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Evaluation of Comparative Effects of the Exposure of Gasoline Fumes/Vapors on the Blood and Urine Picture of Gasoline Filling Workers of Multan City, a Populous City of Southern Punjab, Pakistan

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Abstract

Background: Gasoline workers are continuously exposed to many kinds of harmful fumes at their working places. These fumes contain terribly dangerous pollutants which can seriously alter the urinary and hematological profiles. This could be pathological in many conditions.

Aim and Objective: The aim of this study was to determine the effects (if any) of the exposure of gasoline station workers to the gasoline with special focus on CBC parameters and urinary pattern.

Results: In hematological examinations of group-I, WBC Counts, EO, BA, and platelets with indices PCT and MPV were significantly increased ($p<0.05$). In Group-II, WBC counts, LY, EO, BA and platelets with indices PCT and MPV were significantly increased, whereas RBCs and HGB with their indices HCT, MCV and were significantly decreased. Significant changes in specific gravity, PH-values, Nitrate, Protein, Ketone, UGB and Bilirubin were also investigated. Significant weak negative correlations were found among RBC ($r=-0.3$, $p=0.003$), HGB ($r=-0.3$, $p=0.003$), HCT ($r=-0.3$, $p=0.003$) and MCV ($r=-0.22$, $p=0.03$) with the exposure length. Significant weak positive correlation of MO ($r=0.23$, $p=0.03$), BA ($r=0.22$, $p=0.04$) were observed with the exposure length in gasoline filling workers

Conclusion: The present study concluded that prolong exposure of gasoline fumes cause massive alteration in hematological parameters particularly WBC counts with differentials LY, EO, BA, RBC and HGB with their indices HCT, MCV, PLT, PCT and MPV as well as Nitrate, UGB and Bilirubin in urine. These alterations in hematology and urine composition due to toxicities of gasoline fume produce high levels of many serious abnormalities and illness in human health like bone marrow depression, immune depressing and lymphatic leukemia.

Keywords: Gasoline fumes; Occupational exposure; Hematology changes; Urine composition; Passive inhalers

Introduction

Worldwide, health risk that happens because a consequence of work related to a numeral of gasoline hydrocarbon substances has been identified by World Health Organization (WHO) as amajor giver to mortality and morbidity [1-3]. Gasoline is one of the biggest volume commercial merchandise in the world and is largely a combination of branched chain hydrocarbon, toxics, organic volatile substances with low boiling point and composition that depends on its chemical processing and primary raw materials. The toxicity of hydrocarbons, benzene, toluene and xylene in gasoline has been investigated in both short and long-term exposures of worker that work on gasoline station [4,5]. Benzene, toluene and xylene are noted to be major key dangerous sorts of said hydrocarbons in gasoline [6]. Gasoline contains low boiling points, volatile and it is rapidly disappeared by evaporation in ordinary ecological circumstance [5]. Occurrence of it's in the bulk petroleum products, its volatility and dermal permeability, benzene, 1-3-butadien, toluene and xylene present a significant multiple health threat to fuel workers like fuel station attendants [5,7]. Furthermore, exposure to gasoline fumes/vapors can produce an extensive variety of deleterious consequences on the hematological investigation [8-11], renal function demolition [12,13], hepatic [14-18] central nervous systems [19,20], and lung functions [21,22]. Evidences from previous studies demonstrated that gasoline fume having toxicity carcinogen cause to happen involves many mechanisms like to oxidative stress (Figure 1), as dermatological problems [23,24], respiratory disturbances [25-31], cytogenetic effects [32], structural and numerical abnormalities of DNA [33,34], immune system [35,36]. Some studies have also illustrated threats of leukemia, lymphoma and other types of cancer with connection to occupational gasoline fume/vapors revelation in a few countries [37-45]. WHO, EPA and IARC have declared that carcinogen is present gasoline fume/vapors [45,46]. In Pakistan, the majority of the filling workers fill up fuel into automobiles without taking any protecting apparatus to avoid their exposure to the fumes/vapors. Therefore, gasoline filling stations are a cause of great exposure of their workers to gasoline fumes/vapors. No health protection related precautions/warnings are provided to this

class of workers. They are neither checked for any health problems nor they are passed through any regular medical checkup. Even the possibility of explosions at such places is ignored. Complete blood count is a fundamental diagnostics test for observation of hematological investigations [47]. CBC is generally identified to be rapid, approachable for observing unfavorable effects of gasoline fumes/vapors containing toxic substances benzene, toluene and xylene [10]. Therefore, CBC is provided absolute pictures of varies kinds of blood cells. In this view, the study was planned to examine the outcome of exposure to gasoline fumes/vapors on hematology and urine compositions of a seemingly healthy volunteer individuals in district Multan, Punjab province, Pakistan.

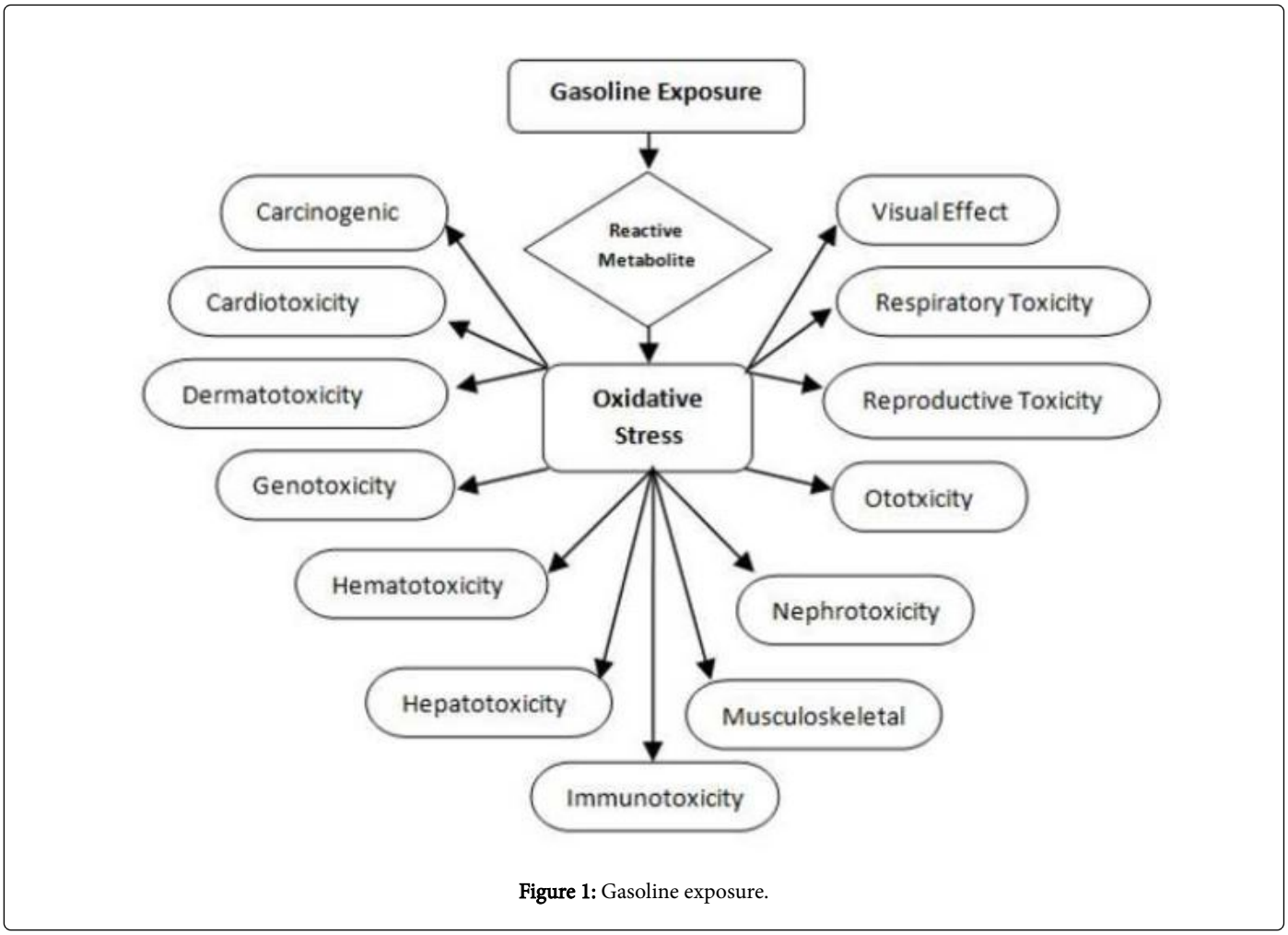

Figure 1: Gasoline exposure.

Materials and Methods

Subjects

Forty-two normal volunteers subjects (all males) from the employees of different vary busy gasoline stations of Multan City were included for the study. The age of these individuals ranged between 20-50 years, and their working durations between 1-18 years. Multan is a populous city of southern Punjab, Pakistan, where environmental pollution is very alarming. On the basis of duration of exposure the subjects were divided into two groups: Group-I comprised the individuals exposed from one to nine years. Group-II included those volunteers who were exposed to gasoline from ten to eighteen years.

Inclusion/Exclusion criteria

The study was limited to only filling fuel workers that were exposed to gasoline fumes/vapors and never took smoking, alcoholic food and other addiction.

Experimental design

The study was designed cross sectional. There were randomly selected study participants from the public gasoline stations which were very busy for twenty-four hours. Before collecting the blood samples from every volunteer individuals, information regarding importance of research on general health and safety precaution for working at filling fuel station were given them and obtained consents.

Study area

The study was carried out in Multan city, which is the most heavily populated of south Punjab, Pakistan. The climate of Multan city is arid, hot summers and the temperature of Multan city is one the most extreme temperature in Pakistan [48]. Its geological location is latitude 30° 9'26.8488" N, longitude 71° 31' 29.694" E and 129 meters high from sea level [49].

Ethical clearance

This study was performed by the approval of institution ethical board and was supervised throughout the length of the study work. Instructions and strategy for sample collection from individuals and procedure of research purpose were followed by the board.

Blood sample collection

Venous blood (2 ml) was drawn by venipuncture through BD syringes, Precision Glide needle 23 G 1 W (Becton, Dickinson Company, Made in Singapore) immediately to Lavender BD, K2-EDTA [18] vacutainer® (5.4 mg, 3.0 ml, Becton Drive, Franklin Lakes, USA) with suitable safety precautions by single qualified phlebotomist. The blood samples drawn were reserved immediately in temperature-controlled container to evade any impacts of ambient temperature for proceeding to hematological examination.

Urine sample collection

Urine samples (40 ml) were taken from volunteers and willing subjects using sterilized sample cups and transferred to laboratory for examination of urine composition at MINAR, Nishtar, Hospital, Multan.

Hematological analysis

Complete blood count measurements were performed on automatic Celltac G MEK-9100 (Nihon Kohden Corporation, Japan) within 2 hours after collection of blood [50]. Blood samples were done hodgepodge on automatic mixer machine before measurements of CBC for an appropriate quantity of time to bring the temperature suggested by the manufacturer. Calibration of the analyzer were done with appropriate standard references material and conformed with utilization of standard control with concentration (low, medium and high) by the recommendation procedure of manufacture [50]. WBC counts with sub-population neutrophils, lymphocytes, eosinophils, monocytes and basophiles, RBC and HGB with their indices HCT, MCHC, RDWCV, RDW-SD, PLT, PCT and PMV parameters were taken into present study.

Urine composition analysis

Examination of urine composition was done by using urine strip analysis (Combur 10 Test, Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305, Mannheim, Germany). The urine strips were sink into urine cup and taken out with in 50 second. Colour urbanized on the urine test strip and was compared with standard colour indicating on strip analysis box to read them.

Statistical analysis

Statistical analysis was performed in Microsoft excel and verified in IBM SPSS-Statistics 24. P value and deviation of measurements of all parameters of CBC and numerical parameters of urine composition were calculated by Paired t test after verification of normal distribution by the D'Agostino Pearson test between the study and control groups. Wilcoxon Rank sum test was executed for those parameters that had non- normal distribution. Fisher exact test analysis was used to access the nominal data of urine composition. Regression and correlation analysis were done to find the potential connection between the time duration of fumes exposure and hematological parameters. $P<0.05$ was taken for considerable result.

Results

In Group-I our observations were following: In hematological examinations, WBC Counts, EO, BA, and platelets with indices PCT and MPV were significantly increased ($p<0.05$). Significant changes in specific gravity, PH-values, Nitrate, Ketone, UGB and Bilirubin were also observed. In Group-II, WBC counts, LY, EO, BA and platelets with indices PCT and MPV were significantly increased, whereas RBCs and HGB with their indices HCT, MCV and MCH were significantly decreased. Significant changes in specific gravity, PH, Nitrate, Protein, Ketone, UGB and Bilirubin were also investigated. Urine examination also showed complete non-appearance in leucocytes, HGB, glucose in both exposure groups.

Significant weak negative correlations were found among RBC ($r=-0.3$, $p=0.003$), HGB ($r=-0.3$, $p=0.003$), HCT ($r=-0.3$, $p=0.003$) and MCV ($r=-0.22$, $p=0.03$) with the exposure length. Significant weak positive correlation of MO ($r=0.23$, $p=0.03$), BA ($r=0.22$, $p=0.04$) were observed with the exposure length in gasoline filling workers.

Discussion

Gasoline is a huge mixture of hydrocarbons consists of n-paraffin, naphthalene, olefin and aromatics [51]. Gasoline fume is an identified as carcinogenic properties, there is no secure concentration presents in gasoline for exposure [41,52]. In our hematological findings, significantly increased results become in WBC with differentials LY, EO and BA that are alike to some other studies showed increased result in WBC, EO, while disagree with in decreased result in BA and LY [14,53]. Study done by Mohammed et al. [54] also showed significantly decreased in EO for prolong gasoline exposure. Only one study also concluded that there is no significant difference in deferential WBC counts of study group with control [55]. Results of some studies done by Ray et al. [55], Avogbe et al. [56], Dede et al. [57], Ismail et al. [58], Christian et al. [59] and D'Andrea et al. [15] also support to our findings in WBC counts. Medicina et al. reported basophiles increased with increasing exposure of benzene which is similar to our study findings [60]. One of the main important finding of our study is significantly increasing in LY, EO and BA in prolong exposure of gasoline. An extended proportion of lymphocyte, eosinophil and basophiles in blood may be risk of leukemia in cumulative exposure of gasoline fume. This threat of leukemia remained elevated for latency period of more than 10 years. Our finding appears to verify that leukemia is related with low exposure to gasoline (Benzene), as prediction from AJEM based on specialist opinion [61]. Results of RBC, HGB, HCT, MCV, MCH and RDW-SD in present study were found significantly decreased in exposure group II when compared with the control ($p<0.05$). Gasoline fumes cause a reduction in hematological indices on prolong exposure concluded by Okoro et al. [62] is alike to ours study. Results of HGB levels, RBC counts, and their indices in studies done by Christian et al. [59], Opute et al. [4] are also similar to our results in exposure group.

In present study, results of PLT, PCT and MPV were investigated significantly increased in both study groups with control. The present findings inconsistent to studies done by Mohammed et al. [55] and Christian et al. [59], while consistent with D'Andrea et al. [15]. Study done by Aqeel et al. [10] demonstrated that PLT were increased significantly in non-smoker fuel filling workers while were decreased in smoker fuel filling workers and vice versa in MPV. In our study shows the same result in platelet but contradict with MPV. In urine

analysis, results of PH-values and specific gravity were significantly different of both study groups with control in Table 1.

Parameters (unit)	Control (mean ± std)	Study group 1 (mean ± std)	P-value	Study group II (mean ± std)	P-value
[a]WBC × (10^3/µL)	7.99 ± 1.21	9.01 ± 1.17	0.020*	9.87 ± 2.10	0.001*
[b]NE × (10^3/µL)	4.35 ± 1.00	4.81 ± 0.96	0.305	4.84 ± 0.78	0.085
[b]LY × (10^3/µL)	2.85 ± 0.59	2.97 ± 0.48	0.357	3.36 ± 1.00	0.034*
[b]EO × (10^3/µL)	0.27 ± 0.26	0.63 ± 0.35	0.003*	0.68 ± 0.31	0.002*
[b]MO × (10^3/µL)	0.44 ± 0.17	0.58 ± 0.56	0.9	0.74 ± 0.64	0.068
[b]BA × (10^3/µL)	0.06 ± 0.02	0.12 ± 0.10	0.005*	0.15 ± 0.10	0.000*
[a]RBC × (10^6/µL)	5.31 ± 0.57	5.35 ± 0.57	0.53	4.99 ± 0.40	0.043*
[a]HGB (g/dl)	14.79 ± 1.85	14.64 ± 1.61	0.78	13.27 ± 1.18	0.003*
[a]HCT (%)	46.30 ± 5.45	45.78 ± 4.91	0.76	41.73 ± 3.56	0.002*
[b]MCV (fL)	87.12 ± 4.70	84.85 ± 7.73	0.322	82.20 ± 7.20	0.042*
[a]MCH(pg)	27.86 ± 1.90	27.54 ± 5.48	0.86	25.19 ± 3.70	0.019*
[b]MCHC(g/dl)	31.93 ± 0.91	32.18 ± 0.94	0.251	30.05 ± 5.33	0.958
[b]RDW-CV (%)	13.21 ± 0.97	13.35 ± 0.94	0.423	13.59 ± 1.28	0.33
[b]RDW-SD(fL)	45.95 ± 2.94	45.51 ± 3.30	0.821	44.43 ± 3.45	0.07
[b]PLT × (103/µL)	262.06 ± 49.15	291.62 ± 40.64	0.042*	297.81 ± 43.97	0.009*
[a]PCT(%)	0.20 ± 0.04	0.23 ± 0.02	0.009*	0.24 ± 0.057	0.006*
[b]MPV(fL)	7.66 ± 0.62	8.20 ± 0.81	0.022*	8.41 ± 0.67	0.002*
[b]PDW (%)	17.96 ± 0.96	17.77 ± 0.72	0.823	18.17 ± 0.52	0.149
Urine					
[a]Specific Gravity	1.017 ± 0.00	1.027 ± 0.00	0.000*	1.028 ± 0.00	0.000*
[b]PH	5.66 ± 0.48	5.00 ± 0.00	0.000*	5.00 ± 0.00	0.000*
[a]Normal Distribution, [b]Non-Distribution, *significant result					

Table 1: Summary of hematological parameters.

Nitrate, Urobilinogen, Ketone, erythrocyte and Bilirubin were positive in participants of both groups. Results of urine composition investigation showed high occurrence of nitrates in urine of participants of both groups in Table 2. Usually, urine and urinary tract are free of pathogen. Positive Nitrate indicates urinary tract infection due to presence of bacteria in UTI [63]. This surveillance that is investigative of likely microorganism infection in these personals, furthermore, verifies immune demoralize effect of gasoline fumes exposure of said participants. In exposure group two, ERY was found significantly positive. Several diseases of the urinary tract and kidney in addition to trauma, medications and smoking can create hematuria [64]. The results of UGB and bilirubin were investigated significantly positive showed in Table 2. Bilirubin is a squander product which is taken away from the blood circulation. Liver constructs bilirubin from the hemoglobin of RBCs. The appearance of bilirubin in urine composition can take place before clinical indication for jaundice buildup and is early marker of liver disease.

Group (subjects=21)	Nitrate		Protein		Ketene		UGB		ERY		Bilirubin	
Group I												
	N	**P**	**N**	**P**	**N**	**P**	**N**	**P**	**N**	**P**	**N**	**P**
Control	21	0	21	0	21	0	21	0	21	0	18	3
Study	15	6	17	4	16	5	8	13	19	2	4	17
P Value	0.021*		0.10		0.04*		0.000*		0.40		0.00*	
Group II												
	N	**P**	**N**	**P**	**N**	**P**	**N**	**P**	**N**	**P**	**N**	**P**
Control	21	0	21	0	21	0	21	0	21	0	18	3
Study	15	6	17	4	16	5	8	13	19	2	4	17
P Value	0.021*		0.10		0.04*		0.000*		0.40		0.00*	

Table 2: Summary of urine composition.

UGB is usually appeared in low proportion in urine. It is made in the intestine from bilirubin, and a fraction of it is taken up rear into the blood. Positive results assist to identify liver disease like hepatitis, cirrhosis and such situation related with increased RBC demolition in hemolytic anemia [65]. The appearance in bilirubin and UGB in urine marks to probable red cell fracture particularly in participant's withprolong duration of exposure to gasoline fumes (Table 3).

Parameter	Correlation	[a]WBC	[b]NE	[b]LY	[b]EO	[b]MO	[b]BA	[a]RBC	[a] HGB	[a] HCT	[a]MCV	[a]MCH	[a]MCHC	[b]RDW-CV	[b]RDW-SD	[b]PLT	[a]PCT	[b]MPV	[b]PDW
Duration (Years) 1 to 18	R-Value	0.2	0.04	0.17	0.08	0.23	0.22	-0.32	-0.32	-0.32	-0.13	-0.2	-0.1	0.05	-0.17	0	-0.01	0.17	0.14
	P-Value	0.06	0.71	0.12	0.47	0.03*	0.04*	0.003*	0.003*	0.003*	0.22	0.03*	0.26	0.5	0.11	0.8	0.9	0.13	0.19
	N	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42
[a]Normal distribution and Pearson Correlation, [b]Non-Distribution and Spearman Correlation, *significance results																			

Table 3: Correlation of CBC parameter with exposure length of gasoline.

Conclusion

The findings of this study presented approaching into the consequences of gasoline fume exposure on hematological parameters as well as on urine in Multan city, Pakistan who were constantly inhaled passively of fumes to dispense fuel in vehicles daily. The present study indicates that significant reduction and negative correlation of HGB, RBC and HCT with exposure of prolong gasoline fumes/vapors length cause bone marrow depression. Significant reduction in HGB, RBC and HCT, negative correlation of exposure length with them and appearance of UGB in urine cause anemia with high alarming. Finally, from our findings and insight through pervious study writings, exposure of gasoline fumes/vapors produces adverse effects on human health related to oxidative stress, bio-activation and reactive metabolites in critical steps.

Recommendations

From our study findings, it is suggested that personnel at gasoline filling stations must be protected from exposure to fumes/vapors by training with the equipment, wearing protective devices to avoid possibly of any abnormalities and clinical check may be done in regular basis.

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