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Study of Biochemical Changes after Plateletpheresis in Healthy Male Donors

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Declaration of Interest

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Summary

BACKGROUND: There is relatively little information about endogenous biochemical changes in a response to plateletpheresis in healthy donors. We aimed to investigate the changes in different biochemical parameters including glycemic status, insulin resistance, iron status, lipid profile and inflammatory markers after plateletpheresis in healthy male donors with normal glycemic status. **METHODS:** In this study we enrolled 10 male subjects. The glycemic status in all subjects was assessed using an oral glucose tolerance test pre- and post-plateletpheresis at different time intervals (1, 8 and 22 days). Different biochemical parameters including glucose, HbA1c, insulin, lipids, uric acid, transferrin, ferritin, C-reactive protein and insulin resistance were measured. Repeated ANOVA was utilized for the purpose of statistical comparison of means between different days. **RESULTS:** Fasting glucose, transferrin, cholesterol, triglycerides, HDL-C, and LDL-C were significantly altered (-3.9%, $p<0.05$; -2.7%, $p<0.05$; -3.9%, $p<0.05$; 23.9%, $p<0.05$; -5.5%, $p<0.01$; and -9.2%, $p<0.05$ respectively) at day 1 following plateletpheresis. There was a gradual reduction in HbA1c and ferritin levels during the time-course of the study, and by day 22, both were significantly lower (-2.0%, $p<0.01$; -18.1%, $p<0.05$ respectively) when compared to the pre-plateletpheresis levels. **CONCLUSIONS:** Post-plateletpheresis, several biochemical parameters may change significantly in healthy donors. The changes were particularly evident one and 22 days after donation. The potential effects of plateletpheresis need to be considered when interpreting biochemical tests.

Key words: Hb1Ac, Iron, Insulin resistance, Plateletpheresis, HOMA-IR

INTRODUCTION

Platelet transfusions are routinely needed as an essential part of treatment for patients undergoing cancer therapy, as well as for those with bleeding disorders, and for patients undergoing open-heart surgery, or organ transplantation[1]. Platelets can be harvested using plateletpheresis, a process whereby whole blood is removed from a donor, separating the blood into its components, keeping the platelets and returning the remaining blood components to the donor; or by separating platelets from units of whole blood [2, 3]. Platelets must be transfused within five days of collection due to their very short shelf-life [4]. Therfore there is a need for constant replenishment of the hospital supply. It is reported that platelet donations can be made up to 24 times per year [5]. However, there is relatively little information about the biochemical and physiological changes in response to plateletpheresis in healthy donors, especially because citrate is routinely used for the collection process to inhibit coagulation, and may lead to metabolic alkalosis. Indeed, it was reported that exposure to citrate during the apheresis procedure may acutely affect mineral and bone metabolism, leading to an increase in serum parathyroid hormone, and a fall in serum albumin, calcium and 25 hydroxy vitamin D levels. This has been shown to compromise bone mineral density at the lumbar spine. [2]. Over the years, the thrombopoietic response to plateletpheresis has been investigated [3,4,5]. After plateletpheresis, platelet counts were reported to increase by some [3,4], and was reported to fall in one study [5]. In addition, serum ferritin levels of the female donors, but not male donors, has been reported to be decreased with increasing frequency of apheresis donation [5].

A more recent study has investigated the effects of phlebotomy on insulin sensitivity and cardiovascular risk factors in patients with metabolic syndrome, and concluded that

consecutive reduction of body iron stores, lowered BP and resulted in improvements in markers of cardiovascular risk including low-density lipoprotein/high-density lipoprotein ratio, and glycemic control. [6]. Whether such an effect is produced after plateletpheresis only is not known. Therefore, we aimed to investigate the effects of plateletpheresis on biochemical tests of glycemic status, insulin resistance, lipid profile, inflammatory markers and iron stores in plateletpheresis donors,

MATERIALS AND METHODS

Thirteen male first time donors were recruited from donation area at the apheresis facility. Exclusion criteria included: diagnosed hypertension, treatment with oral hypoglycaemic drugs, insulin, or other drugs likely to modify insulin resistance and coagulation [7]. Subjects were also excluded if test results suggested abnormalities in glucose tolerance, or iron stores, or if they had a current acute symptoms, or if there were other contraindications to apheresis. After applying exclusion criteria three donors were rejected after the first visit due to impaired glucose tolerance. A standard donor questionnaire was used to verify eligibility for platelet donation, and a pre-procedure CBC is done to ensure that the donor platelet count is >150,000 per mm^3, with donor consent being obtained before donation as usual.

The study was approved by King Abdullah International Medical Research Center Ethics Committee, Jeddah, Saudi Arabia (Study number RCJ0611-183). Written informed consent was obtained from all participating subjects. No participants with missing visits were included. Each subject attended the apheresis area four times during the study

period, making the total number of visits by the ten subjects forty. Each subject was under venipuncture for eight times (pre and post 75 g oral glucose tolerance).

On each visit subjects were weighed, their height measured, and body mass index calculated as weight (kg)/height (m^2). There was no significant difference between BMI taken at the first visit (26.4 + 1.2 kg/m2) when compared to the last visit (26.4 + 1.1 kg/m2), p = 0.91.

At the baseline visit (visit A), venous blood samples were taken after overnight fasting (12 hours) immediately prior to platelet donation. Blood samples were collected into ethylenediaminetetra-acetic acid (EDTA), fluoride oxalate (grey top), and plain tubes. Oral glucose tolerance test (OGTT) was performed on each subject between 8 and 10 am, with further blood samples being collected 2 hours post glucose load into plain and grey top tubes. Within 10 minutes of collection, blood samples were centrifuged (3000 ×g) for 10 minutes. Plasma glucose was measured immediately. Serum specimens for specialized tests, namely: insulin, lipid profile (total cholesterol, HDL-C, triglyceride), iron, ferritin and transferrin were stored at −80 °C until analysis. Once the results of the OGTT were available the glycaemic status of each participant was categorized according to WHO criteria [8]. All ten subjects were classified as having normal glucose tolerance following the first OGTT. Each subject also had their blood pressure measured using an automated equipment (V100 DINAMAP, General Electric) before blood collection.Plateletpheresis was undertaken later the same day using a standard protocol at the apheresis facility.

To monitor the effects of plateletpheresis on biochemical parameters, subjects returned for a second visit (visit B) 24 hours after plateletpheresis. A second OGTT was carried out as described at the first visit. Eight days later a third OGTT was performed (visit C), and 22 days after the first visit a fourth OGTT was performed (visit D). All participants were instructed to maintain their normal lifestyle and food intake before and between visits, to avoid potential affects on the results of biochemical tests independently of plateletpheresis. In addition, they were instructed not to take any medication or supplements during the period of the study. The plateletphereses were performed over a period of 60 to 90 minutes, utilizing the Trima Accel, "Automated Blood Collection System", (manufactured by TerumoBCT, Lakewood, Co, USA), at the blood bank (apheresis area), Department of Pathology and Laboratory Sciences, King Abdulaziz Medical City, Jeddah, which has been accredited by the American Association of Blood Bank (AABB) since 2007.

Assays

All biochemical tests were made using Architect autoanalyser (Abbott, USA) at the main laboratory of Jeddah, National Guard Hospital. The laboratory is accredited by the College of American Pathologists since 2002. The assays for glucose, CRP, cholesterol, HDL-cholesterol, triglycerides, uric acid, iron and transferrin were undertaken on an Architect c8000. LDL-cholesterol was calculated using the Friedewald formula [9]. Glycated hemoglobin (HbA1c) test was performed using a high-pressure liquid chromatography (HPLC) method on a G8-TOSOH Bioscience, Inc instrument. The principle of chemiluminescent immunoassay on Architect i1000 was utilized to measure insulin and ferritin levels in serum.

Indices of insulin resistance

The index ISI-gly (insulin sensitivity index-glycaemia) was calculated based on the fasting and two hour glucose and insulin values using the following formula [10]:

$$\text{ISI-gly} = 2/[(\text{insulin}\rho \times \text{glucose}\rho) + 1]$$

Where insulinρ and glucoseρ represent the sum of measurements of insulin (mU/L) and glucose mg/dL) taken at baseline and 2 hours during OGTT.

Homeostasis model assessment-insulin resistance (HOMA-IR) and β-cell activity (HOMA-β) were calculated based on fasting levels only using the following two formula [11]:

$$\text{HOMA-IR} = \text{Fasting insulin (mU/L)} \times \text{Fasting glucose (mmol/L)} / 22.5$$

$$\beta\text{-cell function (\%)} = 20 \times \text{Fasting insulin(mU/L)} / [\text{Fasting glucose (mmol/L)} - 3.5]$$

Statistics

The study was designed to detect mainly the difference between the baseline visit and other visits. Results are expressed as mean± SD or otherwise indicated. Data were transformed using natural logarithm (Ln) to approximate a normal distribution if necessary. Comparisons between means were performed using Paired Student's *t*-test at 95% confidence interval. Repeated measures one way ANOVA was used to compare means between different visits for the same individuals. Mauchly's test statistic was evaluated for sphericity violation before concluding if there are significant differences between the variance of differences. P values <0.05 were considered significant. SPSS software (version 24) was used for statistical analysis.

RESULTS

All 10 male subjects were from a Saudi population and had a normal glucose tolerance (NGT). The age and body mass index (BMI) and other anthropometric measurements are shown in **Table 1**. Subjects were all under 50 years of age, and either of normal BMI, or overweight.

Changes in different biochemical parameters before and after blood donation are shown in **Table 2**.

Glucose and insulin resistance

Compared to the baseline levels, fasting plasma glucose (5.1 ± 0.6 mmol/L) was significantly lower (4.9 ± 0.5 mmol/L; $p < 0.05$) at visit B **(Table 2)**. Using one way repeated ANOVA, fasting glucose changes from A to D visits were not significant; $F = 1.03$, $p = 0.351$. Changes in post load (2 hours) plasma glucose levels were not significantly different from the baseline throughout the study. Although fasting insulin increased by approximately 10.3% at visit B (10.7 ± 4.0 μIU/mL) compared to the baseline level (9.7 ± 4.5 μIU/mL), this failed to reach statistical significance. There was a negative, but statistically insignificant, effect on the index of insulin resistance (HOMA-IR) at visit D, (-13.8%, $p = 0.16$) when compared to the baseline level. The insulin sensitivity index of ISI-gly increased following plateletpheresis by +5.8% at visit B but this increase was not significant ($p = 0.43$). At visit D, the ISI-gly returned almost to the baseline level.

Glycated haemoglobin (HbA1c)

HbA1c fell in almost all subjects at visit D with a maximum reduction of -5.8%, increasing by +1.7 % in one subject only **(Figure 1)**, and the mean level was significantly lower (5.37% ± 0.38, p<0.01) when compared to the baseline level (5.48% ± 0.32) **(Figure 2, A).** Moreover, the difference across the study period was highly significant; using repeated ANOVA; *p*= 0.008, *F* = 4.99 **(Table 2)**.

Lipid profile

Table 2 Cholesterol fell at visit B by -3.9% (p<0.05), and continued throughout visit C (-10.2%, p = 0.15) and visit D (-1.8%, p = 0.67). However, repeated ANOVA was not significant (F = 1.35, p =0.281). Similar trend of reduced levels was noted in LDL-C test at visit B (-9.2%, p<0.05), C (-19.8%, p = 0.21) and D (-4.5%, p = 0.19), and similarly repeated ANOVA did not show a significant effect. Serum triglycerides showed a trend in the opposite direction, with levels increasing throughout the visits of B (23.9%, P<0.05), C (28.3% P = 0.34) and D (1.9%, p= 0.88), but with no significant differences using repeated ANOVA (F = 1.01, p = 0.36). The level of HDL-C decreased significantly at visit B, (-5.5%, p<0.01) but not at visit C (-5.5%, p = 0.19) or D (2.4%, p = 0.33) when compared to the baseline level. However, using repeated ANOVA, the changes in HDL-C levels were not significant, F = 3.06, p = 0.075.

Iron profile

Iron levels fluctuated throughout the study, showing an increase at visits B (19.5%), and D (12.1%), but not C (-7.5%) when compared to the baseline level (visit A). Differences

throughout different visits were not significant using repeated ANOVA ($F = 2.11$, $p = 0.12$).

Serum transferrin was significantly lower at visit B (-2.7%, $p<0.05$) but not C (-4.6%, $p= 0.12$) or D (2.3%, $p=0.32$).

Following plateletpheresis iron stores which were assessed using serum ferritin, gradually decreased throughout different visits (B, -3.2%, $p = 0.65$; C, -9.6%, $p = 0.44$; D, -18.1%, $p <0.05$) **(Figure 2, B).** However, there were no significant changes using repeated ANOVA ($F = 2.16$, $p = 0.12$).

Other indices

After plateletpheresis, there was no change in levels of serum uric acid at any of the visits **(Table 2)**. The inflammatory marker, CRP, increased at visit B (27.1%) and C (40.9%). At visit D the level became fell to near the baseline level (-9.1%). CRP was not significantly different using repeated ANOVA ($F = 0.67$, $p = 0.44$).

DISCUSSION

Previous studies on the changes in biochemical parameters after plateletpheresis in healthy individuals are limited; our findings show some potentially important effects. A reduction in iron stores after repeated plateletpheresis in males and females, and a correlation between iron depletion and frequency of pheresis donation in male donors has been noted previously [12]. However, this was done by comparing stores in donors and non-donors, not by investigating the effect on the individual donors. In another study carried out on Japanese donors, serum ferritin levels were found to fall with donation-frequency in female donors, [13]. In our study on first time male donors there was a

gradual decrease in iron stores, measured by ferritin level, following plateletpheresis over the study period, with statistical significance being noted at visit D. However, no values were found outside the reference range. This is the first report in literature of decreased iron stores following one single donation. Difference between our results and that of other studies could be due to difference in research design, as well as differences in studied population.

The effect of plateletpheresis on glucose homeostasis and glycemic control is difficult to interpret. Levels of glucose (fasting and post glucose load) fluctuated throughout the study, with significantly ($p < 0.05$) decreased fasting levels noted only at visit B, coinciding with non significant increases in fasting insulin level (10.3%), β-cell activity (42.9%), and ISI-gly (5.8%). In addition, a significant effect was noted on A1c, with levels at visit D being significantly lower ($p < 0.01$) than at visit A, coinciding with a non significant decrease in the index of insulin resistance (HOMA- IR) of 13.8.

The non-significant changes in insulin resistance across different visits could be due to the limitation of the simple methods (i.e HOMA-IR and ISI-gly) utilized in this study and/or the small sample size. The use of reference methods such as euglycemic clamp or frequently sampled intravenous glucose tolerance (FSIVGT) might be more sensitive to detect such changes.

The overall decrease in the mean of HbA1c percentage over the study period suggests an improvement in one of the main diagnostic test of diabetes. A decrease in HbA1c with a maximal reduction at the fourth week following whole blood donation was obtained by Starkman et al., [14]. However, this is the first time to show that, not only whole blood

donation but even plateletpheresis can reduce HbA1c towards a lower level. Such an effect was expected after whole blood donation but not after plateletpheresis because the amount of whole blood lost in this process is limited (80 to 100 mL) compared to whole blood donation (450 mL). The possible reason behind this decrease is unclear but it could be due to other artefactual effects. For example, the status of RBCs viability, integrity and lysis might be affected by the process of cell separation and citrate addition (as inhibitor) before the RBCs are returned to the donor. If the old RBCs are affected by such factors then the new released RBCs to the circulation will be for the purpose of selective removal of the old ones. Unfortunately no previous study has discussed such effects as this may need further investigations. Another possible reason is the mobilization effect of plateletpheresis [15, 16] on different blood components including RBCs not only the platelet.

To interpret the value of the change in A1c, the effect of reference change values (RCV) must be discussed. RCV is defined as the critically significant changes between two results. It is important for determining whether apparent changes in serial results reflect real changes or can be accounted for by biological variation alone [17]. RCV of ±5.4% was previously reported for HbA1c in healthy individuals using the same HPLC method but without any intervention [18]. However, the RCV in our population was not determined, and despite the statistically significant decrease in HbA1c at visit D in our participants, the total change in percentage compared to baseline was only approximately -2.0%, hence does not exceed the previously reported RCV of ±5.4%.

Changes in HbA1c in each involved participant did not exceed the limit of RCV of ±5.4% except in one subject where the change was -5.8%. Variations in HbA1c between different individuals after whole blood donation was investigated before and it was

concluded that it may depend on the effectiveness of erythropoiesis which subsequently depends on sufficient iron stores [19]. Therefore, the same conclusion may be applied on HbA1c variability between different subjects after plateletpheresis. Whether our findings of a decrease in HbA1c reflects a real effect of plateletpheresis in reducing HbA1c levels in non-diabetic individuals is worthy of further investigation, especially in type 2 diabetic donors. Our findings may also suggest that lower ferritin levels at visit D compared to baseline could contribute to enhanced insulin sensitivity for HOMA-IR. This observation is in line with the previously published results about whole blood donation [20]. Hence, it could be suggested that platletpheresis may increase the removal of free transition metals, including iron from the body. This may lead to a reduction in the oxidation susceptibility of end products of serum glycated proteins, which are thought to have potential toxic effects resulting in increased insulin resistance [21, 22]. However, if plateletpheresis is associated with the selective removal of old RBCs, due to the reasons suggested above, then this may lead to the mobilization of iron stores to make new RBCs.

At the first visit 24 hours after plateletpheresis (visit B), total cholesterol, and its estimated fractions were significantly decreased, in contrast to triglycerides which was significantly increased. Such changes may be important to consider if the patient is being treated with lipid lowering agents. In addition, the medical assessment of new donors recruited to platletpheresis program should include a venous blood sample taken prior to initial donation. This trend of decrease in blood cholesterol and LDL-C after plateletpheresis is consistent with the control subjects involved in Houschyar et.al, study [6]. As triglycerides level is diet dependent then the increased level can be related to the timing of the last meal of each individual subject.

When the RCV principle is applied to lipid parameters using the same methods and auto-analyser (Architect c8000), the RCVs for glucose, total cholesterol, triglyceride and HDL-C and were reported as ±14.63%, ±14.19%, ±46.62% and ±20.51% respectively [23]. Based on these values, changes in the analytes were not considered clinically significant.

After plateletpheresis, no significant changes were observed for serum urate. Furthermore, although a progressive increase in serum CRP was noticed after plateletpheresis for up to 8 days, the difference was not significant from the baseline level. This increased level of CRP could be due to the induced citrate solution during the process of platletpheresis, but this point may need further investigation.

One of the main limitations of the present study is the relatively small number of involved subjects. However, the numbers were sufficient to detect changes in this pilot study, and to stimulate further studies. The second limitation was the use of simple surrogate indices of insulin resistance only, which are appropriate for preliminary investigations but they do suffer from some important limitations that have been discussed previously [24, 25].

In summary, although changes in glycaemic control, serum lipids and iron stores are not likely to be clinically significant, they do suggest that there needs to be a careful interpretation of the blood test results of individuals after plateletpheresis. Such potential changes can be detected at 1 and 22 days after donation. Future larger studies with more

than one episode of platelet donation may show a significant clinical change for the same parameters

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Declaration of interest:

The authors report no conflict of interest.

Figure 1. HbA1c changes from the baseline (visit A) until day 22 post plateletpheresis (visit D). Horizontal line represents each of 10 individual subjects. Vertical line is the HbA1c level (%). Each bar represents 95% confidence interval (CI). Circle in the middle of the bar is the mean of HbA1c obtained from the four visits (A, B, C and D). The number on top of each bar represents the difference in percentage between HbA1c level at visit A when compared to visit D for this particular subject.

Figure 2. Changes in the mean of both tests, HbA1c (Fig. A) and ferritin (Fig. B) before (visit A) and after (visits B, C and D) plateletpheresis. *$p<0.05$, **$p<0.01$ when compared to the baseline level (visit A). Bars represent 95%CI.

REFERENCES

1. Estcourt LJ. Why has demand for platelet components increased? A review. Transfus Med 2014; 24:260-8 (PMID: 25327286).

2. Amrein K, Katschnig C, Sipurzynski S, et al. Apheresis affects bone and mineral metabolism. Bone 2010; 46:789-95 (PMID: 19922822).

3. Chen Y, Lin Y, Lin H, et al. Regular plateletpheresis increased basal concentrations of soluble P-selectin in healthy donors: Possible involvement of endothelial cell activation? Clin Chim Acta 2016; 458:18-22 (PMID: 27108199).

4. Pontes TB, Moreira-Nunes Cde F, Maues JH, et al. The miRNA Profile of Platelets Stored in a Blood Bank and Its Relation to Cellular Damage from Storage. PloS one 2015; 10:e0129399 (PMID: 26121269).

5. Beth H. Shaz CDH, Mikhail Roshal, Charles S. Abrams. Transfusion Medicine and Hemostasis: Clinical and Laboratory Aspects. 2013:46. https://www.elsevier.com/books/transfusion-medicine-and-hemostasis/shaz/978-0-12-397164-7

6. Houschyar KS, Ludtke R, Dobos GJ, et al. Effects of phlebotomy-induced reduction of body iron stores on metabolic syndrome: results from a randomized clinical trial. BMC Med 2012; 10:54 (PMID: 22647517).

7. Cagnacci A, Paoletti AM, Renzi A, et al. Glucose metabolism and insulin resistance in women with polycystic ovary syndrome during therapy with oral contraceptives containing cyproterone acetate or desogestrel. J Clin Endocrinol Metab 2003; 88:3621-5 (PMID: 12915645).

8. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998; 15:539-53 (PMID: 9686693).

9. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18:499-502 (PMID: 4337382).

10. Belfiore F, Iannello S, Camuto M, Fagone S, Cavaleri A. Insulin sensitivity of blood glucose versus insulin sensitivity of blood free fatty acids in normal, obese, and obese-diabetic subjects. Metabolism 2001; 50:573-82 (PMID: 11319720).

11. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28:412-9 (PMID: 3899825).

12. Page EA, Coppock JE, Harrison JF. Study of iron stores in regular plateletpheresis donors. Transfus Med 2010; 20:22-9 (PMID: 19903323).

13. Furuta M, Shimizu T, Mizuno S, et al. Clinical evaluation of repeat apheresis donors in Japan. Vox Sang 1999; 77:17-23 (PMID: 10474086).

14. Starkman HS, Wacks M, Soeldner JS, Kim A. Effect of acute blood loss on glycosylated hemoglobin determinations in normal subjects. Diabetes care 1983; 6:291-4 (PMID: 6872811).

15. Gyongyossy-Issa MI, Miranda J, Devine DV. Generation of reticulated platelets in response to whole blood donation or plateletpheresis. Transfusion 2001; 41:1234-40 (PMID: 11606821).

16. H G Heuft LG, J Martens, A Schmitt-Thomssen, R Blasczyk. The mobilization Effect of Plateletpheresis Transfusion 2006; 46:6A. https://www.researchgate.net/publication/295288123_The_mobilization_effect_of_plateletpheresis

17. C.G. Fraser. Biological Variation: From Principles to Practice, AACC press,Washington,

DC. Aacc press 2001:1–145. https://books.google.com.sa/books?hl=en&lr=&id=1YsDPMABuwEC&oi=fnd&pg=PR11&dq=Biological+Variation:+From+Principles+to+Practice+AACC&ots=Fg3jNZCwnk&sig=StLTsugU1G0M-8KwgP21ifXT_YM&redir_esc=y#v=onepage&q=Biological%20Variation%3A%20From%20Principles%20to%20Practice%20AACC&f=false

18. Ucar F, Erden G, Ginis Z, et al. Estimation of biological variation and reference change value of glycated hemoglobin (HbA(1c)) when two analytical methods are used. Clin Biochem 2013; 46:1548-53 (PMID: 23732479).

19. Dijkstra A, Lenters-Westra E, de Kort W, et al. Whole Blood Donation Affects the Interpretation of Hemoglobin A1c. PloS one 2017; 12:e0170802 (PMID: 28118412).

20. Fernandez-Real JM, Penarroja G, Castro A, Garcia-Bragado F, Hernandez-Aguado I, Ricart W. Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and beta-cell function. Diabetes 2002; 51:1000-4 (PMID: 11916918).

21. Qian M, Liu M, Eaton JW. Transition metals bind to glycated proteins forming redox active "glycochelates": implications for the pathogenesis of certain diabetic complications. Biochem Biophys Res Commun 1998; 250:385-9 (PMID: 9753639).

22. Simcox JA, McClain DA. Iron and diabetes risk. Cell Metab 2013; 17:329-41 (PMID: 23473030).

23. Bugdayci G, Oguzman H, Arattan HY, Sasmaz G. The use of reference change values in clinical laboratories. Clin Lab 2015; 61:251-7 (PMID: 25974990).

24. Borai A, Livingstone C, Kaddam I, Ferns G. Selection of the appropriate method for the assessment of insulin resistance. BMC Med Res Methodol 2011; 11:158 (PMID: 22112229).

25. Borai A, Livingstone C, Ferns GA. The biochemical assessment of insulin resistance. Ann Clin Biochem 2007; 44:324-42 (PMID: 17594780).