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Assessment of Liver Functions among Male Filling Station Workers in Dhaka City

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Abstract
***Background:** Filling station workers are occupationally exposed to petroleum products that contain volatile hydrocarbons notably benzene, toluene, ethylbenzene and xylene (BTEX). The liver is the principal organ for biotransformation of these compounds and is therefore a key target for chemical injury. This study assessed liver function in male filling station workers in Dhaka city. **Methods:** This cross-sectional research took place at Dhaka Medical College in 2022 involving 80 males aged 20–55 years, comprising 40 filling station employees with at least 2 years of exposure and 40 controls matched for age and BMI. Workers were categorized based on the length of exposure (2–<5, 5–10, and >10 years). Liver function indicators (ALT, AST, ALP, total bilirubin, and total protein) were assessed and evaluated through t-test, ANOVA with Bonferroni post-hoc analysis, and Pearson's correlation ($p<0.05$). **Results:** Mean serum ALT (37.50±12.64 vs 28.05±6.13 U/L) and AST (30.75±14.84 vs 25.03±7.35 U/L) levels were notably elevated in filling station workers compared to controls ($p<0.001$ and $p=0.003$, respectively). Both parameters rose notably with extended exposure time (ANOVA, $p<0.001$). ALT ($r=+0.819$, $p<0.001$) and AST ($r=+0.803$, $p<0.001$) showed significant positive correlations with the length of exposure. Nevertheless, mean ALP, total bilirubin, and total protein levels exhibited no statistically significant differences among the groups and displayed only weak, non-significant positive correlations with the duration of exposure. **Conclusion:** Increased ALT and AST levels among filling station employees indicate liver dysfunction related to petroleum exposure; routine screening and the use of personal protective equipment are advised.*
***Keywords:** Petroleum products; Filling station workers; Liver function tests; Alanine aminotransferase; Aspartate aminotransferase; Occupational exposure; Bangladesh.*

I. INTRODUCTION

An occupational disease is any illness contracted primarily as a result of exposure to risk factors arising from work activity. The International Labour Organization (ILO) groups occupational diseases into those caused by exposure to chemical, physical and biological agents; those affecting specific target organ systems; occupational cancers; and other illnesses [1]. Globally, an estimated 2.78 million workers die each year from occupational accidents and diseases, and around 374 million sustain non-fatal occupational injuries; work-related illness alone accounts for the majority of these deaths [2].

Rapid economic development, globalisation and urbanisation have increased the number of motor vehicles and, consequently, national fuel consumption. As a result, filling stations have proliferated in both urban and rural areas [3]. Because self-service is uncommon at these stations in many low- and middle-income countries, fuel-filling attendants are employed to dispense fuel; in addition to dispensing, they unload fuel and check storage-tank levels, and are therefore exposed to petroleum products throughout the working day [4,5]. Occupational disease

among filling station workers is a long-recognised global problem, and the true burden in developing countries is thought to be considerably greater than reported [6,7].

Inhalation through the respiratory tract is the main route of petroleum-product exposure, although absorption can also occur through the skin and gastrointestinal tract [8]. Low concentrations of inhaled vapour can cause irritation of the nose and throat, headache, dizziness, nausea, vomiting, confusion and breathing difficulty, while skin contact may produce rashes, redness and swelling. Chronic exposure has been associated with renal, haematological, genetic and hepatic complications, including elevation of liver enzymes, fatty liver and raised creatinine [9,3].

Benzene, toluene, ethylbenzene and xylene (BTEX) are the aliphatic and aromatic hydrocarbon constituents of petrol regarded as most harmful to human health [10]. Chronic benzene exposure can cause liver and kidney failure, myeloid leukaemia, reduced erythropoiesis, weakened immunity and central nervous system damage, and it alters the activity of many hepatic enzymes [11]. Toluene is metabolised by hepatic cytochrome P450 to benzoic acid, which is conjugated with glycine to form hippuric acid and excreted in urine [12]; toluene and xylene have both been reported to cause liver and kidney damage [13].

The mechanisms of petrol-induced hepatotoxicity include direct cellular damage, down-regulation of gene expression and induction of oxidative stress. Reactive metabolites of gasoline (for example 1,2,4-benzenetriol and benzoquinone) combine with hepatocyte membrane lipids to generate lipid peroxides and reactive oxygen species (ROS), which damage biological membranes, cause leakage of hepatic enzymes into the circulation and raise their serum concentrations. Reduced CYP450 activity can decrease bile acid-independent bile flow and promote cholestatic reactions, raising serum bilirubin and ALP [14]. An imbalance between pro-oxidants and antioxidants from excess ROS, antioxidant deficiency, or both sustains the oxidative cellular damage observed in petrol-pump workers [15].

The liver is central to nutrient metabolism, secretion of bile, metabolism of bilirubin, storage of carbohydrate and, importantly, detoxification of endogenous and exogenous compounds [8,16]. Liver function tests (LFTs) including ALT, AST, ALP, bilirubin and total protein are among the most frequently requested screening investigations and provide information across a range of disease processes. ALT is a sensitive and relatively specific marker of hepatocellular injury; AST, a mitochondrial isoenzyme of hepatocytes, typically rises alongside ALT in hepatic injury; and ALP, produced by the cells lining the bile ducts and canaliculi, is released in response to cholestasis [17].

Several studies from other countries have shown that prolonged exposure to petroleum products alters hepatic function in filling station workers. In Bangladesh, however, very little information is available on the health hazards faced by this occupational group, and to the authors' knowledge no previous study has specifically evaluated their liver function. The present study was therefore designed to assess liver function among male filling station workers in Dhaka city, so that appropriate preventive measures can be considered.

II. MATERIALS AND METHODS

Study design, setting and period

This cross-sectional analytical study was conducted in the Department of Physiology, Dhaka Medical College, Dhaka, Bangladesh, from January 2022 to December 2022. Biochemical analyses were performed in the Department of Laboratory Medicine, Dhaka Medical College Hospital, Dhaka.

Study population and sampling

The study population comprised male filling station workers in Dhaka city. The sample size was calculated using the two-mean comparison formula $n = (Z\alpha + Z\beta)^2 (\sigma_1^2 + \sigma_2^2) / (\mu_1 - \mu_2)^2$, taking ALT means and standard deviations from a previous comparable study [8], a 5% level of significance and 80% power; this yielded a minimum of 39 subjects per group, and 40 per group were enrolled for convenience. Subjects were recruited by purposive sampling.

A total of 80 men aged 20–55 years were studied. The study group (Group A, n=40) comprised filling station workers from several stations in Dhaka city with at least two years of occupational exposure to petroleum products. The control group (Group B, n=40) comprised apparently healthy men of the same age range, recruited through personal contact, who were not occupationally exposed to petroleum products and were matched to Group A for age and BMI. To examine the effect of exposure duration, Group A was sub-divided into Group A1 (2–<5 years, n=11), Group A2 (5–10 years, n=15) and Group A3 (>10 years, n=14).

Inclusion and exclusion criteria

Inclusion criteria for the study group were male sex, age 20–55 years, and occupational exposure to petroleum products for at least two years; control subjects met the same age criterion but had no occupational petroleum exposure. Subjects with known liver disease, kidney disease, diabetes mellitus or hypertension were excluded, as were those with a history of anticoagulant use, malignancy or recent blood transfusion. Smokers, alcohol users and tobacco users were excluded from both groups.

Data collection and anthropometry

After the nature, purpose and benefits of the study had been explained and written informed consent obtained, a detailed personal, family and medical history was recorded on a structured questionnaire. Height, weight and body mass index (BMI) were measured, and pulse and blood pressure were recorded for all participants.

Blood sampling and biochemical analysis

Under aseptic precautions, 4 mL of venous blood was collected from the antecubital vein of each subject using a disposable plastic syringe and transferred into a clean, dry test tube. After clot formation, the sample was centrifuged at 3,000 rpm for 10 minutes; the separated serum was stored in labelled microcentrifuge tubes at −20 °C until analysis. Serum ALT, AST, ALP, total bilirubin and total protein were estimated in the Department of Laboratory Medicine, Dhaka Medical College Hospital. Serum creatinine was also measured to exclude renal disease.

Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 26. Results are expressed as mean ± standard deviation (SD). The unpaired Student's t-test was used to compare the two groups, one-way analysis of variance (ANOVA) followed by the Bonferroni post-hoc test was used to compare the three exposure sub-groups, and Pearson's correlation coefficient (r) was used to assess the relationship between liver function parameters and duration of exposure. A p-value <0.05 was considered statistically significant.

Ethical considerations

Ethical approval was obtained from the Ethical Review Committee of Dhaka Medical College, Dhaka, and permission was obtained from the relevant departments and station managers. Written informed consent in Bangla covering the aims and objectives, reasons for invitation, expectations, risks and benefits, and confidentiality of data was obtained from every participant. Participation was voluntary.

III. RESULTS

General characteristics of the study population

Table 1 shows eighty male subjects aged 20–55 years were studied. The mean age of Group A and Group B was 39.60±9.92 and 35.70±8.60 years, respectively, and mean BMI was 24.31±3.44 and 23.57±3.49 kg/m², respectively; neither differed significantly between groups, confirming that the groups were matched for age and BMI. Mean systolic and diastolic blood pressures were also comparable between groups.

Table 1. General characteristics of the subjects in Group A and Group B (n=80)

Variable	Group A (n=40)	Group B (n=40)	p-value
Age (years)	39.60 ± 9.92 (20–55)	35.70 ± 8.60 (20–55)	0.064
BMI (kg/m²)	24.31 ± 3.44 (17.0–33.4)	23.57 ± 3.49 (17.3–36.4)	0.344
Systolic BP (mmHg)	121.38 ± 8.32 (100–135)	122.00 ± 8.90 (100–135)	0.751
Diastolic BP (mmHg)	79.63 ± 5.48 (60–85)	79.75 ± 4.80 (65–85)	0.914

Values are mean ± SD; figures in parentheses indicate range. Analysis by unpaired Student's t-test; p<0.05 considered significant. BMI, body mass index; BP, blood pressure.

Comparison of liver function parameters between exposed and control groups

Table 2 presents mean serum ALT was 37.50±12.64 U/L in Group A versus 28.05±6.13 U/L in Group B, and mean serum AST was 30.75±14.84 U/L versus 25.03±7.35 U/L, respectively. Both ALT (p<0.001) and AST (p=0.003) were significantly higher in the exposed group. In contrast, mean serum ALP (66.20±23.67 vs 66.63±16.49 U/L; p=0.926), total bilirubin (0.59±0.25 vs 0.55±0.21 mg/dL; p=0.412) and total protein (6.98±0.49 vs 7.09±0.55 g/dL; p=0.343) were almost identical between the two groups, with no statistically significant differences.

Table 2. Comparison of liver function parameters between Group A and Group B (n=80)

Parameter	Group A (n=40)	Group B (n=40)	p-value
ALT (U/L)	37.50 ± 12.64 (17–70)	28.05 ± 6.13 (17–39)	<0.001 *
AST (U/L)	30.75 ± 14.84 (14–75)	25.03 ± 7.35 (14–40)	0.003 *
ALP (U/L)	66.20 ± 23.67 (19–120)	66.63 ± 16.49 (44–113)	0.926
Total bilirubin (mg/dL)	0.59 ± 0.25 (0.29–1.30)	0.55 ± 0.21 (0.20–0.92)	0.412
Total protein (g/dL)	6.98 ± 0.49 (5.93–7.81)	7.09 ± 0.55 (6.12–7.90)	0.343

*Values are mean ± SD. Analysis by unpaired Student's t-test; * p<0.05 considered statistically significant. ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase.*

Liver function parameters by duration of exposure

Table 3 shows among the exposure sub-groups, mean serum ALT was 25.91±10.29, 33.53±4.39 and 50.86±7.26 U/L in Groups A1, A2 and A3, respectively, and mean serum AST was 24.09±11.20, 23.87±4.61 and 43.36±16.66 U/L, respectively. One-way ANOVA showed a significant difference across sub-groups for both ALT and AST ($p<0.001$). On Bonferroni post-hoc testing, ALT was significantly higher in Group A2 than Group A1 ($p=0.040$) and in Group A3 than both Group A1 and Group A2 ($p<0.001$ for both); AST was significantly higher in Group A3 than Group A1 ($p=0.001$) and Group A2 ($p<0.001$), but did not differ significantly between Groups A1 and A2 ($p=1.000$). Mean serum ALP, total bilirubin and total protein did not differ significantly across the exposure sub-groups.

Table 3. Comparison of liver function parameters among exposure sub-groups of Group A (n=40)

Parameter	Group A1 (2–<5 y, n=11)	Group A2 (5–10 y, n=15)	Group A3 (>10 y, n=14)	p-value
ALT (U/L)	25.91 ± 10.29	33.53 ± 4.39	50.86 ± 7.26	<0.001 *
AST (U/L)	24.09 ± 11.20	23.87 ± 4.61	43.36 ± 16.66	<0.001 *
ALP (U/L)	63.27 ± 27.12	61.13 ± 14.02	73.93 ± 28.33	0.317
Total bilirubin (mg/dL)	0.63 ± 0.27	0.59 ± 0.28	0.56 ± 0.17	0.781
Total protein (g/dL)	6.93 ± 0.43	6.97 ± 0.40	7.03 ± 0.62	0.880

*Values are mean ± SD. Analysis by one-way ANOVA; * $p<0.05$ considered statistically significant. Bonferroni post-hoc comparisons for ALT: A1 vs A2, $p=0.040$; A1 vs A3, $p<0.001$; A2 vs A3, $p<0.001$. For AST: A1 vs A2, $p=1.000$; A1 vs A3, $p=0.001$; A2 vs A3, $p<0.001$.*

Correlation of liver function parameters with duration of exposure

Table 4 presents in Group A, serum ALT ($r=+0.819$; $p<0.001$) and serum AST ($r=+0.803$; $p<0.001$) showed strong, statistically significant positive correlations with duration of occupational exposure. Serum ALP ($r=+0.210$; $p=0.193$), total bilirubin ($r=+0.308$; $p=0.053$) and total protein ($r=+0.055$; $p=0.737$) showed weak positive correlations that were not statistically significant.

Table 4. Correlation of liver function parameters with duration of exposure in Group A (n=40)

Parameter	Correlation coefficient (r)	p-value
ALT (U/L)	+0.819	<0.001 *
AST (U/L)	+0.803	<0.001 *
ALP (U/L)	+0.210	0.193
Total bilirubin (mg/dL)	+0.308	0.053
Total protein (g/dL)	+0.055	0.737

*Analysis by Pearson's correlation coefficient (r); * $p<0.05$ considered statistically significant.*

Although the group mean values of ALT and AST remained within the conventional reference range, abnormal individual values were observed in 37.5% of workers for ALT, 20% for AST and 2.5% for total bilirubin, suggesting that prolonged exposure to petroleum products may exert deleterious effects on the liver in a proportion of exposed workers.

IV. DISCUSSION

This cross-sectional analytical study assessed liver function serum ALT, AST, ALP, total bilirubin and total protein in male filling station workers in Dhaka city and compared them with apparently healthy, age- and BMI-matched unexposed men. In the control group, all parameters lay within physiological limits, consistent with observations by several investigators in other settings [11,13,18,19]. The two groups were comparable in age, BMI, pulse and blood pressure, minimising the influence of these potential confounders.

The principal finding was that mean serum ALT and AST were significantly higher in exposed workers than in controls. This is consistent with reports from Libya, Egypt, Ethiopia, India, Sudan and Iran, where filling station or petrol-pump workers showed significantly raised aminotransferases relative to unexposed controls [5,8,11,13,19,20]. Eze et al. reported significantly higher AST but, in contrast, lower ALT in exposed workers [21]. A minority of studies found no significant difference in aminotransferases between exposed and unexposed groups [10,18,22,23], a discrepancy likely to reflect differences in exposure intensity and duration, analytical methods, handling of confounders such as age, BMI and personal habits, and the extent of personal protective equipment use.

Elevated aminotransferases in exposed workers are biologically plausible. Petroleum products contain hepatotoxic hydrocarbons benzene, toluene and xylene that enter the body mainly by inhalation and are metabolised in the liver by CYP450 (particularly CYP2E1) oxidative pathways. This generates free radicals and

reactive metabolites such as phenol, hydroquinone, benzoquinone and 1,2,4-benzenetriol, which promote lipid peroxidation, damage hepatocyte membranes and cause leakage of intracellular enzymes into the circulation [14,15]. ALT, being relatively specific to hepatocytes, and AST, a mitochondrial isoenzyme responsive to membrane stress, are sensitive indicators of this process [17].

In contrast, mean serum ALP, total bilirubin and total protein did not differ significantly between exposed and unexposed groups. The ALP finding agrees with several reports [5,13,18,22], whereas other investigators have described significantly higher ALP in exposed workers [10,24,25]. The absence of a significant difference in total bilirubin is consistent with several studies [19,22,26,27], although raised bilirubin has been reported elsewhere [28,29]. Similarly, the lack of a significant difference in total protein is in agreement with some authors [22,26,30] but not others, who found reduced total protein in exposed workers [11,19]. These divergent findings again point to differences in exposure characteristics, study populations and methodology.

When workers were stratified by duration of exposure, mean serum ALT and AST were significantly higher in those exposed for more than 10 years than in those exposed for 2–<5 years or 5–10 years, and ALT was also significantly higher at 5–10 years than at 2–<5 years. Comparable duration-dependent increases in aminotransferases have been reported by other investigators [11,19]. Serum ALP, total bilirubin and total protein did not vary significantly across exposure sub-groups, in keeping with some previous reports [27].

Pearson's correlation analysis reinforced these findings: ALT and AST correlated strongly and significantly with duration of exposure ($r=+0.819$ and $r=+0.803$, respectively), while ALP, total bilirubin and total protein showed only weak, non-significant positive correlations. A significant positive correlation of aminotransferases with exposure duration has likewise been documented elsewhere [19,20], although one study found no significant correlation for ALT, bilirubin or total protein [27]. The dose–response pattern observed here strengthens the inference that the aminotransferase elevation is attributable to cumulative occupational exposure rather than chance.

Although group mean ALT and AST remained within the reference range, abnormal individual values were found in a substantial minority of workers (37.5% for ALT, 20% for AST). This indicates that a clinically meaningful subset of exposed workers may already have subclinical hepatocellular stress, underscoring the value of periodic biochemical screening even when group averages appear normal.

V. LIMITATIONS

This study has some limitations. It was conducted in a small number of selected filling stations in Dhaka city, so the findings may not represent the wider working population. Female workers were not included, as women are very rarely employed in filling stations in Bangladesh. The cross-sectional design precludes firm conclusions about causality, and ambient hydrocarbon concentrations and individual biomarkers of exposure were not measured.

VI. CONCLUSION

Serum ALT and AST were significantly elevated in male filling station workers compared with unexposed controls and increased significantly with longer duration of occupational exposure, while ALP, total bilirubin and total protein were not significantly affected. These findings indicate that chronic occupational exposure to petroleum products may compromise hepatocellular integrity in this workforce. Periodic liver function screening, health education, and consistent use of personal protective equipment together with larger multicentre studies including additional markers of hepatic synthetic function are recommended to protect the health of filling station workers.

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