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Diet and Time Related Changes in Hemoglobin and Hematocrit Levels in Blood Donors

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ABSTRACT

Hemoglobin is the important indicator of functional iron so to find out the hemoglobin level and hematocrit percentage in blood donors, a study was carried out in two different localities rural and urban areas in Pakistan, to predict the effect of diet on hemoglobin and hematocrit level in blood donors and also to carry out the result of variation in hemoglobin level with time interval. In this study primary data was collected. A sample of 100 donors was collected from Khyber Teaching Hospital of urban area and DHQ of rural area through a well-structured questionnaire. The studies also find out the descriptive statistics of personal attributes of sample donors, which are age and area groups that explore the descriptive statistics of major variables. The mean hemoglobin level of blood donors before donation in urban areas was 13.88 ± 1.85 g/dl and after donation it drops to 12.82 ± 1.80 g/dl. While in rural areas the mean was 13.00 ± 1.52 g/dl before blood donation and after blood donation it drops to 12.81 ± 1.48 g/dl. The major finding of the study shows that 1.12 ± 0.20 g/dl hemoglobin decline in 450 ml of blood after each blood donation. After each blood donation the body takes 50 to 60 minutes to regain its blood volume. The results also reveal that diet counseling has no effect on hemoglobin after a month probably due to already diet consciousness and hematocrit is almost 3 time of hemoglobin. The study also suggests that after blood donation iron rich source of diet must be consumed.

Abbreviations: RBCs: Red Blood Cells; WBCs: White Blood Cells; Hb: Hemoglobin; Hct: Hematocrit

Introduction

Blood is a specialized connective tissue consisting of formed elements i.e., Red Blood Cells (RBCs), White Blood Cells (WBCs), plate lets, and a liquid portion called plasma. Blood has nutritive, respiratory, excretory, buffering, transport, defensive, maintenance and homeostatic functions. Blood is a viscous fluid composed of cells and plasma. More than 99 percent of blood is composed of RBCs. The average body blood volume of a normal adult is almost 5,000ml [1]. The RBCs, WBCs, and Platelets are 500: 1: 30 presents in the blood. RBCs are the most important part of the blood. Each Red blood cell contains 65 % water, 35 % solid (33 % is hemoglobin and 2 % is meshwork of proteins. Hemoglobin is an iron containing pigment in the red blood cells of blood. It is composed of four globins chains each surrounding an iron containing porphyrin molecule termed as heam. The iron in the hemoglobin is in the ferrous form (F+2) which binds oxygen. Hemoglobin works as a transportation of respiratory gases that binds towards oxygen to form oxyhemoglobin. This affinity of hemoglobin is affected by pH, temperature and concentration in the red cell. When blood is exposed to oxidizing agents like drugs it is converted to methemoglobin. Carbon monoxide reacts to hemoglobin to form carboxyhemoglobin. Hemoglobin is a conjugated protein consisting of hem and protein. It is a protein specially adopted for oxygen transport [2].

The red, oxygen carrying pigment in the red blood cells of vertebrates is Hemoglobin. Heam portion of hemoglobin is synthesis mainly from acetic acid and glycin and that most of these syntheses occur in mitochondria. The acetic acid is changed in the kerb cycle to succinyl-co A, and then two molecules succinyl-co A combine with two molecules of glycine to form a pyrrole compound. In turn four

pyrrol compounds combine to form a protoporyrin compound. One of the protopyrrins known as protopyrrin ix, then combines with iron to form the heam molecule [3]. The life span of RBC is 120 days after which they are phagocytes by reticulate endothelial system. Amino acids from globin chain are recycled and iron is removed from haem for reuse in hemoglobin synthesis [2]. The amino acid sequences in polypeptide chains of hemoglobin are determined by globin genes. There are two types of inherited disorders of hemoglobin in humans collectively called hemoglobinopathies. Disorders of hemoglobin can be broadly divided into two categories. Decrease concentration of hemoglobin (Anemia) and increase concentration of hemoglobin (Poycythemia) [3]. The average normal hemoglobin content of the blood is 16gm/dl in male 14gm/dl in female per 100ml. Normal hemoglobin value in male is >13.5 and in female is >11.5 [4]. Blood contains an average of 15 grams of hemoglobin per 100 ml [3]. Hemoglobin has a molecule weight of 64,458 delton.

Each gram of pure hemoglobin is capable of combining with approximately 1.39 ml of oxygen. Therefore, in normal male over 21ml of oxygen can be carried in combination with hemoglobin in each deciliter of blood while in normal female 19 ml of oxygen can be carried. A healthy male having weight of about 70 kg, contains about 900 gm of hemoglobin, in which 0.3gm is destroyed and 0.3gm is synthesized hourly basis. A hemoglobin level greater than 900gm of normal (adult females 165g/L or haematocrite > 0.48 and adult males 180g/L or haematocrit > 0.52) may be due to in increases number of red blood cells (true poycythaemia) or a reduction in the plasma volume (relative or apparent polycythaemia. Currently, a minimum capillary hemoglobin of 12.5 gm/dl is the sole requirement for donor qualification in the US as far as iron levels are concerned, yet it is known that hemoglobin level is a poor surrogate for low iron. Blood transfusion is considered when hemoglobin level is lower than 7 g/dl [5]. Hematocrit is the percentage of red blood cells in the blood determined by centrifuging blood in a hematocrit tube. The true hematocrit is approximately 40 for a normal male and 36 for a normal female. The hematocrit averages about 96 % of the measure hematocrit. Between 200 to 250 mg of the functional iron is removed with each and every whole blood donation, reflecting losses from the hemoglobin in red blood cells.

This amount represents approximately 25 % of the average iron stores in male and almost 75 % of the iron stores in female [6]. Typically, a healthy person can endure a loss of 10–15 % of the total blood volume without serious medical difficulties, and blood donation typically takes 8–10 % of the donor's blood volume. Treatment is done by dietary counseling, correction of the underlying cause and if patient complaint of angina, heart failure or evidence of cerebral hypoxia blood transfusion is considered [6]. The present study is designed in response to assess timely change in hemoglobin and hematocrit levels in blood donors and the time to regain blood volume and the relationship between hemoglobin and hematocrit level. To observe the effect of diet counseling on hemoglobin level after blood donation.

Materials and Methods

Sample Size and Selection Criteria

A total of 231 individuals were entertained of which 200 donors complete the study. A representative sample size of 200 individuals was selected for better assumption in blood donation in this study. All healthy individuals free of any complications with clear medical and family history, of any age, physical activities and anthropometry receiving no iron supplementation were enrolled in the study after signed written consent form. When the patients were unable to give consent due to depressed conscious status, consent was taken from their legal guardian.

Research Plan

Data for the first three objectives was collected during the time of subject registration and during the process of blood donation. A specifically designed questionnaire clearly containing all the required history of the subjects was filled in. Blood biochemistry for Hemoglobin and hematocrit was done on one automatic analyzer "Mission Hb" to minimize the systematic error. Blood biochemistry of the subject for Hemoglobin and hematocrit was done before blood donation and after blood donation frequently the test was done after required time interval. For 4th one objective the subjects were registered for 30 days to see the effect of diet related changes on hemoglobin level and hematocrit percentage. Dietary counseling of 100 subjects was done accordingly and 100 subjects were kept blind of proper diet plain. Food frequency was record twice in this duration at 15th days interval. After 30th day of blood donation once again blood biochemistry for hemoglobin and hematocirt was done and after completing the required sample size, comparison was done by SPSS applying paired T-test of both categories for getting the result of any effect of diet on hemoglobin and hematocrit.

Study Design

The study was hospital based cross sectional study and convenient sampling was used. All volunteers and easily accessible blood donors with clear medical history were enrolled for this study.

Data Collection

Data was collected on a well-planned questionnaire designed for recording personal history, demographic and socioeconomic status and medical history of the subjects. The study was also be approved by the medical superintendents of respective health facilities.

Biochemistry for the Hemoglobin

The biochemical parameters including hemoglobin and hematocrit was analyzed at the spot, by using an automatic analyzer "Mission Hb" (Acon Laboratorie made in Germany)

Mission Hb

The "Mission Hb" Hemoglobin Testing System is intended for the quantities testing System of Hemoglobin (Hb) and calculated Hematocrit (Hct) in capillary and venous human whole blood. The easy to operate system consists of a portable meter that analyzes the intensity and color of light reflected from the reagent area of a strip, ensuring quick and accurate results.

Test Strip

The mission Hb Hemoglobin test Strips which contain a chemical reagent system which works with the Mission Hb Hemoglobin Meter to measure the Hemoglobin (Hb) concentration in capillary and venous whole blood. The strip contains a specimen Application area. For the best filling results the Specimen Area with the correct amount of blood specimen.

Procedure

A small prick on the ring finger, with a disposable lancet, let some blood out of the finger. A control strip will be inserted into the Mission Hb meter. 10 µL of blood will be dropped on the control strip with the help of a specified capillary tube. After 15 seconds the meter will show the exact hemoglobin and hematocrit percentage on the digital screen. This process will be repeated every time on measuring subjects' hemoglobin and hematocrit percentages. Biochemical data will be recorded on a record sheet.

Data Analysis

Data was checked for its distribution and errors by using descriptive statistics. Paired t-test used to find difference in mean at 0.05 % level of significance.

Results and Discussion

This study reveals the key finding of the research and clarifies the finding to the reader. The expected results are interpreted and explained with reference to proceeding research studies. The empirical results of various models and tests in the study are presented in the light of set study objectives.

Personal Attribute of Sampled Donors

This portion of the study shows various socioeconomics characteristics of the interviewed respondents. These are area and age.

Area

Area is an important aspect of selecting sample because there is climatic change, Activity level and diet culture in area wise which can affect the hemoglobin level. From the result it is clear that in urban area the minimum valve of hemoglobin level before blood donation was 9.80g/dl while their maximum is 17.40g/dl having mean of 13.88g/dl. While the hemoglobin level of blood after donation ranging from 9.00g/dl to 16.30g/dl having means of 12.82g/dl. Similarly, in the rural area the hemoglobin level blood before donation ranging from 11.80g/dl to 17.60g/dl with a mean of 12.81 g/dl while the hemoglobin level after blood donation in the rural area ranging from 10.70g/dl to 16.20 g/dl with a mean of 12.81 g/dl. (Table 1) However, our present study result is contrary to who indicated that the serum ferratin level in non-settled area will be slightly high as compared to the serum feritin level of the settled areas. It investigated in their research that there are a lot of factors which can bring change in the human blood composition in which climatic condition, environments drinking water cultural diet routine has great effect on blood biochemistry.

Table 1: Hemoglobin status in relation to area.

Area	Hemoglobin Status Means ± S. D	
	Before Blood Donation	After Blood Donation
Urban Area	13.88 ± 1.85	12.82 ± 1.80
Rural Area	13.00 ± 1.52	12.81 ± 1.48

Note: S.D stands for standard deviation

Age

Age is an attribute which can also affect the hemoglobin level of blood. The total blood donors sample size was divided into four age groups. The groups were under 20 years, 20-30 years group, 31-40 years and above 40 years of age. These groups were named group A, B, C and D respectively. Group A was age below 20 years, group B was between 20-30 years, group C was range between 31 to 40 years and group D was above 40 years of age. Each of these groups contains 50 sample sizes. Patel [7] also done research work to find out the influence of hemoglobin level in the different life stage of humans and found that the middle stage is most suitable stage of life for high hemoglobin level and for blood donation. The middle stage in the research work ranged between 25-40 years of age (Figure 1). After analyzing the collected data, the observation and findings is that hemoglobin level before blood donation of group ranges from 11.80 g/dl to 17.60 g/dl having mean of 14.09 g/dl, while after blood donation the hemoglobin level range from 10.70 g/dl to 16.20 g/dl having mean of 12.91g/dl. Similarly, the hemoglobin level before blood donation of Group B had range of 12.30g/dl to 17.30 g/dl with mean of 12.91g/dl while after blood donation ranging from 11.00g/dl to 15.80g/dl with a mean of 13.10 g/dl. From the result it is also concluded that the hemoglobin level before blood donation of Group C having a range of 10.70g/dl to 16.10g/dl with a mean of 13.40g/dl g/dl while after blood donation at group ranging from 9.40 g/dl to 15.00 g/dl with a mean of 12.42 g/dl.

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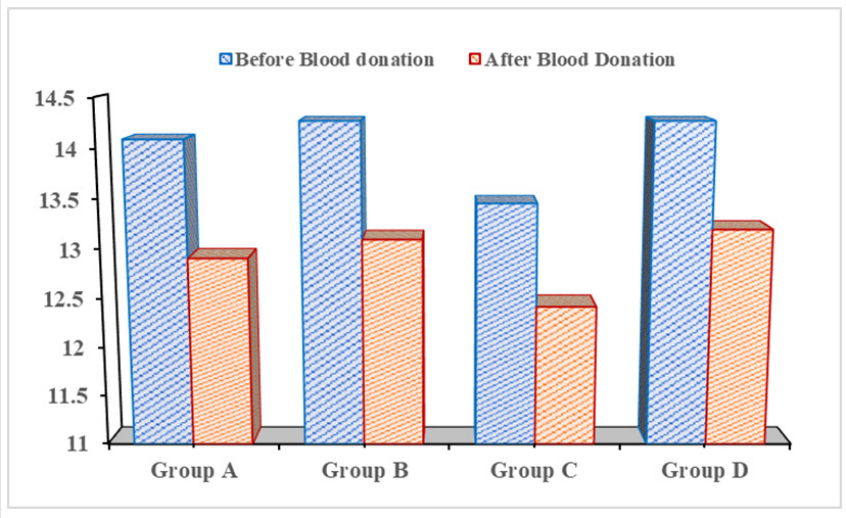

Figure 1: Hemoglobin status in relation to age and blood donors.

Note: Group A=Age <20 years, Group B=20-30 years, Group C= 31-40 years and Group D= > 40 years

The hemoglobin level before donation of Group D ranges from 9.80 g/dl to 17.40 g/dl having mean of 14.27 g/dl while hemoglobin after blood donation at same age ranging from 9.00 g/dl to 16.00 g/dl having mean of 13.20 g/dl. Studies performed by Finch and colleagues in the 1950 found that iron absorption is influenced by both the rate of erythropoiesis and on the size of iron store. The regulator of iron store has the capacity to increase iron absorption up to about 2 mg per day, while the capacity of the erythroid regulator is much greater, perhaps up to 40 mg per day in the patients in severe anemia receiving oral iron supplementations. The erythroid regulator has the capacity to drive continued dietary iron absorption, even in the presence of severe iron overload, to protect against iron deficiency erythropoiesis. This is the best demonstration in patients with beta-thalassemia, who has a greatly expanded erythroid compartment and continued dietary iron absorption despite total body iron overload. Since hepcidin production is inappropriately low in these patients, it is through that the erythoid regulator mediates its activity, at least in part, by decreasing hepcidin production (Figure 2). This concept is supported by studies of mice undergoing phlebotomy in the presence of inhibitors of erythropoiesis which have shown that increased erythropoiesis, as opposed to anemia, tissues hypoxia or erythropoietin is necessary to reduce hepcidin synthesis and increase iron available for synthesis of new red blood cells.

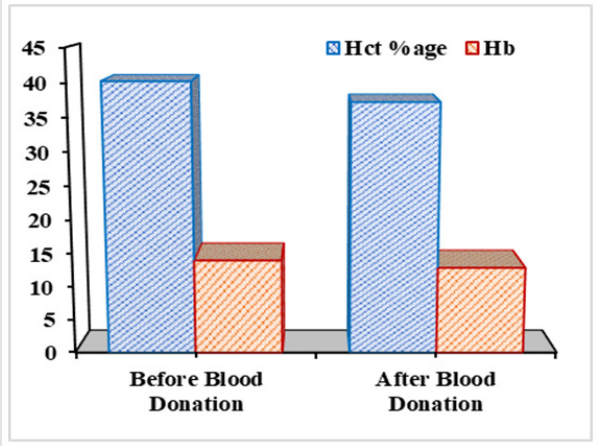

Figure 2: Pair T-test of hemoglobin and hematocrit level before and after blood donation.

Pair T-test of Hemoglobin level

From the above results it is clear that the hemoglobin level decreases 1.12 g/dl and there is statistically difference between hemoglobin level before blood donation and after blood donation also followed a self-asked question in their research work, that by how much does a single unit transfusion increase the recipient's hemoglobin? They found that the effect of a single unit RBC transfusion on the recipient's Hemoglobin was recorded in 123 patients who received only one unit of stored RBC (allogeneic or predonated autologous) during their hospital stay. Their study also finds that approximately 1 g/dl hemoglobin increases with each and a single blood transfusion, in the case females experience more increase in the hemoglobin than males. The above results are statistically significant at .01percentage of significance level. While the mean differences are 2.98 percentage which are given in table below 4.2.3. So, in this case we fail to accept null hypothesis and accept alternative hypothesis. From the above results it's clear that the hematocrit percentage decrease 2.98 percent and there is statistically difference between hemoglobin level before and after donation. Mast et al.2012 conducted a study on impact of HFE mutations on hemoglobin and iron status in individuals experiencing repeated iron loss through blood donation and found that measure of iron status such as ferritin and reticulocyte hemoglobin content are useful predicators of venous hemoglobin level in frequent blood donors. Which was somewhat similar to our study.

From the major variable descriptive shows that the hemoglobin level before donation falls in range from 9.80g/dl to 17.60 g/dl with a mean of 14.03 g/dl while the hemoglobin level after blood donation ranging from 9.00 g/dl to 16.30 g/dl with a mean of 12.91 g/dl. The descriptive statistics of their mean differences ranging from 0.80 g/dl to 1.30 g/dl with a mean of 1.11g/dl. The major variable descriptive shows that the hematocrit percentage before donation falls in range of 31.20 to 49.70 with a mean of 40.02, while the hematocrit percentage after donation ranges from 28.50 to 46.40 with a mean of 37.24. The descriptive of their difference ranging from 2.70 to 3.30 with a mean of 3.00.

Hemoglobin Means Difference After One Month

From the results it is clear that the hemoglobin level decreases 0.05 g/dl and there is statistically no difference between hemoglobin level before and after 30 days of blood donation (Table 2). Similarly, the hemoglobin level of non-dietary counseling group shows that the t value is 0.58 which is insignificant at 01percentage and 05percentage of significance level and also the mean difference is 0.02g/dl which means that there is not statistically difference of non-dietary counseling group after 30 days. From the above statistics it is clear that the hemoglobin level of dietary counseling group and non-dietary counseling group before blood donation and after a month have no significant difference.

Table 2: The difference in hemoglobin after one month of counseled and non-diet counseled groups.

Pair Samples Test		
Groups	**Means±SD**	**P-Value**
Diet counseled	0.05±0.19	0.078
Non-Diet counseled	0.24±0.288	0.56

Note: SD=Standard deviation, P-Value=Probability value

Correlation of Food Groups with Dietary Counseling and Non-Dietary Counseling Groups

The study shows the correlation of food groups with dietary counseling and non-dietary counseling groups. Meat and meat products, fruits and vegetables were positively correlated with both dietary counseling and non-dietary counseling groups (Figure 3). Bacon, et al. 2011 carried out a study and reported that meat and meat products and milk and milk products and cereals were positively correlated with functional iron like hemoglobin which was in line with our findings.

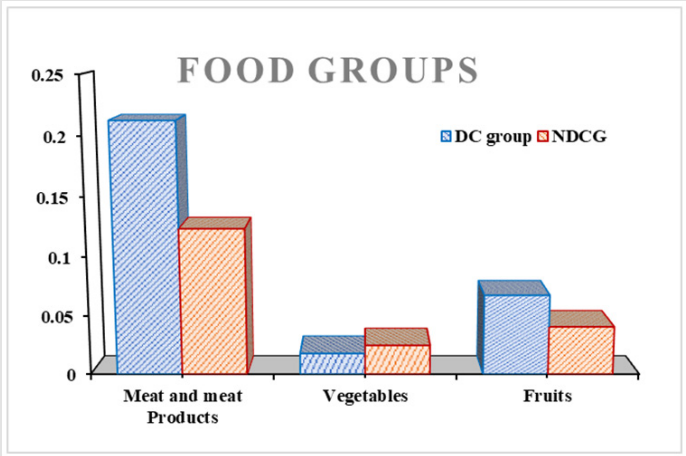

Figure 3: Correlation of food groups meat and meat products, vegetables and fruits with dietary counseling and non-dietary counseling groups.

Time Interval Variation in Hemoglobin Level

After analyzing the data, the obtained results show that before blood donation the hemoglobin level have a mean of 14.03gm/dl while after ten minutes of blood donation it reaches to 14.54g/dl. After 20 minutes of blood donation the hemoglobin level of blood drops to 13.03g/dl. After more ten minutes the hemoglobin level further drops to 13.63g/dl, after 40 minutes it reaches 13.22g/dl. Then after 50 minutes it reaches 12.91g/dl and at 60 minutes after blood donation it reaches 12.92g/dl. From the descriptive statistics it is clear that after 10 minutes of blood donation the hemoglobin level 1st increases by 0.51 g/dl and after 20 minutes it decreases by same ratio and after 30 minutes it decreases by 0.40 g/dl. After 40 minutes it still decreases upto 0.41g/dl and after 50 minutes it decreases to 0.31 g/dl and at last after 50 to 60 minutes after blood donation their seen no or negligible change in hemoglobin level. So, the study showed that the body takes 50- 60 min to complete its blood volume after 450 ml of blood donation. The above descriptive results are mentioned (Figure 4).

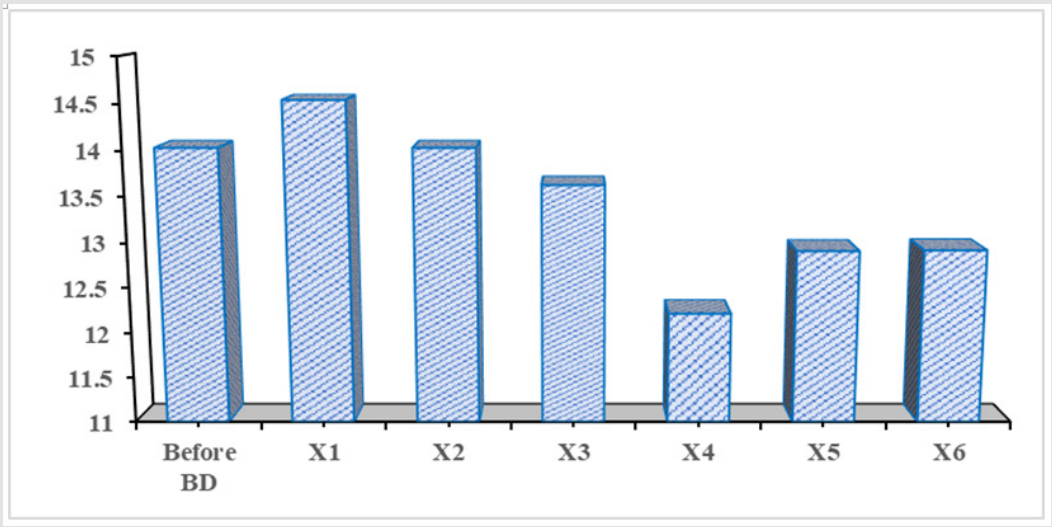

Figure 4: Time change in hemoglobin after blood donation.

Summary, Conclusion and Recommendation

The proposed study was conducted in the urban and rural areas of Pakistan to know the exact blood loss by biochemistry in various cases. The time required for the body to regain its blood volume, the relation between hemoglobin and hematocrit and the effect of diet on hemoglobin after blood donation. For the study 200 individual were selected. All the selected subjects were free of any medical problems. The selected willing individual Hemoglobin level and hematocrit percentage were done before the blood donation and were recorded on the record sheet. After blood donation the same test for hemoglobin and hematocrit percentage were done in a time interval of 10 mints to see any effect on Hemoglobin and hematocrit percentage. These sample sizes were divided into two groups: dietary counseling and non-counseling group. The same test was repeated twice in a month with 15 days interval. From this study the major findings were obtained and discussed. The personal attributes were analyzed through descriptive statistics which are age and area. The mean hemoglobin level of blood donors before donation in urban areas was 13.88 ± 1.85g/dl and after donation it drops to 12.82 ± 1.80g/dl. While in rural areas the mean was 12.81 ± 1.52g/dl before blood donation and after blood donation it drops to 13.81±1.48g/dl. The hemoglobin level of different ages of group A, B, C and d have mean of 14.09 ± 1.64, 14.27±1.42, 13.36±1.41 and 14.27±2.14g/dl before blood donation while after donation its mean decrease to 12.91±1.57, 13.10 ± 1.40, 12.42 ± 1.48 and 13.20 ± 2.02g/dl.

By using pair t test on hemoglobin level before and after donation the mean difference was 1.11 ± 0.20 g/dl which is statistically significant at 1 percentage while hematocrit percent is statistically significant at 1 percentage with a mean difference of 2.98 ± 0.38 percent before and after donation. The descriptive of major variable of hemoglobin before, after blood donation and differences means are 14.03, 12.91 and 1.11 while Hematocrit percent have 40.21, 37.24 and 2.98. By comparing mean difference between hemoglobin before and after 30 days of blood donation the study found 0.037 differences which are statistically significant at 1 percentage level. The study also finds out the time interval variation of hemoglobin level of before blood donation, After 10, 20, 30, 40, 50 and 60 minutes through descriptive which have mean of 14.03 ±1.69, 14.54±1.69, 14.03±1.69, 13.63±1.68, 13.22±1.67, 12.91±1.64 and 12.92±1.69g/dl.

Conclusion

After analyzing and brief discussion of the study it is concluded that an average of 1.12g/dl hemoglobin level and 3 percent of hematocrit declines in each and every 450ml of blood donation. Then a normal human body takes about 50 to 60 min to regain its blood volume. Hematocrit percentage is approximately 3 times of hemoglobin level. There is no effect of diet counseling after blood donation. This can be possible that all the volunteer individuals for blood donation were health and diet conscious.

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