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Effect of Environmental Contamination on Cell Division of Chick Embryos

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Abstract: This study aimed to use chick embryos as a biological indicator for assessing the genotoxic activity of environmental pollutants and used it as a living test system or a useful tool when a study on the target species is difficult or impracticable. Fertilized eggs were collected from AL-Twietha(contains the Iraqi nuclear reactor)represents a control group . Cytogenetic analysis for chick embryos have been done by studying Blast index (BI), and Mitotic index (MI), Replicative index (RI) and Sister Chromated Exchange (SCE) for embryonic cell in 2-7 days age. Results were compared with those that were held to chick embryos for eggs collected from AL-Kadhimiya and AL-Baya as a control group. Result showed significant increase in MI and RI in embryonic cells for chicks collected from AL-Twietha with age were as embryonic cells for chicks collected from AL-Kadhimiya and AL-Baya that decreased as age increase. SCE values showed a significant increase for eggs collected from AL- Tuwaitha area. These results show a defect in the genetic material and inability of the cell to repair the DNA damage, which resulted in a rise in SCE value. From this study it can be conclude that the possibility of using chick embryos as a biological indicator to detect the effect of pollutants and adoption as an alternative method for *in vitro* blood cultures for being easy ,accurate, and economic method.

Key word: Sister chromatid exchange (SCE), cell proliferation, chick embryos, environmental pollutants.

تأثير التلوث البيئي في الانقسامات الخلوية لأجنة الدجاج

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الخلاصة: تهدف الدراسة استخدام أجنة الدجاج كمؤشر بيولوجي للكشف عن تأثير التلوث البيئي على المادة الوراثية واعتمادها كنظام اختباري حي وطريقة بديلة للنماذج التي يتعذر تجربة تأثير الملوثات عليها. جمع بيض ملقح من منطقة التويثة (تحتوي على المفاعل النووي العراقي). أجريت التحليلات الوراثية الخلوية لأجنة الدجاج بدراسة معامل الأرومة (blast index)(BI) ومعامل الانقسام (mitotic index)(MI) ومعامل التضاعف (replecative index)(RI)، والتبادل الكروماتيدي الشقيقي (Sister Chromated Exchange)(SCE) للخلايا الجنينية وبعمر 2−7 أيام وقورنت النتائج مع تلك التي أجريت لأجنة دجاج من بيض تم جمعه من منطقتي الكاظمية والبياع كمجموعة سيطرة. وتم حساب معامل الانقسام MI ومعامل التضاعف RI والتبادل الكروماتيدي الشقيقيSCE. بينت النتائج إن هناك ارتفاعا معنويا في معامل الانقسام الخلوي MI ومعامل التضاعف RI في الخلايا الجنينية لأجنة الدجاج لمنطقة التويثة مقارنة بقيمها في الخلايا الجنينية لأجنة الدجاج لمنطقتي الكاظمية والبياع والتي انخفضت فيها قيمة معامل الانقسام MI ومعامل التضاعف RI بتقدم العمر. وتمت دراسة فحص التبادل الكروماتيدي الشقيقي (SCE) ولوحظ زيادة في قيمة(SCE) في أجنة البيض لمنطقة التويثة. توضح النتائج حدوث خلل في المادة الوراثية وعدم قدرة الخلية على إصلاح جزيء ال DNA والذي نتج عنه الارتفاع في قيمة (SCE). نستنتج من الدراسة إمكانية استخدام أجنة الدواجن كمؤشر بيولوجي للكشف عن تأثير الملوثات واعتمادها كطريقة بديلة لمزارع الدم في الزجاج *in vitro* لكونها طريقة سهله ودقيقة واقتصادية.

Introduction

A lot of studies went to accomplish the best means to detect environmental contaminants effect. Cytogenetic analysis in chick embryos is biological methods use globally, provides a good approach for assessing the genotoxic activity of environmental chemicals and is a useful tool when a study on the target species is difficult or impracticable (1). Chick embryonic considered as developing test system used as an acceptable model for detection genotoxic activity (2, 3) because it is an in vivo assays considered efficient and sensitive for the testing of environmental chemicals(3). Direct and indirect-acting mutagens may be detected early in development (4).

Directly labeling DNA in proliferating cells is an important technique used by many researchers(5). BrdU ((5- bromo-2-deoxyuridine)), a thymine analogue that incorporated into the proliferating DNA during S-phase of cell division(5). Various authors have suggested that sister chromatid exchange (SCE) analysis using the BrdU probe offers a cytogenetic technique for determining the potential genetic hazards of chemicals in the environment (2, 6),it is a sensitive cytogenetic assay for detecting genotoxic effects of chemical mutagens and carcinogens (7).

Evidence is mounting that SCE analysis is a far more sensitive indicator of chemically-induced chromosome damage than the traditional chromosome breakage studies and are easier to see and score than chromosome gaps and breaks (8). The frequency of SCE and the frequency of chromosomal aberrations are the genetic end points that can be used (2).

Chick embryos are used widely as test system for studies in cell biology, virology, immunology, cancer biology experimental embryology and teratology as well as for genotoxicity testing (9,10) and have adapted as cytogenetic test system for the study of the genotoxic activity of pesticides(8). Current study was conducted in AL-Tuwaitha, this region contains the Iraqi nuclear reactor, materials and supplies for nuclear reactors, and the most important material (Yellow cake) depleted uranium which was stealing. Looters emptied the depleted uranium barrels in agricultural land and tables, as well as using the empty barrels for domestic purposes, so we collected the eggs from the surrounding, neighboring areas as a test group and from far field(AL-Kadhimya,AL-Baya) to be control group. The objective of this study to detect the effect of pollution by using cytogenetic tests in chick embryos.

Materials and Methods

Wild type strain chicken eggs were used in the present study. The eggs were set in an incubator with automatic hourly rotation of eggs at a temperature of 37.80c and at a relative humidity of

about 65%. After 3 days and 7 days of incubation, 100µl (150 µg/embryo,100µg/embryo) of BrdU were injected into the air sace as shown in fig(1). The hole was sealed with wax. Embryos were removed on day 4, 7 of incubation (at 97 h, 144h) after a 2-h exposure to a 0.05% colchicine solution (11). Eggs were collected from three areas in Baghdad, and were divided into two groups the first group which collected from AL-Tuwaitha as a test group. The second group which collected from AL-Kadhimiya, AL-Baya represents the control group.Each egg weight separately before introduced to the Hatcher.

Chromosome preparations were made by a solid tissue technique (12). The slides were stained by a modified fluorescence plus Giemsa technique for differential chromatid staining as previously reported (13), using the fluorescent dye Hoechst 33258 (Fluka), followed by Giemsa stain (R66, Gurr, Poole, UK). SCEwere scored in the first five pairs of macro-chromosomes. A total of 30–40 second-division cells were examined from each embryo and 5-10 embryos per area were included.

Cell cycle progression analysis

The frequencies of first (M1), second (M2), and third (M3) mitoses were determined by analysis of staining patterns (14); 200 metaphases (8–10 embryos) per treatment selected at random were analyzed. The mitotic index was determined on 1000 consecutive cells.

Slides stained with Hoechst 33258 to calculate replicated index (RI) and sister chromatid exchange (SCE) according to (15) method.

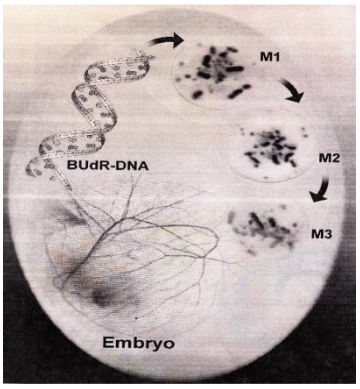
$$RI = \frac{\%M_1 X_1 + \%M_2 X_2 + \%M_3 X_3}{100}$$

Figure (1) scheme for cytogenetic analysis method on chick embryos

The eggs were laid in the brooder at 37.8° C and humidity of 65% for fetal growth

↓

Eggs were examined optically, and injected with Brdu 150µg/0.1ml for embryos aged 3 days and150 µg /0.1 ml for embryos aged 7 days



↓

After 24 h. eggs injected with 5µg/0.1ml colchicine solution

↓

Blood collection from AIlantoic fluid 0.1-0.2ml

↓

Cell harvesting

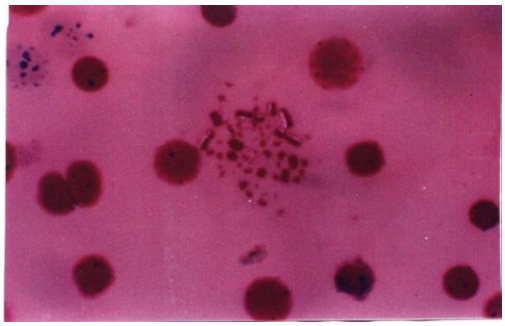
↓

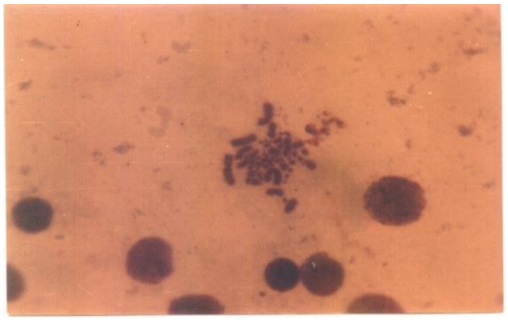
SCE, RI

MI

Slides stained with Hoechst

Slides stained with Gemza





Statistical Analysis

Data were analyzed using the SPSS/PC+ statistical package (SPSS Inc., Chicago, IL) Control and experimental data were compared by one-way analysis of variance, followed by Duncan's multiple comparison test and by linear regression analysis.

Results and Discussion

The statistical analysis for the results showed significantly increase in the values of MI index and RI index of embryonic cells along with age($p \leq 0.01$) for eggs collected from AL-Tuwaitha were as the opposite was observed in cells for embryonic eggs from the regions of AL-Kadhimiya, AL-Baya which decreased with age increase Table (1). The decrease in the coefficient of division in embryos, especially in the seventh day of the age occur in the natural state, because the divisions occur in the first few days of growth and then cut down the growth with a discrepancy in this reduction depends on the type of tissue developing (16,17) that was observed in the blood cells of chick embryos belonging to the two areas of AL-Kadhimiya, and AL-Baya. Significant inhibition of cell cycle progression was result from exposure to environmental chemical (8), and this was also confirmed by (18), by contrast, aclear increase in cell cycle progression in cells for chick embryo from the regions of AL-Tuwaitha were observed may be indicative of damage activity due to enyironmental contamination with depleted uranium.

Table (1): Percentage of MI, RI of chick embryos according the age in different regions

RI%	M3%	M2%	MI%	MI	Age(day)	
2.14	39	35	26	5.9	2	AL- Kadhimiya region
2.02	28	40	32	5.6	3	
1.94	39	29	32	6.1	4	
2.00	35	30	35	6.3	5	
2.02	27	34	39	5.6	6	
2.00	38	32	30	5.0	7	
2.14	39	36	26	8.5	2	AL-Baya region
1.98	30	28	32	5.9	3	
2.02	27	39	34	6.4	4	
1.94	33	28	39	6.1	5	
1.02	30	32	38	6.1	6	
.001	30	30	35	6.2	7	
2.04	38	28	34	6.6	2	AL-Tuwaitha reagion
2.05	33	38	29	6.8	3	
2.03	32	39	29	7.2	4	
2.01	31	40	29	6.8	5	
2.96	38	30	32	6.4	6	
2.98	34	36	30	7.8	7	

Note: Had been study ten models of every age

Duncan s value for RI :	Duncan s value for MI
0.05=0.433, 0.455	0.05=0.654, 0.687
0.01=0.582, 0.607	0.01 = 0.878, 0.915

The SCE is associated with abnormal DNA replication as a result of which DNA repair mechanisms are initiated. Sister chromatid exchanges appear in cells due to incorrectly or inefficiently functioning mechanisms of DNA damage repair(6).The result of SCE investigation are shown in table (2) the rate of SCE in chick embryos cells of the areas under study (AL-Kadhimiya , AL-Baya , and AL-Tuwaitha)revealed that there is a significance rise($p\leq o.o5$, and at $p\leq 0.01$) in the values of SCE in the cells of chick embryos in AL-Tuwaitha from the regions of AL- Kadhimiya , and AL-Baya This means the damage in the DNA molecule which lead to increase the frequency of SCE and this was confirmed by (6,19,20) from the frequency of SCE means to repeat the damage in a DNA molecule and it depends on the quality and quantity of the radiation dose that have been exposed to it.

The increase in the SCE lead to a decline in the cell cycle progression and this was resulting from exposure to chemical pollutants (2). The decline or decrease in the rate of SCE means that there is a high level and accurate in the process of repair of the DNA molecule, which referred to increasing in the division and percentage of cells in the second stage M2, where as the rise in rates of SCE attributed to the inability of the cell to repair DNA and the appearance of a high rate .Since in case of a decline in the rates of SCE means the death of cells bearing damage in the DNA division and cell cycle reflecting the inference of the impact of radioactive contamination on the genetic material (21,19) .

This demonstrates that the effect on chick embryos from AL-Tuwaitha more sensitivity than that of chick embryos of AL- Kadhimiya and AL-Baya.This result is consistent with result confirmed in the previous study that the animals grazing(sheep) in AL-Tuwaitha be more sensitive than that graze in other areas (not exposed to contamination) through a rise in coefficient of cell division , and an increase in the value (SCE) as a result of exposure to radioactive materials (22), and the results indicate the presence of DNA damage , and this was confirmed by Al-Sheikh (2003) from exposure to ionizing radiation lead to the damage in the DNA molecule , and the inability to repair the DNA molecule (2002). Many researchers pointed to the use of embryonic cells as an easy, accurate and economical way to study the effect of mutagenic and carcinogenic substances in the genetic material of embryos (2). The SCE test makes it possible to detect chromosome

instability that corresponds with elevated vulnerability of the organism to genotoxic factors of a mutagenic and carcinogenic nature (6). The results prove the usefulness of the SCE test and cell cycle progretion in chick embryo for the detection of chromosome instability caused by environmental factors with mutagenic and genotoxic properties such as depleted uranium, materials and supplies for nuclear reactors which was destroyed after 2003 event, and the test
employed in the present study is also a useful cytogenetic tool, with application as a biomarker in the monitoring of environmental contamination.

Conclusion

The possibility of adopting chick embryos as a biological indicator of environmental pollutants, and possible use in the detection of the effect of environmental contamination such as pesticide and gives sufficient evidence for detection of genotoxic effect of some chemical environmental pollutants. As well as the possibility of using chick embryos to detect contamination of feed pesticides. In addition as an alternative method to conduct cytogenetic analyses in vitro as a living test system dose not need to culture media, so it is an economic, easy and accurate, method.

Table (2): the rate of SCE values in embryonic cell in the areas under study (AL-Tuwaitha, AL-Baya,and AL- Kadhimiya)

Embryonic age(day)	SCE/metaphase (X±SD)		
	AL-Tuwaitha	AL-Baya	AL- Kadhimiya
3	0.10± 1.53	0.02±0.91	0.11±0.88
5	0.02± 1.71	0.04±0.89	0.02±0.96
7	0.01±2.24	0.04±1.20	0.10±1.16

Note: Had been study five sample for each age.

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