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Chromosomal Aberrations in Peripheral Blood Lymphocytes in Brick Kilns Workers

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Abstract

This study was conducted on the workers of the brick kilns at Al- Nahrawan area south-east of Baghdad city to investigate the effect of the pollutant emissions (fumes and gases) released from the burned fuel in this kiln. Blood samples were taken from group individuals of workers at this brick kilns and non-working individuals as a control. The influence of these emissions on the workers' health was measured by examining certain chromosomal aberrations among the workers. These aberrations were a formation of: chromosomal rings, dicentric chromosomes, breaks arm and acentric fragments. It has been observed that most of these aberrations were found within workers of age 40 years and above, represented by formations of averages 1.50 and 1.02 for chromosomal rings and fragments respectively, whereas the results of the group of age range 10-19 years old showed chromosomal aberration types (ring 0.05, dicentric 0.2, chromosomal break 0.15 and acentric fragment 0.05) while the control results for all chromosomal aberrations (ring 0.09 dicentric 0.07, chromosome break 0.12 and acentric fragment 0.10). An increase in levels of dicentric chromosomes, chromosome breaks and acentric fragments were observed in the group of age 40 years and above (0.25, 1.50 and 1.25) respectively. All these chromosomal aberrations showed significant differences when compared to the results of groups 10-19 years of age. The results also demonstrated a relation between smoking and serving years of workers, showing (0.27, 0.28 , 1.26 and 1.12) a significant increases in chromosomal aberrations (ring, dicentric, breaks and acentric fragments) at worker group of serving years 16-20 years (0.23, 0.25, 1.28 and 1.30) and group of 21-25 years (0.27, 0.28, 1.26 and 1.12) respectively comparing with healthy individuals of chromosomal aberrations types (ring 0.05, dicentric 0.20, chromosomal breaks 0.15 and acentric 0.05) Respectively. The aim of study is to investigate the effect of gases emission on chromosomes in brick kiln workers.

Keywords: Types of chromosomal aberrations, human blood lymphocytes, brick kiln workers.

الانحرافات الكروموسومية للخلايا اللمفاويه للدم المحيطي للعاملين في معامل الطابوق

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الخلاصه

أجريت هذه الدراسة على العاملين لدى معامل الطابوق في منطقة النهروان جنوب شرقي مدينة بغداد لغرض التحري عن الملوثات المنبعثة نتيجة احراق الوقود في هذه المعامل (ابخره وغازات) جمعت نماذج من الدم من مجاميع من العاملين لدى هذه المعامل اضافة الى نماذج اخرى جمعت من غير العاملين(كمجموعه

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ضابطه) وقد تم قياس تأثير هذه المواد المنبعثة على الماده الوراثية من خلال دراسة التشوهات الكروموسوميه اظهرت النتائج معظم هذه الانحرافات الكروموسوميه ضمن العاملين بأعمار 40 سنه فما فوق متمثلة بظهور معدلات عاليه (1.05 , 1.02) للكروموسوم الحلقي والقطع عديمة المركز على التوالي. في حين سجلت نتائج مجموعة الاعمار 10–19 سنه معدلات الانحرافات (الحلقي 0.05 الثنائي المركز 0.02 والكسور الكروموسوميه 0.15 و القطع عديمة المركز 0.05)، بينما كانت نتائج المجموعة الضابطة لكافة الانحرافات الكروموسوميه هي (الحلقي 0.09 ،ثنائي المركز 0.07 والكسور الكروموسوميه 0.12 والقطع عديمة المركز 0.1 وقد كانت زياده في مستوى الكروموسومات ثنائية المركز والكسور الكروموسوميه وكذلك القطع عديمة المركز في مجموعة العاملين من الاعمار 40 سنه فما فوق (0.05 , 0.5, 0125) على التوالي. وقد أظهرت جميع تلك الانحرافات الكروموسوميه فروقات معنويه لدى مقارنتها مع نتائج مجموعه الاعمار (10–19سنة) وكذلك بينت هذه النتائج وجود علاقه بين المدخنين من العاملين ومدة الخدمة , 1.12,1.26 (0.27 , 0.28) وقد كان هناك زياده معنويه في الانحرفات الكروموسوميه (الكروموسوم الحلقي ،الكروموسومات ثنائية المركز والكسور الكروموسوميه ،قطع الكروموسومات عديمة المركز لدى العاملين من الاعمار 16–20 سنه (0.23, 0.25 , 1.28 , 1.3) وكذلك المجموعه21–25 سنه (0.28 0.12,1.26 0.27, 0. ,) على التوالي، مقارنة مع المجموعة الاصحاء والضابطة (الحلقي 0.05 وثنائية المركز 0.2 والكسور الكروموسوميه 0.15 والقطع عديمة المركز 0.05) لذا هدف البحث الى دراسة تأثير الانبعاثات الغازية على المادة الوراثية من خلال دراسة التشوهات الكروموسومية للعاملين في معامل الطابوق .

Introduction

The air pollution is defined as the presence of substances in certain amounts in the atmosphere as to affect human, vegetation, animals, or material adversely. Large scale burning of fuels, coal, oil, gas etc. To supply energy to industries**.** Air pollution could have local as well as global impacts. All living beings inhaling or exposed to the polluted air gets affected by it. Particulate matters such as smaller dust and smoke particles penetrate deeply into the lungs and get deposited there. The brick kilns emit toxic fumes containing suspended particulate matters rich in carbon particles and high concentration of carbon monoxides and oxides of sulfur (Sox) that are harmful to eye, lungs, throat and also stunt the mental and physical growth of children [1]. The recent scientific report states the combustion of clay and fuels for making bricks in the brickfield produce dioxins and furans as by-products, which first enter in to the air from where the humans, birds and other animals either directly inhale or intake through different contaminated food stuff, both vegetable and animal origin. Condensation of the genetic material at metaphase stage is a crucial event which provide equal segregation of chromosomes between the two new daughter cells during the next step, anaphase, this is followed by relaxation of the genetic content after cell division [2]. Genotoxic chemicals are able to cause damage to DNA but do not inevitably lead to the creation of cancerous cells Peripheral T-cell Lymphoma PTCL [3]. Dicentric chromosomes are product of genome rearrangement that place two centromeres of the same chromosome [4] Depending on the organism, dicentric stability varies after formation. In humans, dicentrics occur naturally in a substantial portion of the population and usually segregate successfully in mitosis and meiosis [4]. Their stability has been attributed to inactivation of one of the two centromeres, creating a functionally monocentric chromosome that can segregate normally during cell division [5]. Dicentric chromosomes have been identified as instigators of the genome instability associated with cancer, but this instability is often resolved by one of a number of different secondary events [6]. Tests for chromosomal aberrations (CA) are usually included in cytogenetic assays to determine the clastogenic properties of xenobiotics. There are three main methods of chromosome visualization applied in CA analysis labeled chromosome band-specific painting probes. Spectral Color Banding (SCAN) analysis simultaneously identifies the origin of chromosome bands by a unique spectrum for each band. SCAN analysis can identify a particular region of a chromosome such as a translocate or deleted region, so that it can be directly assigned to the corresponding band number in G-banding. SCAN is useful for full characterization of chromosomal abnormality that could not be identified by G-banding or Spectral karyotyping SKY analysis. This technique can therefore be expected to become a powerful tool for cancer [7].

Materials and Methods

The study was conducted at Al-Nahrawan area that located 65 km at south- east of Baghdad center. The area includes about 85 brick kilns which represent 17% of the total kilns in Iraq. The workers and the residents at this area are continuously exposed to the pollutants released by the chimneys or the cracks of the walls of the brick kilns and they were suffering of several environmental problems as a result of poor caring and lack of suitable and active infrastructure, the information about workers who exposure to pollutants of brick Kilns were collected using the questionnaire form.

Blood Samples

Four milliliter of peripheral blood was collected under aseptic conditions by vein- puncture using disposable syringe promoted with heparin. The blood was placed in a cool- box and transferred to the laboratory. The samples were used for cytogenetic analysis and micronucleus assay.

Cytogenetic analysis of human blood lymphocytes

Blood cell culture for cytogenetic analysis was done [8]. Briefly human peripheral blood was collected into heparin coated syringe by taking 0.25ml in test tube contains 2ml of Roswell Park Memorial Institute (RPMI -1640), with adding 0.25ml of Phytohaemagglutinin-L (PHA-L) and mixed gently, then incubated at 37^{o}C for 72 hrs. 0.2 ml of colcemid was added before 2hrs of harvesting .The test tubes were centrifuged at speed 2000 rpm for10 min .The supernatant was removed and 5 ml of 0.075 M hypotonic solution was added, and then the test tubes were incubated at 37˚C for 1hr with shaking. The tubes were centrifuged at 2000 rpm for 10 min .The supernatant was removed and the Carney's fixative 3 methanol: 1 Acetic Acid (V/V) was added as drops on the inside wall of the tubes with continuous shaking .The cells were kept at 4˚C for 30 min for fixation. The tubes were re-centrifuged at 2000 rpm for 10 min. The process was repeated 3 times and after that, the cells were suspended in 2 ml of fixative solution. A few drops from the tubes were dropped vertically by the use of Pasteur pipette from the height of 3 feet at a rate of 4 -5 drops to give the chance for chromosomes to be spread well, then the slides were kept at room temperature. The slides were stained with Giemsa stain working solution was prepared by taking: a stock solution of Giemsa stain was prepared by adding 1ml absolute methanol 1.25 ml sodium bicarbonate solution, 0.5 ml distal water (D.W.) 40 ml.for 2min and then washed with D.W. Two slides for each concentration were prepared for cytogenetic assay.

Statistical Analysis

One way analysis of variance was performed to test whether group variance was significant or not, the comparison between groups were used analysis of variance ANOVA.

Results and Discussions

Types of Chromosomal Aberration

The results in Table -1 showed the relationship between smoking and non -smoking in induction of chromosomal aberrations that gave an idea of increasing chromosomal aberration in smoking compared with non- smoking and with control by using LSD value and this results agreed with [9].The tobacco constituents are proven carcinogens and are known to induce significant chromosome aberrations in blood cells of smokers [9] .The table showed increase in ring formation in smoking (0.24± 0.007) and non smoking workers 0.17±0.002 when compared with control(0.05 ± 0.00) , also in the formation of dicentric chromosome showed significant differences between control and smoking (0.25± 0.001) and non smoking (0.20±0.003). The statistical analysis showed significant differences between control and workers of brick kiln but there were no significant differences between group of smoking and non smoking. The results of chromosome breaks showed significant differences between control (0.15± 0.002) and smokers (1.58± 0.04) and non smokers (1.27± 0.04) and the same in the results of acentric fragments . The smoke of cigarettes contain many chemical compounds that caused to DNA damage in addition to many toxic chemicals such as nitrosamine , poly aromatic hydrocarbons and aromatic amines that affect on DNA adducts and then cause damage to it[13] .The smoke of cigarettes lead to increase the oxidative damage and other compounds react with the proteins responsible of repair process and inhibit the repair system in the cell [14]. By high levels of air pollution. The excess of genetic damage observed in somatic cells usually T lymphocytes may imply an increased, albeit quantitatively undefined, cancer risk in other tissues [10].

Table 1- Comparison between smoking and non-smoking in chromosomal aberrations

Group	Mean ± SD			
	Ring	Dicentric	Chromosome break	Acentric fragment
Control	0.05 ± 0.00	0.13± 0.004	0.15± 0.002	0.05± 0.002
Smoking	0.24± 0.007	0.25± 0.001	1.58± 0.04	1.13± 0.005
Non-smoking	0.17±0.002	0.20±0.003	1.27± 0.04	0.93±0.003
LSD	0.092*	0.086*	0.483**	0.528**
P-value	0.0478	0.0514	0.0039	0.0153
*(P < 0.05) ** (P < 0.01).				

Table-2 demonstrated the effect of pollution of brick kilns on induction of chromosomal aberrations of employment and residents. The result showed chromosomal aberrations that represent ring, Acentric fragments of chromosome [10] .The results demonstrate the effect of employee years of workers and its relation on the induction of chromosome aberration, there were an increase in types of chromosomal aberrations that represent ring, Acentric fragments. The employee years between 5 groups showed the highest percentage of ring formation, it gave (0.27 ± 0.008) and the lowest value gave (0.13 ±0.006), the employee years in group 1 ranged 1-5 years, so as group 5 showed significant of differences with the group 2 while group 4 with employee years 16-20 years and group 3 which represent 11-15 years showed no significant differences between the two groups**.** In addition, the results showed the effect of pollution in induction of acentric fragments reveled significantly differences between control and groups of service years and increased with the increase of service years , indicated the highest value in group 4and 5 (1.3±0.02, 1.12±0.09) respectively, and the lowest value in group 2 (0.77±0.2) , this may be due to the exposure duration of employee to the pollutants in brick kiln which accumulated in the body and increase their effects on the induction of chromosome aberrations due to genetic mutation [11] .

Table 2-Effect of service years on induction of chromosomal aberration

Service years	Chromosomal aberrations of workers		
	Control	Ring Mean ±SD	Acentric fragment Mean ± SD
Group 1:(1-5)	0.005±0.00	0.13±0.006	0.77±0.02
Group2: (6-10)	0.05 ± 0.00	0.17 ± 0.003	0.87 ±0.006
Group 3:(11-15)	0.05 ± 0.00	0.20 ± 0.003	0.92 ± 0.05
Group4:(16-20)	0.05 ± 0.00	0.23 ± 0.005	1.3 ± 0.02
Group5:(21-25)	0.05 ± 0.00	0.27 ± 0.008	1.12 ± 0,09
LSD value	0.00NS	0.083	0.392
P-value	1.000	0.0519	0.00246
*(P <0.05), ** (P <0.01).			

In Table-3 the relationship between service years and induction of chromosomal breaks showed significant differences between groups of all service years of brick kiln workers and control. The highest value was in group 4 and 5 and the lowest value was seen in group 1. While there were no significant differences between group 1 and group 3 and it showed significant differences between group 5 and group 1 and group 2.

Table 3- Relations ship between service years and induction of chromosomal aberrations

Service years	Chromosomal aberration workers	
	Control	Ch. Break
Group1:((1-5)	0.15 ± 0.00	0.43±0.005
Group 2:(6-10)	0.15± 0.00	0.56±0.002
Group 3:((11-15)	0.15± 0.00	0.98±0.006
Group4:(16-20)	0.15± 0.00	1.28 ± 0.15
Group5:((21-25)	0.15± 0.00	0.12 ± 1.26
LSD value	0.00 NS	0.428
P-value	1.000	0.00317
*(P<0.05), ** (P<0.01) *		

The results in Table -4 showed other type of chromosome aberrations that is the formation of di centric chromosome as a result of occupational exposure of the workers to the pollutants of brick kiln , it showed increase in its rate with high value in service group 5 that gave(0.280±0.007) and also in group 4 that gave (0.25±0.011). The statistical analysis showed significant differences between group 4 and group 1and 2 while the results showed no significant differences between group 1and 2 and also no significant differences between group 4 and 5.

Table 4- The Relationship of service years on induction of chromosomal aberration (Dicentric Chromosome)

Chromosomal aberrations		
Services years	Control	Dicentric Mean SD
Group 1: (1-5)	0.2 ± 0.00	0.08± 0.003
Group 2:((6-10)	0.2 ± 0.00	0.12± 0.005
Group 3:(11-15)	0.2 ± 0.00	0.17±0.002
Group4:((16-20)	0.2 ± 0.00	0.25±0.011
Group5:((21-25)	0.2 ± 0.00	0.280±0.007
LSD	0.00NS	0.115**
P-Value	1.000	0.0143
	*(P<0.05),(P<0.01)**	

The results in Table-5 showed the aberrations relationship between age group and chromosomal aberrations that indicated increasing in chromosomal aberration with increase of age groups the value of LSD was 0.107 and significantly clear of compares different means with control. There were an increase in all types of chromosomal aberrations that represent ring, dicentric, chromosome breaks , acentric fragments The group: 1 and 2 showed significantly difference with groups 5 in ring chromosome formation which gave (0.09 ± 0.003) and (0.18 ± 0.002) respectively, and (0.30±0.004) in group 5, it suggest that the age group of 40-49 years and over 40 gave high rate of chromosomal aberrations types and show significant between age group 4 which present 40-49 years and group 5 which present over 40 years with the age of group 1which represent 10-19 years old and group 2 which represent 20-29 years old and 30-39 years old. In addition the results showed the effect of pollutants on the formation of dicentric chromosome and show significant differences when compared with control and also with the group ages ,it of group ages gave high value of dicentric formation (0.25± 0.004) in group 5 which showed significant differences with group 1 and 2 (0.07± 0.002 , 0.98± 0.005) respectively. The table also showed other type of chromosomal aberrations is the chromosome breaks and also gave significant differences when compare with control group and the same results with the formation of acentric fragments. Acentric rearrangement with significantly differences between group 5and group 2,1 and also with control groups showed significant differences with all age groups and these results agreed with [12][13] [14]. The causes of chromosomal aberrations are well understood polyploidy from failed cytokinesis , aneuploidy from nondisjunction or premature separation of chromosome, and structural rearrangement from chromosome breakage in addition of host intrinsic and extrinsic factors produce chromosomal aberration in tissue culture cell [15].

Table 5- Comparison between age groups (years) and Chromosomal aberrations in brick kiln workers.

Age groups (yr.)	Workers in brick kilns				
	Control	Ring Mean ± SD	Dicentric Mean ± SD	Ch. break Mean ± SD	Acentric frag Mean ±SD
Group 1:(10-19)	0.05±0.00	0.09 ± 0.003	0.07± 0.002	0.12± 0.004	0.10± 0.003
Group 2:(2o-29)	0.05±0.00	0.18 ± 0.002	0.98± 0.005	0.23± 0.002	0.22± 0.005
Group 3:(30-39)	0.05±0.00	0.26 ± 0.007	0.12± 0.005	0.87± 0.003	0.50± 0.002
Group4:(40-49)	0.05±0.00	0.27± 0.004	0.20± 0.01	0.120± 0.005	1.10± 0.04
Group 5:(Over49)	0.05±0.00	0.30±0.004	0.25± 0.004	0.150± 0.003	1.25± 0.14
LSD value	0.00 NS	0.107**	0.248**	0.531**	0.463**
p-value	1.000	0.0049	0.0136	0.0042	0.01126
** (P< 0.01).					

Conclusions

It is strongly recommended that steps should be taken to reduce exposure residential area by increasing the distance of brick kiln furnace by 200 feet from residential area so that exposure to various gases and Installation of proper chimneys can also be helpful in reducing the exposure to fumes and gases.

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