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# Influence of Lead Exposure in the Expression of Calmodulin – Related Genes: A Preliminary Study on Workers Working in Industry of Batteries, In Iraq

***Ala'a Hassan Mirza Hussain***

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## Abstract:

Most pathological effects of lead on the body are due to ability of lead to bind with important cellular molecules of various tissues and organs leading to formation abnormal molecules and thus to emergence of pathological conditions. To evaluation the risk to the health status of Iraqi workers who work in the batteries industry, expression of three types of calmodulin related genes were examined. Blood samples were collected from worker working in Iraqi industry of batteries (located in Al-Waziriya), then RNAs extraction were done thereby gene expression for Calcium/Calmodulin- dependent protein kinase2 (CaMKK2), C-X-C Chemokine receptor 4 (CXCR4) and mitogen activated protein kinase kinase 6 (MAP2K6) was done for each sample by using RT-qPCR. The study showed that the expression of CXCR4 gene was significantly decrement in the lead exposed workers meanwhile the MAP2K6 gene insignificantly increment in those workers, but no effect appeared on their CaMKK2 gene expression. Conclusion obtained from this study is that lead has ability to impact on calmodulin related genes in the workers working in the batteries manufacture, but this ability is variable according to the type of gene.

**Keywords**: Pb, Calmodulin-related genes, CaMKK2, CXCR4, MAP2K6.

## Introduction:

However lead affects various systems in the body, mainly; immune, cardiac, Hemopoietic, nervous, and digestive systems, but its pathological effects on each system and on the human health are multifactorial (1, 2, 3). Genetic and molecular studies hypothesized that Pb is a genotoxic material. It can disrupt the expression of many kinds of genes and proteins including; glial- fibrillary acidic protein (GFAP), interleukin 6 (IL6), transfer growth factor B1 (TGF-B1) (3) and Ca/Calmodulin- related genes including CaMKK2, MaP2K6 and CXCR4 (4). Calmodulin is a small protein located in the cytosol and cell membrane of all eukaryotic cells and encoded in human by calmodulin 1 gene. Calcium has affinity to bind calmodulin and forms Ca/Calmodulin complex. This complex can activate a large number of enzymes mainly kinases and phosphatase and also can activate ion channels (5, 6). Pb binds calmodulin with many fold higher affinity than Ca and also can displace Ca in the N-terminal domain (7), additionally Pb has "opportunistic binding sites" i.e Pb attaches with other sites than Ca attachment sites.

Department of Basic Science, College of Nursing, University of Baghdad, Baghdad, Iraq.
E-mail: alaa_merza@yahoo.com

These multiple binding sites for Pb produce alteration in the conformation of calmoldulin molecule (4, 7). Therefore the molecular toxicity by Pb is due to its binding with calmodulin and other proteins. So the most pathological effects of Pb in the body are related to mysterious properties of Pb result in its ability to be targeted by multiple molecules which can explain its multi-systemic toxicity (4).

CaMKK2 gene is responsible for encoding CaMKK2 enzyme which is activated by binding calcium with calmodulin molecule. The activated enzyme can phosphorylate and activate other enzymes (CaMK1, CaMKIV and AMP- activated kinase). CaMKK2 is a key regulator for many important physiological and pathological processes such as energy balance, inflammation, development and function of neurons, hemopoiesis, hemostasis of glucose, adiposity (by acting on the hypothalamus) and cancer. Increase expression of CaMKK2 is strongly associated with cancer such as gastrointestinal cancers, hepatocellular carcinoma and prostatic cancer. On the other hand, decrease its expression is related with behavioral disorders including schizophrenia and bipolar disease (8, 9, 10).

The abnormality in the chemokine receptor type 4 (CXCR4) is associated with rare genetic

disease called WHIM syndrome in which the patient complain from formation warts on the hand, hypogammaglobulinemia, infection and myelokathexis (11, 12). However, overexpression of CXCR4 occurs in many invasive cancer cells associated with tumor cell proliferation, new blood vessels formation (angiogenesis), and even metastasis (13, 14). Furthermore CXCR4 with its cytokine play an important role in the progression of tumor and cell survival (15).

Notably the MAP2K6 gene belong kinase genes it responsible for encoding a kinase family enzyme called mitogen activated protein kinase Kinase or also called external signal regulated kinases (16). It plays an important role in both anabolism and catabolism of cartilage (17). Thereby it is implicated in the cases of rheumatic arthritis, osteoarthritis and intervertebral disease (17, 18).

Recently, the factory of batteries in Iraq has resumed its production. It is the main factory for manufacturing batteries which is located in Al-Waziriya in Baghdad and contains different departments, which lacks occupational safety. So the workers are at risk of lead poisoning especially who directly expose to lead via inhalation, ingestion, and directly contact with skin. This study is a preliminary study designed to evaluate the health status of Iraqi workers who work in this factory, by detecting the expression of three genes that have versatile effects on the body and they belong to Ca/ camodulin related genes. These genes are (CaMKK2, MaP2K6 and CXCR4 genes).

## Materials and Methods:

### Sample collection:

The total number of collected blood samples was 30, ten of them were taken from workers in the batteries factory who were exposure to lead directly, the second ten blood samples were collected from workers in the same factory but their duties were exposure to lead indirectly (the exposure to lead in both groups of workers was less than 12 months) while the last ten blood samples collected from workers outside the batteries factory and unrelated to lead exposure. Each sample of blood was collected in EDTA tube. The history about health status of each donor was taken. And also the written constant from the factory's manager and verbal constants from the workers were taken before starting the study.

### RNA Extraction:

Total RNA from blood of persons working at batteries factory (20 workers, ten of them exposed to lead directly and the other ten exposed to lead indirectly) and control (10 persons not related to this factory) was extracted using Trizol reagent (TRIzol$^{R}$ reagent ambion/RNA by life technology). Chloroform was used to purify RNA. Meanwhile RNA was concentrated by isopropanol and rehydrated by 70% ethanol. Then after ethanol evaporation the nucleic acid was eluted by nuclease free water.

### RT –qPCR analysis

Real time analysis was used to detect gene expression in three Ca/ Calmoldulin related genes in workers exposed to lead by using GoTaq 1- Step RT-qPCR kit (Promega USA). RNA extracted from 30 blood samples, twenty of them were lead exposed workers, and 10 from unexposed. Real time was used to analyze the expression of CaMKK2, CXCR4, and MAP2K6 genes, while Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as housekeeping gene. The mixture for Rt-qPCR reaction contained: master mix 5µl, reverse transcriptase mix 0.25 µl, forward primer 0.5 µl, reverse primer 0.5 µl, nuclease free water 1.75 µl and RNA 2 µl. Cycling parameters were as following: 15 minutes at 37$^{o}$ C for conversion RNA to DNA, 3 minutes at 90$^{o}$ C for initial denaturation of c-DNA, then 40 cycles as following: 20 seconds at 95$^{o}$C for denaturation, 20 seconds at 60$^{o}$C for annealing, and 30 seconds at 72$^{o}$C for extension.

Qualification of the mRNA levels of calmodulin related genes were done by measuring the threshold cycle (Ct). At the same time (GAPDH) was assayed by Rt- qPCR as endogenous invariant control. Table (1) shows all primers were used in this study.

Both RNA extraction and RT –qPCR techniques were performed in the laboratory of Advanced Scientific Center.

**Table1. Sequences of primers were used in the study**

| Gene | Sequences of Primer (5'-3') |
|---|---|
| CAMKK2 | F: ACA TCA TGG CTA CCT GAC TTG<br>R: CAC TTC CCG TGG GTG AAT TA |
| CXCR4 | F: AGT GAG GCA GAT GAC AGA TA<br>R: GAC AAT ACC AGG CAG GAT AAG |
| MAP2K6 | F: CAC ACC ACC TCG AGA TTT AG<br>R: CTC GTC CCA GTT CCA TTA TAG |
| GAPDH (House Keeping) | F: AGA AGG CTG GGG CTC ATT TG<br>R: AGG GGC CAT CCA CAG TCT TC |

### Statistical analysis:

T test was used to analyze the data between exposed workers and unexposed group. Whereas, data analyzing among intensive exposed, less

intensive exposed and unexposed groups was done by using one way ANOVA test.

## Results:

According the factory's reports, the level of lead in the blood of workers was within acceptable limit (did not estimated by this study due to that the lead level is periodically measured in the workers) but the workers (especially who exposed to lead directly) suffered from many health problems such as: loss of memory, difficulty in concentration, cerebral stroke, arthritis, numbness in the feet, colonitis, hypersensitivity, and abnormality in the blood pressure (hypotension in some and hypertension in others).

Our results revealed that the lead had an effect on expression of calmodulin related genes (Fig. 1 and Fig. 2). This effect exerted by the following: No differences appeared in CaMKK2 expression neither with t- test nor ANOVA test. Expression of CaMKK2 gene in the workers exposed to lead was 1.22 meanwhile in the unexposed group was 1.17 However, 1.3 was the value of the expression of CaMKK2 in the direct exposed workers, and 1.2 in the indirect exposed workers. On the other hand decrement in the expression of CXCR4 gene was exhibited in all workers in the batteries factory (0.19450) comparing with unexposed group (4.31000) this decrement also appeared among groups (direct exposed, indirect exposed and unexposed) 0.14, 0.25, and 4.31 respectively. Statistical analysis showed that there was a significant difference at level $P \leq 0.05$. With regard to the expression of MAP2KK6 gene revealed increment in both exposed groups. Neither t- test nor ANOVA test showed this increment was significant at level $P \leq 0.05$.

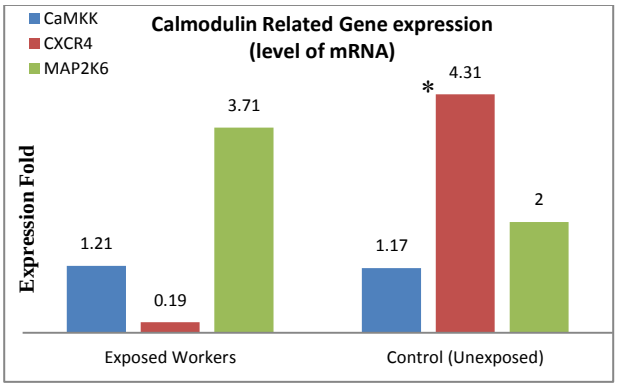


**Figure 1. The level of mRNA of CaMKK2 is unchanged but there is significant decrease in the level of CXCR4 in the factory's workers while the level of mRNA for MAP2K6 insignificant increases. *significant at level ≤ 0.05.**

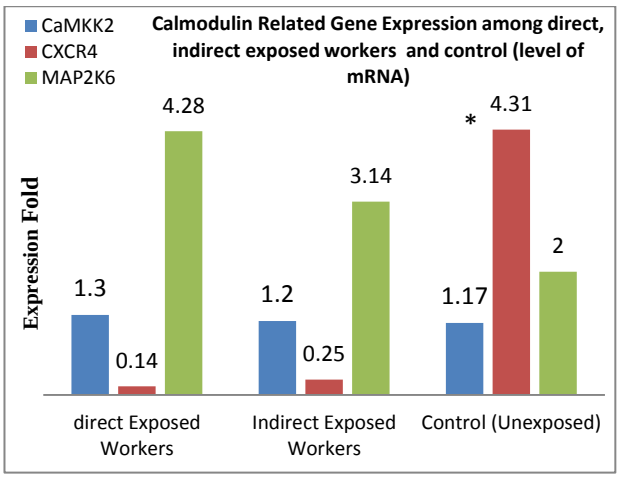


**Figure 2. The level of mRNA for CXCR4 gene in both direct and indirect exposed workers comparing with control, and insignificant increases in the mRNA for MAP2K6 in both groups of workers comparing with control while no changes in the mRNA for CaMKK2 among groups. * significant at level ≤ 0.05.**

## Discussion:

Lead is a heavy metal hazardous to health and enters into many industries and poses a real risk to those who are exposed to it. One of the most important sources of exposure is the batteries factory, so the workers are prone to lead poisoning. Despite the battery production plant in Baghdad has stopped producing batteries for since the war in 2003, recently returned to its production but not in full capacity. Therefor all workers exposed to lead for less than one year and the routine examination of the lead level in the blood of factory's workers is within acceptable limits.

The results of this study exhibited that Pb had the ability to impact calmodulin related genes but the sensitivity of these genes to Pb was variable, therefore the expression of CaMKK2 gene unaffected, meanwhile MAP2K6 gene elevated insignificantly, whereas CXCR4 significantly inhibited. Moreover the same results appeared in both direct exposed and indirect exposed workers comparing with control (persons whose work in other field rather than batteries factory). These findings in somewhat consistent with previous experimental research in which, reported that the expression of CXCR4 gene was inhibited significantly by exposure to lead, but the expressions of both CaMKK2 and MaP2K6 genes were significantly elevated (7). This variance is probably due to that the toxicity of Pb in this study was lesser than in the experimental one.

CXCR4 gene is responsible for encode a G protein coupled receptor called CXCR4 receptor which presents in the cell membrane of various body tissues and it is activated mainly by CXCR12

chemokine and indirectly by CXCR 14 . CXCR4 is expressed abundantly in mature white blood cells, progenitor of hemopoietic stem cells, epithelial cell, tumor cells and brain. This receptor is responsible for cellular emigration and adhesion; it plays a vital role in the immune system, nervous system and vascular system (19). So, the health problems suffered by workers (loss of memory, difficulty in concentration, cerebral stroke, numbness in the feet, disturbances in blood pressure) probably due to abnormality in the expression of this gene. Decrease in the CXCR4 leads to defect in native and acquired immunity by decrease in the number of circulating neutrophils, B cells, natural killer cells, and impaired memory maintenance of CD 8 T cell (20). Thereby the exposed workers may be become more reliable to get infection. And probably more acceptable to gain weight and induce their obesity (21). Moreover, the chance for healing was reduced in the brain traumatic injury and in vascular injury (22, 23).

Despite that the increment in the expression of MAP2K6 gene was insignificant. But some of the workers were complaining from arthritis that may be due to action of this gene (17).

Conclusion obtained from this study is that Pb can produce changes in the expression of calmodulin related genes in workers exposed to lead even with short term exposure but this change is variable according to the type of gene.

## Acknowledgment

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## Conflicts of Interest: None.

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# تأثير التعرض للرصاص في التعبير الجيني للجينات المتعلقة- بالكالموديولين: دراسة تمهيدية حول العمال العاملين في صناعة البطاريات في العراق

**آلاء حسن مرزه حسين**

فرع العلوم الأساسية، كلية التمريض، جامعة بغداد، بغداد، العراق.

**الخلاصة:**

معظم التغييرات المرضية التي يسببها الرصاص ناتجة عن قابلية هذا المعدن في الاتحاد بجزيئات خلوية مهمة توجد في أنسجة واعضاء مختلفة في الجسم محولا اياها الى مركبات غير طبيعية تتسبب في ظهور حالات مرضية متعددة. ولتقييم المخاطر الصحية التي يسببها الرصاص لدى العاملين في مصنع البطاريات في العراق الواقع في الوزيرية في بغداد، تم فحص التعبير الجيني لجينات ثلاثة تندرج ضمن الجينات المتعلقة بالكالموديولين. حيث أُستخلص الحامض النووي الرايبوسومي من عينات دم جُمعت من العاملين في مصنع البطاريات في العراق، ثم تم الكشف عن التعبير الجيني للجينات CaMKK2 , CXCR4, MAP2K6 في كل عينة بإستعمال تفاعل سلسة البلمرة . أظهرت النتائج انخفاضا مهما إحصائيا في التعبير الجيني للجين CXCR4 لدى عمال المصنع وزيادة في الجين MAP2K6 لكن هذه الزيادة كانت غير مهمة أحصائيا بينما لم يتأثر التعبير الجيني للجين CaMKK2 لهؤلاء العمال. نستنتج من هذه الدراسة ان للرصاص قابلية في التاثير في الجينات المتعلقة بالكالموديولين ولكن هذا التأثير متغير حسب نوع الجين.

**الكلمات المفتاحية:** الرصاص، الجينات المتعلقة بالكالموديولين، CaMKK2، CXCR4، MAP2K6