Iraqi Journal of Cancer and Medical Genetics

Genotoxicity of aluminum chloride (AlCl3) on the albino rat *Rattus norvegicus*

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Abstract:

Sixty males of the Albino rat Rattus norvegicus were used in this present study, and divided to four groups . Each group was contained fifteen males. Three groups were injected intraperitoneal for 30 days with different of concentrations Aluminum Chloride AlCl3 and the fourth group was used as control. Animals were killed, and an autopsy was extracted micronuclei from its bone marrow in accordance with method of (Schmid,1975) [12] . And collected mitotic index through number of metaphase per 1000 lymphocyte was extracted from its bone marrow accordance with method of (Brusick,1980) [13].
The results showed significant increased , of micronucleus (MN) in treated rats with increasing concentrations of AlCl3 , in comparison with the control group at ($p<0.05$) .As well was in the mitotic index (MI) in lymphocyte of bone marrow in comparison with the control group at the level of significance ($p<0.05$).

Key words: *aluminum chloride (AlCl3), Rattus norvegicus.*

Introduction:

Aluminium chloride(AlCl3) was known as a neurotoxin that can be cause certain diseases such as Alzheimer disease, dialysis dementia, Parkinsonism, and amyotropic lateral sclerosis In addition [1] to its neurotoxicity, Aluminum affects other body structures like the skeletal system[2], brain tissue, bone , blood cells, liver and kidney[2,3]. AlCl3 accumulation in kidney promotes degeneration in renal tubular cells, inducing nephrotoxicity [4,5]. Salts of aluminum may bind to DNA, RNA; inhibit such enzymes as hexokinase, acid and alkaline phosphatases, phosphodi-esterase and phosphooxydase [6]. The sources of Aluminum are specially corn, yellow cheese, salt, herbs, spices, tea, cosmetics, aluminum ware and containers. Also, Aluminum is widely used in food additives and toothpaste [1]. Aluminum compounds are widely used in medicine e.g., antacids drugs, which are frequently used by pregnant women [7,8].Therefore, several reports indicate the toxic effects of different concentrations of AlCl3 on an embryo/fetus [9]. Phosphate binders, buffered aspirins, vaccines and allergen injections [10]. In addition, aluminum was added to drinking water for purification purposes [6].

Environmental pollution with the different aluminum containing compounds, specially those in industrial waste water, exposed people to higher than normal levels of Aluminum [4]. Particulate matters distributed by cement -producing factories contain high amount of Aluminum, and populations residing in the vicinity are exposed to the pollution [11].Because of the health problems that induced were by many environmental pollutants. The goal of this study, to identify the effects of aluminum chloride on the proliferation of cells.

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Materials and Methods:

Laboratory animals

Males Albino rats (body weight of (150-200 g) were obtained from the Animal House of the Samara drugs industry company(SDIC). The rats were housed in plastic cages at an environmentally controlled room (constant temperature 25-27ºC, with 12h light /dark cycle)and fed with suitable quantity of water and complete diet commercially available with the following materials: Crushed barely 24.5% , Crushed wheat 30 % , Crushed yellow corn 22.5% , Soya bean 15.2% , NaCl 0.45% ,plant oil 0.20% ,Animal protein 7.15% . Rats were ran-

domized and divided into four groups , as follows:

1-Control group: injected with 0.5ml of material control (normal saline).

2-First treatment group: injected with 0.5 ml of Aluminum Chloride concentration (mg/kg10) of the body weight.

3-Second treatment group: injected with 0.5 ml of Aluminum Chloride concentration (mg/kg15) of the body weight.

4-Third treatment group: injected with 0.5 ml of Aluminum Chloride concentration (mg/kg25) of body weight.

Each group was contained fifteen males injected with the items mentioned above for the Intraperitoneal for thirty days and by forty-eight hour, The animals killed per day thirty-one of injection, and an autopsy was extracted micronuclei from its bone marrow in accordance with method of (Schmid,1975) [12], and the mitotic index was measured through number of metaphase per 1000 lymphocyte method of Brusick (1980) [13].

Cytogenetic Analysis

A- Micronucleus test : The experiment was done according to(Schmid,1975) [12] as follows:

The animals were sacrificed by cervical dislocation. The thigh bone stearlized by using 70% ethanol and cleaned form tissues and muscles , then gapped formed the middle with a forceps in a vertical position over the edge of test tubes by a sterile syringe, (2ml) of human plasma was injected so as to wash and drop the bone marrow in the test tube.The test tube were centrifuged at a speed of 1000 rpm 10min, the supernatant was removed and one drop from the pellets taken to make a smear on a clean slide . The slides were kept at room temperature to dry. The slide then fixed with absolute methanol for 5min, then stained with D.W, and left to dry. Three slides for each animal were prepared for micronucleus test.

B-Mitotic Index Test: The experiment was done according to Brusick(1980) [13] As follows:

The animal was injected with (0.25 ml) of colchicine with concentration of (0.5 mg/ml) Intraperitoneal (I.P) 2 hours before scarifying the animal, sacrificed by cervical dislocation . Swabbed with 70% ethanol. Bone was taken and cleaned form the other tissues and muscles , then gapped from the middle with a forceps in a vertical position over the edge of a test tube by a sterile syring , (5ml) of PBS was injected so as to wash and drop the bone marrow in the test tube . Centrifuged at speed of 1000 rpm for 10 min. The supernatant was removed and (5ml) of potassium chloride (KCL) was added as a hypotonic solution at (0.075M) and then the tubes were put in water bath at 37 °C with shaking form time to time , and centrifuged at 1000 rpm for 10min . The supernatant was removed and the fixed solution was added (as drops) on the inside wall of the test tube with continuous shaking, the volume was fixed to (5ml) and the content was shake well. The tubes were kept at 4°C for 10min, to fix the cell. Then centrifuged at 1000 rpm for 10 min, the process was repeated for 3 time and the cells were suspended in 2 ml of the fixative solution. By a pasture pipette, few drops from each tube were dropped vertically on the chilled slide from a high of 3 feet a rate of (4-7) drops to give the chance for the chromosome to spread well. The slides were dried at room temperature , then stained with Gimsa stain and left for 15min then washed with distilled water .Three slides for each animal were prepared for mitotic index assay. The slide were examined under light microscope with 40x, and 1000 of the divided and non-divided cells were counted and the percentage rate was calculated [14] for only the divided (metaphases) ones according the below equation:

MI = No. of mitotic cells / 1000 X 100

Statistical Analysis: The results were expressed as the mean values ± the standard error, and statistical differences between groups were assessed by F- test. Values of $P<0.05$ were considered significantly different.

Results:

The results of our present study showed, the frequency of micronucleus (MN) was significantly increased with increasing the concentrations of AlCl3 (Table 1) in comparison with the control group (Fig.1 and 2)at level ($p<0.05$) . In addition Mitotic index of lymphocyte in treated bone marrow with all concentrations of AlCl3 in comparison with the control group (Table 2), at the level ($p<0.05$).

(Table:1) The percentage of Micronucleus (MN) in Albino rat treated with AlCl3 (Mean ± SE) (N=15)

Group	Percentage of Micronucleus(MN)
Control	52.6 ± 3.26
Treated with 10 mg/kg	106± 3.03**
Treated with 15 mg/kg	106 ± 3.84**
Treated with 25 mg/kg	109± 3.57**

** Difference between experimental groups and the relevant control group was significant, $P<0.05$.

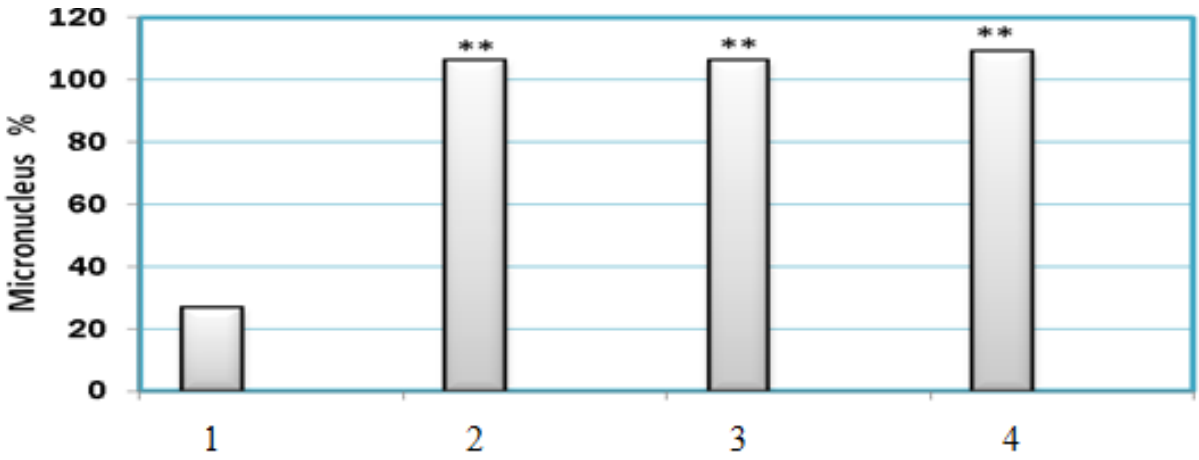

Fig.1.percentage Micronucleus (MN) in studied groups.1: conterl ; 2: treated with 10 mg/kg of AlCl3 ; 3: treated with 15 mg/kg of AlCl3 ; 4: treated with 25 mg/kg of AlCl3

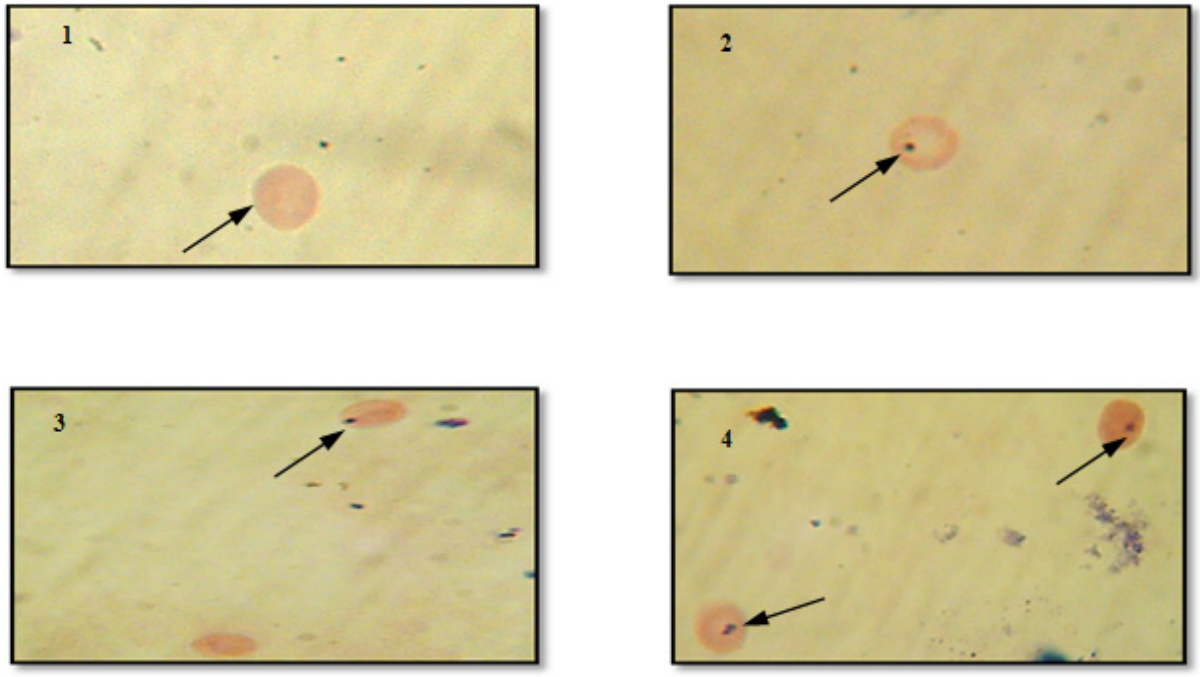

Fig.2. Micronucleus (MN) in studied groups under microscop with magnification 40x.1. Polychromatic erythrocyte normal of bone marrow in rats injected normal saline.2,3,4. Polychromatic erythrocyte abnormal contain micronucleus of bone marrow in rats injected AlCl3

(Table:1) The percentage of Mitotic index (MI) in Albino rat treated with AlCl3 (Mean ± SE) (N=15)

Group	Mitotic index(MI)%
Control	452 ± 4.24
Treated with 10 mg/kg	219 ± 1.58**
Treated with 15 mg/kg	133 ± 4.30**
Treated with 25 mg/kg	26 ± 2.23**

** Difference between experimental groups and the relevant control group was significant, P<0.05.

Discussion:

The findings of this study showed that the Intraperitoneally administration of AlCl3 was caused a significant increase frequency Micronucleus (MN) (Table1). Similarly, Balasubramanyam et al. [15] reported that exposure, AlCl3 increased the frequency of MN in rat peripheral blood cells. Banasik et al. [16] found increasing at micronuclei formation in human peripheral blood lymphocytes treated with AlCl3.Yumei et al. [17] examined the dust derived from an electrolytic aluminum plant and evaluated its genotoxicity by Ames assay, sister chromatid exchange and micronucleus , and found an increasing of in frequency MN . While Tirppi et al. [18] related the aluminum treatment in cell from Parkinson disease patients did not increase micronucleus frequency, indicating that aluminum do not have an amplifier mutagenic effect on the patient.

In other studies relating the mutagenic potential of AlCl3 had indicated by micronucleus (MN), sister chromatid exchange (SCEs) and chromosomal aberration (CA) assay [19,20,21,22]. Botchway et al.[23] observed chromosome breaks in v79-4 Chinese hamster cells irradiated with aluminum ions of low energy. Moreover, Pazy-Mino et al.[24] showed that gaps are indicating of DNA damage caused by aluminum ions, which supported their inclusion in the analysis of chromosomal aberration (CAs).

There are several direct mechanisms have been proposed to explain micronucleus production. Cross-linking of DNA with chromosomal proteins [25] and Aluminum directly binding to nuclear DNA probably occurs to change the structure of chromatin [26]. As well as, nuclear fragmentation and DNA degradation, DNA strand breaks, interaction with microtubule assembly and mitotic spindle functioning, induction of oxidative damage, damage of lysosomal membranes with liberation of DNAase , have been suggested to explain the induction of structural chromosomal aberrations, micronucleus, chromosome loss and formation of oxidized bases in experimental systems[25]. Moreover, the effect of administration of AlCl3 on RNA and protein synthesis through AlCl3 metabolism and free- radical production, and disruption of second was studied [1]. At the same time, Muma et al. [27] reported an decreasing in the expression of mRNA in spinal cord in rabbits of administration of AlCl3 for 7- 14 days. The cytotoxicity of AlCl3 decreased the mitotic index (MI) of lymphocyte in rats injected by all AlCl3 concentrations tested. Dovgaliuk et al. [28,29] Studied the cytogenetic effects of metal toxic salt including aluminum (Al[No3]3) using meristematic cell from Alium cepa and demonstrated the clastogenic and aneugenic effects (mitosis and cytokinesis disturbs) in the cell. While, Toimela et al.[30] did not found cytotoxic effects of AlCl3 at 1µM for 24h exposure in epithelial and neuronal cell line ,respectively.

There are several causes have been proposed to explain cytotoxicity production by aluminum chloride. Aluminum chloride , generates free radicals and reactive oxygen species in cells, which cause fatty acid superoxides and oxidation of cellular membrane proteins, which leads to reduction of membrane fluidity and in consequence to damage of cellular membrane[31].

References:

1. Abbasali, K.M., Zhila ,T. and Farshad,N. ;(2005).Developmental Toxicity of Aluminium from High Doses of AlCl3 Mice. The Journal of Applied Research., 5: 575-579.
2. Mestaghanmi, H.; El-Amrani, S. and Saile, R.; (2002).Effects of aluminum chloride administration during gestation in rat. Stal., 27: 73-81.
3. Stella, M.; Ne'stor, M. ; Marcela, G. ; Maria del Carmen, C. and Maria Monica, E.; (2005). Alterations of the renal function and oxidative stress in renal tissue from rats chronically treated with aluminum during the initial phase of hepatic regeneration . J. of Inorganic. Biochem., 99: 1858-1864.
4. Kloppel, H., Fliedner, A. ;and Kordel, W. ;(1997). Behavior and endotoxicology of aluminum in soil and water. Review of the scientific literature. Chemosphere., 35: 353-363.
5. 5. Suwalsky, M., Ungerer, B.; Villena, F.; Norris , B.; Cardenas, H. and Zatta, P.; (2011). Effects of AlCl3on toad skin, human erythrocytes, and model cell membranes. Brain Res Bull., 55: 203-210.
6. Ochmanski, W. and Barabasz, W.; (2000).Aluminium-occurrence and toxicity for organisms. Przegl. Lek., 57: 665-668.
7. Dominigo, J.L. ,(1995).Reproductive and developmental toxicity of aluminium: a review. Neurotoxicol Teratol. 17:515-521.

8. Agrawal, S.K. ;Ayyash, L.; Gourley, C.S.;Levy, J.; Faber, K. ; Hughes, C.L.(1996) . Evaluation of the developmental neuroendocrine and reproductive toxicology of aluminum. Food Chem Toxicol.;34:49-53.
9. Colomina, M.T.; Esparza, J.L.; Corbella, J.; Domingo, J.L. ,(1998).The effect of maternal restraint on developmental toxicity of aluminium in mice. Neurotoxicol Teratol. 20:651-656.
10. Lione, A., (1985). Aluminum toxicity and the Aluminum containing medication. J. Pharmacol. Ther., 29: 255-285. http://cat.inist.fr/?aModele=afficheN&cpsidt=7862 878
11. Shehla, K.F.; Prabhavathi, P.A.; Padmavathi, P.; and Reddy,P.P. ;(2001). Analysis of chromosomal aberrations in men occupationally exposed to cement dust. Mutat. Res., 490: 179-186. http://cat.inist.fr/?aModele=afficheN&cpsidt=913452
12. Schmid, W. ;(1975). The micronucleus test. Mutat. Res.,31:9-15.
13. Brusick, D. J. ,(1980). Principles of Genetic Toxicology. New York, Plenum Press. P. 24, 33 and 218.
14. King, M.T.; Wild, D.; Gocke, E. and Eckhardt, K.;(1982). 5-Bromodeoxyuridine tablets with improved depot effect for analysis in vivo of SCEs in bone marrow and spermatogonial cells. Mutat. Res., 97:117-129.
15. Balasubremanyam ,A. ; Sailaja, N. ; Mahboob ,M. ; Rahman, M.F. ; Saber, M.H. ; and Paramjit ,G. (2009).In vivo genotoxicity assessment of aluminum oxid nonmaterial's in rat peripheral blood cells using the comet assay and micronucleus test . Mutagenesis 24(3):245-251.
16. Banasik , M.; Chandel, D.; Memon, M.R.; Patel ,T.N. ;(2005). Genotoxic effects of cotreatments of fluoride and aluminum on peripheral blood lymphocyte cultures . Mutat .Res., 327(1-2):17-22.
17. Yumei ,W.; Jinfeng ,J. ; Xiaohong ,Z. ; Baoshan ,Y. ;(1998).Genotoxicity of the dust organic extract and its fraction derived from an aluminum electrolytic plant .Toxicol .Lett. 98(3).147-153.
18. Trippi , F. ; Botto , N. ; Scarpato , R. ; Petrozzi ,L. ; Bonuccelli , U.; Latorraca , S. ; Sorbi , S.; Migliore ,L. ;(2001). Spontaneous and induced chromosome damage in somatic patients .Mutagenesis 16,323-327.
19. Moreno ,E.A.; Rojes ,G .F. Frenk , F.H. ; De La Huerta ,A.O ; Belmeras ,R.Q. ;and Vargas A.R.O. ; (1997) .In vitro induction of abnormal anaphases by contaminating atmospheric dust from the city of Mexicali .Baja California ,Mexico , Arch. Med . Res 28(4),549-553.
20. Elmore, A. R. ,(2013). Finle report on the safety assessment of aluminum silicate ,calcium silicate , magnesium aluminum silicate, magnesium silicate, magnesium trisilicate ,sodium magnesium silicate .zirconium silicate .attapulgite . bentonite , Fullers earth ,hectorite ,kaoline , lithium magnesium silicate, lithium magnesium sodium silicate ,montmorillonite , pyrophyllite and zeolite .Int.J. Toxicol . 22(1),37-102.
21. Synzynys, B.I. ; Sharetskii ,A.N. ; Kharlamova , O. V. ; (2004). Immunotoxicity of aluminum chloride. Gig. Sanit . 4,70-72.
22. Varella, S. D.; Pozetti ,G.L.; Vilegas ,W.; and Varanda ,E.A.;(2004). Mutagenic activity in waste from an aluminum products factory in Salmonnella / microsome assay . Toxicol ,In Vitro 18,895-900.
23. Botchway ,S.W.; Stevens ,D.L. ; Hill ,M.A. ; Jenner ,T.J. and On eill,P.; (1997).Induction and rejoining of DNA double –strand breaks in Chinese hamster V79-4 cell irradiated with characteristic aluminum k and copper L ultra soft X rays . Radiat .Res .148(4),317-324.
24. Pazy-Mino, C. ;Davalos , M. V. ; Sanchez ,M. E. ; Arevalo, M. ;Leone , P.E.; (2002). Should gaps be included in chromosomal aberration analysis ? Evidence based on the comet assay .Mutat .Res .516, 57-61.
25. Aguilar, F.; . Autrup, H. ; Barlow,S.; Castle, L.; Crebelli, R.; Dekant, W.; Engel, K.-H. ; Gontard,N. ; Gott, D.; Grilli, S. ; Gürtler, R. ; Larsen, J.-C.; Leclercq, C. ; Leblanc, J.-C. ; Malcata, F.-X. ; Mennes, W. ; Milana,M.-R. ; Pratt,I.; Rietjens, I.; Tobback, P. Toldrá, F.;(2008). Safety of aluminium from dietary intake . The E.F.S.A. Journal 754, 1-34.
26. Matsumoto H (1988) Changes of the structure of pea chromatin by aluminum. Plant Cell Physiol 29: 281–287.
27. Muma, N.A., Troncoso,J.C. ; Hoffiman, P.N.; Koo, E.H. and Price, D.L. ;(1988). Aluminium neurotoxicity: altered expression of cytoskeletal genes. Molec. Brain Res., 3: 115-122.
28. Dovgaliuk ,A.I. ; Kaliniak ,T. B. ; Blium ,I.B. ;(2011) a. Assessment of phyto and cytotoxic effects of heavy metals and aluminum compound using onion apical root meristem . Tsitol . Genet.35(1).3-9.
29. Dovgaliuk ,A.I. ; Kaliniak ,T. B. ; Blium ,I.B. ;(2001 b). Cytogenetic effects of toxic metals salts on apical meristem cells of Allium cepa L. seed roots .Tsitol. Genet. 35(2) ,3-10.
30. Toimela, T.; Tahti , H.; (2004). Mitochondrial vibility and apoptosis induced by aluminum , mercuric mercury and methylmercury in cell lines of neural origin .Arch. Toxicol ..78(10),549-564.
31. Corain, B.,(2013). The solution state of aluminum (III) as relevant to experimental toxicology: Recent data and new perspectives. Coord. Chem. Rev.,112: 19-32.

السمية الخلوية الوراثية لمادة كلوريد الألمنيوم (AlCl3) على الجرذ الأبيض *Rattus norvegicus*

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الخلاصه:

استخدمت في الدراسة الحالية (60) ذكر من الجرذان المختبرية البيضاء نوع *Rattu norvegicus* والتي قسمت الى اربعة مجاميع احتوت كل مجموعة على (15) ذكر. حقنت ثلاثة مجاميع عن طريق غشاء الخلب لمدة (30) يوم بتراكيز مختلفة من مادة كلوريد الالمنيوم AlCl3 وحقنت المجموعة الرابعة بمادة السيطرة. قتلت الحيوانات، وتم تشريحها واستخرجت النوى الصغيرة من نخاع العظم وفقاً لطريقة[12] (Schmid,1975). كما حسب معامل الانقسام الخلوي من خلال عد الخلايا التي في الطور الاستوائي الى 1000 خلية لمفاوية استخرجت من نخاع العظم وفقا لطريقة [13] (Brusick,1980) .
بينت النتائج زيادة معنوية في النوى الصغيرة للجرذان المعاملة بكلوريد الالمنيوم AlCl3 ولجميع التراكيز مقارنة مع مجموعة السيطرة وعند مستوى معنوية ($p<0.05$). كذلك انخفاض معامل الانقسام الخلوي للخلايا اللمفاوية في نخاع العظم مقارنة مع مجموعة السيطرة وعند مستوى معنوية ($p<0.05$).