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Relationship between blood lead levels and cytogenotoxic effects in human exposed to diesel exhaust

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Abstract:

Diesel exhaust (DE) is a mixture of hundreds organic and inorganic compounds, several of them are cytogenotoxic. This way, the aim of this study was to indicate the relationship between blood lead level and the exposure to cytogenotoxic constituents of DE. Blood samples were collected from 80 nonsmoker healthy men. Sixty of them were exposed directly to DE and the others were negative control. Exposure metrics included blood lead levels (Blls) while cytogenotoxic effects of DE were investigated by using micronucleus assay (MN), sister chromatid exchange assay (SCE) and comet assay. The results showed that the means of Blls in exposed workers to DE (47.40 – 49.50) µg/dl significantly higher (P <0.01) than mean of Blls in control gruop (15.75) µg/dl. When Blls range from 60 to 80 µg/dl , the cytogenotoxic effects were significantly higher (P <0.01) than others Blls groups. So that BLL it can be indictor to exposure to DE which have cytogenotoxic effects on human.

keywords: *diesel exhaust, lead, blood lead levels, sister chromatid exchange, micronucleus assay, comet assay.*

Introduction:

Diesel exhaust is emitted at ground level generated during the combustion process of diesel fuel (1). It is considered as a major component of urban air pollution (2).

United States Environmental Protraction Agency, mentioned primary diesel emissions are a complex mixture containing hundreds of organic and inorganic constituents in the gas and particle phases (3).

Gases including nitrogen N2, oxygen O2 , carbon dioxide CO2 , and volatile hydrocarbon species (4, 3, 5).

The diesel exhaust particles (DEPs) contain carbon nuclei, which absorb a vast number of organic compounds such as polycyclic aromatic hydrocarbons (PAH), nitroaromatic hydrocarbons, heterocyclics, quinones, aldehydes, and aliphatic hydrocarbons, and traces of heavy metals such as iron, copper, chromium, and nickel (6, 7 , 8, 9).

Lead was one of the first metals used by human, which have unique properties like softness, malleability, low melting point, and resistance to corrosion which make it one of the most widely used metals, and one of the oldest known occupational and environmental poisons (10, 11). According to statistics, millions of tons of lead are produced or processed every year and its use is the fifth highest metal used throughout the world (12). Lead occurs naturally throughout the world and is found in soils, oceans, and air (13). Generally, contamination by lead from natural sources is very small and man's exposure from these sources is negligible (14 , 13). Al-Saleh mentioned the annual anthropogenic lead emission exceeded the natural rates by approximately 1814% (14). It can cause many health problems especially for children (15, 11). Urban air lead levels are typically between 0.15 and 0.5 µg/m3 in most European cities (16). Nowadays, lead is used in several building materials, acid batteries, munitions and protection against X-rays, metallic alloy to produce solders, fuses, plastic, ceramics, paints, glazes, insecticides and petrol, among others (17, 18, 11). There are numerous ways for the entry of lead into the human body , which include inhalation, ingestion and through the skin (19). Lead absorption in the human body is slow and it is influenced by a large number of factors. These include variations in dose, age, nutritional status and physiological and genetic differences (11). It can distribute in the body by three main parts of blood, soft tissues, and bone (12). It causes damage especially in three systems: the neurological, the hematopoietic and the renal (20). Lead has carcinogenesis and mutagenesis effects on human (11). It is also caused le-

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sions in other systems, mainly at human gastrointestinal tract, circulatory (21), respiratory , immune system , endocrine , and reproductive system (18). In children lead may impairment and disturbed vitamin D metabolism (16). The toxicity of lead is mainly done via influencing the enzymatic systems of cells (12).

Materials & Methods:

Sampling blood

Ten milliliters of blood samples were collected from 80 non-smoker healthy men. Their age around from 20 – 35 years. Sixty persons of them were workers exposed directly to diesel exhaust in Baghdad city. Thirty of them exposed to diesel exhaust less than 3 years, while the others exposed to diesel exhaust more than 3 years and the last 20 persons were control negative.

Measurement the levels of lead in blood samples

Sampling

Six milliliters of blood with EDTA was collected as mentioned previously. The blood samples were kept frozen at -20ºC until analyzed to measure the concentration of heavy metals in blood.

Sample preparation

The protocol of National Institute for Occupational Safety and Health (NIOSH) (22) was preformed modified as follows:

Samples were allowed to thaw to room temperature.

Three milliliters of each blood sample were transferred to clean beaker.

Ten milliliters of digestion acid (3 nitric acid : 1 perchloric acid) were added to each blood sample and heated at 110ºC for two hours on a hotplate. Reagent blanks were started at this step.

Hotplate temperature was increased to 250ºC and heated until remained 1 ml.

Samples beakers were allowed to cool.

Each beaker contents were transferred to 10 ml volumetric flask, and completed to the mark with deionized water.

Measurement

Standards and samples were analyzed by using flameless atomic absorption. (Ibn Sina Stat Company / Ministry of Industry and Minerals) Standard was also analyzed once every ten samples.

Calculations

C = CsVs - CbVb

Cs= concentration of heavy metals in blood samples (µg/ml).

Cb= concentration of heavy metals in blank (µg/ml).

Vs= final solution volume of sample (ml).

Vb= final solution volume of blank (ml). (22)

Sister chromatid exchange assay

This technique was applied according to the protocol (23).

One half of milliliter was added to culture tube media contain 4.5 ml of media RPMI -1640 with fetal calf serum (FCS) with phytohemagglutinin. Bromodeoxyuridine BrdU 10 µg/ml was added to each culture tube with final concentration 10 µg/ml. Twenty minutes before ending of 72 hours incubating, 100 µl of colcemid were added to each culture tube. After 20 mins all culture tube were centrifuged at 1500 rpm for 10 min. The supernatant was discarded and the cells left in bottom of the tube were resuspended in 10 ml hypotonic solution (KCl 0.075 M) , and then incubated for 30 min at 37 ºC. Culture tubes were centrifuged at 1500 rpm for 10 min. Cells were fixed with freshly made fixative (1 glacial acetic acid : 3 methanol). Then cells were dropped on clean slides. A few drops of Hoechst 33258 solution were placed on each slide. The slides were put on PBS and exposed to ultraviolet light at a distance of 20 to 25 cm from the source for 2 hours. Then stained with giemsa stain and examined with light microscope.

Calculations

Sister chromatid exchange(SCE/cell) = Totalnumberof-SCEat25cellsinM2/25

(24).

Micronucleus assay

It was done through the same steps for sister chromatid exchange assay , other than that 4.5 µg/ml of Cytochalasin-B was added to each blood tube culture instead of BrdU after 44 hours of added PHA (25). Slide prepared by dropping few drops of cells on each slides. Each slide was stained with giemsa stain and examined with light microscope.

Calculations

NDI= 1(M1) + 2(M2) + 3(M3) + 4(M4)/N

M1, M2, M3, and M4 represent the number of cells with one, two, three, and four nuclei respectively.

N = The number of scoring cells which are at least 1000 cells.

MN= 1(MN1) + 2(MN2) + 3(MN3) + 4(MN4)/N

MN1, MN2, MN3, and MN4 represent different numbers of micronucleus form one to four respectively in binucleate cells (25).

Neutral Comet Assay for whole blood

It was done according to (26). Ten microliter of heparinized blood sample were diluted with PBS and then added equal volume of diluted blood and low melting point agarose 1%. This mixture was putted on coated slide with normal melting point agarose 1%. Slides were dipped in cold lysing solution and keep it at 4º C for two hours in dark place. Then slides was putted in electrophoresis tank contain neutralization buffer pH 7.5 at 24 volts for 30 min. Slides were drained then stained with Ethidium Bromide (2 µg/ml) and examined with florescent microscope. Then assay was completed by using software program (cometscore15) to get final results.

Statistical analysis

Analysis of variance (ANOVA) was used for the statistical analysis of the results and to look for the differences which statistically significant at the level (0.01% or 0.5%). These calculations were carried out according to program GraphPad PRISM version 6.0 (2012).

Results:

Blood lead levels

The results in (fig 1) showed that the highest mean ± SD of blood lead level was (49.50 ± 15.04) µg/dl at the group of people exposed to diesel exhaust more than 3 years with no statistically significant differences (P > 0.01) compared with the group exposed to diesel exhaust less than 3 years (47.40 ± 15.14) µg/dl.

The lowest mean ± SD blood lead level was (15.75 ± 6.851) µg/dl at control group with very high significant differences (P <0.01) when compared with both of tow exposed groups.

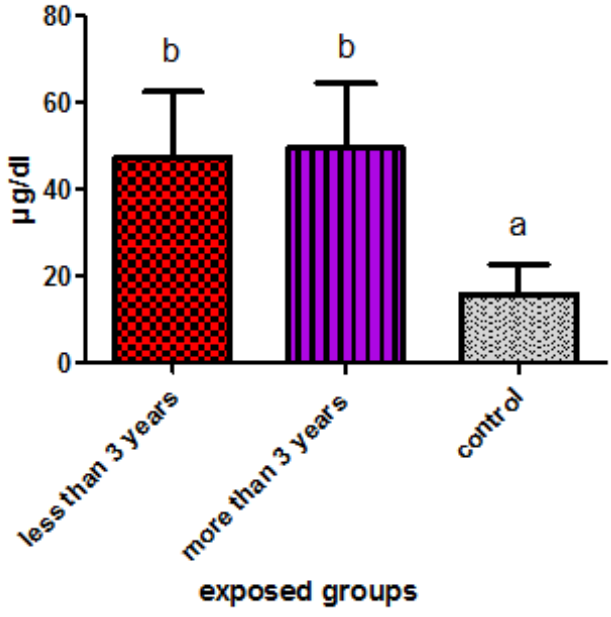


Figure (1) Blood lead levels
* The different small letters refer to find significant difference among results groups (P <0.01).

Blood lead levels and cytogenotoxic effects

The results in table (1) referred that there were a relationship between blood lead levels of people exposed to diesel exhaust and cytotoxic effects as show in fig. (2,3).

Table (1) Cytotoxic effects of blood lead levels

Cytotoxic effects / Paramaters	Blood lead levels µg/dL				P value
	20-40	40-60	60-80	Control	
**MN frequency ± SD	15.81±2.182 * b	22.05±3.885 c	29.18±4.142 d	12.25±4.051 A	<0.05
***NDI±SD	1.641±0.3804 a	1.638±0.2154 a	1.679±0.2408 a	1.530±0.1736 a	<0.05
****SCE/cell ±SD	6.229±0.59 b	7.518±1.063 c	8.488±0.584 d	3.945±1.502 a	<0.05

* The different small letters refer to find significant difference among results (P <0.05).
** MN frequency = Micronucleus frequency
*** NDI = Nuclear division index
**** SCE/cell = Sister chromatid exchange / cell

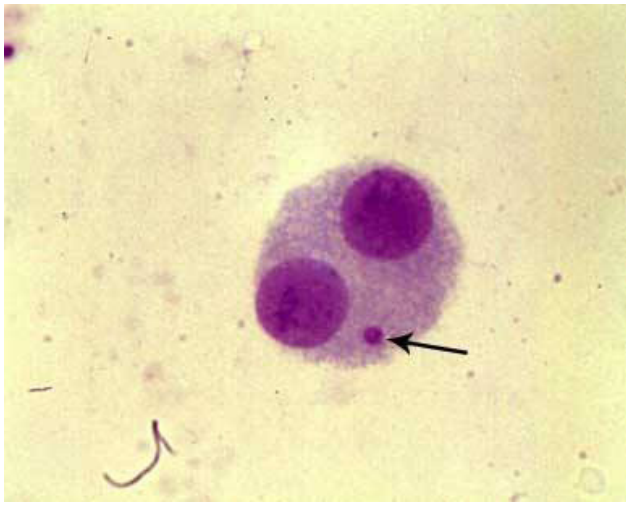


Figure (2) One micronuclei in binucleated lymphocyte cells of human with high level of lead blood (100x)

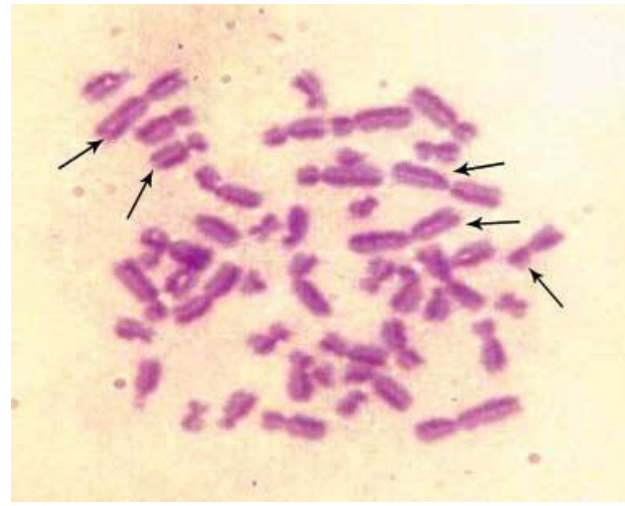


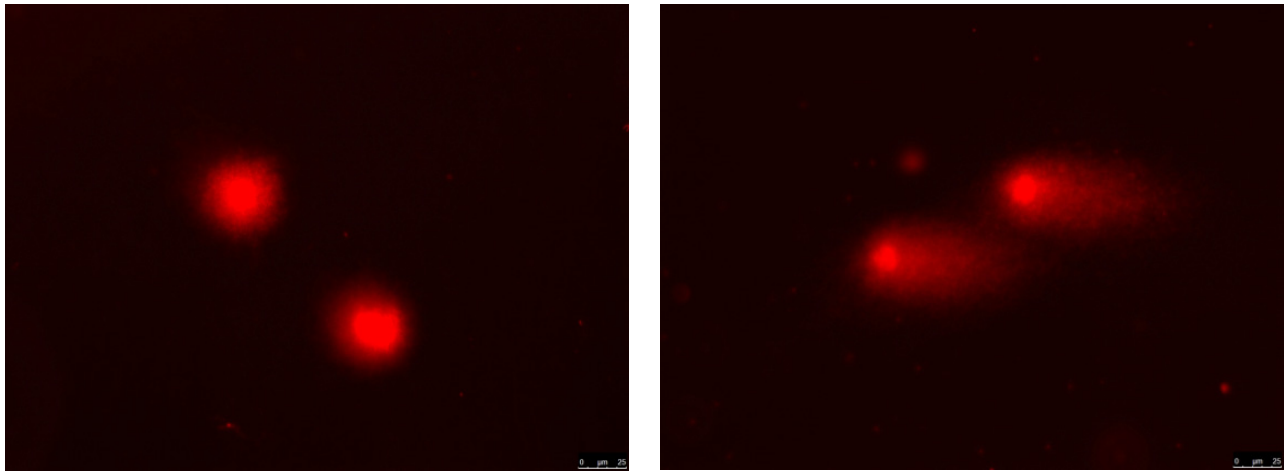
Figure (3) Sister chromatid exchanges in blood lymphocyte of human with high level of lead blood (100X)

The result also showed in table (2) that the lead blood levels increased the DNA damage in cells by increased the parameters of comet assay as exhibit below fig. (4)

Table (2) DNA damage effects of blood lead levels

Comet assay Paramaters 20-40	Blood lead levels µg/dL				P value
	40-60	60-80	Control		
Tail length of comet ±SD**	24.38±11.48 b*	55.41±13.45 c	78.71±14.73 d	11.70±10.48 a	<0.05
Tail DNA*±SD**	22.95±7.263 b	25.33±12.87 b	35.06±15.79 c	13.75±6.008 a	<0.05
Olive tail moment** ±SD**	5.033±0.8966 b	7.40±1.139 c	13.13±2.783 d	2.870±1.884 a	<0.01

* The different small letters refer to find significant difference among results (P<0.05).
** Tail Length is the distance of DNA migration from the body of the nuclear core and it is used to evaluate the extent of DNa damage.
*** Percent of DNA in the Tail.
**** Olive tail moment is defined as the product of the tail length and the fraction of total DNA in the tail.



A- Normal DNA B- Damaged DNA

Figure (4) DNA of peripheral blood lymphocytes after comet assay (40X)

Discussion:

The results referred that the means of blood lead level in both exposed groups (49.50 – 47.40) µg/dl were higher than normal limited value 0 –20 µg/dl and less than maximum toxic value 80 µg/dl (16).

Once absorbed in the blood stream, lead is primarily distributed among two compartments. The more rapid turnover pool with distribution to the soft tissues such as the liver, lung, spleen, and kidney, and slower turnover pool with distribution to skeleton (14 , 27). Blood lead level (BLL) is a common biomarker for lead. It is reflects the equilibrium between absorption, excretion, and deposition in tissues. There is linear relationship between BLL and exposure to lead. (19).

Exposure to lead in the environmental and occupational settings continues to be a serious public health problem (28). The results demonstrated that there were significant relationships between blood lead levels and cytogenotoxic effects on human exposed to diesel exhaust.

According to epidemiological studies of occupational exposure to lead is associated with increased rate of abnormal mitosis and increased incidence of chromosomal aberrations and sister chromatid exchange, at BLL ranging from 22 to 89 µg/dL (29). There are some studies that point out indirect mechanisms of genotoxicity such as inhibition of DNA repair or production of free radicals (30).

Conclusion

Blood lead level it can be indictor to exposure to diesel exhaust which have cytogenotoxic effects on human.

Recommendations

More researches should be done on the effects of diesel exhaust and heavy metal on the environment in general and specially on human health.

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العلاقة بين مستويات الرصاص في الدم والتأثيرات السمية الخلوية الجينية لدى الأشخاص المعرضين لعادم الديزل

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الخلاصه:

يعد عادم الديزل خليط من مئات المركبات العضوية والغير عضوية والتي لبعضها سمية خلوية جينية. أن الهدف من هذه الدراسة هو لأثبات العلاقة بين مستويات الرصاص في الدم ومدى التعرض للمركبات السمية الخلوية الجينية الموجودة في عادم الديزل. جُمعت عينات الدم من 80 رجل غير مدخن سليم البنية. كان قد تعرض ستون منهم بصورة مباشرة الى دخان الديزل ، اما العشرون الآخرون فيعدون سيطرة سالبة. تضمنت مقاييس التعرض لعادم الديزل هو قياس مستويات الرصاص في الدم فضلا عن التأثيرات السمية الخلوية الجينية ، أذ تم تحديدها بأستعمال أختبار الأنوية الصغيرة وأختبار التبادل الكروماتيدي الشقيق وأختبار المذنب. أظهرت النتائج أن متوسطات مستويات الرصاص في الدم لدى العمال المعرضين لعادم الديزل هي (47,40 – 49،50) مايكروغرام / ديسيليتر والتي كانت أعلى وبصورة معنوية عند مستوى الاحتمالية أقل من 0,01 بالمقارنة مع مستويات الرصاص في الدم عند مجموعة السيطرة والتي كانت 15،75 مايكوغرام / ديسيليتر. إن التأثيرات السمية الخلوية الجينية لدى المجموعة التي تراوحت مستويات الرصاص في الدم لديهم بين 60 – 8- مايكروغران / ديسيليتر كانت أعلى وبصورة معنوية الاحتمالية أقل من 0,01 من مستويات الرصاص في الدم الأخرى. وبهذا يمكن الأعتماد على مستويات الرصاص في الدم كمؤشر للتعرض لعادم الديزل الذي له تأثيرات سمية خلوية جينية على الأنسان.