DOI: 10.7860/JCDR/2018/37181.12050

Original Article

Physiology Section

Lymphocyte Apoptosis in Third Trimester of Pregnancy

HANAN L AL-OMARY[1], ZAINAB M ALAWAD[2], SHOUB YASSIN HUSSEIN[3]

ABSTRACT

Introduction: During pregnancy, the female's body undergoes many adaptations, including changes in the number and survival of White Blood Cells (WBCs). Programmed deaths of the cells are an important regulatory process that happens under a variety of physiological and pathological conditions.

Aim: To measure the percentage of apoptosis of lymphocytes in peripheral blood of pregnant females and healthy non pregnant females, and to compare lymphocytes apoptosis between them in term of blood pressure and blood sugar. Also, to relate this apoptosis to blood sugar levels and blood lead levels.

Materials and Methods: Fourty two pregnant females in their third trimester had been enrolled in the prospective study and were considered as the patients group. They were recruited from the labour room of the Baghdad Teaching Hospital, Baghdad, Iraq. The control group consisted of 15 healthy non pregnant women with matched age. Blood samples were collected from both groups and subjected to lymphocytes apoptosis estimation; blood sugar level and blood lead level measurements. Blood lead level was measured using blood lead analyser kit. Lymphocytes examination and separation was done. Data analysis was done using SPSS version 20.0. ANOVA test, Independent sample t-test and Pearson correlation test were also used.

Results: There was no significant difference in age and blood lead level between patients and control groups. Lymphocyte apoptosis was significantly higher in the patients group than the control group (3.50±2.24 vs. 1.49±0.66). Lead level correlated positively and significantly with lymphocyte apoptosis in both groups. Apoptosis level was not significantly higher in hypertensive patients however, it was significantly higher in diabetic patients.

Conclusion: Lymphocytes apoptosis is significantly higher in third trimester of pregnancy and it is higher in diabetic and hypertensive pregnant women than healthy non pregnant women. The increase in blood lead level may cause a significant increase in lymphocytes apoptosis in pregnant and non pregnant women.

Keywords: Diabetes, Hypertension, Lead

INTRODUCTION

Normal pregnancy involves a mixed and complicated activity of many immunoregulatory processes that protect the foetus from the action of the mother's immune system [1], and it is characterised by a lot of haematological changes, which may seem to be pathological in the non pregnant conditions [2].

The mother immune system responses to foetal antigens actually play an essential role. There is evidence showing that T lymphocytes play an important role in stopping foetal rejection [3].

In haematopoietic system, the cells that undergo programmed cell death or apoptosis shrink and have nuclear destruction, and then they are engulfed by a phagocyte, without arousing the inflammatory response [4].

Lymphocyte apoptosis is important for good immune function. It clears away developing lymphocytes that cannot show an antigen receptor, and it removes lymphocytes with antigen receptors that perceive autoantigens. Apoptosis also arranges the size and duration of immune reactions; stimulated lymphocytes are killed when an infection is abolished successfully [5]. The dysregulation of apoptosis can cause different immune alterations, including immunodeficiency and autoimmunity [6].

Environmental lead exposure is considered a public health problem worldwide especially in the developing countries [7]. In pregnant women, a blood lead level of 5 µg/dL or more needs counselling, follow up, and advice to be away from the source of lead in the environment [8]. Physiological stress occurring in pregnancy can raise bone turnover of lead causing shift of lead to maternal blood; thus, pregnant women are susceptible to have higher blood lead level compared to non pregnant women [9].

Increasing evidence shows that the immune system plays an important role in the pathogenesis of cardiovascular diseases including hypertension [10]. It was suggested that improper activation of the immune system can result in pre-eclampsia [3].

High blood sugar level was claimed to affect WBCs apoptosis due to the fact that there is impairment of the immune system during diabetic state [11].

Studies showed inconsistent results regarding lymphocytes apoptosis in pregnancy, since some studies observed an increase in lymphocytes apoptosis during pregnancy [1,12], whereas another evidence suggested that lymphocytes proliferation overcomes apoptosis in pregnancy [13]. This study was done to reach further understanding about factors affecting this process in pregnancy.

The aim of the study was to measure the percentage of apoptosis of lymphocytes in peripheral blood of young pregnant females during their third trimester and healthy non pregnant females, and to compare lymphocytes apoptosis between them in term of blood pressure, and blood sugar. Also, to relate apoptosis to blood sugar levels and blood lead levels.

MATERIALS AND METHODS

A total of 57 women participated in this prospective study and were randomly selected regarding their age, occupation and residency. They were divided into two groups (patient group and control group). Fourty two pregnant women (considered as the patients group) aged 26±5.40 years were compared to the control group which comprised 15 non pregnant healthy women with matched age (25±4.81 years). Participants were recruited from the labor room of Baghdad Teaching Hospital (the patients group) and from the staff of the Medical College, University of Baghdad (control group), in the period between September 2017 and April 2018. The study was approved by the Ethical Committee of Medical College

of University of Baghdad and Baghdad Teaching Hospital. It was in accordance with the Helsinki Declaration of 1975 that was revised in 2000. Informed consents of the women participated in this study were taken. All pregnancies were singleton.

The patient group was further subdivided into those having hypertension, those having diabetes mellitus and those who had no hypertension or diabetes. The blood pressure was considered high when it exceeded 140/90 mmHg [14]. And the blood sugar was considered high when fasting and pre-meal glucose levels were above 105 mg/dL and HbA1c above 6% [15].

Thorough history was obtained from the women and careful examination was done by an Obstetrics and Gynaecology specialist. Blood samples (5 milliliter) were taken from them by venipuncture in sterile states and put in heparinized tubes. Blood sugar and whole blood lead levels were measured. Blood lead was measured using lead care analyser which contains the lead care kit and the (lead care analyser device) version 3.3 (ESA, INC, USA). Blood lead levels of less than 10 µg/dL in adults and less than 5 µg/dL in children are usually not causing poisoning, but it can be harmful if the exposure continues for long time [16].

About 2 millilitres of blood was withdrawn in the tubes supplied by the kit. The tubes were covered and the blood was mixed with the reagent, until the colour of the mixture became brown. Then they were left in upright position for a minute permitting the mixture to drain down to the bottom of the tube. Using pipette, a 35 µL of the mixture was drawn and placed to the sensor of the device after calibration, and then analysis was started.

Total WBCs count was measured using haemocytometer counting chamber and special pipette for the procedure. The blood was diluted with WBCs solution (glacial acetic acid and gentian violet or methyl blue). Then the mixture was put on the chamber and WBC was counted under the light microscope.

Lymphocyte examination and separation was done through the following method: About 2 millilitres of blood was withdrawn in Ethylene Diamine Tetraacetic Acid (EDTA) tubes and diluted 1:1 in freshly prepared phosphate buffer saline (pH-7.2). Then 2 millilitres of mixture were carefully layered over the 4 mL ficoll 400 (Pharmacia fine chemicals) which was disposed in 10 millilitres siliconised glass centrifuged tubes, then it was centrifuged in cold centrifuge (2100 rpm), for 25 minutes. After centrifugation, the lymphocytes forms a white buffy coat at the interface between plasma and separating media, which was aspirated by Pasteur pipette and transferred into another tube. The aspirated lymphocyte layer was washed by phosphate buffer solution and centrifuged (2500 rpm) until a pellet was formed. The supernatant was discarded and this procedure was repeated for three times. Finally the lymphocyte pellet was resuspended in 0.5 mL of phosphate buffer. The number and morphology were detected using haemocytometer counting chamber.

Trypan blue exclusion test was done to assess cell viability. The viable cells exclude the trypan blue dye, while the dead cells accept it. A known volume of the suspension (100 microliter) was mixed with same volume of the dye and examined immediately under light microscope [17].

STATISTICAL ANALYSIS

Statistical analysis was done by using SPSS version 20.0. All values were presented in Mean±Standard Deviation (SD) and unpaired t-test and ANOVA test were used wherever appropriate. A p-value of <0.05 was considered significant. The correlations were tested using Pearson correlation test [18].

RESULTS

The mean age of the patients group was 26±5.40 years ranging between 18 and 35 years. The mean age of the control group was 25±4.81 years ranging between 17 and 33 years. The mean age of hypertensive and diabetic patients group was 23.61±2.92 years and 21.5±3.24 years respectively. There was no statistical difference between the groups (p>0.05).

The number of hypertensive (blood pressure >140/90 mmHg) pregnant women was eight and the number of diabetic (fasting and pre-meal glucose levels >105 mg/dL and HbA1c >6%) pregnant women was 11.

Lead levels in the blood were measured in patients group, control group, hypertensive group and diabetic group and it was noticed that there was no significant difference between the groups [Table/Fig-1].

Variables	Patients (=42)	Control (n=15)	Hypertensive patients group (n=8)	Diabetic patients group (n=11)	p-value
Age (years)	26±5.40	25±4.81	23.61±2.92	21.5±3.24	>0.05
Blood Lead level (µg/dL)	5.03±3.62	4.93±1.66	6.21±2.3	6.55±4.1	>0.05

[Table/Fig-1]: Comparison of age and lead level between patients and control groups.
ANOVA test was used for data analysis

Total WBC count was measured, finding that their count was not statistically different between patients and control group. On the other hand, the lymphocyte count was higher in patients than control group with a significant difference. After processing and measuring the percentage of lymphocyte apoptosis, it was obvious that the apoptosis was significantly higher in patients group than control group [Table/Fig-2]. ANOVA test was used for data analysis in [Table/Fig-1] and Independent sample t-test was used for data analysis in [Table/Fig-2].

Variables	Patients (n=42)	Control (n=15)	p-value
Total white blood cells (number/dL)	10671±2513	8440±1557	>0.05
Lymphocyte number (number/dL)	3310±1440	2200±770	≤0.05
Lymphocytes apoptosis (%)	3.50±2.24	1.49±0.66	≤0.05

[Table/Fig-2]: Comparison of total white blood cells number, lymphocytes number and lymphocytes apoptosis between patients and control group.
Independent sample t-test was used for data analysis

A significant positive correlation was found between blood lead level and lymphocytes apoptosis in both the groups [Table/Fig-3].

The increase in blood sugar was associated with an increase in apoptosis of lymphocytes and this correlation was significant only for patients group [Table/Fig-4]. Pearson correlation test was used for data analysis in [Table/Fig-3,4].

Comparing the lymphocytes apoptosis in hypertensive pregnant patients and control groups showed that there was no significant difference between them, although the percentage was higher in hypertensive group [Table/Fig-5].

Group		Lead level (µg/dL)	
		r	p-value
Patient	Apoptosis	0.84	≤0.05
Control		0.91	≤0.05

[Table/Fig-3]: Correlation between lead level and lymphocytes apoptosis in patients and control groups.
Pearson correlation test was used for data analysis

Group		Blood sugar (mg/dL)	
		r	p-value
Patient	Apoptosis	0.69	≤0.05
Control		0.31	>0.05

[Table/Fig-4]: Correlation between blood sugar level and lymphocytes apoptosis in patients and control groups.
Pearson correlation test was used for data analysis

Group	Hypertensive patients (n=8)	Control (n=15)	p-value
Lymphocytes apoptosis (%)	4.94±2.42	1.49±0.66	>0.05

[Table/Fig-5]: Comparison of lymphocytes apoptosis between patients with hypertension and control groups.
Independent sample t-test was used for data analysis

Lymphocyte apoptosis was also compared between hypertensive and non hypertensive patients and there was no significant difference [Table/Fig-6].

Group	Hypertensive patients (number=8)	Non hypertensive patients (number=34)	p-value
Lymphocytes apoptosis (%)	4.94±2.42	3.21±2.12	>0.05

[Table/Fig-6]: Comparison of lymphocytes apoptosis between patients with hypertension and patients with no hypertension.
Independent sample t-test was used for data analysis

Concerning diabetic patients, they had significantly higher lymphocyte apoptosis than the control group [Table/Fig-7]. Comparing diabetic group to the non diabetic group revealed no significant difference [Table/Fig-8]. Independent sample t-test was used for data analysis in [Table/Fig-5-8].

Group	Diabetic patients (number=11)	Control (number=15)	p-value
Lymphocytes apoptosis (%)	6.04±1.79	1.49±0.66	≤0.05

[Table/Fig-7]: Comparison of lymphocytes apoptosis between patients with diabetes and control groups.
Independent sample t-test was used for data analysis

Group	Diabetic patients (number =11)	Non diabetic patients (number =31)	p-value
Lymphocytes apoptosis (%)	6.04±1.79	2.93±1.88	>0.05

[Table/Fig-8]: Comparison of lymphocytes apoptosis between patients with diabetes and patients with no diabetes.
Independent sample t-test was used for data analysis

DISCUSSION

Lymphocytes apoptosis in pregnancy (third trimester) was measured in this study, evaluating the effects of blood sugar levels, blood lead levels and hypertension on this state.

Lead is highly polluting the environment of Iraq (mean of blood lead level in Iraq is 7.3±2.8 µg/dL and the toxic level is >10 µg/dL) [19]. Lead exposure may cause blood changes to people at any age. Studies found that apoptosis may result in oxidative stress and DNA destruction [20]. To eliminate the differences in the effect of lead on patients and control groups, we compared the blood lead level in all groups and there was of no significant difference since the participants are all living in the same environment.

Total WBC count was of no significant difference between patients and control groups although it was slightly higher in patients' group. This result goes with other studies which found that WBC-count is raised in pregnancy with the lower limit of the reference range being about 6,000/dL. The physiological stress during pregnancy is thought to be the cause of this slight leukocytosis [2]. Another study found a significant increase in WBC count in pregnant women compared with non pregnant women [21].

Lymphocyte count was significantly higher in pregnant women, which had agreement with other studies who also found that the percentage of lymphocytes was elevated during the third trimester of pregnancy [21,22], but this result was not shown by some studies since that the behavior of various types of lymphocytes during pregnancy has not yet been determined with any precision. There were studies showing decreased numbers of lymphocytes [13,23] and studies which observed slight increase of lymphocytes [2,24,25].

In this study, lymphocytes apoptosis in pregnant women was found to be significantly higher than non pregnant women. This result is consistent with other studies which reported that the total lymphocytes apoptosis was significantly higher in pregnant women (in all trimesters) when compared to non pregnant women [3]. It had been revealed that exosomes of placenta are important in controlling T cell activation by stimulating lymphocyte apoptosis [26]. In pregnancy, it has been documented that the regeneration and tolerance factor are manifested during T lymphocyte activation and it has a role in T lymphocyte apoptosis [27].

In pregnant and non pregnant women, apoptosis has been shown to correlate positively with the lead level, which goes with the results found by Zhao D et al., who postulated that exposure to lead can induce apoptosis of lymphocytes as seen in chicken splenic lymphocytes [28]. Also, other researchers found that lead stimulates oxidative stress in blood lymphocytes and oxidative stress plays an essential role in apoptosis due to the high production of free radicals [29,30]. It has been observed that lead causes structural nuclear and cytoplasm abnormalities to WBCs [9].

The lymphocytes apoptosis was non significantly higher in hypertensive pregnant women than non pregnant women. Some studies showed that apoptosis is abnormally enhanced in animals and humans with arterial hypertension [31-33]. Active T lymphocytes regulate blood pressure through the release of reactive oxygen species and activated vessels' cytokines, thus changing the inflammatory state in the wall of the vessel and the kidney, so mice who do not have T lymphocytes are resistant to blood pressure increase, which explains that lymphocytes apoptosis to some extent associated with hypertension [10]. While other studies showed a low percentage of apoptosis in patients with hypertension and cardiovascular pathology [34,35].

Diabetic pregnant women demonstrated a significant higher apoptosis than non pregnant women. This was in agreement with other studies which have reported that the elevation of oxidative stress in diabetic patients induces lymphocyte apoptosis [36,37]. Other studies also found that T lymphocytes were reduced mainly in diabetic patients with poor control [38].

In diabetes, there is decrease in lymphocytes count which is possibly a clinical consequence of apoptosis [11]. In a study done by Lien E and Ingalls RR, blood peripheral lymphocytes taken from poorly controlled diabetic pregnant had higher DNA fragmentation in comparison to cells collected from healthy people [39].

LIMITATION

A relatively small sample size was used in the study. However, we should have worked on fresh blood samples for measuring apoptosis which made it difficult to increase sample size in a limited period.

CONCLUSION

Lymphocytes apoptosis is significantly higher in third trimester of pregnancy and it is higher in diabetic and hypertensive pregnant women than healthy non pregnant women. The lead levels correlate positively and significantly with lymphocytes apoptosis in both patients and control groups.

ACKNOWLEDGEMENTS

We are thankful to the staff of labour room at Baghdad Teaching Hospital and staff of the Medical College, University of Baghdad, Baghdad, Iraq, for their assistance in sample collection and for making things possible.

REFERENCES

[1] Darmochwal-Kolarz D, Sobczak E, Pozarowski P, Kolarz B, Rolinski J, Oleszczuk J. T CD3+CD8+ lymphocytes are more susceptible for apoptosis in the first trimester of normal human pregnancy. J Immunol Res. 2014;2014:670524.
[2] Chandra S, Tripathi AK, Mishra S, Amzarul M, Vaish AK. Physiological changes in haematological parameters during pregnancy. Indian J Hematol Blood Transfus. 2012;28(3):144-46.

[3] Darmochwal-Kolarz D, Saito S, Tabarkiewicz J, Kolarz B, Rolinski J, Leszczynska-Gorzelak B, et al. Apoptosis signaling is altered in CD4+ CD25+ FoxP3+ T regulatory lymphocytes in pre-eclampsia. Int J Mol Sci. 2012;13(6):6548-60.
[4] Squier MK, Sehnert AJ, Cohen JJ. Apoptosis in leukocytes. J Leukoc Biol. 1995;57(1):2-10.
[5] Newton K, Strasser A. Cell death control in lymphocytes. Adv Immunol. 2000;76:179-226.
[6] Zhang N, Hartig H, Dzhagalov I, Draper D, He YW. The role of apoptosis in the development and function of T lymphocytes. Cell Res. 2005;15(10):749-69.
[7] Tong S, von Schirnding YE, Prapamontol T. Environmental lead exposure: a public health problem of global dimensions. Bull World Health Organ. 2000;78:1068-77.
[8] American College of Obstetricians and Gynaecologists. Lead screening during pregnancy and lactation. Committee opinion. 2012;(533).
[9] Sharma R, Panwar K, Mogra S. Effects of prenatal and neonatal exposure to lead on white blood cells in Swiss mice. J Cell Mol Biol. 2012;10(1):33-40.
[10] Zhang J, Crowley SD. Role of T lymphocytes in hypertension. Curr Opin Pharmacol. 2015;21:14-19.
[11] Otton R, Soriano FG, Verlengia R, Curi R. Diabetes induces apoptosis in lymphocytes. J Endocrinol. 2004;182(1):145-56.
[12] Kopcow HD, Rosetti F, Leung Y, Allan DS, Kutok JL, Strominger JL. T cell apoptosis at the maternal–fetal interface in early human pregnancy, involvement of galectin-1. Proc Natl Acad Sci USA. 2008;105(47):18472-77.
[13] Norton MT, Fortner KA, Bizargity P, Bonney EA. Pregnancy alters the proliferation and apoptosis of mouse splenic erythroid lineage cells and leukocytes. Biol Reprod. 2009;81(3):457-64.
[14] Carson MP, Chen KK. Hypertension in a woman planning pregnancy. CMAJ. 2014;186(2):129-30.
[15] Riskin-Mashiah S, Damti A, Younes G, Auslander R. Normal fasting plasma glucose levels during pregnancy: a hospital-based study. J Perinat Med. 2011;39(2):209-11.
[16] Kao LW, Rusyniak DE. Chronic poisoning: trace metals and others. In: Goldman L, Schafer AI, editors. Goldman's Cecil Medicine. 25th ed. Philadelphia: Elsevier Saunders; chap 22. 2016.
[17] Abbassi-Ghanavati M, Greer LG, Cunningham FG. Pregnancy and laboratory studies: a reference table for clinicians. Obstet Gynaecol. 2009;114(6):1326-31.
[18] Conover WJ, Iman RL. Rank transformations as a bridge between parametric and nonparametric statistics. Am Stat. 1981;35(3):124-29.
[19] Al-Dosky AH, Al-Timimi DJ, Al-Dabbag SA. Lead exposure among the general population of Duhok governorate, Kurdistan region, Iraq/Exposition au plomb dans la population générale du gouvernorat de Duhok, dans la région du Kurdistan (Iraq). East Mediterr Health J. 2012;18(9):974-79.
[20] Yedjou CG, Milner JN, Howard CB, Tchounwou PB. Basic apoptotic mechanisms of lead toxicity in human leukemia (HL-60) cells. Int J Environ Res Public Health. 2010;7(5):2008-17.
[21] Kühnert M, Strohmeier R, Stegmüller M, Halberstadt E. Changes in lymphocyte subsets during normal pregnancy. Eur J Obstet Gynaecol Reprod Biolo. 1998;76(2):147-51.
[22] Monif GR. Interpretation of the maternal white blood cell count in the postpartum period. Infect Med. 2002;19(3):106-08.
[23] Bulmer R, Hancock KW. Depletion of circulating T lymphocytes in pregnancy. Clin Exp Immunol. 1977;28(2):302-05.
[24] Somerset DA, Zheng Y, Kilby MD, Sansom DM, Drayson MT. Normal human pregnancy is associated with an elevation in the immune suppressive CD25+ CD4+ regulatory T-cell subset. Immunology. 2004;112(1):38-43.
[25] Newport A, Carter J. Changes in T and B lymphocyte populations in the lymph nodes draining the uterus in pregnant mice. J Reprod Fertil. 1983;67:433-40.
[26] Sabapatha A, Taylor GC, Taylor DD. Specific isolation of placenta derived exosomes from the circulation of pregnant women and their immunoregulatory consequences. Am J Reprod Immunol. 2006;56(5-6):345-55.
[27] Boomer JS, Derks RA, Lee GW, DuChateau BK, Gilman-Sachs A, Beaman KD. Regeneration and tolerance factor is expressed during T-lymphocyte activation and plays a role in apoptosis. Hum Immunol. 2001;62(6):577-88.
[28] Zhao D, Zhang X. Selenium antagonizes the lead-induced apoptosis of chicken splenic lymphocytes in vitro by activating the PI3K/Akt pathway. Biol Trace Elem Res. 2018;182(1):119-29.
[29] Xu J, Lian LJ, Wu C, Wang XF, Fu WY, Xu LH. Lead induces oxidative stress, DNA damage and alteration of p53, Bax and Bcl-2 expressions in mice. Food Chem Toxicol. 2008;46(5):1488-94.
[30] Kannan K, Jain SK. Oxidative stress and apoptosis. Pathophysiology. 2000;7(3):153-63.
[31] González A, Fortuño MA, Querejeta R, Ravassa S, López B, López N, et al. Cardiomyocyte apoptosis in hypertensive cardiomyopathy. Cardiovasc Res. 2003;59(3):549-62.
[32] Mahmoud F, Omu A, Abul H, El-Rayes S, Haines D. Lymphocyte subpopulations in pregnancy complicated by hypertension. J Obstet Gynaecol. 2003;23(1):20-26.
[33] Sacks GP, Studena K, Sargent IL, Redman CW. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. Am J Obstet Gynaecol. 1998;179(1):80-86.
[34] Revnic C, Revnic F. Evaluation of lymphocyte apoptosis from hypertensive patients of different ages with stroke. Alzheimers Dement. 2012;8(4):294-95.
[35] Intengan HD, Schiffrin EL. Vascular remodeling in hypertension: roles of apoptosis, inflammation, and fibrosis. Hypertension. 2001;38(3):581-87.
[36] Arya AK, Garg S, Kumar S, Meena LP, Tripathi K. Estimation of lymphocyte apoptosis in patients with chronic non-healing diabetic foot ulcer. Int J Med Sci Public Health. 2013;2(4):766-68.
[37] Gligor-Dinca FS, Gligor R, Lazar-Leordean V, Mocanu CP, Zdremţan D, Puşchiţă M. Lymphocytes values in type 1 diabetes mellitus newly diagnosed in children. Proc Rom Acad., Series B. 2015;17(3):229-32.
[38] Arya AK, Pokharia D, Tripathi K. Relationship between oxidative stress and apoptotic markers in lymphocytes of diabetic patients with chronic non healing wound. Diabetes Res Clin Pract. 2011;94(3):377-84.
[39] Lien E, Ingalls RR. Toll-like receptors. Crit Care Med. 2002;30(1):S1-11.

PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Physiology, Medical College, University of Baghdad, Baghdad, Iraq.
2. Assistant Lecturer, Department of Physiology, Medical College, University of Baghdad, Baghdad, Iraq.
3. Assistant Lecturer, Department of Biology/Anatomy, Medical College, University of Baghdad, Baghdad, Iraq.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Zainab M Alawad,
Assistant Lecturer, Department of Physiology, College of Medicine, University of Baghdad, Bab Al Muadam,
P.O.Box-61023, Mail Code-12114, Baghdad, Iraq.
E-mail: z_m_ch@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **May 17, 2018**
Date of Peer Review: **Jun 16, 2018**
Date of Acceptance: **Jul 07, 2018**
Date of Publishing: **Sep 01, 2018**