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Study of Some Hematological Parameters in Offpring of Heperlipidemic Female Laboratory Rats After Slmvastatln Treatment During Lactation

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Abstract

The present study was designed to evaluate some of the haematological parameters in offspring of hyperlpidemic female rats after simvastatin treatment during lactation. Twenty four female rat were used in the current study were matted with fertile males, twelve of females are induced for hypercholesterolemia during pregnancy, other twelve were not, all animals were divided into 4 equal groups after delivery: **G**.1 (Control) normal animals given only 0.5 ml/kg animal of Dimethyl Sulphoxide (DMSO). **G**.2 hypercholestrolemic animals given only 0.5 ml/ animal of Dimethyl Sulphoxide (DMSO). **G**.3 (S1) hypercholestrolemic animals treated with 20 mg/kg/day (0.02ml/ animal) of simvastatine . **G**.4 normal animals treated with 20 mg/kg/day (0.02ml/ animal) of simvastatine. The treatment of mothers were continued for 30 days after delivery. Offspring (only the males)of these animals were sacrificed at their puberty and blood samples were collected in EDTA tubes for measuring of some hematological parameters. The present study revealed that a significantly decreased($P \leq 0.05$) of RBC count, hemoglobin concentration (Hb), platelets count and MPV levels in offspring (male and female) of G3 and G4 groups treated with simvastatin , except RBC count in G3 were not significantly differ compare with control group. Treatment with statins significantly reduce of some hematological parameters such as RBC, Hb, platelets and MPV in offspring of females rat that treated with these drugs during lactation irrespective of cholesterol levels.

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دراسة بعض المعايير الدمية في مواليد الجرذان البيضاء من امهات المفرطة الكوليستيرول بعد معاملتها بالسمفاستاتين اثناء فترة الرضاعة.

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الخلاصة

تعد المعايير الدموية والمصلية من الشروط الاساسية لمعرفة وظائف الجسم الطبيعية وللتأكد من الطبيعة الغير سمية للمواد الدوائية. صممت الدراسة الحالية لتحديد بعض المعايير الدموية في مواليد الجرذان البيضاء المفرطة الكولسترول بعد معاملة الامهات بمادة السمفاستاتين . يعتبر الستاتينات مثل السمفاستاتين والسمفاستاتين واللوفاستاتين وغيرها من الادوية التي تثبط عمل انزيم (HMG-COA reductase) المهم في تكوين الكوليستيرول وهذه الادوية تستعمل عادة كمادة علاجية مخفضة للكولستيرول في الجسم وتعالج امراض القلب وتصلب الشرايين وتقلل من نسبة تخثر الدم و حدوث الجلطة . استعملت في هذه الدراسة اربعة وعشرون من أناث الجرذان . بعد الولادة قسمت الإناث إلى 4 مجاميع كل مجموعة تتكون من 6 حيوانات تم استحثاث الكوليستيرول في 12 حيوان وتركت 12 حيوان بدون كولسترول هي: المجموعة الأولى:مجموعة السيطرة (control) حيوانات سليمة أعطيت (DMSO) بجرعة 0.25 مل / حيوان. المجموعة الثانية مفرطة الكولسترول اعطيت فقط DMSO بجرعة 0.5 مل/ حيوان , المجموعة الثالثة: مفرطة الكولستيرول : اعطيت سمفاستاتين بجرعة 0.02 ملغم/ حيوان :المجموعة الرابعة: اعطيت االسمفاستاتين بجرعة 0.02 ملغم/ حيوان ,

استمرت المعالجة يوميا لمدة 30 يوم. بعد ذلك تمت التضحية بالمواليد وجمعت عينات الدم في انابيب تحتوي مادة EDTA لإجراء الفحوصات المختبرية لبعض المعايير الدموية .

اظهرت نتائج انخفاضا معنويا في تعداد كريات الدم الحمر ونسبة الهيموغلوبين واعداد الصفائح الدموية ومعدل حجم الصفائح في ذكور واناث المواليد المجموعتين G3,G4 مقارنة بمجموعة السيطرة , ما عدا اعداد كريات الدم الحمر في المجموعة G3 لم تختلف معنويا عن مجموعة السيطرة .نستنتج من الدراسة الحالية ان المعالجة بالسمفاستاتين يقلل معنويا من بعض المعايير الدموية في مواليد الجردان المعاملة بتلك المادة اثناء فترة الرضاعة.

Introduction

Hematological parameters is a prerequisite to understand the normal functioning of the system and to confirm the nontoxic nature of the administered drug. Hematological parameters such as red cell distribution width [1], mean platelet volume (MPV) [2], are associated with the increased risk for Cardio Vascular Disease(CVD). However, whether other parameters of the complete blood count such as erythrocyte and its related parameters, such as hemoglobin (Hb), is also associated with the risk for CVD and whether the combination of these parameters with blood lipids can improve the ability to predict the risk for CVD are still unclear. Mean platelet volume (MPV) which is a simple measure of platelet activation, has recently become an interesting topic in cardiovascular research. When platelets become activated (MPV) increases and change

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from quiescent discs to swollen spheres. Large platelets are more adhesive and likely to aggregate than small ones [3,4].

Statins are the inhibitors of hydroxymethyl glutaryl coenzyme A (HMG CoA) reductase. They are mostly used to treat hyperlipidaemia [5]. Statins that currently carried for clinical use include Atorvastatin, Fluvastatin, Lovastatin, Pravastatin, and Simvastatin. Lowering of low-density lipoprotein (LDL) plasma levels has been shown to reduce primary and secondary cardiovascular events including myocardial infarction (MI), stroke, and all-cause mortality [6]. They also have favorable effects on platelet activation, endothelial function, inflammation, and coagulation cascade [7,8]. The present study was designed to evaluate some hematological parameters in rat offspring from rats treated during lactation period with simvastatin that currently present in market with certain side effects like rhabdomylysis, muscle weakness etc..

MATERIALS AND METHODS

The present study was carried out in the animal house and laboratories of the College of Veterinary Medicine/ University of Basrah to investigate of some haematological parameters in offspring of hyperlpidemic female laboratory rats after simvastatin treatment during lactation.

Experimental Animals: Twenty four female rat with (9-10) weeks old-and weighting (190-220 gm) were used in the current study . Animals were housed in plastic cages with metal covers, containing bedding materials of fine wood which was kept dry and changed twice weekly. The animals were maintained under controlled optimum conditions light dark cycle (12/12) hours, at a temperature (22±3°C). The diet was offered *ad Libitum*, and presented with tap water.

Induction of hyperlipidemia : These females were matted with fertile males, during pregnancy these animals were induced for hypercholesterolemia by administration of cholesterol powder at dose 2.5 ml /kg (1%) daily dissolved in coconut oil given by oral gavages [9], was continued for 30 days.

Experimental Groups: Twelve of females are induced for hypercholesterolemia during pregnancy, other twelve were not, all animals were divided into 4 equal groups after delivery: **G**.1 (Control) normal animals given only 0.5 ml/Kg animal of Dimethyl Sulphoxide (DMSO). **G**.2 hypercholestrolemic animals given only 0.5 ml/ animal of Dimethyl Sulphoxide (DMSO). **G**.3 (S1) hypercholestrolemic animals treated with 20 mg/kg/day (0.02ml/ animal) of simvastatine . **G**.4 normal animals treated with 20

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mg/kg/day (0.02ml/ animal) of simvastatine. The treatment of these animals were continued for 30 days after delivery. At the end of the experiment, offspring of these animals were sacrificed at their puberty (55 days old) and blood samples were collected in in clean dry Eppendorf tubes containing EDTA as anticoagulant to be used for hematological studies. The following parameters RBCs, Hb, , Platelets count and MPV were all determined by a Celltac- α Hematology analyzer.

Statistical Analysis: One-way ANOVA-test was used to determine the significant difference between groups. Differences between data were compared by least significant difference (LSD). All data were expressed as mean ± standard deviation. All statistical tests were done by using statistical program SPSS (version 21.0) the level significant set on $p \leq 0.05$. (Bryman and Cramer, 2012).

RESULTS

Results of male offspring revealed that RBC count, hemoglobin concentration (Hb), platelets count and MPV levels in groups treated with simvastatin of G3 and G4 were significantly ($P \leq 0.05$) decreased than those in control group except RBC count in G3 were not significantly differ from those in control group , as illustrated in table (1)**.** treated with simvastatin G3 and G4 were significantly ($P \leq 0.05$) decreased in female

than those in control group except RBC count in G3 and platelet count in G4 were significantly differ from those in control group, as illustrated in table (2)**.**

Table (1): Hematological parameters for male offspring at puberty .

Group / Par.	G1: Control	G2: HC Hyperchol.	G3: Hyperchol. + Simvastatin	G4: Simvastatin
RBC count 10 12/L	6.21 ± 1.67 a	5.56 ± 0.93 b	5.44 ± 0.66 ab	4.70 ± 0.21 b
Hb (g/L)	12.36 ± 0.32 a	11.80 ± 1.82 ab	10.93 ± 2.73 b	9.56 ± 1.25 c
Plt. (× 10 9/L)	584.33 ± 83.54 a	410.66 ± 84.5 ab	384.33 ±23.11 b	193±11.26 c

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MPV (fL)	**10.40± 0.36 a**	**9.76± 0.61 ab**	**9.43± 0.30 b**	**8.96± 1.01 c**

***RBC, red blood cell; Hb, hemoglobin;; Plt, platelet; MPV, mean platelet volume.**
***Different letters refer to the significant differences at (P ≤ 0.05).**

Table (2): Hematological parameters for female offspring at puberty .

Group	G1: Control	G2: HC [yperchol.	G3: Hyperchol. + Simvastatin	G4: Simvastatin
RBC count 10 12/L	**6.27 ± 0.9 a**	**6.22 ± 0.10 ab**	**5.37 ± 0.34 ab**	**5.49 ± 0.77 b**
Hb (g/L)	**10.76 ± 0.23 a**	**11.79 ± 0.45 ab**	**11.80 ± 2.02 b**	**10.20 ± 1.02 c**
Plt. (× 10 9/L)	**477.33 ± 7.7 a**	**391.70 ± 1.5 ab**	**284.66 ±4.9 b**	**171.6 ±6.65 c**
MPV (fL)	**10.5 ± 1.12 a**	**8.5 ± 1.13 bc**	**7.5 ± 1.10 c**	**8.73 ± 0.40 b**

***RBC, red blood cell; Hb, hemoglobin;; Plt, platelet; MPV, mean platelet volume.**
***Different letters refer to the significant differences at (P ≤ 0.05).**

DISCUSSION

The present study showed significantly decreasing RBC count and concomitant lowering hemoglobin, this may due to deformation of red blood cell (RBC) that may be related to ATP release from cells [11]. Also the present study showed, after treatment

with statins, MPV significantly reduced, this means that statins may affect the platelet function. Statins have anti-platelet activation effect, and can prevent or delay the

occurrence or development of CVD in patients with hypertension, coronary heart disease, hyperlipidemia or diabetes [12].Membrane cholesterol has been shown to alter the properties of cell membrane such as fluidity and bending stiffness [13], and membrane cholesterol increase has been observed in some CVD. Therefore, one of the mechanisms underlying the antithrombotic effect of statins may be ascribed to the decrease in erythrocyte membrane lipid composition which increases red blood cell deformability, and then decreases RBC aggregation [14].

Statins have various effects through inhibiting the mevalonate pathway. It has been reported [15] that statins can lower the cholesterol content in serum, liver and aorta as well as the VLDL-Ch and LDL-Ch in hyperlipemia patients. In past decades, many large case-control studies have confirmed that statins are effective for the prevention and treatment of CVD [16]. Hyperlipidemia is a risk factor of systemic atherosclerosis [17]. The platelet aggregation and cholesterol-rich lipoproteins have been found in the atherosclerotic plaques, which suggest that such lipoproteins and activated platelets are involved in the pathogenesis of atherosclerosis [18]. Therefore, the blood lipid-related indicators and hematological parameters should be regularly monitored for patients with a risk for atherosclerosis. In the present study, the effects of simvastatin on RBC, Hb , PLT and MPVwere investigated, which may be helpful to guide the clinical therapy of these patients. For this search in groups G3,and G4 all these hematological parameters blood cell count, hemoglobin, platelets and MPV were significantly decreased after simvastatin treatment. It is well known that MPV is an indicator of platelet activation and has been widely studied in CVD [19]. The platelets transform from static dish into globular swelling shape after activation, resulting in MPV increase. The adhesion and aggregation of platelets increase significantly after activation. However, the platelets are basically at the rest state in the blood circulation of health subjects. In the prothrombotic state, platelets at rest state may be activated by various factors, which significantly increase the incidence of coronary heart complications and thrombosis. Therefore, reducing platelet activity is very important for the clinical treatment of coronary heart disease and the prevention of cardiovascular events. It is well known that statins have antithrombotic effect. For hyperlipidemia patients, the inhibition of statins on the platelet-dependent thrombosis is not ascribed to its lipid lowering effect, indicating that statins have some biological effects independent of their lipid lowering effects, i.e., anti-platelet aggregation [20].

Conclusion: treatment with statins significantly reduce some hematological parameters such as RBC, Hb, platelets and MPV levels in offspring of females rat that treated with these drugs during lactation irrespective of cholesterol levels.

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References

1- **Borne, Y.;Smith, J.; Melander, O. and Engstrom, G. (2014).** Red cell distribution width in relation to incidence of coronary events and case fatality rates: a population-based cohort study. *Heart Journal*; 100: 1119-1124.

2- **Lance, M.; Sloep, M.; Henskens, Y. and Marcus, M.(2012)**.Mean platelet volume as a diagnostic marker for cardiovascular disease: drawbacks of preanalytical conditions and measuring techniques. *Clin. Appl. Thromb. Hemost*.; 18: 561-568.

3- Martin, J.F.; Trowbridge, E.A,.; Salmon G . (1983). The biological significance of platelet volume: its relationship to bleeding time, thromboxane B2 production and megakaryocyte nuclear DNA concentration. *Thromb Res*, 32: 443–460.

4. **Sivri, N.; Tekin,G.; Yalta, K.; Aksoy,Y, Senen, K. and Yetkin, E. (2013)** .Statins decrease mean platelett volume irrespective of cholesterol lowering effect . *Cardiologia Polska* . 71, 10: 1042–1047;

5- Winterfeld, U.; Allignol, A.; Panchaud, A.; Rothuizen, L. ; Merlob, P. ; Cuppers-Maarschalkerweerd, B. ; Vial, T.; Stephens, S.; Clementi, M.; De Santins, M.; Pistelli, A.; Berlin, M.; Eleftheriou, G.; Manakova, E. and Buclin T. (2012). Pregnancy outcome following maternal exposure to statins. *An International Journal of Obstetrics and Gynaecology*. 10: 1-9.

6- National Cholesterol Education Program (NCEP) (2002). Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation*, 106: 3143–3421.

7- Undas, A.; Brummel-Ziedins, K.E.; Potaczek, D.P *et al*.(2006). Atorvastatin and quinapril inhibit blood coagulation in patients with coronary artery disease following 28 days of therapy. J Thromb Haemost, , 11: 2397–2404.

8- Zhou, Q. and Liao, J.K.(2010) Pleiotropic effects of statins. *Circ J*, ; 74: 818–826 .

9- Swati, R.; Malvika, S. and Vilasrao J. (2014). Standardization of cholesterol suspension induced hyperlipidemia in rats . *W. J. Pharma. Pharmaceu. Sci..;* 3,(12):- 1107-1123.

10. Bryman, A. and Cramer, D. (2012). Quantitative Data Analysis With IBM SPSS (21): A Guide For Social Scientists Rutledge.

11- Wan, J.; Forsyth, A.M and Stone, H.A. **(2011).** Red blood cell dynamics: from cell deformation to ATP release. *Integr Biol (Camb);* 3: 972-981.

12- Sikora, J.; Kostka, B.; Marczyk, I, Krajewska, U.; Chalubinski M. and Broncel, M.(2013). Effect of statins on platelet function in patients with hyperlipidemia. *Arch Med Sci*; 9: 622-628.

13- Forsyth, A.M; Braunmuller. S; Wan, J; Franke, T and Stone, HA.(2012). The effects of membrane cholesterol and simvastatin on red blood cell deformability and ATP release. *Microvasc Res* 2012; 83: 347-351.

14- Jian, B. ; Jia, F.; Yu-Chun, C. and Yu-Wei, Y.(2015). Effects of simvastatin and atorvastatin on biochemical and hematological markers in patients with risk of cardiovascular diseases. *Int J Clin Exp Med* ,8(8):13983-13989.

15-Macedo, A.F.; Taylor, F.C.; Casas, J.P.; Adler ,A.; Prieto- Merino D and Ebrahim, S. (2014). Unintended effects of statins from observational studies in the general population: systematic review and meta-analysis. *BMC Med*. ; 12: 51.

16- Zhang, X,.; Xiang, C, Zhou, Y.H, Jiang, A, Qin, Y.Y and He, J. (2014). Effect of statins on cardiovascular events in patients with mild to moderate chronic kidney disease: a systematic review and meta-analysis of randomized clinical trials. *BMC Cardiovasc Disord* ; 14: 19.

17- Soehnlein, O. and Swirski, F.K.(2013). Hyperchole-sterolemia links hematopoiesis with atherosclerosis. *Trends Endocrinol Metab*; 24: 129-136.

18- Badimon, L, Vilahur, G. and Padro, T.(2009). Lipoproteins, platelets and atherothrombosis. *Rev Esp Cardiol* 2009; 62: 1161-1178.

19- Sun, X. and Jia Z. (2012). A brief review of biomarkers for preventing and treating cardiovascular diseases. *J Cardiovasc Dis Res* , 3: 251-254.

20- Desai, C.S, Martin, S.S and Blumenthal, R.S.(2014). Non-cardiovascular effects associated with statins. *BMJ*; 349: g3743.