A Study of some physiological changes in patients with inherited thrombosthenia

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الخلاصة:

طريقة العمل: شملت هذه الدراسة ٣٠ (١٥ذكر و١٥ أنثى) مريضا و الذين تم إدخالهم إلى مركز نزف الدم الوراثي في مستشفى بابل للولادة والأطفال في بابل ومستشفى الزهراء للولادة والأطفال في النجف. يتراوح أعمار هؤلاء المرضى من ٣-٤ ١٨ سنة (معدل اعمار هم ١١±٥ سنة). تم تقسيم هؤلاء المرضى إلى مجموعتين حسب العمر كالآتي: المجموعة الأولى تتراوح أعمار هم بين ٣-٤٠ سنة؛ و المجموعة الثانية تتراوح أعمار هم بين ١٠-١٨ سنة وكل مجموعة عمرية تم تقسيمها إلى فئتين وهي فئة الذكور وفئة الإناث. وشملت هذه الدراسة على ٣٠ (١٠

النتائج: تميز المرضى المصابين فيما يخص المتغيرات الدموية بانخفاض معنوي لكل من خضاب الدم(Hb) وحجم الخلايا المضغوط (PCV) ومحتويات خلية الدم الحمراء (MCHC, MCH, MCV) في معظم المرضى مقارنة مع مجاميع السيطرة.

فيما يتعلق بالمتغيرات الكيموحيوية فقد لوحظ هناك انخفاض معنوي في قيمة الحديد المصلي(serum iron) وخزين الحديد المصلي (serum ferritin) بينما توجد زيادة معنوية في قيمة سعة الحديد الكلية في مصل الدم (serum TIBC) في معظم المرضى مقارنة مع مجاميع السيطرة.

الاستنتاجات: إن التغيرات الحاصلة في المعايير الدموية و الكيميوحيوية الحاصلة لمرضى وهن الصفيحات الدموية الوراثي يمكن أن تعزى بصورة رئيسية إلى نزف الدم المتكرر وهي احد العلامات الرئيسية للمرض.

Abstract

Aim: This study was designated to investigate the changes of some hematological and biochemical parameters in patients affected with hereditary thrombosthenia.

Method: The study included 30 patients (males & females) with hereditary thrombosthenia who admitted to the hereditary bleeding disorder center at Babylon maternity and pediatrics teaching hospital in Hilla city and at the hereditary bleeding disorder center in AL-Zahraa maternity and pediatrics teaching hospital in AL-Najaf city. Their age was ranged $3-\le 18$ years (mean age 11 years \pm SE 5 years). The patients were classified into 2 groups according to the age which are group I ($3-\le 10$ years) and group II ($10-\le 18$ years) and each age group was subdivided into males and females subgroups. Thirty apparently healthy subjects (15 males and 15 females) of matched age and gender were also included in this study.

Results: Concerning the hematological parameters, it was found that hemoglobin (Hb), packed corpuscular volume(PCV), means corpuscular volume (MCV), means corpuscular hemoglobin (MCH), and means corpuscular hemoglobin concentration (MCHC) show significant decrease in most groups of hereditary thrombosthenia in comparison with the control groups.

Regarding the biochemical parameters, it was found that serum iron and serum ferritin are significantly decreased and serum total iron binding capacity(TIBC) are significantly increased in most groups of hereditary thrombosthenia compared to the control groups.

Conclusion: In view of the changes summarized, the increase or decrease in some hematological and biochemical parameters may be attributed to recurrent blood loss which is the most important manifestation of the diseases.

Introduction

Thrombosthenia is the functional impairment of platelets leading to bleeding⁽¹⁾. Thrombosthenia can be classified into hereditary thrombosthenia and acquired type. The hereditary thrombosthenia includes: Bernard Soliuer syndrome and Glanzmann thrombosthenia(GT). The aquired thrombosthenia includes: stem cell disorders, drugs induced like indumethacin and midazole, dysproteinaemia like multiple myeloma and macroglobinaemia, uraemia and miscellaneous like antibodies, DIC, post-transfusion etc.^(2,3)

Bernard Soliuer syndrome also called hemorrhagiparous thrombocytic dystrophy, is a rare autosomal recessive bleeding disorder that causes a deficiency of platelet glycoprotein Ib (GpIb), the receptor for von willbrand factor, which is important in clot formation.^(1,4) It is characterized by prolonged bleeding time, thrombocytopenia, giant platelets, and decreased platelet survival. Children with thrombasthenia may have purpura, epistaxis, gingival bleeding, GI bleeding, and menorrhagia.^(4,5)

Glanzmann thrombosthenia(GT) is an autosomal recessive disorder and heterozygous individuals are asymptomatic. It is caused by an abnormality of the platelets.^(2,3) It is an extremely rare coagulopathy, in which the platelets lack glycoprotein IIb/IIIa. Hence, no fibrinogen bridging can occur, and bleeding time is significantly prolonged. Patients suffering from Glanzmann's thrombasthenia thus have platelets less able to adhere to each other and to the underlying tissue of damaged blood vessels.⁽⁶⁾

Patients with GT are classified as having type 1, type 2, or variant type based on the degree of GP IIb-IIIa deficiency, fibrinogen binding, and clot retraction.⁽⁷⁾ Patients with type 1, the most severe form of the disease, have less than 5% of the normal amount of GP IIb-IIIa present on their platelets. Additionally, they have absent fibringen binding and clot retraction. Individuals with type 2 have 10-20% of GP IIb-IIIa, can bind fibrinogen, and have normal-to-moderately deficient clot retraction capability. Persons with the variant type of thrombasthenia have more than 50% of the normal amount of GPIIb-IIIa; however, fibrinogen binding and clot retraction widely vary ^(2,5). Patients with GT are typically diagnosed in infancy or early childhood. However, age of diagnosis can range from birth to adulthood. Neonatal purpura typically suggests type 1 thrombasthenia. Epistaxis and GI bleeding are frequent presenting signs of GT and are more severe in children, especially those aged 4-10 years. Menorrhagia may be a presenting sign of GT in adolescent females and can be a critical problem. The severity and frequency of bleeding usually decreases with age.⁽⁸⁾ Severe menorrhagia is a common problem that requires careful observation and treatment with oral contraceptive pills. It is usually associated with an excessively proliferative endometrium that reflects estrogen dominance.⁽⁹⁾

This study aims to estimate some hematological and biochemical changes among hereditary thrombosthenic patients. The parameters studied include PCV, Hb, MCV, MCH, MCH, serum iron, serum total iron binding capacity and serum ferritin.

Materials and Methods

This study was carried out over 6 months in the hereditary bleeding disorder center at Babylon maternity and pediatrics teaching hospital in Hilla city and at the hereditary bleeding disorder center in AL-Zahraa maternity and pediatrics teaching hospital in AL-Najaf city. The study included 30 patients (mean age 11 years \pm SE 5 years)affected with hereditary thrombosthenia.The patients are classified into 2 groups according to the age which are group I (3- \leq 10 years) and group II (10- \leq 18 years) and each age group

was subdivided into males and females subgroups.^(10,11) Thirty healthy controls(15males and 15 females) of matched age and gender were also included in this study.

The collection of blood was done in the hereditary bleeding disorder centers at 9 A.M. Eight ml of blood are drawn for each hematological and biochemical studies. Two groups of labeled tubes were used; the first tubes contain EDTA as anti- coagulants to prevent clotting of blood to be used for hematological studies. The second group tubes were without anti-coagulant as plain tubes, for blood to be used for preparing sera for subsequent biochemical tests.⁽¹²⁾ Each sample was labeled and given a serial number together with the patient name. The serum samples were kept frozen at -20°C for biochemical analysis.⁽¹³⁾

A cyanomethemoglobin method was used to estimate the hemoglobin contents of the blood. The method was based on Drabkins cyanide- ferriccyanide solution.⁽¹⁴⁾

Microhematocrit method was used to determine PCV. Heparinized capillary tubes used. $^{\rm (13)}$

Regarding the measurement of RBCs indices which include the following:-

i) The mean corpuscular volume(MCV)was calculated as the following:

Packed corpuscular volume

ii) The mean corpuscular hemoglobin (MCH) was calculated as the following : Hemoglobin in g/dL

MCH = _____ = picogram (pg)

RBCs count* 10¹²

iii) The mean corpuscular hemoglobin concentration (MCHC) was calculated as following :

Hemoglobin gm/dL

$$MCHC = \frac{gm/dL \ RBCs^{(13)}}{PCV}$$

The determination of serum iron was according to procedure recommended by the serum iron from Biomaghreb company, Tunis. Where the iron dissociated from transferrin-iron complex by a solution of guandine acetate and reduced by ascorbic acid reacts with ferrozine to give a pink complex. The intensity of the color was measured photometrically by using spectrophotometer at 562 nm wave length.⁽¹⁵⁾

The determination of serum total iron binding capacity(TIBC) was according to procedure recommended by the serum total iron binding capacity from Biomaghreb company, Tunis. Where an excess of iron is added to the serum to saturate the transferrin. The unbound iron is precipitated with basic magnesium carbonate.⁽¹⁶⁾

The measurement of serum ferritin quantitative test is based on a solid phase enzyme linked immunosorbent assay (ELISA). The concentration of ferritin is directly proportional to the color intensity of the test sample (according to procedure recommended by the serum ferritin from Human company,Germany).⁽¹⁷⁾

SPSS program was used in this study. All values were expressed as mean \pm standard error (SE). Independent t-test was used to estimate differences between groups. The differences were considered significant when the probability (P) was less than 0.05 (P<0.05) and highly significant when the probability (P) was less than 0.01 (P<0.01).⁽¹⁸⁾

The results:-

(1):-Hematological studies:-

(1-1):- Level of hemoglobin (Hb) in blood:-

The mean and standard error of Hb for males subgroup of group I was: $10.32 \pm 0.46489 \text{ g/dL}$ and it was significantly (P<0.01) lower than controls ($12.175 \pm 0.14608 \text{ g/dL}$) as well as the mean and standard error of Hb for females subgroups of group I was: $9.674\pm0.8793 \text{ g/dL}$ and it was significantly (P<0.01) lower than controls ($12.0\pm0.22136 \text{ g/dL}$) (figure 1).



Figure 1: The means and standard errors of hemoglobin for males and females subgroups of group I of hereditary thrombosthenic patients and controls. -****P**<**0.01**

The mean and standard error of Hb for males subgroup of group II was: 12.452 ± 0.983 g/dL and it was insignificantly (P>0.05) lower than controls(12.72 ± 0.21746 g/dL) as well as the mean and standard error of Hb for females subgroups of group II was: 8.932 ± 0.0658 g/dL and it was significantly (P<0.01) lower than controls(11.982 ± 0.8932 g/dL) (figure 2).



Figure 2: The means and standard errors of hemoglobin for males and females subgroups of group II of hereditary thrombosthenic patients and controls. -****P**<**0.01**

(1-2):-Packed corpuscular volume (PCV):-

The mean and standard error of PCV for males subgroup of group I was: 31.282 ± 0.6723 and it was significantly (P<0.01) lower than controls(38.56 \pm 0.6054) as well as the mean and standard error of PCV for females subgroups of group I was: 28.345 ± 0.265 and it was significantly (P<0.01) lower than controls(37.21 ± 0.289) (figure 3).



Figure 3: The means and standard errors of packed cells volume for males and females subgroups of group I of hereditary thrombosthenic patients and controls. -****P**<**0.01**

The mean and standard error of Hb for males subgroup of group II was: 39.121 ± 0.2032 L/L and it was significantly (P>0.05) lower than controls(39.32 ± 0.0834 L/L) as well as the mean and standard error of Hb for females subgroups of group II was: 28.673 ± 0.0153 L/L and it was significantly (P<0.01) lower than controls(35.945 ± 0.0342 L/L) (figure 4).



Figure 4: The means and standard errors of packed cells volume for males and females subgroups of group II of hereditary thrombosthenic patients and controls. -****P**<**0.01**

(1-3):-The mean corpuscular volume(MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC):-

The mean and standard error of MCV for males subgroup of group I was: 72.523 \pm 2.6726 fL and it was significantly (P<0.01) lower than controls (81.987 \pm 1.22379 fL) and the means and standard errors of MCH and MCHC for males subgroup of group I was: 24.35 \pm 0.80132 pg and 30.04 \pm 0.77486 mg/dL respectively and they were significantly (P<0.05) lower than controls (26.85 \pm 0.4532 pg and 32.07 \pm 0.3104 mg/dL respectively) as well as The mean and standard error of MCV, MCH and MCHC for females subgroup of group I were: 76.56 \pm 3.432 fL, 22.98 \pm 0.84758 pg and 29.986 \pm 1.11786 and they were significantly (P<0.05) lower than controls (P<0.05) lower than controls (P<0.05) lower than controls (9.05) low



Figure 5: The means and standard errors of MCV, MCH and MGHC for males and females subgroups of group I of hereditary thrombosthenic patients and controls. -*P<0.05 -**P<0.0

The mean and standard error of MCV, MCH and MCHC for males subgroup of group II were: 80. 66 ± 1.05195 fL, 26.28 ± 1.11597 pg and 32.06 ± 0.68088 mg/dL respectively and they were insignificantly (P>0.05) lower than controls (80.99 ± 0.68602 fL, 26.3 ± 0.71809 pg and 32.2 ± 0.50271 mg/dL respectively) as well as The mean and standard error of MCV, MCH and MCHC for females subgroup of group II were: 70.62 ± 2.27978 fL, 21.6 ± 0.625 pg and 28.25 ± 0.67480 mg/dL and they were significantly (P<0.01) lower than controls(79.8 ± 0.55710 fL, 26.7 ± 0.70532 pg and 32.12 ± 0.29806 mg/dL respectively) (figure 6).



Figure 6: The means and standard errors of MCV, MCH and MGHC for males and females subgroups of group II of hereditary thrombosthenic patients and controls. -****P**<**0.01**

(2):-Biochemical studies:-

(2-1):-Serum iron:-

The mean and standard error of serum iron for males subgroup of group I was: $15.345 \pm 0.2648 \ \mu mol/L$ and it was significantly (P<0.01) lower than controls(21.3402 $\pm 0.398 \ \mu mol/L$) as well as the mean and standard error of serum iron for females subgroups of group I was: $16.254 \pm 0.9234 \ \mu mol/L$ and it was significantly (P<0.01) lower than controls(20.992 $\pm 0.3156 \ \mu mol/L$) (figure 7).



Figure 7: The means and standard errors of serum iron for males and females subgroups of group I of hereditary thrombosthenic patients and controls. -****P**<**0.01**

The mean and standard error of serum iron for males subgroup of group II was: 19.698 $\pm 0.982 \ \mu mol/L$ and it was insignificantly (P>0.05) lower than controls (20.213 $\pm 0.983 \ \mu mol/L$) as well as the mean and standard error of serum iron for females subgroups of group II was: 12.754 $\pm 1.0376 \ \mu mol/L$ and it was significantly (P<0.01) lower than controls (18.943 $\pm 0.5636 \ \mu mol/L$) (figure 8).



Figure 8: The means and standard errors of serum iron for males and females subgroups of group II of hereditary thrombosthenic patients and controls. -****P**<**0.01**

(2-2):-Serum total iron binding capacity(TIBC):-

The mean and standard error of serum TIBC for females subgroup of group I was: 88.032 \pm 3.245 µmol/L and it was significantly (P<0.01) higher than controls(59.365 \pm 1.349 µmol/L) as well as the mean and standard error of serum iron for females subgroups of group I was: 96.143 \pm 4.254 µmol/L and it was significantly (P<0.01) higher than controls (63.195 \pm 3.398 µmol/L) (figure 9).



Figure 9: The means and standard errors of serum TIBC for males and females subgroups of group I of hereditary thrombosthenic patients and controls. -****P**<**0.01**

The mean and standard error of serum TIBC for males subgroup of group II was: 54.287 $\pm 2.187 \mu mol/L$ and it was insignificantly (P>0.05) higher than controls (51.984 \pm 3.163 $\mu mol/L$) as well as the mean and standard error of serum TIBC for females subgroups of group II was: 99.193 \pm 1.0376 $\mu mol/L$ and it was significantly (P<0.01) higher than controls (69.153 \pm 2.187 $\mu mol/L$) (figure 10).



Figure 10: The means and standard errors of serum TIBC for males and females subgroups of group II of hereditary thrombosthenic patients and controls. -****P**<**0.01**

(2-3):-Serum ferritin:-

The mean and standard error of serum ferritin for females subgroup of group I was: 17.451 ± 4.0923 ng/mL and it was significantly (P<0.01) lower than controls(48.231 \pm 5.183 ng/mL) as well as the mean and standard error of serum ferritin for females subgroups of group I was: 14.325 ± 4.284 ng/mL and it was significantly (P<0.01) lower than controls(39.285 ± 3.162 ng/mL)(figure 11).



Figure 11: The means and standard errors of serum ferritin for males and females subgroups of group I of hereditary thrombosthenic patients and controls. -**P<0.01

The mean and standard error of serum ferritin for males subgroup of group II was: 47.592 ± 2.376 ng/mL and it was insignificantly (P>0.05) lower than controls (49.810 ± 2.820 ng/mL) as well as the mean and standard error of serum ferritin for females subgroup of group II was: 13.9508 ± 2.1054 ng/mL and it was significantly (P<0.01) lower than controls (42.471 ± 2.436 ng/mL) (figure 12).



Figure 12: The means and standard errors of serum ferritin for males and females subgroups of group II of hereditary thrombosthenic patients and controls. -****P**<**0.01**

Discussion:-

The present study investigated the relationship between the hereditary thrombosthenia which associated with recurrent bleeding episodes and some hematological and biochemical parameters. Regarding group I of males and females subgroups, it was found that the majority of the patients had significant decrease in hematological parameters (Hb, PCV, MCV, MCH and MCHC). These results are in agreement with other study.⁽¹⁹⁾

Sajid *et al.*, (²⁰⁾ showed that the patient with inherited bleeding disorder are liable for recurrent bleeding episodes even with trivial trauma which may leads to prolonged bleeding and subsequently leads to anemia especially if there is no replacement therapy for this bleeding. The MCV is an extremely useful value in classification of anemia, but the MCH and MCHC often do not add significant, clinically relevant information. ^(21,22) However, the MCH and MCHC play an important role in laboratory quality control because these values will remain stable for a given specimen over time. ⁽²³⁾ Both microcytosis and hypochromia are sensitive indicators of iron deficiency in the absence of chronic disease or co-existent B12 or folate deficiency .⁽²⁴⁾ Microcytosis and hypochromia are also present in some cases of anemia of chronic disease.

Moreover, Provan & O'Shaughnessy,(1999) ⁽²⁵⁾ recorded that the thrombosthenic patients may have decrease in MCV, MCH, MCHC which may indicate that those patients may have iron deficiency anemia which attributed to recurrent bleeding especially in the severe form of the disease.

Regarding the serum iron, serum TIBC and serum ferritin, majority of the patients had significant decrease in serum iron and serum ferritin and significant increase in serum

TIBC. These results may suggest that those patients have iron deficiency anemia and these results are in agreement with other study.⁽²⁵⁾

Kouides, (2008)⁽²⁶⁾ suggested that, those patients may have iron deficiency anemia due to frequent blood loss for example frequent epistaxis. The serum markers of iron deficiency are low ferritin, low iron, raised total iron binding capacity, raised red cell protoporhyrin, and increased transferrin binding receptors (sTfR). Serum ferritin is the most powerful test for iron deficiency.^(27,28)

Regarding group II of females subgroups, it was found that the majority of the patients had significant decrease in hematological parameters (Hb, PCV, MCV, MCH and MCHC) and significant decrease in serum iron and serum ferritin and significant increase of serum TIBC. These results may suggest that those patients may have iron deficiency anemia and the results of our study are in agreement with other studies. ^(8,29,30) which state that iron deficiency anemia is frequently encountered in patients with thrombosthenic females after puberty because of menorrhagia which makes the patients loss large amount of blood monthly because of defect in the primary hemostasis.

Kouides, (2008)⁽²⁰⁾ explained that the iron deficiency anemia is the usual complication of thrombosthenia especially if the patients not compensated for blood loss because those patients are liable for frequent bleeding episode especially female after puberty.

Regarding group II of males subgroups, it was found that the majority of the patients had insignificant decrease in hematological parameters (Hb, PCV, MCV, MCH and MCHC) and insignificant changes in biochemical parameters (serum iron and serum ferritin and serum TIBC). These results occurred because the patients had mild form of the disease and these result are supported by other studies. ^(5,31).

Conclusion:-

Chronic and recurrent blood loss especially females after menarche considered the main causes for changing in our results regarding the hematological and biochemical parameters (PCV, Hb, MCV,MCH,MCHC, serum iron, serum TIBC and serum ferritin).

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