# Changes in the hematological profile among workers at patrol stations in Babil Province/Iraq

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**Abstract:**

Exposure to the high levels of benzene and its metabolites are commonly reported to induce hematotoxicity. However, the effects of exposure to low levels are still obscure. In addition, these possible effects occurred in haemopoietic components could potentially lead to initiate harmful effects in different parts of the body later in the life course. This study was designed to assess the potential effects of benzene exposure on the hematological profile among workers at filling stations and compared them with office's workers in Babil Province/Iraq. 24 blood samples (exposed group) and another 14 blood samples (non-exposed to benzene) were collected from public patrol stations. Full analysis of blood picture was performed using fully automated hematology analyzer. Results indicated that most of hematological biomarkers (14 out of 15 biomarkers) were not significantly changed in both groups. However, Hemoglobin (Hb) and Packed cell volume (PCV %) were significantly increased in smoker workers compared to non-smokers at patrol stations, while the total number of platelets PLT was significantly reduced. There are no significant associations between the duration of benzene, personal age and all measured hematological parameters. Conclusions: The findings of this study revealed that benzene exposure has a potential to induce hematological among smoking people working at filling stations in Babil province/ Iraq. The hematological changes include increased Hb and PCV% and decreased the number of PLT. However, our results did not find any significant changes in most of hematological biomarkers in workers at filling stations compared to the control group in Babil Province.

**Keywords:** Filling stations, hematological parameters, smoking, benzene.

## Introduction:

Gasoline is a chemical mixture of different types of hydrocarbon molecules. Benzene is considered to be one of the most hazardous kinds of these hydrocarbons (e.g. xylene and toluene)[1]. Chemically, Benzene is the aromatic hydrocarbons composing of six carbon atoms connected as a ring with one atom of hydrogen[2]. Physically, it is characterized as a colorless with non-corrosive and strongly flammable liquid. It is also known to be easily evaporated under normal environmental conditions due to having low boiling point[3]. Benzene is routinely utilized as a general solvent in many of chemical and pharmaceutical factories. It is also used as an initiative substance participating in the synthesis of different sorts of chemical compounds[4]. As a result, it is represented as a main factor for air pollution [5,6] International Agency for Research on Cancer has classified Benzene as a strong carcinogenic and later the World Health Organization has ranked it as a first class material [7]. Data collected from USA indicated that production of benzene increased enormously of about 7.2 million metric tons in 2002 compared to 5.4 metric tons recorded in 1992[8]. Scientific research also indicated that the benzene appears as an intrinsic ingredient of cigarette and hence that the smokers tend to have more benzene in their bodies compered to non-smokers people[2]. Observational studies suggested that some people are more targeted to be exposed to gasoline than others; these involve the workers at filling station, attendants at service station, drivers of gasoline trucks, and refinery workers[9]. In patrol stations, workers are experienced high concentration of evaporated benzene. The vast majority of its poisonous effects can be belonging to inhaling exhaust fumes, evaporative and refueling revivals[10]. Pervious research indicated that half of breathed benzene is usually absorbed into the human body. One way for benzene to be absorbed is skin, in which the rate of its absorption is exponentially increased if the human's skin is not in a good condition (cracking, blistering and abrading skin) [11]. Absorption can also be happened during ingestion of direct liquid benzene and this is achieved by the activity of mucosa layer of digestive system[10]. It has been suggested that the harmful impacts results from benzene can be vary and constrained by the quantity of benzene exposure, way of absorption, duration time of exposure, and age of exposed individual[4]. Once it is absorbed into the human body, it is metabolized and giving further metabolites excreting in the urine. It has been proposed that the adverse consequences of benzene exposure might be stemmed from these chemical molecules [12]. Exposure to benzene has been historically associated with serious medical problems targeting different human's systems and organs. These systems include respiratory, haemopoietic, nervous systems and immunity. Inhaling few amounts of benzene vapors causes different sorts of respiratory disturbances[13]. Contacting human skin with benzene may also be responsible for promoting many dermatological problems such as rash, redness, and swelling[14]. Many human's organs like liver, heart, and kidneys are also badly influenced by exposure to benzene through triggering the cellular maintenance and inducing cell death[15]. One of the possible mechanisms explaining the adverse effect of benzene is related to its impacts on the bone marrow, leading to the failure of blood formation. The metabolites derived from benzene are found to destroy the hematopoietic components in the bone marrow. It has been shown that the exposure to benzene is mostly linked to the different types of blood deformation starting with aplastic anemia and ended up with leukemia and other chromosomal deletion[11]. Moreover, exposure to benzene could also lead to increase the potential tendency of bleeding and enhance the infectious rate [15]. Despite the fact that the high levels of benzene metabolites are commonly reported to induce hematotoxicity, the effects of exposure to low levels are still obscure. In addition, these possible effects occurred in bone morrow could potentially lead to initiate harmful effects later in the life course[16]. Therefore, scientific research is urgently needed to explore the hematological changes of exposure to low levels of benzene. Regular assessments of biological markers responding to benzene exposure would also be essential for lowering the potential risk factors for such exposure[16]. Complete blood picture (CBP) is an important approach to inspect the changes in the hematological profile. CBP is usually known to be quick, easy test and attainable checking for monitoring the adverse effects of benzene on the hematological status[17]. Therefore, CBP will provide us with comprehensive picture regarding to the numbers of various sorts of blood cells. Appling this kind of the test could also help to figure out the causes of some hematological disturbances such as anemia, specific types of cancers and some of immune disorders[17]. The measured variables in this test include; the total number of white blood cells (WBC), basophil, acidophil, neutrophils, lymphocytes, monocytes, total number of red blood cells (RBC), Quantity of hemoglobin (HGB), Packed cell volume % (PCV), Mean Corpuscular Volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration

(MCHC), Red blood cell distribution width (RDW). Total number of platelets (PLT), and Mean platelet volume (MPV). Our study was designed to shed the light about the possible changes in the hematological profile among workers in patrol stations and compare them with those working at the offices (not directly contact with benzene) in Babil province/Iraq. In addition, this study aimed to investigate the possible effects of the duration time of benzene exposure, smoking, and personal age on the measured parameters in both groups.

## Material and Methods:

### Experimental design:

This study was represented as a cross-sectional study. Twenty four filling workers at public patrol stations across Babil province/Iraq were randomly assigned in this study. Another fourteen workers at the station's offices were also randomly selected and used as a control group. Prior to conduct this research, all participants are formally informed regarding to importance of this research on the general health and safety of all workers in patrol stations and hence consents were obtained. All participants were also told that all given information were kept confidential. Data were obtained according to the structured questionnaire. The questionnaire covered name, age, duration time of an employment for filling and office's workers, smoking status, and any history of medical problems. Those workers with chronic diseases were totally excluded from this study.

### Collection of samples:

After the consent being taken form the participants, Five milliliters of blood was withdrawn from each worker using a vein puncture approach and then collected into two tubes. Around 2 ml of blood was collected in EDTA tube and used for the hematological measurements. The rest of blood was contained in a plain tube for serum preparation for another study. To avoid any effects of ambient temperatures on the samples, collected blood samples were always stored in cold conditions using a wet-ice box until the time of measurement. All laboratory work was carried out in a medical lab center in Department of Biology at the college of Science/Babylon University. Comprehensive analysis of blood picture was made using an automated haematology analyzer (Mythic, manufactured in Orphee-Swiss). Results were recorded in a file sheet with normal ranges for each hematological biomarker. Reminder blood samples were centrifuged at 3000 round per minute (RPM) for 5 minutes and serum were manually separated and kept in -20C freezer for later analysis.

### Statistical Analysis:

Prior to perform an appropriate type of analysis, all data were tested for normality by using a Shapiro-Wilk test and transformation of the data were used if necessary ensuring that the data were normally distributed. Independent T-test was applied to explore the significant differences in hematological biomarkers among groups. The same test was also used to examine the effect of smoking on the measured biomarkers among filling workers. Pearson correlation was used to test the possible correlations between the duration of benzene exposure (indicated by how many years of employment for a specific work place e.g.: office or filling station), age on the measured variables. Results were expressed as mean ± standard error. Differences were indicated significance if the P values equal 0.05 or less. All Statistical Analyses were performed using Minitab, version 17 (Minitab Inc., State College, PA, USA).

### Results and Discussion:

Independent-T-test revealed that the mean of worker's age at filling stations was not significantly different than the ones observed in control group (see table1). The mean of employment duration in workers at filling stations was also matched to those found in control group. The number of smokers for the workers at filling stations was 15 subjects compared to 9 ones in non-smokers. However, the picture was in opposite direction in control group, in which the number of non-smoker workers 10 subjects compared to 4 ones in smoker workers (see table 1)

Table1: Demographical Characteristics of the study groups

| Parameters | Filling workers (N=24) | Office's workers (N=14) | P=values |
|---|---|---|---|
| | Mean± SE | Mean± SE | |
| Duration Time (years) | 10.21±0.98 | 10.71±2.2 | P=0.75 |
| Age | 39.29±1.4 | 37.57±2.3 | P=0.52 |
| Smokers | 15 | 4 | |
| Non-smokers | 9 | 10 | |
| Gender/male | 24 | 14 | |

No significant differences were found in the means of RBC counts, Hb, PCV%, MCV, MCH, MCHC, RDW, WBC counts, differential counts of WBC, PLT counts between workers exposed to benzene (filling workers) and non-exposed (office's workers). However, only one biomarker was significantly influenced by exposure to benzene, in which the mean of platelet volume (MPV) was significantly increased (p=0.05) in the filling workers compared to those unexposed to benzene (control group) (see table 2).

Table2: Comparison of hematological profile between filling workers and office's workers in patrol stations at Babil Province

| Parameters | Filling workers | Office's workers | P=values |
|---|---|---|---|
| | N=24 | N=14 | |
| | Mean± SE | Mean± SE | |
| RBC (10*6/μL) | 5.96±0.26 | 5.6±0.16 | *P=0.31* |
| Hb (gm/dl) | 16.45±0.71 | 15.94±0.53 | *P=0.98* |
| PCV% | 55±2.3 | 53.12±1.7 | *P=0.58* |
| MCV | 93±2.1 | 95.14±1.9 | *P=0.99* |
| MCH | 27.79±0.66 | 28.51±0.69 | *P=0.79* |
| MCHC | 29.85±0.14 | 29.95±0.17 | *P=0.66* |
| RDW% | 13.18±0.19 | 13.35±0.19 | *P=0.39* |
| WBC (10*3/μL) | 8.4±0.53 | 8.01±0.47 | *P=0.61* |
| Basophils % | 0.7±0.04 | 0.69±0.05 | *P=0.87* |
| Eosinophil % | 2.24±0.11 | 2.2±0.12 | *P=0.79* |
| Neutrophils % | 63.85±1.3 | 62.79±2.4 | *P=0.68* |
| Lymphocytes | 27.63±1.1 | 28.43±2 | *P=0.71* |
| Monocytes% | 8.53±0.42 | 8.78±0.72 | *P=0.75* |
| PLT (10*3/μL) | 184.3±20 | 201±15 | *P=0.50* |
| MPV | 8±0.15* | 7.5±0.11 | *P=0.05* |

In this table, an independent-T-test was performed to explore the possible differences between variables. RBC:red blood cells; Hb: Hemoglobin: PCV:packed cell volume; MCV: Mean Corpuscular Volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; WBC:White blood cells; PLT: Total number of platelets; MPV: Mean platelet volume.SE: standard error.

In this study, only mean MPV value was significantly higher in workers in the filling stations. However, the rest of hematological profile did not experience any significant changes between workers in the filling stations and control group. These results are supported by many previous findings. Previous research indicated that changes in the number of blood cells are not especially in parallel with the benzene impacts [4,11].These findings were further supported by experimental studies showing that experiencing to low doses of a one of the benzene metabolites namely, hydroquinone, had failed to induce any changes in total

number of RBCs and WBCs[18,19]. Experimental studies on small animals exposed to low levels of benzene were also in the line of our findings. Exposure of male Sprague-Dawley rats to benzene for low doses on daily bases did not incur any significant alternations in hematological profile[20]. One possible reason behind the lack of changes observed in this study is that the exposure to benzene may not have been reached to the point that can trigger significant disturbances in different types of blood cells [20]. Another explanation for this absence could be related to the idea that the effect of such level of benzene exposure in the current study may be more pronounced in other systems that were not measured here. Previous literature on the effect of benzene exposure indicated that the immune system is also targeted and many of immunological changes were associated to the benzene exposure[4,21,22]. Moroa and his colleagues measured different immunological biomarkers among workers at gas stations in Brazil and found that the workers had increased their innate immune system such as interleukin 6 and decreased the adaptive one such as expression of the molecules (CD80 and CD86) activating T-cell activation[4]. Another study conducted in Croatia also demonstrated that the number of B-lymphocytes was significantly decreased in workers at patrol stations compared to the control group[21]. Levels of immunoglobulin such as IgG1 and IgG2 were also found to be negatively influenced by benzene exposure[22]

Table3: Comparison of hematological profile between smokers and non-smokers filling workers in patrol stations at Babil Province

| Parameters | Smoker workers at the filling stations  N=15 | Non-smoker workers at the filling stations N=9 | P=values |
|---|---|---|---|
| | Mean± SE | Mean± SE | |
| RBC (10*6/µL) | 6.14±0.34 | 5.67±0.38 | P=0.36 |
| Hb(gm/dl) | 17.49*±0.98 | 14.71±0.67 | **P=0.02** |
| PCV% | 58.2*±3.2 | 49.66±2.3 | **P=0.03** |
| MCV | 95.21±7.48 | 89.4±4.4 | P=0.39 |
| MCH | 28.57±0.68 | 26.48±0.13 | P=0.18 |
| MCHC | 29.97±0.18 | 29.64±0.2 | P=0.19 |
| RDW% | 13.07±0.18 | 13.37±0.43 | P=0.77 |
| WBC (10*3/µL) | 8.99±0.78 | 7.43±0.55 | P=0.3 |
| Basophils % | 0.78±0.05 | 0.57±0.01 | P=0.003 |
| Eosinophil % | 2.3±0.13 | 2.13±0.13 | P=0.37 |
| Neutrophils % | 64.56±1.7 | 62.66±2.2 | P=0.50 |
| Lymphocytes % | 26.61±1.33 | 29.32±2 | P=0.2 |
| Monocytes% | 8.83±0.59 | 8.02±0.52 | P=0.31 |
| PLT (10*3/µL) | 147.1*±19 | 246±36 | **P=0.03** |
| MPV | 8.24±0.18 | 7.61±0.35 | **P=0.09** |

Table 3 revealed the effect of cigarette smoking on the hematological biomarkers in workers at the filling stations. There was a significant effect of smoking on the Hb, PCV%, and PLT counts. Hb levels were shown to be significantly increased in the smoker workers compared to non-smoker workers (p=0.02). The same trend was also observed in the mean percentage of PCV of smoker workers. The changes in the PLT counts were found in opposite direction to what we have seen in the Hb and PCV%, in which the mean of the PLT counts was significantly decreased in the smoker workers compared to those in non-smokers group. No significant correlations with duration of employment (benzene exposure) and personal age in one side and all measured hematological biomarkers on the other ones were found in Pearson correlation (see table 4 just only for duration of employment).

Table4: Correlations matrix of the effect of duration time of benzene exposure on the hematological profile among filling workers in patrol stations at Babil Province

| Parameters | Pearson correlations with duration time of benzene exposure (years) N=24 | |
|---|---|---|
| | R2 | P values |
| RBC (10*6/μL) | R2= 0.2 | *P=0.34* |
| Hb(gm/dl) | R2= 0.3 | *P=0.16* |
| PCV% | R2= 0.31 | *P=0.14* |
| MCV | R2= 0.13 | *P=0.53* |
| MCH | R2= 0.13 | *P=0.55* |
| MCHC | R2= 0.07 | *P=0.77* |
| RDW% | R2= 0.04 | *P=0.84* |
| WBC (10*3/μL) | R2= 0.09 | *P=0.65* |
| Basophils % | R2= 0.01 | *P=0.99* |
| Eosinophil % | R2= 0.03 | *P=0.89* |
| Neutrophils % | R2= 0.13 | *P=0.54* |
| Lymphocytes | R2= -0.08 | *P=0.71* |
| Monocytes% | R2= -0.2 | *P=0.35* |
| PLT (10*3μ/L) | R2= -0.15 | *P=0.47* |
| MPV | R2= -0.01 | *P=0.95* |

In this table, an independent-T-test was used to determine the effects of cigarette on the hematological parameters among filling workers. RBC: red blood cells; Hb: Hemoglobin: PCV: packed cell volume; MCV: Mean Corpuscular Volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; WBC: White blood cells; PLT: Total number of platelets; MPV: Mean platelet volume.SE: standard error.

The results indicated that cigarette smoking had strong impacts on the levels of Hb, PCV%, and PLT counts in workers at filling stations. These findings are in an agreement with a previous study conducted in Baghdad[11]. The elevation of Hb and PCV levels observed in the smokers group could be partially attributed to the synergistic effect of smoking with benzene exposure[11]. Lower production of platelets cells observed in our study may be traced back to the formation of the condition, namely, pancytopenia. This condition may be interfering with the normal blood clotting and thus leading to raise the potential possibility of bleeding. Smoking can also target the platelets formation in indirect way by mediating oxidative stress. Oxidative stress is a condition in which the production of reactive oxygen species (ROS) exceeds the level of total antioxidants[23]. It has been shown that the cigarette smoking has hundreds of chemical molecules (benzene included) that induce the oxidative stress among different tissues[24,25]. It is believed that the function of platelets and coagulation process are negatively influenced by increasing the ROS levels as well as down-regulation in the antioxidant levels, leading to increase the oxidative stress[26]. Previous works on the link between benzene exposure and oxidative stress have shown that there is an inverse association between them. Levels of main enzymatic antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) were found down-regulated among workers at gas stations compared to the non-exposed individuals[27]. Other studies found that oxidative damage to the biological molecules (protein, lipids, and DNA) were higher among exposed workers [22,27–29]. Therefore, exposure to benzene 's vapor and cigarette smoking could be serious factors for down regulation of platelets production [13].

The lack of findings any significant correlations between duration of benzene exposure , personal age and the measured hematological biomarkers in our study are in line with the findings of other studies [19, 12]. The absence of such correlations could be explained by the idea that the workers with different exposure periods are still having an effective compensatory system to cope with possible changes that might be found during the exposed levels and thus ending up with good hemostasis [13].

## Conclusions

(1) Benzene exposure has a potential effect to induce hematological alterations among smoking people working at filling stations in Babil province/ Iraq.

(2) Smoker workers at filling stations had significantly higher levels of Hb and PCV%, but decreased levels of total number of PLT compared to their levels in non-smokers.

(3) Our results did not find any significant changes in hematological biomarkers in workers at filling stations compared to the control group in Babil Province.

(4) Further studies are needed to investigate more biochemical changes in workers exposed to benzene and compare them with non-exposed ones (e.g. organ functions, oxidative stress, and repairing pathways of biological molecules).

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