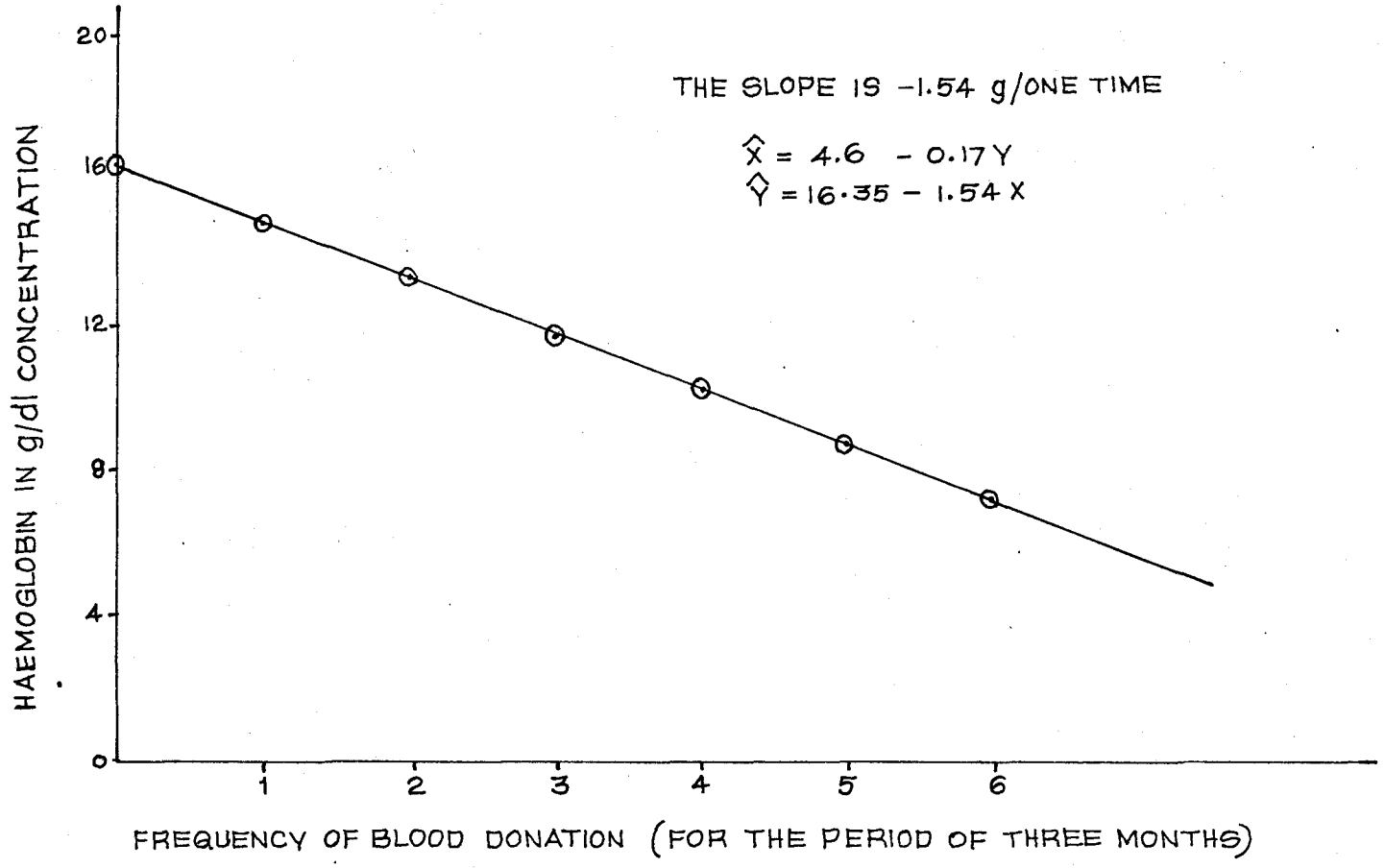
Subjects selected for the study	Number of subjects	Haemoglobin g/dl Mean ± S.D.	Groups compared	'E' (CD) value
Non donors (a)	20	15.4 ± 1.9 ($\bar{a}1$)	a1 vs b1	1.6NS
			a1 vs c1	0.51NS
			a1 vs d1	10.2**
Volunteer donors (b)	58	16.3 ± 2.6 ($\bar{b}1$)	b1 vs a1	1.6NS
			b1 vs c1	2.2*
			b1 vs d1	13.8**
Relative donors(c)	33	15.1 ± 2.4 ($\bar{c}1$)	c1 vs a1	0.51NS
			c1 vs b1	2.2*
			c1 vs d1	9.8**
Professional donors (d)	67	10.5 ± 1.9 ($\bar{d}1$)	d1 vs a1	10.2**
			d1 vs b1	13.8**
			d1 vs c1	9.8**

**Significant at 1 per cent level
*Significant at 5 per cent level
NS - Not significant

The Table Iv depicts the haemoglobin levels of volunteer, relative and professional blood donors and non-donors. Figure. 2 indicates the same.

The mean haemoglobin levels of the volunteer, relative professional blood donors and non donors were found to be 16.3 ± 2.6 g/dl, 15.1 ± 2.4 g/dl, 10.5 ± 1.9 g/dl and 15.4 ± 1.9g/dl respectively.

The Haemoglobin levels of the volunteer donors were found to be significantly high at 5 per cent level when



Graph.1. HAEMOGLOBIN VALUES OF BLOOD DONORS (VOLUNTEER, RELATIVES, AND PROFESSIONAL) PLOTTED AGAINST THE FREQUENCY OF BLOOD DONATION WITH THE LINE OF LEAST SQUARE REGRESSION

compared to the relative donors. The level of haemoglobin in the professional donors were found to be significantly low at one per cent level when compared to the volunteer and relative blood donors and non donors. There were no significant difference in haemoglobin levels between volunteers and non-donors, relatives and non-donors.

The hemoglobin levels of professional donors were found to be below the WHO reference value (12 g/dl). The low haemoglobin level could leads to the iron deficiency among the professional blood donors mainly this associated with low standard of living and ignorance about nutritional facts, malnutrition lowers blood donors resistance to diseases resulting in higher morbidity apathy, lethargy, sluggishness and poor working efficiency, would leads to the further blood donation for the hunger removal.

Haemoglobin concentration of the professional donor was found to be 10.2 g/dl and for volunteer donors 15.8 g/dl as per Nutritional News (1986). The results from the above study was similar to that of the present findings.

The higher haemoglobin level (16.3 $\pm$ 2.6 g/dl) in the volunteer blood donors might be due to the better dietary intake and limited amount of blood donated within a stipulated period. The haemoglobin level in the professional donors were found to be significantly lower than volunteers, which

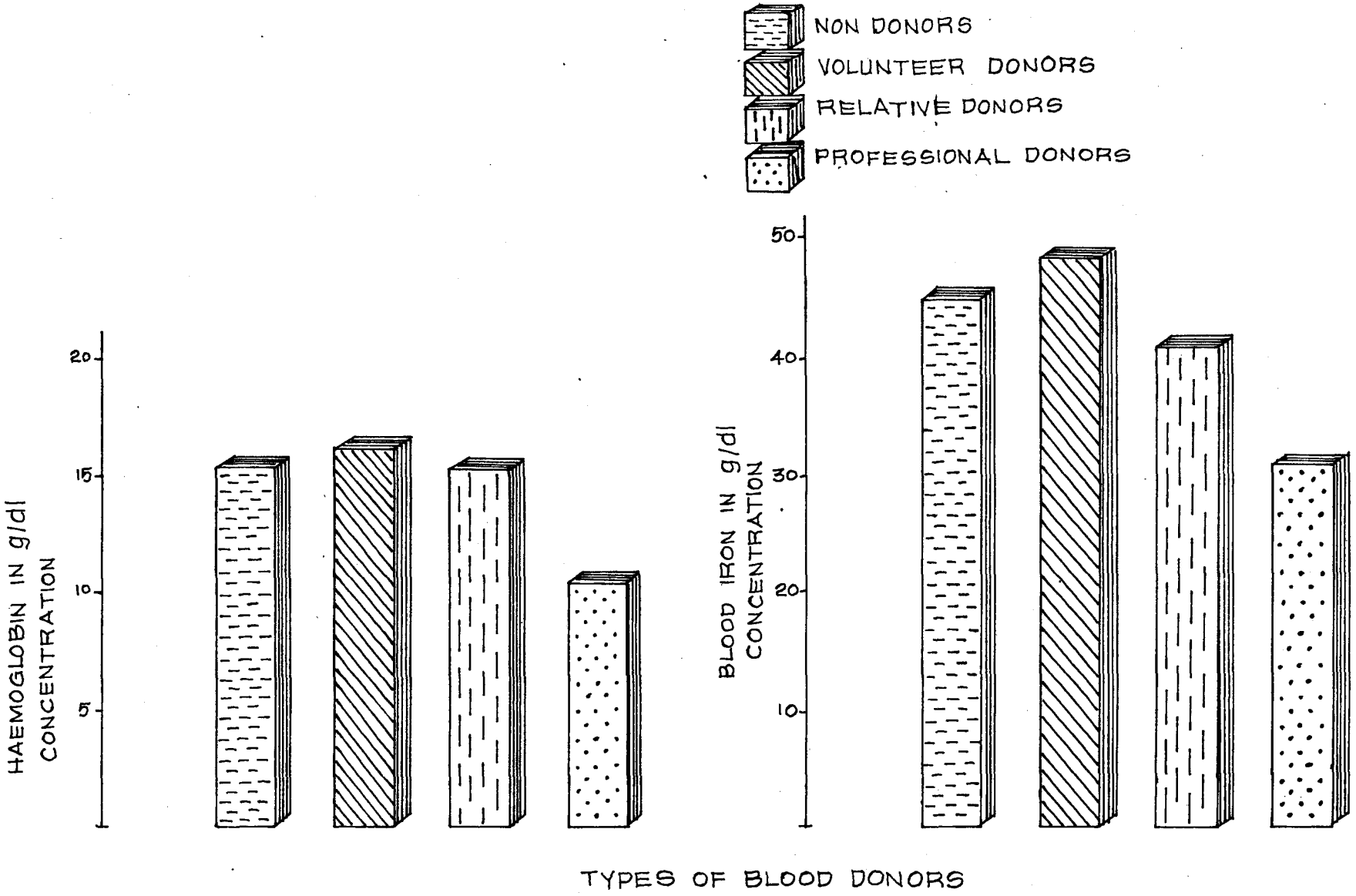


Figure. 2 LEVELS OF HAEMOGLOBIN IN NON DONOR AND BLOOD DONORS

Figure. 3 LEVEL OF BLOOD IRON IN NON DONORS AND BLOOD DONORS

The Table V presents the mean blood iron levels of volunteer, relative and professional blood donors and non donors. Figure.3 gives the same.

Blood iron levels indicate the mobile body iron stores in individuals and a low blood iron levels indicate iron deficiency anaemia.

The mean blood iron levels of volunteer, relative, professional blood donors and non donors were found to be 48.0 ± 7.3 g/dl, 43.9 ± 7.3 g/dl, 31.1 ± 5.6 g/dl and 45.2 ± 5.7 g/dl respectively. The blood iron levels in the Volunteer blood donors were found to be significantly higher when compared to the relatives and professional blood donors at 5 per cent and one per cent level respectively. The professional donors blood iron level was found to be lower (significant at 1%) when level compared to the two other groups (volunteer and relative donors). There were no significant difference in the blood iron level between the volunteer donors and non-donors and relative donors and non donors.

The high socio economic status and the rare donation of the blood might be the reason for the increased level of blood iron 48.0 ± 7.3 g/dl in the volunteer donors. Among relative donors low blood iron (43.9 ± 7.3g/dl) might be due to the accidental donation without proper nutrient

supplementation. The professional donorsblood irondeficiency might be due to the frequent blood donation and poor food intakes. The dietary intake data is discussed in the latter section of this study.

The blood donation would lead to the significant loss of total body iron (Penington et al.,1979, Wintrobe et al., 1981). This trend was also noticed in the present study.

6. Red blood cell count of selected volunteer, relative and professional blood donors and nondonors:

TABLE VI

RED BLOOD CELL COUNT OF SELECTED VOLUNTEER, RELATIVE, PROFESSIONAL BLOOD DONORS AND NON DONORS

Subjects selected for the study	Number of subjects	RBC count (millions/cmm) Mean ± S.D.	Groups compared	'Z' (CD) value
Non donors (a)	20	5.9 ± 0.93 ($\bar{a}1$)	a1 vs b1	0.091NS
			a1 vs c1	1.8NS
			a1 vs d1	9.5**
Volunteer donors (b)	58	5.88 ± 0.71 ($\bar{b}1$)	b1 vs a1	0.091NS
			b1 vs c1	1.8 NS
			b1 vs d1	16.2**
Relative donors (c)	33	5.54 ± 0.99 ($\bar{c}1$)	c1 vs a1	1.8NS
			c1 vs b1	1.8NS
			c1 vs d1	9.7**
Professional donors (d)	67	3.9 ± 0.69 ($\bar{d}1$)	d1 vs a1	9.5**
			d1 vs b1	16.2**
			d1 vs c1	9.7**

**Significant at 1 per cent level
NS - Not significant

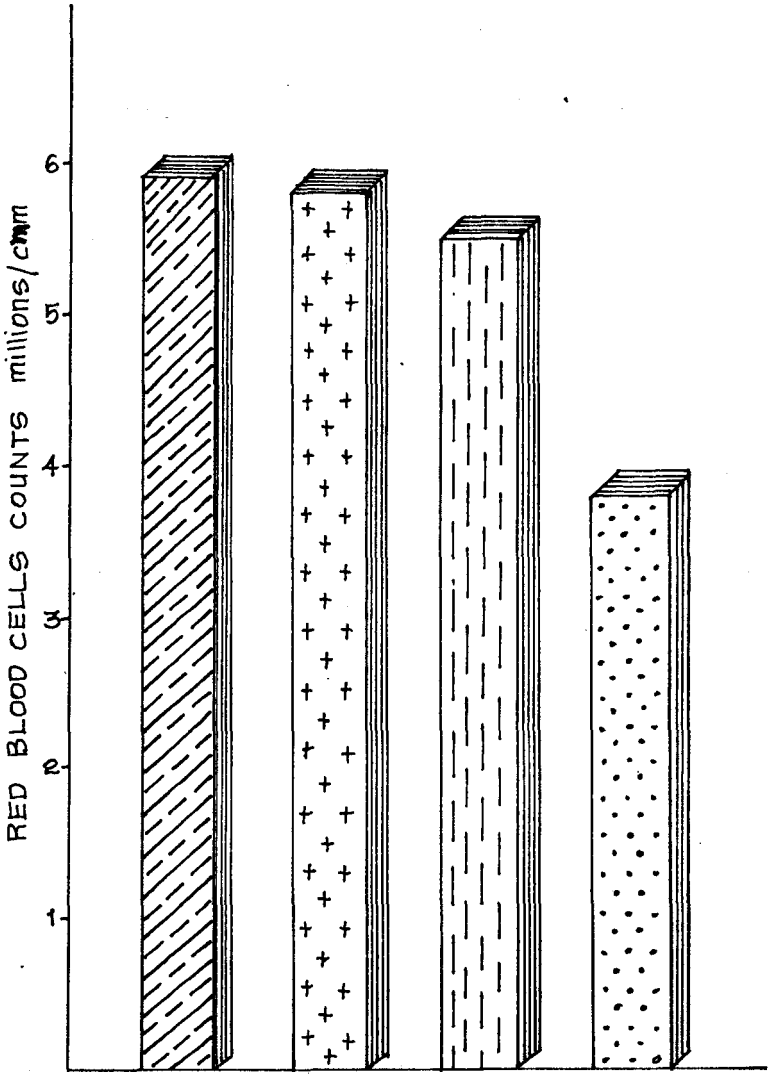


Figure. 4 RED BLOOD CELL COUNT IN NON DONOR AND BLOOD DONORS

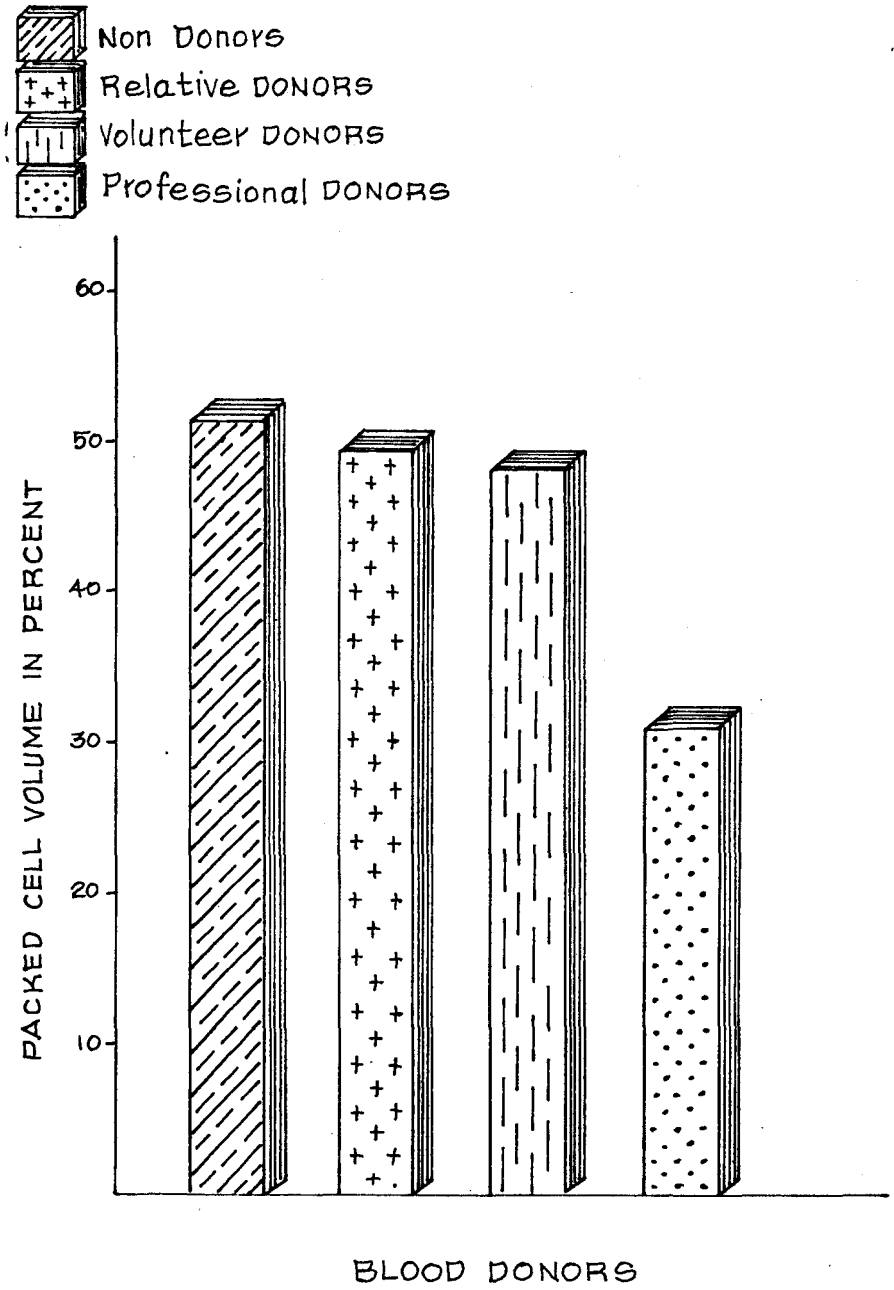


Figure. 5 LEVEL OF PACKED CELL VOLUME IN NON DONORS AND BLOOD DONORS

The Table VI presents red blood cell count of selected volunteer, relative and professional blood donors and non donors. Figure.4 gives the same.

The mean Red blood cell count of the volunteer, relative and professional blood donors and non-donors were found to be 5.88 ± 0.71, 5.54 ± 0.94, 3.9 ± 0.69 and 5.9 ± 0.93 (Millions/cmm) respectively.

The RBC count of the non-donors were found to be greater (5.9 ± 0.93 m/cmm) when compared to the volunteer relative and profession blood donors. The RBC count of the professional blood donors was lower when compared to volunteer and relative blood donors, it was reduced at one per cent level.

The reduced red blood cell count might be due to the iron deficiency in the professional donors. The present result was in accordance with Brain (1982), Kaplan and Pesce (1984). They had reported that the normal Red blood cell count was reduced in the blood donors due to iron deficiency (4.0 millions/cmm).

7. Packed cell volume of selected volunteer, relative and professional blood donors and non-donors:

There were no significant differences between the volunteer donors and non donors, and Relative and non donors.

The haemotocrit value indicated the ratio of the volume of erythrocyte to that of the whole blood. The present study indicated a decreased erythrocyte among the professional blood donors. The result of the present study coincide with the study of Blum et al., (1986), Nutritional News (1986), who have reported that the haemotocrit value was significantly reduced in professional donors (33.48 per cent).

8. MCV, MCH, MCHC levels of selected volunteer, relative and professional blood donors and non donors:

The table VIII depicts the mean M.C.V., M.C.H., M.C.H.C. of volunteer, relative and professional blood donors and non donors:

TABLE VIII

LEVELS OF M.C.V., M.C.H., M.C.H.C. IN NON DONORS AND VOLUNTEER, RELATIVE, PROFESSIONAL BLOOD DONORS

Subjects selected for the study	Number of subjects	M.C.V. (Cubic micron) Mean ± S.D.	M.C.H. (Micro micro gram) Mean ± S.D.	M.C.H.C. (Percent) Mean ± S.D.	Groups compared	'Z' (CD) values
Non donors (a)	20	85.0 ± 0.63 ($\bar{a}1$)	28.5±1.4 ($\bar{a}2$)	34.4 ± 5.6 ($\bar{a}3$)	a1 vs b1	0.29NS
					a1 vs c1	1.05NS
					a1 vs d1	7.4**
					b1 vs c1	1.1NS
					b1 vs d1	1.4NS
					c1 vs d1	3.7**
Volunteer donors (b)	58	85.1 ± 2.4 ($\bar{b}1$)	28.6± 6.1 ($\bar{b}2$)	33.9± 1.6 ($\bar{b}3$)	a2 vs b2	0.27NS
					a2 vs c2	0.0NS
					a2 vs d2	1.3NS
					b2 vs c2	1.36NS
					b2 vs d2	1.4NS
					c2 vs d2	1.3NS
Relative donors (c)	33	84.4 ± 3.2 ($\bar{c}1$)	28.5± 1.16 ($\bar{c}2$)	33.7 ± 1.1 ($\bar{c}3$)	a3 vs b3	0.37NS
					a3 vs c3	0.58NS
					a3 vs d3	0.0NS
Professional donors (d)	67	81.8±3.4 ($\bar{d}1$)	27.4 ±6.4 ($\bar{d}2$)	34.4±1.9 ($\bar{d}3$)	b3 vs c3	0.96NS
					b3 vs d3	1.14NS
					c3 vs d3	2.3*

** - Significant at 1 per cent level
* - Significant at 5 per cent level
NS - Not significant

The mean M.C.V. of volunteer, relative and professional donors and non donors were found to be 85 ± 2.43 cubic micron 84.4 ± 3.2 cubic micron, 81.8 ± 3.4 cubic micron and 85.0 ± 0.83 cubic micron respectively. The mean corpuscular volume had decreased in professional donors at oneper cent level (81.8 ± 3.4 cubic micron) when compared to volunteer, relative blood donors and non donors. The above in accordance with that of Cooks (1982) who had reported 80.0 cubic micron in professional donors. There were no significant differences between the volunteer donors and non donors, and relative donors and non donors.

Mean MCH level (Micro Micro grams) of volunteer, relative and professional blood donors and non donors were found to be 28.6 ± 1.6, 28.5 ± 1.16, 27.4 ± 6.7 and 28.5 ± 1.4 respectively. The M.C.H. was found to be normal in all three types of blood donors (volunteer, relative and professional) and non donors. There were no significant differences between the blood donors and non donors. The result coincides with the findings of Michael (1986) who reported in professional donors 27.6 micro mirco grams.

Mean M.C.H.C. of the three groups of blood donors (volunteer, relative and professional) and non donors were found to be 33.9 ± 1.6 per cent, 33.7 ± 1.1 per cent, 34.4 ± 1.9 per cent and 34.4 ± 5.6 per cent respectively. The M.C.H.C. level of relative donors were low when compared

to professional donors. The difference was significant at 5 per cent level. There were no significant differences between the Volunteer and relative and non donors and relatives.

The above results were in accordance with that of cook (1982) and Flynn et al., (1974) who had reported that the first and second stage of iron deficiency was not associated with any alteration in the M.C.V., M.C.H. and M.C.H.C. levels. But the third stage of iron deficiency was associated with reduced red cell indices. These values were estimated mainly because they could be used as a rough estimate of iron deficiency. Hence this has not been taken as an index of iron status while blood donation.

9. Serum iron level of selected volunteer, relative and professional blood donors and non donors:

The serum iron levels in professional donors which was significant at 1 per cent level were found to be low (73.3 ± 13.2 µg/dl) when compared to the volunteer, relative blood donors and non donors. There were no significant difference between the volunteer and non donors and relative and non donors.

It was evident from the above study the iron level of professional donors were significantly reduced. This might be due to the fact that the food consumed by the professional donors were of low in iron content, and frequent donation of blood with in a stipulated period. The iron loss in the professional donors would lead to iron deficiency anaemia.

Due to low socio economic status and their laziness towards hard working, they had donated their blood often for money. The blood bank authorities does not care the health of the donors. This might be due requirement of blood stock in the banks. The transfusion with these type of blood would cause the ill effects to the patients to whom the transfusion was carried out.

Thus a suggestion emerges from the present study to the blood bank authorities, that while collecting blood from the professional donors they should screen the blood donors carefully in terms of health and their weight, HB and iron level. It was found out from the enquiry that the

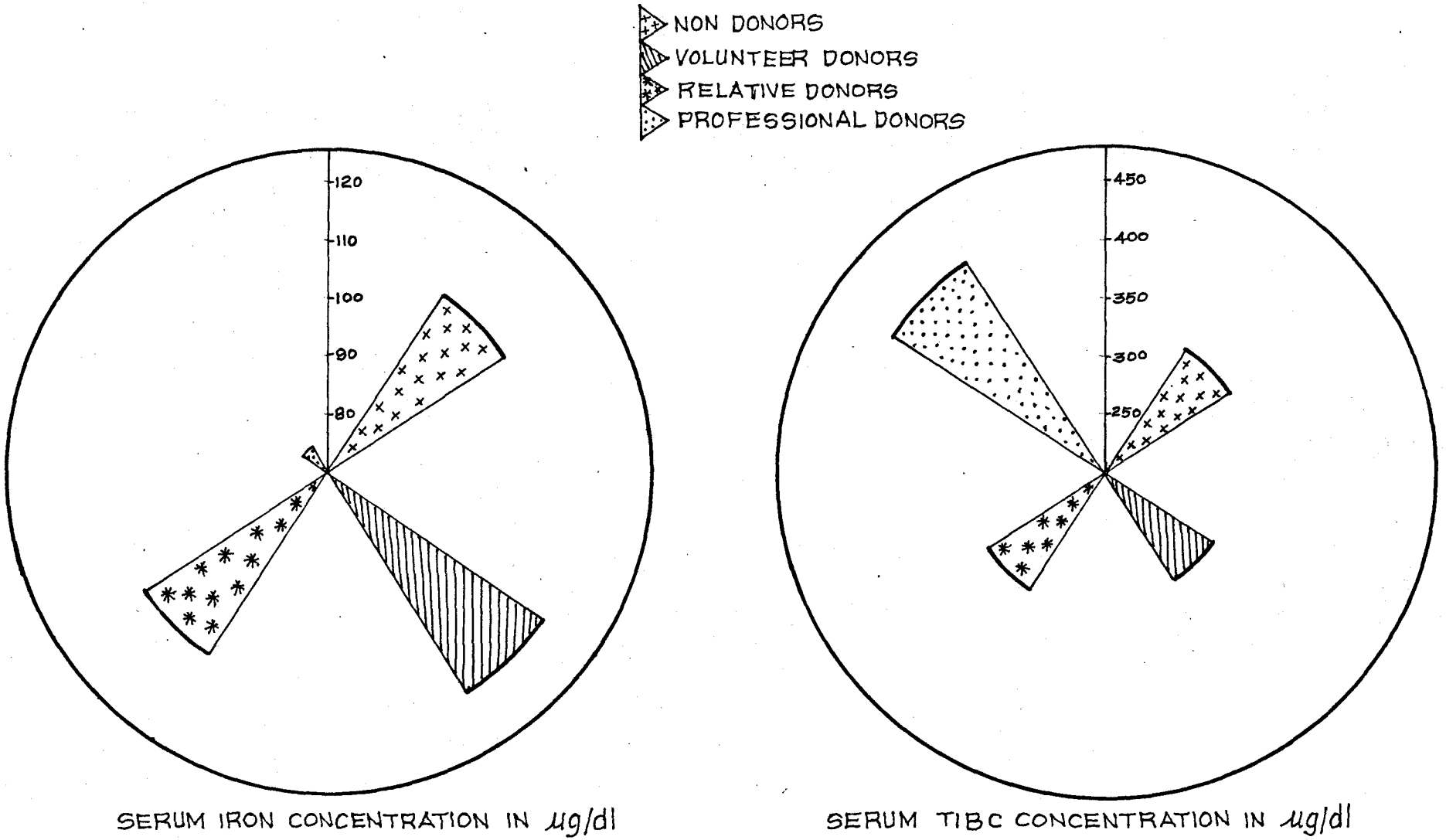


Figure. 6 LEVEL OF SERUM IRON IN NON DONORS AND VOLUNTEER, RELATIVE AND PROFESSIONAL BLOOD DONORS

Figure. 7 SERUM TIBC OF NON DONORS AND VOLUNTEER RELATIVE, AND PROFESSIONAL BLOOD DONORS

incidence of post transfusion hepatitis increased with increasing number of units of blood transfused and transfursion of commercial donor blood (Patwari et al.,1986).

The above results coincides with the findings of Ona et al.,(1978), Morck et al.,(1983) and Nutritional News (1986) who had reported that the professional blood donors forms an important group of developing from deficiency anaemia. It was suggested that they should be given iron supplementation for 3 to 6 months after each donation. Iron deficiency was more prevalent among the low socio economic group when compared to the high income group.

10. Serum TIBC levels of selected volunteer, relative and professional blood donors and non donors:

TABLE X

LEVELS OF TIBC IN VOLUNTEER, RELATIVE, PROFESSIONAL BLOOD DONORS AND NON DONORS

Subjects selected for the study	Number of subjects	TIBC (µg/dl) Mean ± S.D.	Groups compared	'Z' (Cp) value
Non donors (a)	20	338 ± 90 ($\bar{a}1$)	a1 vs b1	1.7NS
			a1 vs c1	1.1NS
			a1 vs d1	3.8**
Volunteer donors(b)	58	302.0±47.9 ($\bar{b}1$)	b1 vs a1	1.7NS
			b1 vs c1	0.85NS
			b1 vs d1	14.2**
Relative donors(c)	33	313.5±65.6 ($\bar{c}1$)	c1 vs a1	1.1NS
			c1 vs b1	0.85NS
			c1 vs d1	8.32**
Professional donors (d)	67	417.5±42.8 ($\bar{d}1$)	d1 vs a1	3.8**
			d1 vs b1	14.2**
			d1 vs c1	8.3**

** -Significant at 1 per cent level
NS - Not significant

Table X and Fig.7 indicates the TIBC levels of volunteer, relative and professional blood donors and non donors. Figure.7 indicates the same.

The mean TIBC of volunteer blood donor, relative blood donor and professional blood donor and non donors were found to be 302.5 ± 47.9 µg/dl, 313.5 ± 65.6 µg/dl, 417.5 ± 42.8 µg/dl and 338 ± 90 µg/dl respectively.

Mean TIBC (Total iron binding capacity) level in the professional donors were found to be significantly increased

(417.5 ± 42.8 µg/dl) at one per cent level when compared to non donors (338.0 ± 90 µg/dl) and also with volunteer and relative donors. There were no significant differences between the volunteer and relative donors when compared to non donors.

The above result was in accordance with Neumann and Weguer (1973), Brain (1982), Lieden (1973). They had reported a low value for serum iron and high for TIBC in the professional donors. Decreases in serum or plasma iron levels are generally due to a deficiency in the total amount of iron present in the body.

11. Percentage transferring saturation of selected volunteer, relative and professional blood donors and non donors:

TABLE XI

SATURATION

LEVEL OF TRANSFERRIN IN VOLUNTEER, RELATIVE, PROFESSIONAL BLOOD DONORS AND NON DONORS

Subjects selected for the study	Number of subjects	Transferrin saturation (Per cent) Mean ± S.D.	Groups compared	'Z' (CD) value
Non donors (a)	20	34.6 ± 7.2 ($\bar{a}1$)	a1 vs b1	1.7NS
			a1 vs c1	0.26NS
			a1 vs d1	9.5**
Volunteer donors (b)	58	38.5 ± 10.9 ($\bar{b}1$)	b1 vs a1	1.7NS
			b1 vs c1	1.5NS
			b1 vs d1	12.5**
Relative donors (c)	33	35.2 ± 9.7 ($\bar{c}$)	c1 vs a1	0.26NS
			c1 vs b1	1.5NS
			c1 vs d1	9.3**
Professional donors (d)	67	18.5 ± 5.5 ($\bar{d}$)	d1 vs a1	9.5**
			d1 vs b1	12.5**
			d1 vs c1	9.3**

** - Significant at 1 per cent level
NS - Not significant

The values of transferring saturation in volunteer, relative and professional blood donors and non donors were depicted in Table XI.

Mean transferrin saturation of volunteer, relative and professional blood donors and non donors were found to be 38.5 ± 10.9 per cent, 35.2 ± 9.7 per cent, 18.5 ± 5.5 per cent and 34.6 ± 7.2 per cent respectively.

Transferrin saturation of volunteer and relative blood donors were not statistically significant when compared

to non donors. The transferrin saturation of professional blood donors was found to be significantly low (18.5± 5.5 per cent) when compared to non donors and volunteer and relative blood donors. The reduced level was significant at one per cent level.

The iron nutritional status was defined as function of percentage transferrin saturation. The present study result was in acceptance with the result of Walters et al., (1973), who had reported that the blood donors (professional) transferrin saturation was consistently below 15 per cent. When compared to non donors. This trend coincides with the present investigation. The reduced level of serum iron coincides with the reduced transferrin saturation.

12. Serum ferritin level of selected volunter and relative and professional blood donors and non donors:

TABLE XII

LEVELS OF SERUM FERRITIN IN VOLUNTEER, RELATIVE, PROFESSIONAL BLOOD DONORS AND NON DONORS

Subjects selected for the study	Number of subjects	Ferritin (ng/ml) Mean ± S.D.	Groups compared	't' value
Non donors (a)	20	46.0 ± 2.0 (a_1)	a1 vs b1	2.1NS
			a1 vs c1	2.9*
			a1 vs d1	59.3**
Volunteer donors (b)	58	35.0 ± 6.1 (b_1)	b1 vs a1	2.1*
			b1 vs c1	1.56NS
			b1 vs d1	23.4**
Relative donors (c)	33	26.8 ± 9.9 (c_1)	c1 vs a1	2.9*
			c1 vs b1	1.56NS
			c1 vs d1	10.7**
Professional donors (d)	67	2.26 ± 0.39 (d_1)	d1 vs a1	59.3**
			d1 vs b1	23.4**
			d1 vs c1	10.7**

** - Significant at 1 per cent level
* - Significant at 5 per cent level
NS - Not significant

Table XII presents the level of serum ferritin of selected volunteer, relative and professional blood donors and non donors. Fig.8 gives the levels of Ferritin in donors and non donors.

The ferritin molecule consist of a protein shell (MW 450,000) and a core of iron. High concentrations are found in liver cells and in erythrocyte recyling centres of the liver, spleen and bone marrow. Ferritin is also

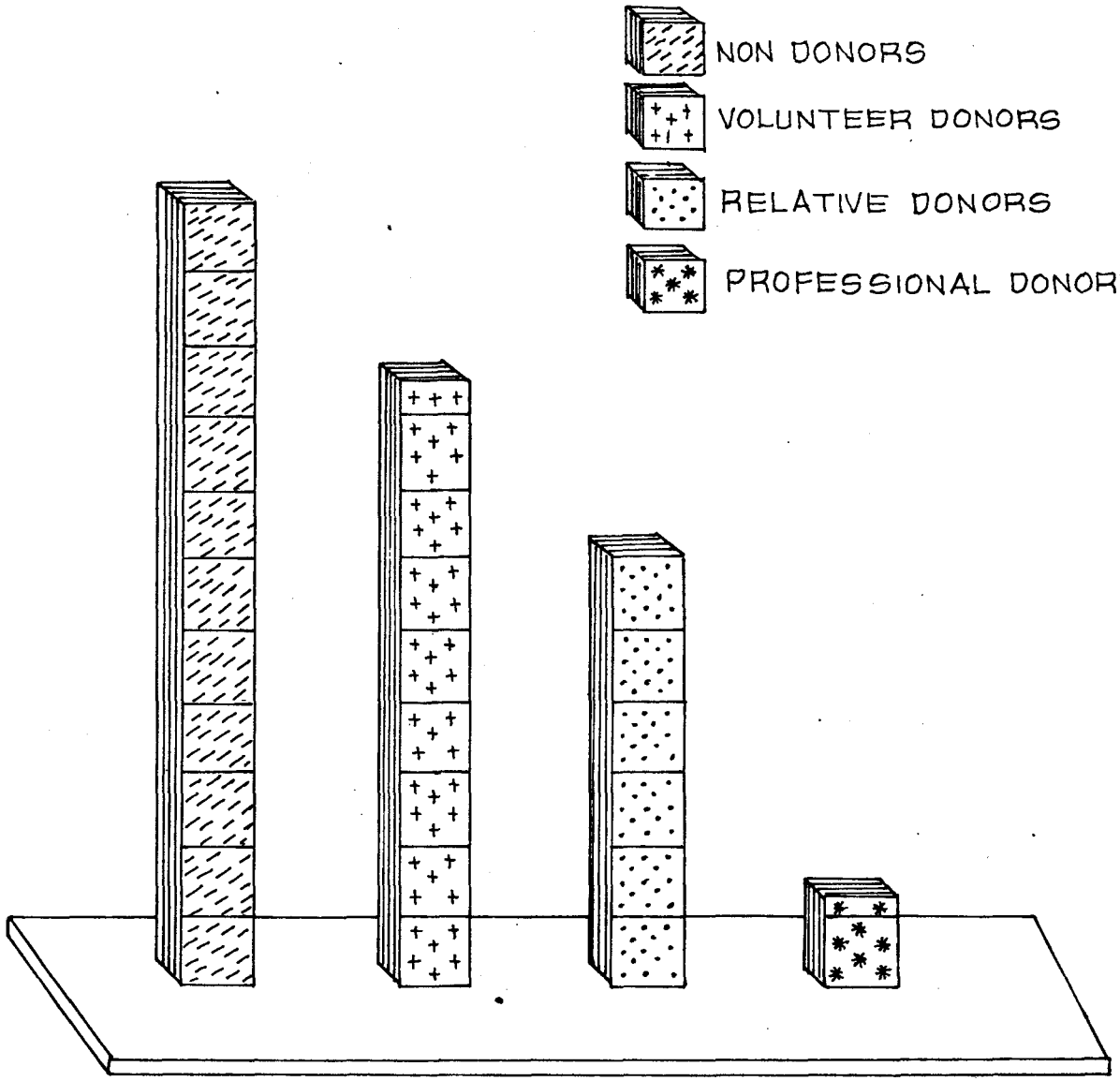


Figure.8 SERUM FERRITIN LEVELS IN NON DONORS AND VOLUNTEER, RELATIVE AND PROFESSIONAL BLOOD DONORS

present in human plasma and serum, where its concentration was normally a satisfactory index of body iron stores as measured by quantitative phlebotomy, iron absorption studies, liver biopsy and the microscopic examination of bone marrow aspiraties for stainable iron deposits.

Serum ferritin was estimated by double antibody radio immuno assay technique. Among the three group of blood donors (volunteer, relative, professional) and non donors only 2-7 participants were selected for ferritin estimation, because the method was more sophisticated and costly. (Graph II indicates the standard line from which the Ferritin concentration was calculated).

Mean ferritin levels of volunteer, relative and professional blood donors and non donors were found to be 35.0 $\pm$ 6.1 ng/ml, 26.8 $\pm$ 9.9 ng/ml, 2.26 $\pm$ 0.39 ng/ml and 46.0 $\pm$ 2.0 ng/ml respectively.

In the professional blood donors the serum, ferritin was found to be significantly low (2.26 $\pm$ 0.39 ng/ml) when compared to non donors (46.0 $\pm$ 2.0 ng/ml) and two other blood donors groups (Volunteer - 35.0 $\pm$ 6.1 ng/ml and relative - 26.8 $\pm$ 9.9 ng/ml). The level was significantly low at one per cent level. Mean ferritin levels in volunteer and relative donors were found to be low which was significantly at 5 per cent level (35.0 $\pm$ 6.1ng/ml,

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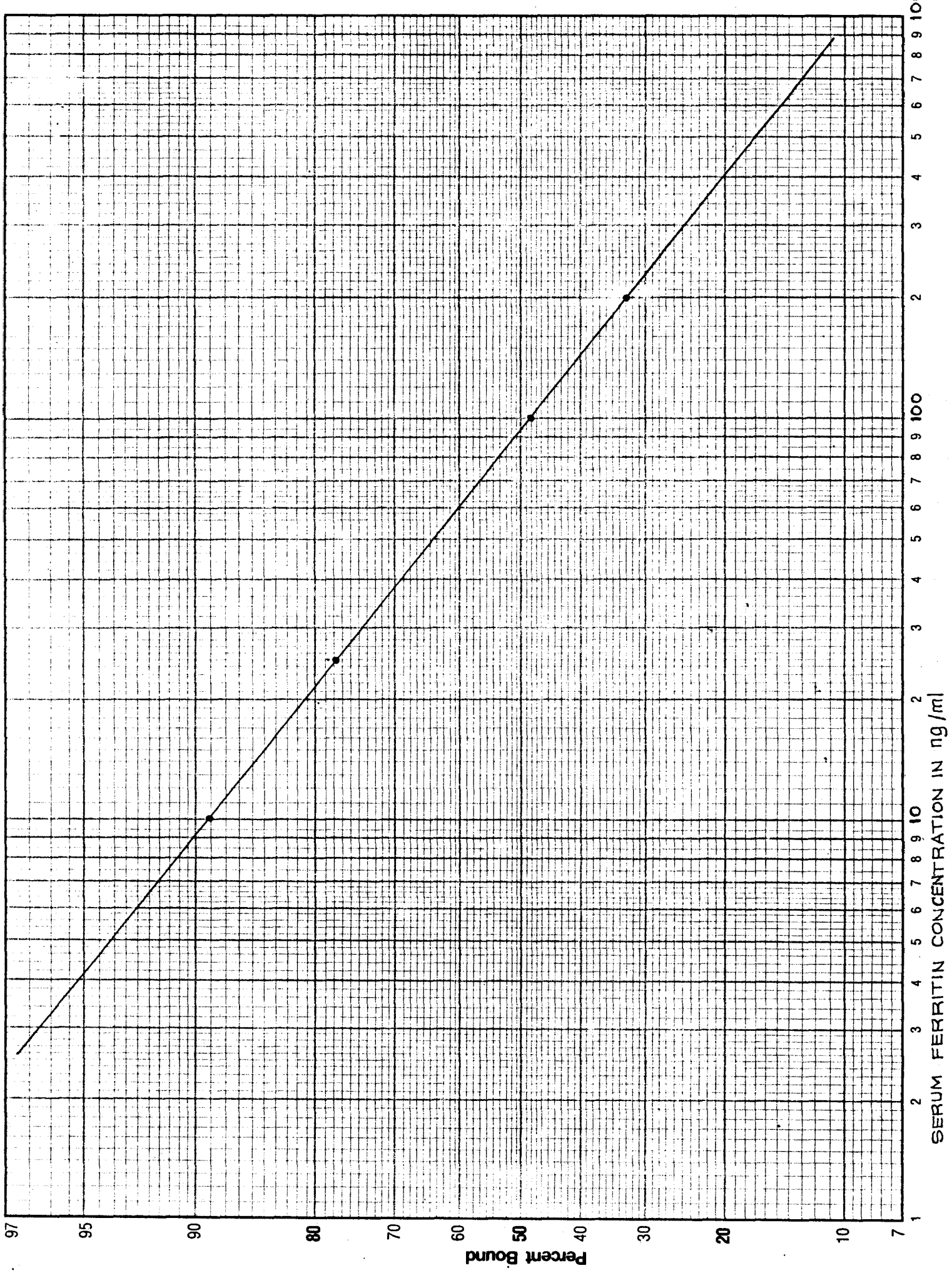
Kit:_____ Assay Date: _____

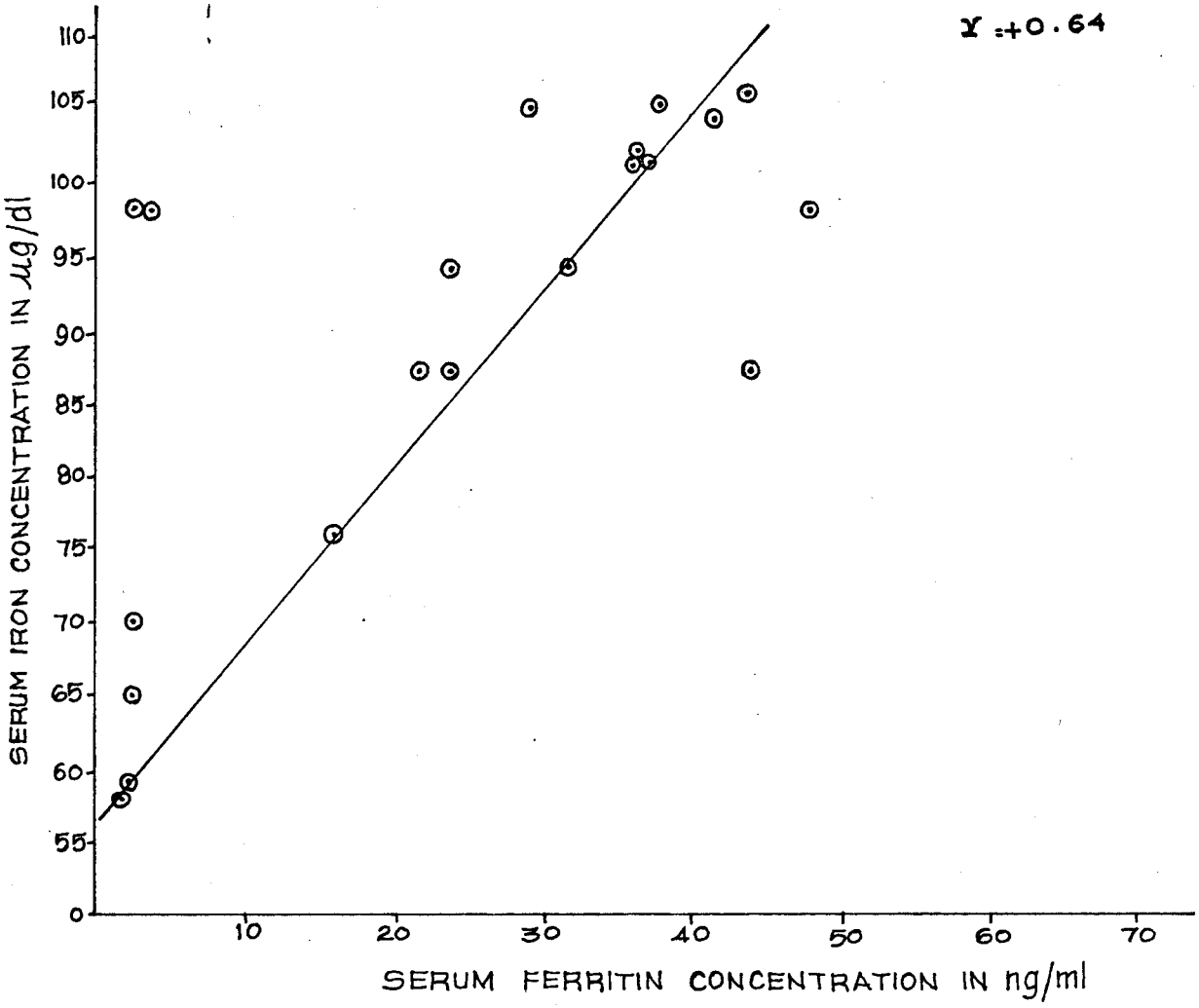


26.8 ± 9.9 ng/ml) when compared to non donors (46.0 ± 2.0 ng/ml). There were significant difference in the ferritin levels between volunteers and relative donors.

Serum ferritin concentration was found to be the best index compared to other parameters (Haemoglobin, serum iron, RBC count, and transferrin saturation) for the determination of iron status in blood donors. It is also recommended for the blood bank authorities to assay the serum ferritin concentration of donors along with the other routine blood estimation.

The mean ferritin was significantly lowered in the blood donors than in non donors after 6 to 8 phlebotomies. The serum ferritin levels were 42.9 µg/l and 14.6 µg/l in volunteer and professional blood donors respectively (Nutritional News, 1986). The above trend was noticed in the present study.

Tea and coffee intake had a significant negative correlation with serum ferritin level and positive correlation with Haemoglobin (Galan et al.,1985 and Bramen et al., 1982). The significant reduction in the ferritin level of profession donors might be due to the heavy coffee and tea intake and frequent blood donation (data obtained from diety survey).



Graph.3 CORRELATION BETWEEN SERUM IRON AND FERRITIN IN VOLUNTEER, RELATIVE AND PROFESSIONAL BLOOD DONORS AND NON DONORS

A positive correlation between the serum iron and ferritin levels were observed in volunteer, relative and professional blood donors and non donors, which is represented in Graph III, (γ +0.64).

13. Plasma ascorbic acid level of selected Volunteer, relative, professional blood donors and non donors:

TABLE XIII

LEVELS OF PLASMA ASCORBIC ACID IN VOLUNTEER, RELATIVE, PROFESSIONAL BLOOD DONORS AND NON DONORS

Subjects selected for the study	Number of subjects	Ascorbic acid (mg/dl) Mean ± S.D.	Groups compared	't' value
Non donors (a)	20	0.9 ± 0.2 ($\bar{a}1$)	a1 vs b1	1.55NS
			a1 vs c1	0.52NS
			a1 vs d1	6.8**
Volunteer donors (b)	58	1.04 ± 0.15 ($\bar{b}1$)	b1 vs a1	1.55NS
			b1 vs c1	1.7NS
			b1 vs d1	27.8**
Relative donors (c)	33	0.99 ± 0.2 ($\bar{c}1$)	c1 vs a1	0.52NS
			c1 vs b1	1.7 NS
			c1 vs d1	12.3**
Professional (d) donors	67	0.78 ± 0.14 ($\bar{d}1$)	d1 vs a1	6.8**
			d1 vs b1	27.8**
			d1 vs c1	12.3**

** - Significant at 1 per cent level
NS - Not significant

The levels of plasma ascorbic acid in volunteer relative and professional donors and non donors were depicted in Table XIII.

Volunteer blood donors, relative donors and professional blodd donors and non donors had mean plasma ascorbic acid levels of 1.04 ± 0.15 mg/dl, 0.99 ± 0.2mg/dl, 0.88 ± 0.14 mg/dl and 0.9 ± 0.2 mg/dl respectively.

The professional donors were found to have low level of plasma ascorbic acid compared to the volunteer, relative and non donors. The significant reduction in level of ascorbic acid in professional donors were at 1 per cent level.

14. Dietary intake of selected volunteer, relative, professional blood donors and non donors:

TABLE XIV

MEAN DAILY NUTRIENT INTAKE BY THE VOLUNTEER, RELATIVE, PROFESSIONAL BLOOD DONORS AND NON DONORS AND PERCENTAGE DIFFERENCE OF THEIR INTAKE WITH RECOMMENDED DIETARY ALLOWANCE AS PER ICMR, 1984

Nutrients	ICMR Recommended allowances (1984)	Daily Nutrient intake							
		Non donors	Percentage difference	Volunteer donors	Percentage difference	Relative donor	Percentage difference	Professional donors	Percentage difference
Energy (kcal)	2800	2800	0	3807	+35%	2607	-6.8	1560	-44
Protein (g)	55	45	-18.1	68	+23	49	-10	30	-45
Iron (mg)	34	39	+14.7	42	+23	36	+5.8	21	-38
Ascorbic acid(mg)	40	50	+25	56	+40	52	+30	30	-25

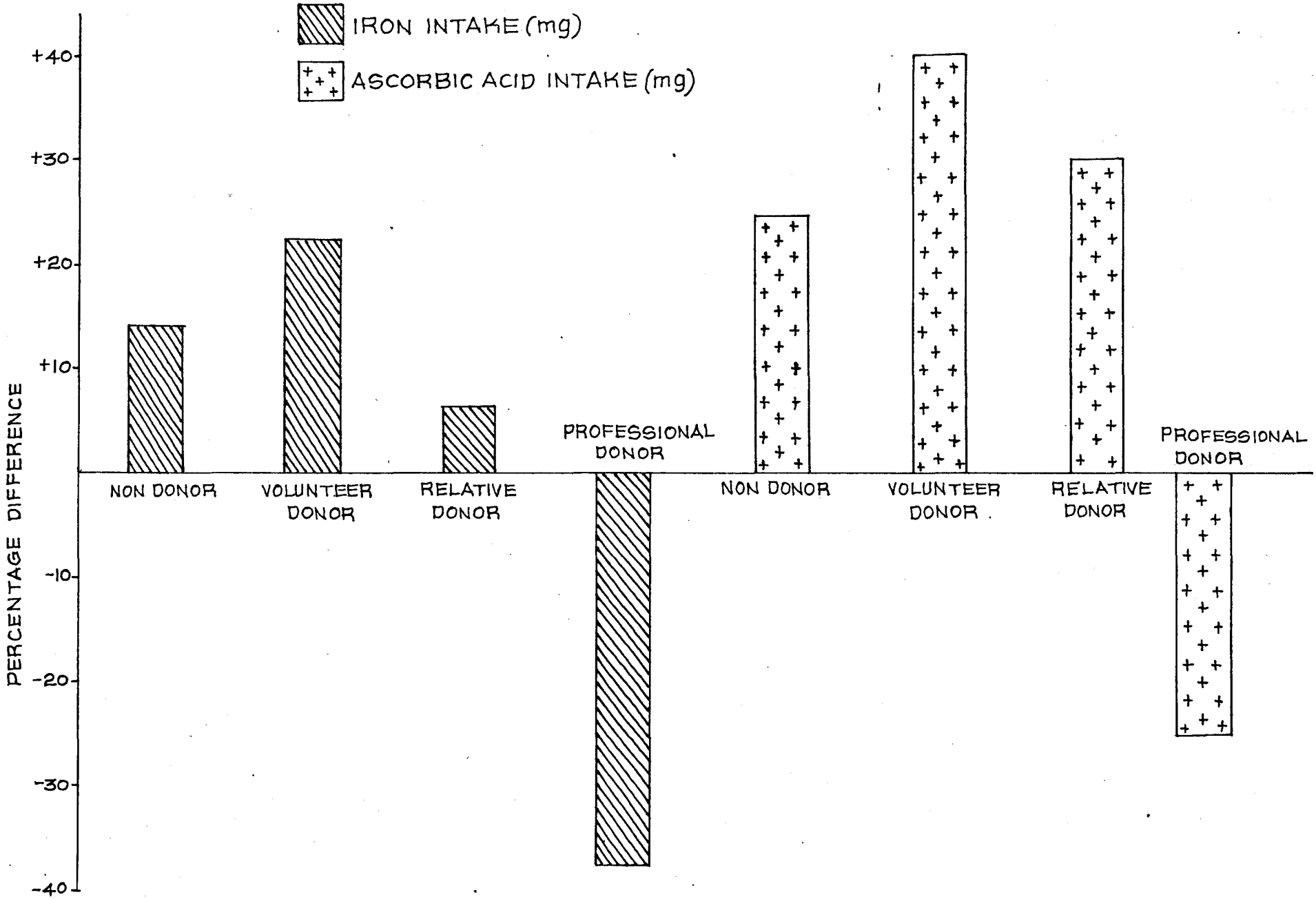


Figure. 9 COMPARISON OF THE IRON AND ASCORBIC ACID INTAKE BETWEEN THE PARTICIPANTS AND RDA (ICMR 1984)

Table XIV presents the mean nutrient intake of volunteer, relative and professional blood donors and non donors. Fig.9 gives the comparison of the iron and ascorbic acid intake between the participants and RDA (ICMR, 1984).

The mean energy intake by the non donor, and volunteer relative and professional blood donors were found to be 2800 kcal, 3807 kcal, 2607 kcal, and 1560 kcal respectively. The energy intake by the volunteer donors were found to be high (35 per cent high) compared to the relative and professional blood donors and ICMR (1984) recommended daily allowances. Notably, there was a low intake of energy by the professional donors (44 per cent) than volunteer donors (6.8%) when compared to ICMR recommended daily allowances.

The protein intake by the volunteer, relative and professional blood donors and non donors were found to be 68g, 49g, 30g and 45g respectively. The protein intake by the volunteer donors were found to be high (23 per cent) when compared to ICMR recommended daily allowance. The protein intake by the professional donors, relative donors and non-donors were found to be low (45 per cent, 10 per cent, 18.1 per cent respectively) when compared to ICMR recommended daily allowance.

The iron nutrient intake by the volunteer, relative and professional donors and non donors were found to be 42mg, 36mg, 21mg and 39mg respectively. The iron nutrient

intake by the professional donors were found to be low (38 per cent) when compared to ICMR recommended allowances, where as in volunteer donors, iron nutrient intake was found to be high (23 per cent) when compared to the relative and professional donors. The above fact might be one of the reason for the increased level of serum iron in the volunteer donors. Reilly (1985) has remarked that the daily dietary intake of iron could very from 11 to 6mg.

Ascorbic acid intake of the volunteer, relative and professional donors and non donors were found to be 56mg, 52mg, 30mg and 50mg respectively. The low ascorbic acid intake was found out only among the professional donors (25 per cent) when compared to ICMR recommended allowances. The reduction of the plasma ascorbic acid level in professional donors might be due to the less intake of ascorbic acid nutrients. Among the blood donors, iron deficiency and anaemia were found out only among the professional donors mainly associated with low standard of living, and ignorance about nutritional facts. Malnutrition lowers the blood donor's (professional) resistance to diseases resulting in higher morbidity, apathy, lethargy, sluggishness and poor working efficiency. These factors lead to low income, low standard of living, poverty and back to malnutrition and infection.

Summary and Conclusion

V. SUMMARY AND CONCLUSION

The present study was undertaken to find out the iron nutritional status of blood donors which may be of diagnostic importance to find out the iron deficiency in the different types of blood donors (volunteer, relative and professional blood donors). The study also attempted to find out whether there are any possible biochemical indices which could be useful to find out the extent of iron deficiency among the professional donors.

Totally one fifty eight blood donors participated in this study. Out of them sixty seven were professional donors (fee paid according to the amount of blood donated) fifty eight volunteer donors (not paid) and thirty three relative donors (blood donated to the family members or relatives) 10 were women, of whom eight were volunteer and two were relatives and twenty apparently normal individuals served as controls (non donors). Five ml of blood samples were collected from each individuals for the biochemical estimation. The samples were obtained from Coimbatore Medical College Hospital blood bank and two other private blood banks in Coimbatore City.

The age group of volunteer, relative and professional blood donors and non donors ranged between 19 and 58 years. The mean body weight of non donors, volunteer, relative

and professional donors were 64.7 ± 10.1kg, 62.9 ± 5.8kg, 58.5 ± 11.0kg and 54.2 ± 5.7kg respectively. The mean body weight of non donors were found to be greater than the three other groups (volunteer, relative and professional blood donors). The mean body weight of professional donors was low compared to three other groups. Thus the professional donors were poor in body weight. In spite of this fact poverty compelled them to sell their blood. There were no significant difference in blood pressure between the four different groups.

The frequency of blood donation is an important consideration among the blood donors. The frequency was recorded for a period of three months. The mean frequency of blood donated by the volunteer, relative and professional blood donors were found to be 1.4 ± 0.64, 2.0 ± 1.3 and 5.0 ± 1.1 respectively. The frequency of blood donated by the professional donors were found to be greater (5.0 times with in a period of three months) when compared to volunteer and relative donors.

The mean haemoglobin levels of the volunteer, relative and professional blood donors and non donors were found to be 16.3 ± 2.6g/dl, 15.1 ± 2.4g/dl, 10.5 ± 1.9g/dl and 15.4 ± 1.9g/dl respectively. The haemoglobin levels in the professional blood donors were significantly low at one per cent level compared to volunteer, relative blood

donors and non donors. Thus in spite of severe anaemia, the professional donors continue to give blood.

A low blood iron indicates iron deficiency anaemia. The mean blood iron levels of volunteer, relative and professional blood donors and non donors were found to be 48.0 ± 7.3g/dl, 43.9 ± 7.3g/dl, 31.1 ± 5.6g/dl and 45.2 ± 5.7g/dl respectively. The blood iron in relative and professional donors were significantly low compared to volunteer and non donors, at five per cent and one per cent levels respectively.

Mean red blood cell count of volunteer, relative and professional blood donors and non donors were found to be 5.88 ± 0.71 millions/cmm, 5.54 ± 0.94 millions/cmm, 3.9 ± 0.69 millions/cmm and 5.9 ± 0.93 million/ccm respectively. The non donors red blood cell count was found to be greater (5.9 ± 0.93 millions/cmm) compared to three other groups. The red cell count of professional donors were found to be reduced at one per cent level compared to volunteer, relative blood donors and non donors. Thus the rapid erythropoises rate in blood losses which is a well established homeostasis mechanism did not catch up with the professional blood donors.

The mean packed cell volume (PCV) of volunteer, relative and professional blood donors and non-donors

were found to be 48.86 ± 7.7 per cent 48.6 ± 7.4 per cent 30.2 ± 5.6 per cent and 50.9 ± 0.2 per cent respectively. The PCV levels in professional donors were reduced significantly at one per cent level compared to volunteer, relative and non donors. There were no significant differencesbetween the volunteer and non donors and relative and non donors. Thus the haemoglobin pattern of the target participants was reflected in the data of PCV too.

The levels of mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were altered among the three groups of blood donors (volunteer, relative and professional). But there were no significant difference in mean corpuscular haemoglobin (MCH) levels among the three groups of blood donors and non donors. The mean MCV of volunteer, relative and professional blood donors and non donors were found to be 85.1 ± 2.4, 84.4 ± 3.2, 81.1 ± 3.4 and 85.0 ± 0.63 cubic micron respectively. MCV was found to be low in professional donors compared to three other groups, it was significantly low at one per cent level. MCH of volunteer, relative, professional blood donors and non donors were found to be 33.9 ± 1.6 per cent, 33.7 ± 1.1 per cent, 34.4 ± 1.9 per cent and 34.4 ± 5.6 per cent respectively. The MCHC level of relative and professional donors were found to be significantly low when compared to volunteer donors and non donors.

The serum iron estimation would give the mobile tissue pools in man. The mean serum iron of three groups of blood donors (volunteer, relative and professional) and non donors were found to be 115.2 ± 18 µg/dl, 108.4 ± 16.9 µg/dl, 73.3 ± 13.3 µg/dl and 107.1±11.6µg/dl respectively. The serum iron level in the professional donors were significantly low at one per cent level (73.3 ± 13.2µg/dl) compared to three other groups.

The total iron binding capacity (TIBC) estimation gives the measure of transferrin which is the transport protein for iron. The iron status in turn defined as a fuction of percentage saturation. Mean TIBC level of professional donors were found to be significantly increased at one per cent level (417.5 ± 42.8µg/dl) compared to volunteer donors (302.5 ± 47.9 µg/dl) relative donors (313.5 ± 65.6 µg/dl) and non donors (238 ± 90 µg/dl) respectively. Mean transferrin saturation of volunteer relative and professional donors and non donors were found to be 38.5 ± 10.9 per cent, 35.2 ± 9.7 per cent, 18.5 ± 5.5 per cent and 34.6 ± 7.2 per cent respectively. In the professional blood donors transferring saturation was found to be significantly low (18.5 ± 5.5 per cent) compared to others.

The serum ferritin was a satisfactory measure of body iron stores. Serum ferritin was estimated by double antibody radio immunoassay technique. Mean ferritin levels of volunteer, relative and professional blood donors and non donors were found to be 35.0 ± 6.1ng/ml, 26.8±9.9mg/ml 2.26 ± 0.39 ng/ml and 46.0 ± 2.0ng/ml respectively. In the professional blood donors serum ferritin was found to be significantly low (2.26 ± 0.39ng/ml) when compared to non donors, volunteer and relative blood donors. The reduction in the value was found to be significant at one per cent level. Mean ferritin levels of volunteer and relative donors significantly low at 5 per cent level when compared to non donors.

Iron deficiency condition were some times reported to be associated with the reduced plasma ascorbic acid levels. The mean ascorbic acid levels of volunteer, relative and professional blood donors and non donors were found to be 1.04 ± 0.15mg/dl, 0.99 ± 0.2 mg/dl, 0.88 ± 0.14 mg/dl and 0.9 ± 0.2mg/dl respectively.

Professional donors were found to have a significantly low level of ascorbic acid compared to the volunteer, relative blood donors, and non donors. The reduction might be due to the low ascorbic acid content food intake by the professional donors.

Iron and haemoglobin levels in blood might be considered as a direct result of dietary intake. Hence daily nutrient intake by the volunteer, relative and professional donors and non donors were calculated in the present study by a dietary survey. The mean dietary energy intake per head per day in the volunteer, relative and professional blood donors and non donors were found to be 3807kcal, 2607kcal, 1560kcal and 2800kcal respectively. Mean dietary protein intake by the three groups of blood donors (volunteer, relative and professional) and non donors were found to be 68g, 49g, 30g and 45g respectively per day, the mean iron intake by the volunteer, relative and professional donors and non donors were found to be 42mg, 36mg, 21mg and 30mg respectively. The ascorbic acid intake of the blood donors (volunteers, relative and professional) were found to be 56mg, 52mg, 30mg and non donors ascorbic acid intake was found to be 50mg. Thus the levels of all nutrient intake by the volunteer and relative donors and non donors were found to be high compared to recommended ICMR allowances. The professional blood donors were found to consume less amount of nutrients compared to recommended ICMR allowance.

The dietary iron intake among the professional donors were 38 per cent less than RDA ICMR (1984). The volunteer and relative blood donors dietary iron intake

were 23 per cent and 5.8 per cent higher than RDA ICMR (1984). The non donors dietary iron intake was 14.7 per cent higher than RDA ICMR (1984).

The results showed that, the blood iron, serum iron, , ferritin and other haemotological parameters (RBC, PCV and MCV) levels of professional donors decreased, while the TIBC level increased with increasing the iron deficiency. Mean corpucular haemoglobin content was not altered in the professional and other two groups of blood donors (volunteer and relative) and non donors. The ferritin was found to be a good index to findout the iron deficiency in the blood donors and non donors. The volunteer and relative blood donors iron level and other haemotological parameters were found to remain normal.

Of the three groups of blood donors (Professional, volunteer,and relative) professional donors forms an important group in donating the blood regularly as well as during emergency conditions. But they are at risk of developing iron deficiency anaemia due to fequent blood donations.

Thus in the present study, the anaemia and its various dimension (Haemoglobin, haematocrit, blood and serum iron, serum ferritin, serum transferrin saturation) were found to be very severe among the professional donors.

Inspite of a requisite for screening the Haemoglobin and Haematocrit, to eliminate anaemic individuals from blood donations, poverty seemed to have driven the professional donors in this study to donate blood as much as 5 times within a period of three months. So blood bank and hospitals need to enforce the minimum levels of health and nutrition of the blood donors. The blood bank authorities should also regulate the donation practices by the frequent professional blood donors.

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Appendices

APPENDIX I

QUESTIONNAIRE FOR ELICITING THE PROFILE OF THE PARTICIPANTS

1. Name of the interviewee ..
2. Name of the interviewer ..
3. Age ..
4. Sex ..
5. Weight ..
6. Occupation ..
7. Monthly income ..
8. Blood pressure noted ..
9. Blood group tested ..
10. Rho type ..
11. Pattern of blood donation..

Number of times donated	Frequency (within a period of three months)

12. Dietary pattern (Amount and type of cooked foods as consumed by the participants for 3 consecutive days)

	Early morning	Breakfast	Lunch	Tea	Dinner
i.					
ii.					
iii.					

APPENDIX II

ESTIMATION OF HAEMOGLOBIN BY CYANMETHEMOGLOBIN METHOD (Samuel, 1986)

Estimationof hemoglobin by this method is recommended by 10th international Haematology congress and world Health Or-ganisation Expert Committee on Nutritional Anaemias.

This method measured not only oxyhemoglobin but also carbon monoxide, hemoglobin and methenoglobin except sulphemoglobin. With filter type photo electirc colorimeters the single relatively broad hand of cyanethemoglobin in the green spectral region, has a distinct advantage.

Reagents

Drabkin's Diluent solution:

Sodium Bicarbonate: 1.0g

Potassium Cyanide: 0.05g

Potassium Ferricyanide: 0.02g

Distilled water to make 1.0 litre

This solution should not be used after it forms a precipitate on the bottom of the storage bottle. The solution was stored under cold conditions. Its preparation and handling was done with great care.

Procedure

1. Exactly 5.0ml of Drabkins dilnent solution was measured into a dry test tube from pipette.

2. Exactly 0.02ml of blood was transferred from a standardized hemoglobin pipette into the diluent solution. Usual care in filling the pipette was taken.

3. The pipette was rinsed three times with the diluent solution without allowing the formation of airbubbles in the solution.

4. The blood and the diluent were thoroughly mixed by rotating the tube.

5. 10 minutes time was allowed for the formation of cyanmethemoglobin.

6. 5.0ml of the diluent solution was used as blank.

7. With green filter No.540 the readings were taken in a photoelectric colorimeter.

Calibration procedure

This determination gives absolute amount of haemoglobin.

Exactly 0.02ml of the known blood was treated as above and measured as standard.

$$\frac{\text{Optical density of test}}{\text{Optical density of standard}} \times \text{Concentration of standard} \times 0.251$$

= grams of Haemoglobin 100ml.

APPENDIX III

ESTIMATION OF BLOOD IRON AND HAEMOGLOBIN BY WONG'S METHOD (Varley, 1980)

Principle

The iron is detached from the haemoglobin molecules by treatment with concentrated sulphuric acid in the presence of potassium per-sulphate witout heating. After removal of the proteins by tungstic acid the iron in the filtrate is determined colorimetrically. From the total iron content the haemoglobin content is readily obtained, Since the haemoglobin content is 0.34% of iron and only 1% to 2% or less of the total blood iron is non-haemoglobin iron.

Reagents

1. 10 per eent sodium tungstate
2. Satured potassium persulphate solution
3. 3N potassium cyanaide solution: 146g of potassium thiocyhdte in 500ml of water.
4. Standard iron solution 70.2mg of ferrous ammonium sulphate in 100ml of water
5. Working standard

Diluted 10ml of stock standard to 100ml with water. 1.0ml of this solution contains 10µg of iron.

Procedure

With micropipette accurately transferred 0.5ml of

well mixed oxalated whole blood into a 50ml volumetric flask. Added 2ml of saturated potassium persulphate solution. Mixed and diluted to about 25ml with water. Added 2ml of 10 per cent sodium tungstate solution. Mixed and cooled to room temperature under the tap and diluted to volume with water. Stoppered and mixed by inversion filtered through a dry, paper collected the filtrate in a dry flask. Prepared a standard in a second 50ml volumetric flask by addition of 2ml of concentrated sulphuric acid, 2ml of saturated potassium per sulphate and 2.5ml of standard iron solution containing 0.1mg of ferric iron per ml.

Cooled to room temperature, diluted with water to the mark and mixed. Prepared a blank similar to the standard but omitting the standard iron solution.

Measured 20ml of unknown filtrate standard and blank, if necessary into separate test tubes. To each added 0.5ml of saturated persulphate solution followed by 2.0ml of 3N potassium thiocyanate solution. Mixed by inversion and read with in the next thirty minutes, setting the colorimeter to zero density with the blank at 480mμ.

Calculation

$$\frac{\text{Density of unknown}}{\text{Density of standard}} \times 0.25 \times \frac{100}{0.5} \times \frac{1}{3.4}$$

= grams of Haemoglobin per 100ml of blood.

APPENDIX IV

ESTIMATION OF RED BLOOD CELL COUNT BY THE METHOD OF Henry (1986)

Total count of Red blood cell

Apparatus

Microscope, RBC pipette, Neubawer counting chamber.

RBC pipette

It has got marking 0.5 and 1.0cm the stem and so the dilution made is 1/200 and 1/00 respectively.

Slide: Neubawer rulling

The counting slide consisted of a thick glass slide with a central platform divided by a short transverse gutter into two portions each of whihh is ruled with the counting grid. The counting plateform for the Neubawer thype is engraned with rulling which measured 3 x 3mm. This area was divided into five large squares. First divided by five horizontal and five vertical lines that 25 x 6400 used by the first five lines and then four lines. So the capacity of the smallsquare was 1/20x1/20cm and the depth of the counting chamber between the conver glass and the ruling was 1/10mm.

For total RBC count one each at the corner and the centre (that was 8 small squares).

Red blood cell (Erythrocyte)

The mature erythrocyte was a biconcave disc circular in shape centrally unstained and periphery stained pink in colour size 7.2 microns in average diameter. It contains haemoglobin.

RBC diluting fluid

(a) Formal citrate solution

Trisodium citrate - 3.0g

Distilled water - 99ml

Formalin - 1.0ml

(b) Hayem's fluid

Sodium chloride - 0.5g

Sodium sulphate - 2.5g

Mercuric chloride - 0.125g

Distilled water - 100ml

Procedure

Cleaned the finger tip with spirit. Allowed it to dry. Pricked the finger with surgical needle. A sudden prick was given so that a free flow of blood was obtained. Drew the blood upto 0.5 mark and diluted upto the mark. The pipette was held firmly by its ends between the four fingers and thumb and rotated so that the content was mixed well. The mixture was then applied to the narrow slit

between the counting chamber and the corver slip. The fluid runs under the cover slip was set aside for 2 minutes for the cells to set.

Counting

Counting was done under a high power objective. Each medium sized square conains 16 small squares. Counted the number of RBC's in 80 small squares (4 squares of 16 at the four corners and one of 16 at centre) should not counted the cells touching the lower and right hand lines but the cells touching the upper and left hand lines were counted.

Calculation

The total number of cells in 80 small squares were counted. The area of a small square was 1/400 sqmm. The depth of the counting chamber was 1/10mm. Therefore the volume of a small squarewas 1/400x 1/10 = 1/4000cmm.
The dilution of the blood was 1/200

$$\text{Total RBC's} = \frac{\text{counts million per cmm}}{30} \times \frac{4000}{1} \times \frac{200}{1}$$

APPENDIX V

ESTIMATION OF HAEMATOCRIT BY MACROMETHOD OF WINTROBE (Henry, 1986)

Equipments

The wintrobe haematocrit tube is a thick walled glass tube with a uniform internal bore and a flattened bottom. It is graduated in millimeters from 0 to 105 and has a rubber cap to prevent evaporation during the long period of centrifugation. A disposable capillary (Pasteur) pipette with a rubber bulk is used to fill the tube.

The essential requirement of centrifuge was that it had to generate a centrifugal field of not less than 2500g at the bottom of the cup.

Procedure

After adequate mixing the sample, after blood was collected to ensure even distribution and oxygeneration of red cells, the haematocrit tube was filled. The tip of the pipette was introduced to the bottom of the tube. As filling procedes the tip of the pipette was raised, but it remained under the rising blood meniscus in order to avoid foaming. The level of the blood was noted and the tubes capped to avoid evaporation during the required centrifugation for 30 minutes at 2500g.

Reading was done without disturbing the specimen. The result was calculated.

The original column of blood in the tube being 100mm. The volume of packed cells can be read directly as a percentage.

APPENDIX VI

ESTIMATION OF M.C.V., M.C.H. AND M.C.H.C. BY THE METHOD OF Samuel (1986)

Mean corpuscular volume (M.C.V.)

The average volume of a single red cell in cubic microns was determined by estimating the mean corposcular volume.

Calculation

$$\frac{\text{Packed cell volume} \times 10}{\text{Red blood cells in million per cmm}} = \text{M.C.M. (cubic microns)}$$

Mean corpuscular Haemoglobin (M.C.H.)

Average haemoglobin content of a single red cell in micro-micrograms was determined by estimating the mean corpuscular Haemoglobin.

Calculation

$$\frac{\text{Haemoglobin} \times 10}{\text{RBC's in million per c.mm}} = \text{M.C.H. (micro-micrograms of haemoglobin)}$$

Mean corpuscular haemoglobin concentration (M.C.H.C.)

The haemoglobin content of 100ml of packed cell as a percentage as opposed to the percentage of haemoglobin of whole blood by determining the mean carpuscular haemoglobin concentration.

Calculation

$$\frac{\text{Haemoglobin} \times 100}{\text{Packed cell volume}} = \text{M.C.H.C. (\%)}$$

APPENDIX VII

ESTIMATION OF SERUM IRON BY DIPYRIDYL METHOD (Varley, 1969)

Principle

Ferrous iron gives a pink colour with 2.2 dipridyl. A solution of dipyridyl in acetic acid is added to serum followed by a reducing agent. Proteins are removed by heating in boiling water and then centrifuged.

Reagent

1. 2,2 dipyridyl 0.1% in acetic acid 3% v/v
2. Sodium sulphate 0.1m
3. Chloroform
4. Standard solution containing 200 micrograms of iron /ml
5. Working standard - dilute 3ml of the stock solution to 100ml with water to obtain a solution containing 3µg/ml.

Procedure

Mixed equal volumes of serum, sodium sulphate and dipyridyl reagent in glass stoppered tube which can be centrifuged. Heated in boiling water bath for 5 minutes cooled and added 0.1ml of chloroform, stoppered and shaked vigourously for five minutes at 300rpm. If the supernatant was not completely clear, repeat the shaking the centrifuging. Read at 520 mµ standard was also read in the same way.

Calculation

$$\text{Micrograms of iron per 100ml of the serum} = \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 300$$

APPENDIX VIII

DETERMINATION OF TOTAL IRON BINDING CAPACITY BY RAMSAY'S DIPYRIDYL METHOD (Varley, 1969)

Reagent

1. Ferric chloride solution - 5mg iron/ml in 0.005 N HCl. Prepared a stock solution containing 145mg of $Fecl_2$ per 100N acid. Diluted one to 100ml with distilled water.

2. Magnesium carbonate - light for adsorption

3. Sodium sulphite 0.2m; 2.52g of the anhydrous salt/ 100ml.

4. 2.2 dipyridyl 0.2 per cent in acetic acid 3 per cent

5. Chloroform and standard solutions as for the method of serum.

Technique

Added 4ml of the ferric chloride solution to 2ml of serum. After allowing for 5 minutes added 400g of mg_2Co_3 (100mg for each ml of ferric chloride) shaked frequently and vigorously for 30-60 seconds. Centrifuged and pipetted out 4ml of the supernatant fluid for iron determination. If the dipydridyl method was used, added one ml each of the 0.2m sulphite and 0.2 per cent dipyridyl and proceeded as described previously for determining serum iron. The result gave the total iron binding capacity. If the serum iron was determined at the same time that per cent saturation was easily calculated.

Calculation

Total iron finding capacity in µg per 100ml serum

$$= \frac{\text{Reading the unknown}}{\text{Reading of standard}} \times \frac{100}{133} \times 6$$

$$= \frac{\text{Reading of unknown}}{\text{Reading of standard}} \times 450$$

APPENDIX IX

ESTIMATION OF FERRITIN BY DOUBLE ANTIBODY RADIOIMMUNO ASSAY (Arosio, 1981)

Introduction

In the double Antibody Ferritin procedure ^{125}I - labelled ferritin competed with ferritin in the patient sample for sites on ferritin - specific antibody. After incubation for a fixed time separation of bound from free is achieved by the PEG (Poly ethyleneglycol) accelarted double antibody method. The tube is then counted in a gamma counter. The counts being inversely related to the amount of ferritin present in the patient sample.

Material required and initial preparation

1. Ferritin antiserum

One vial (two vials*) of lyophilized ferritin antiserum reconstituted each vial by adding 10ml distilled water. Store - refrigerated; stable at 2-8°C for at least 30 days after reconstitution colour: blue.

2. Ferritin

One vial (2 vials*) containing 10ml of iodinated ferritin in liquid form ready to use. Store refrigerated, stable at 2-8 degree for at least 30 days after opening or until the expiration date marked on the vial. Do not freeze.

3. Ferritin calibrators

One set of seven vials, labelled A through G of ferritin calibrators in a protein base. The zero calibrator vial A contains 6.0ml; the remaining vials B through G each contain 2.0ml. Store-refrigerated, stable at 2 to 8°C for at least 30 days after opening.

4. Precipitating solution

One 110ml vial (two 110ml vials)* of precipitating solution containing goat anti-rabbit gamma globulin (GARGG) and dilute polyethylene glycol (PEG) in saline. The precipitating solution was supplied in liquid form ready to use. Store-refrigerated stable at 2-8°C for at least 30 days after opening. Since a fine precipitate may form after, refrigeration, the precipitating solution should be thoroughly mixed before use without foaming.

*Pertains to the 200 tubes $KFED_2$ Kit

Procedure

1. Labelled eighteen tubes in duplicate: T (total counts) NSB (non specific binding) A (maximum binding) and B through G labelled additional tubes, also in duplicate for serum samples and controls.

Calibrator	ng/ml
A (MB)	0
B	10
C	25
D	100
E	200
F	500
G	1000

2. Pipetted out 100µl of the zero calibrator A into the NSB and A tubes and 100µl of each of the remaining calibrators B through G into correspondingly labelled tubes. Pipetted 100ml of each patient sample (serum plasma) and each control, into the tubes prepared. High samples should be diluted with the kit's zero calibrator.

3. Added 100µl of (^{125}I)Ferritin to all tubes

4. Added 100µl of Ferritin antiserum to all the tubes. Except the NSB and T tubes. Vortex.

5. Incubated for one hour at 37°C

6. Added 1.0ml of well mixed cold precipitating solution to all tubes. Vortex

7. Centrifuged for 15 minutes at 3000xg.

8. Decanted the supernatant, retaining the precipitate for counting

9. Count each tube for 1 minute.

Calculation of Results

To calculate ferritin concentration from a logit-log representation of the calibration curve, first calculated for each pair of tubes the average ^{N}SB - corrected counts per minute.

Net count = Average ^{C}PM minus NS^{B},CPM then determined the binding of each pair of tubes as a percent of maximum binding (MB) with the ^{N}SB - corrected counts of the A tube taken as 100%

$$\text{Percent Bound} = \frac{\text{Net counts}}{\text{Net MB counts}} \times 100$$

using the logit-log graph paper provided with the kit plot per cent bound on the vertical axis against concentration on the horizontal axis for each of the calibrators, B through G and draw a straight line approximating the path of these six points. Ferritin concentrations for the unknown might then be estimated from the line by interpolation.

Although other approaches are accepted data reduction by the logit-log method just described has certain advantages in this context - for example in allowing easier recognition of deviant calibration points, since the double antibody ferritin procedure has been optimised for linearity in the representation.

Example: The figures tabulated below are for illustration:

Tube	Duplicate CPM	Average CPM	Net CPM	Percent Bound	Ferritin ng/ml
T	37,648 37,667	37,658			
NSB	918 916	917	0		
A (MB)	17,873 17,811	17,842	16,925	100%	0
B	15,816 16,193	16,005	15.088	89.17%	10
C	14,252 14,230	14,241	13,324	78.7%	25
D	8,998 9,148	9,073	8,156	48.2%	100
E	6,416 6,469	6,440	5,523	32.6%	200
F	3,851 3,827	3,839	2,922	17.3%	500
G	2,446 2,418	2,432	1,515	9.0%	1000
Unknows:					
X_1	12,204 12,234	12,219	11,302	66.8%	45
X_2	7,321 7,233	7,277	6,360	37.6%	159
X_3	4,366 4,103	4,230	3,313	19.6%	40?

Quality control parameters: T = 37,658 CPM

20% intercept = 396 ng/ml
% NSB = 2.4%

50% intercept = 94ng/ml
% MB = 45%

80% intercept = 22 ng/ml

APPENDIX X

ESTIMATION OF ASCORBIC ACID BY THE METHOD OF ROE AND KUTHER (Tietz ,1976)

Principle

Ascorbic acid is oxidised to dehydroascorbic acid in a strongly acid solution. This dehydroascorbic acid reacts with 2,4 dinitro phenylhydrozine to form a dinitrophenyl hydrozons. The hydrazone in the presence of strong sulphuric acid solction, develops a red colour which can be measured spectrophotometrically. Thiourea is added to the dinitrophenyl hydrozine reagent to prevent the oxidation of the dinitrophenyl hudrozine reagent by interefering substances.

Reagent

1. 4 per cent oxalic acid solution (4.0g/100m) dissolved 4.0g of oxalic acid in distilled water and made upto 100ml with the same.

2. Sulphuric acid 4.5 molar.

Added slowly 250ml of concentrated sulphuric acid to 500ml of cold water in a liter flask and brought to a final volume of the one litre with distilled water.

3. Sulphuric acid 12 molar.

Added 650ml of concentrated sulphuric acid to 300ml of cold water in a one litre flask. Cooled and brought to a final volume of one litre with distilled water refrigerated.

4. 2,4 dinitrophenyl hydrazine reagent

2.0g per 100ml in 4.5 molar sulphuric acid. Dissolved 10g of 2,4 dinitrophenyl hydrozine in 4.5 molar sulphuric acid and diluted to a final volume of 500ml. Let it stand in the refrigerator overnight and then filtered.

5. Thiourea solution

Dissolved 5g thiourea in glass distilled water and diluted to a final volume of 100ml, stable for one month at $4^{o}C$.

6. Copper sulphate solution (0.6 g/100ml)

Dissolved 0.6g of anhydrous copper sulphate in glass distilled water and diluted to a final volume of 100ml.

7. Dinitrophenyl hydrqzine thiourea - copper sulphate (DTCS) reagent

Combined 5.0ml of the thiourea solution 5.0ml of copper sulphate solution and 100ml of 2,4 dinitrophenyl hyrazine reagent stored in a bottle at $4^{o}C$ for a maximum of 1 week.

8. Standard

All ascorbic acid standard should be prepared fresh daily.

(a) Ascorbic acid stock standard

Dissolved 50mg of ascorbic acid in 4 per cent oxalic acid and brought to a final volume of 100ml with 4 per cent oxalic acid.

(b) Working standards

In to a series of 25.0ml of volumetric flask pipetted the following amounts 0.5, 2.0, 4.0, 6.0 and 10.0ml of stock standard. Brought to a final volume of 25.0ml with oxalic acid (4.0g/100ml) to yield working standards with concentrations of 0.1, 0.4, 0.8, 1.2 and 2.0mg/100ml.

Procedure

1. Added 0.5ml of the sample to 2.0ml of freshly prepared oxalic acid. Centrifuged for 10 minutes at 25000rpm. Pipetted 1.2ml of the clear supernatant.

2. Added 1.2ml of each concentration of working standards and 1.2ml of oxalic acid for blank.

3. Added 0.4ml of DTCS reagents to all tubes capped the tubes mixed the contents and incubated the tubes in a water bath at 37°C for 3 hours.

4. Removed the tubes from the water bath and chilled for 10 minutes in a ide bath while mixing slowly added to all tubes 2.0ml of cold 12 molar sulphuric acid and mixed.

5. Adjusted to blank in a spectrophotometer at 520nm and read the standard and unknown, plotted the concentration of each working standard vs absorbance values. The standard curve obey's Beer's law upto an ascorbic acid concentration of 2.0mg/100ml.

Calculation

The concentration of the samples were obtained from the standard curve and were multiplied by 5 (to correct for dilution of the samples by oxalic acid) to give the concentration of arcobic acid per 100ml of the sample.