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Original article

Genotoxic Effects on Gas Station Attendants in South-southeastern México due to Prolonged and Chronic Exposure to Gasoline

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ABSTRACT

Background: Gasoline, a complex mixture of volatile organic compounds is classified as possibly carcinogenic to humans. Gasoline station attendants, consistently exposed to its hazardous components, may face genotoxic effects. This study aimed to assess the influence of varying work shift durations on DNA damage in gasoline station attendants.

Methods: Ninety individuals from three locations in southern México were studied. Peripheral blood mononuclear cells (PBMCs) were isolated, and DNA damage was assessed using the comet assay. Demographic, occupational, and lifestyle data were collected. Statistical analyses included *t*-tests, ANOVA, and Pearson correlation.

Results: Significant differences in DNA damage parameters were observed between exposed and unexposed groups. The impact of tobacco, alcohol, and exercise on DNA damage was negligible. Extended work shifts (12 and 24 hours) showed heightened DNA damage compared to 8-hour shifts and the unexposed group. A novel finding revealed a modest but significant correlation between DNA damage and job seniority.

Conclusion: The study highlights the intricate relationship between occupational exposure to gasoline components, DNA damage, and work shift lengths. Extended shifts correlate with heightened genotoxic effects, emphasizing the importance of personalized safety measures. The significant correlation between DNA damage and job seniority introduces occupational longevity as a determinant in the genetic health of gasoline station attendants. This discovery has implications for implementing targeted interventions and preventive strategies to safeguard workers' genetic integrity throughout their years of service. The study calls for further exploration of unconsidered factors in understanding the multifactorial nature of DNA damage in this occupational setting.

1. Introduction

Gasoline, a byproduct of petroleum refining, comprises a complex mixture of alkanes, aromatics, olefins, and other volatile organic compounds (VOCs), with notable constituents being Benzene, Toluene, ethylbenzene, and Xylene isomers (BTEX) [1–3]. The International Agency for Research on Cancer (IARC) classified gasoline as a Group 2B substance in 1987, indicating it as possibly carcinogenic to humans [4]. Among the most dangerous components of gasoline for health is benzene, which is a defined

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carcinogen (Group 1). Exposure to these compounds in gasoline can be highly harmful to health since they promote damage through the fragmentation of DNA molecules causing DNA damage [5–7]. Prolonged exposure to xylene and toluene, prevalent components of gasoline, adversely affects the respiratory and central nervous systems, as noted by the Agency for Toxic Substances and Disease Registry (ATSDR) [8,9]. Gasoline station attendants are continuously exposed to these hazardous compounds released during fuel dispensing [10]. The complex mixture of VOCs in gasoline raises concerns about its potential genotoxic effects, particularly in individuals consistently exposed to these compounds.

While previous studies have associated petroleum-derived compounds with adverse biological effects, including genotoxic potential [,1,3,10–12], there is a gap that prevents us from understanding how different work conditions and durations can contribute to DNA damage in gas station attendants. The current study aims to address this gap by specifically investigating the influence of varying work shift durations—8-hour shifts, 12-hour shifts with rotating schedules, and 24-hour shifts with the same rest period—on the magnitude of DNA damage in gasoline station attendants. It is essential to evaluate these varying work conditions comprehensively, as the potential health impacts may extend beyond the immediate exposure to VOCs of gasoline. The choice of these durations is based on the common work patterns observed in this occupational setting, and assessing these durations allows for a nuanced understanding of the potential cumulative effects.

In this study, we utilized the comet assay, a reliable method for human biomonitoring, to assess DNA damage in gasoline station attendants. This assay allows the analysis of DNA migration due to the fragmentation of genetic material. The extent of damage is assessed according to the number of affected cells, the length of the tail, and the fluorescence intensity of the fragments [13]. This evaluation was performed in three locations in southern México: Campeche, Tabasco, and Chiapas. The primary aim was to determine how different work shift durations influence the magnitude of DNA damage. As a result of this study, we obtained the first evidence of the effect of the length of the working day with exposure to gasoline components and genotoxic damage shown by gasoline station attendants.

2. Methods

2.1. Study population and sample collection

This study was carried out on individuals working in a total of nine service stations in the states of Campeche, Chiapas, and Tabasco, all in the south-southeast of Mexico. The control group consisted of individuals from the same states without periodic or occupational exposure to petroleum gasoline or with similar scenarios. A total of 90 individuals, including men and women, were involved in the study, with 82 being gasoline station attendants and 8 forming the unexposed control group. Blood samples were collected from all participants at the onset of their workday. To ensure consistency, samples were taken under similar conditions for both the exposed and control groups. Demographic and occupational data for all participants were gathered through structured interviews, providing valuable insights into variables such as work shift duration, seniority, and lifestyle factors.

2.2. Isolation of PBMCs

Blood samples were obtained by venipuncture and collected into BD Vacutainer® CPT™ Cell Preparation Tubes containing sodium heparin as an anticoagulant. Direct isolation of peripheral blood mononuclear cells (PBMCs) from these tubes was performed by centrifugation at 1800 × g for 30 minutes, the interface was transferred to a new tube and centrifuged again to pellet the mononuclear cells at 400 × g, for 40 minutes, discarding the supernatant and resuspending the PBMCs in phosphate-buffered saline (PBS, pH 7.4, Sigma-Aldrich®) to the target concentration. This procedure was performed for each blood sample, and PBMCs were obtained individually from each participant. To determine the number of viable cells in the suspension, 0.4% trypan blue (Sigma-Aldrich®) was used; The viable cell concentration and volume for the comet assay were adjusted to 200,000 PBMC/mL.

2.3. Comet assay

The alkaline comet assay technique described by Singh et al. [14], with modifications suggested by Tice et al. [15], was used to determine DNA damage. The standardization implications of the technique described by Ersson and Möller [16] were also considered. The technique was applied to the 90 samples of PBMCs extracted as described above. For comparative purposes, age groups were organized by quinquennium, the entity where the service station was located, length of the working day, lifestyle indicators such as exercise and tobacco consumption, as well as working seniority (Table 1).

Each sample was prepared on microscope slides using a mixture of 5 μL of PBMCs and 75 μL of 0.5% low-melting agarose (Sigma-Aldrich®). This mixture was expanded on microscope slides coated with 180 μL of 0.5% low-melting agarose and allowed to solidify at 4°C for 5 minutes. To ensure coverage, an additional layer of 180 μL of 0.5% low-melting agarose was added and allowed to solidify at 4°C for 5 minutes. Subsequently, the slides were immersed in a lysis solution containing trypsin (20 mg/mL, Sigma-Aldrich®) in PBS, pH 7, and 1% Triton X-100 (Sigma-Aldrich®), for 30 minutes at 37°C. The released DNA was electrophoresed in an alkaline solution (0.3M NaOH (CTR® Scientific), 1mM disodium EDTA (Aldrich® Chemistry), pH 13) at 1.5 V/cm, 300 mA, at 10°C for 20 minutes in total darkness. Then, the samples were neutralized using Trizma® base 0.4M, pH 7.5 (Sigma® Life Science), dried and stored at room temperature until microscopic analysis. DNA fragmentation was visualized by incorporating ethidium bromide (0.05mM, Sigma®) into the DNA, and the samples were analyzed under a fluorescence microscope. For each sample, fifty images of cells showing both comets and normal cells were captured. All images obtained from the comet assay were digitized using AxioVision 4.8.2 and analyzed with TriTek CometScore v1.5™ software, collecting parameters such as Olive Tail Moment (OTM), Tail Length (TL), and the percentage of fragmented DNA (% Tail DNA).

2.4. Statistical analysis

The statistical analyses were conducted using the JMP 10™ program. The results obtained from the study were analyzed using Student's *t*-tests and Analysis of Variance (ANOVA). Additionally, Pearson correlation tests were performed. Tables summarizing the data were created using Excel™ for Mac 16.16.27.

2.5. Research ethics criteria

The present research was approved by the Research Ethics Committee (CEI) of El Colegio de la Frontera Sur (ECOSUR) following the regulations established for this purpose. Participants were informed of their freedom to participate, as well as the confidentiality of the results, utilizing a letter of informed consent that was applied to the participating individuals.

Table 1
Demographic characteristics of the studied population in San Cristóbal de las Casas (SCLC), Villa Hermosa (VH), Ciudad del Carmen (CDC) and the unexposed group

	Exposed ($n = 82$)									Unexposed ($n = 8$)		
	SCLC			VH			CDC					
	%	n	M ± ES	%	n	M ± ES	%	n	M ± ES	%	N	M ± ES
Age	100	28	32.1 (2.3)	100	28	29.5 (2.3)	100	26	39.8 (2.4)	100	8	25.1 (3.4)
Seniority in the job in gas station	100	28	5.6 (2.1)	100	28	4.5 (2.1)	100	26	6.5 (2.2)	N/A	–	
Gender												
Male	92.9	26		92.9	26		76.9	20		50.0	4	
Female	7.1	2		7.1	2		23.1	6		50.0	4	
Tobacco consumption												
Yes	71.4	20		64.3	18		15.4	4		25	2	
Not	28.6	8		35.7	10		84.6	22		75	6	
Alcohol consumption												
Yes	64.3	18		57.1	16		69.2	18		50	4	
Not	35.7	10		42.9	12		30.8	8		50	4	
Physical exercise												
Yes	35.7	10		78.6	22		23.1	6		25	2	
Not	64.3	18		21.4	6		76.9	20		75	6	
Length of the working day exposed												
0 h										100	8	
8 h	28.6	8		57	16		15.4	4		N/A	–	
12 h	N/A	N/A		36	10		53.8	14		N/A	–	
24 h	71.4	20		7	2		30.8	8		N/A	–	

3. Results

Table 1 provides insights into the composition and characteristics of the study population, offering a snapshot of its demographic structure and health-related behaviors. Most of the population (84.4%) consisted of men, with women representing 15.6%. Notably, gas station attendants in Ciudad del Carmen (CDC) had an average age of approximately 39.8 years, while the Villahermosa (VH) group exhibited a notably lower average age of around 29.5 years, indicating an age difference compared to the CDC group. In addition, it was observed that the VH group exercises more compared to the other groups, and that they have the least seniority in terms of work as gasoline station attendants. During the present study, the stations located in the municipality of San Cristóbal de las Casas (SCLC) did not have a 12-hour workday programmed, so the gas station attendants only operated on 8 and 24-hour shifts.

Table 2 displays data on OTM, TL, and % Tail DNA obtained by service station, compared to the non-exposed group. They were observed significant differences between the non-exposed group and the study groups.

In the comparison of the factors of tobacco consumption, alcohol consumption, and physical exercise, no significant differences were observed in the evaluated parameters (OTM, TL, and % Tail DNA), as detailed in Table 3.

When comparing the populations according to the length of the working day, statistically significant differences in OTM values were observed between the groups working in 12- and 24-hour shifts compared to the control group. No significant differences were found in this parameter between the control group and gas station attendants with 8-hour shifts. Regarding TL in comets, a significant difference was evident in the groups with longer workdays (12 and 24 hours) compared to the control group and the groups with an 8-hour workday. Furthermore, the % DNA in the tail showed a significant difference in all exposed groups, regardless of their length of the workday, compared to the control group of unexposed individuals (Fig. 1).

The analysis of the correlation between DNA damage and biological age did not reveal statistically significant differences in the study population. However, the correlation coefficient between DNA damage and job seniority, albeit modest, was statistically significant (Table 4).

4. Discussion

4.1. Demographic characteristics

Significant distinctions in demographic and occupational characteristics were observed among the study groups. The CDC group displays the highest average age, followed by SCLC and VH, while

Table 2
Comet assay results by service station and unexposed group. Olive Tail Moment (OTM), Tail Length (TL), and percentage of tail DNA (% Tail DNA)

		OTM (μm)	TL (μm)	% Tail DNA
Unexposed		5.04 ± 0.58^{a}	12.38 ± 2.43^{a}	10.66 ± 2.25^{a}
Service station	SCLC	7.24 ± 0.51^{b}	26.99 ± 2.25^{b}	27.55 ± 2.24^{b}
	VH	7.25 ± 0.53^{b}	21.56 ± 2.31^{b}	20.48 ± 2.16^{b}
	CDC	7.17 ± 0.53^{b}	24.15 ± 2.34^{b}	24.63 ± 2.24^{b}

*Data indicate mean ± standard error. Statistical analysis by Student's test (*t*-test). Superscript letters 'a' and 'b' indicate statistical comparisons between groups. Different superscript letters denote a statistically significant differences ($p < 0.05$) comparing each group of service station with the unexposed group.

Table 3
Impact of alcohol and tobacco consumption, and physical exercise on DNA damage parameters (OTM, TL, and % Tail DNA)

	Alcohol consumption			Tobacco consumption			Exercise		
	OTM	TL	% Tail DNA	OTM	TL	% DNA tail	OTM	TL	% Tail DNA
Yes	6.86±0.3[a]	22.37±1.76[a]	23.89±1.77[a]	7.68±0.57[a]	21.35±1.58[a]	23.8±2.8[a]	5.66±0.31[a]	20.21±1.85[a]	22.85±2.11[a]
Not	6.861±0.48[a]	22.23±2.27[a]	25.84±2.41[a]	6.87±0.45[a]	20.6±1.27[a]	24.83±1.65[a]	6.31±0.51[a]	19.96±2.04[a]	25.98±1.9[a]

*Data indicate mean ± standard error. Statistical analysis by Student's test (*t*-test). Superscript letters 'a' and 'b' indicate statistical comparisons. Different superscript letters denote statistically significant differences ($p < 0.05$).

the control group had the lowest average age. This demographic pattern could potentially influence the observed results, as it has been demonstrated that age can be a determining factor in susceptibility to genetic damage. A similar trend was noticed in job seniority, with the CDC having the longest, followed by SCLC and VH. This finding holds implications, suggesting that job seniority may be linked to cumulative exposure to gasoline components, influencing the extent of DNA damage (see Table 3).

The gender distribution is noteworthy, with 84% male and 15.6% female participants, compared to a gender balance in the control group. While this gender imbalance could introduce variations in susceptibility to genetic damage, considering hormonal and metabolic differences [17], it is important to note that the control group exhibited lower DNA damage when compared to the study groups.

Disparities in health habits were evident, particularly in tobacco consumption and physical exercise frequency. While the majority of the control and CDC groups reported non-smoking, in contrast, the SCLC and VH groups had a higher proportion of tobacco consumers. Additionally, the VH group stood out for a greater percentage engaging in physical exercise, which could influence the body's ability to cope with oxidative stress and genetic damage. However, the comparison of genetic damage analyzed by comet assay across these factors did not show significant differences (see Table 2).

The SCLC group showed a notable prevalence of 24-hour shifts, while VH and CDC exhibited more diverse work patterns, including 8- and 12-hour shifts. Despite potential contributions to disparities in DNA damage due to differences in work patterns, no significant differences were observed in DNA damage between service stations (see Table 2). These demographic and occupational disparities underscore the complexity of assessing the impact of the occupational environment on the genetic health of gas station attendants.

4.2. Comparison between service stations and the non-exposed group

Table 2 provides a comparison of the OTM, TL, and % Tail DNA parameters between the service stations (SCLC, VH, CDC) and the unexposed group. Statistical tests revealed significant differences, indicating a noticeable impact of occupational exposure on genetic damage levels. These findings underscore the importance of considering variability in working conditions when assessing DNA damage in gas station workers. Furthermore, the presence of significant differences emphasizes the need for implementing specific safety and protection measures in these occupations to mitigate the harmful effects of exposure to gasoline components. The tendency of higher values shown by the exposed group in comparison with the control group aligns with previous genotoxicity studies utilizing the comet assay within the same occupational groups [6].

4.3. Factors influencing DNA damage

Analyzing factors like tobacco and alcohol consumption, and physical exercise among the study groups, we sought to elucidate their potential impact on observed DNA damage (Table 3). Surprisingly, the findings revealed no statistically significant differences in DNA damage based on these lifestyle factors. Both smokers and non-smokers exhibited similar levels of DNA damage, and the same trend was observed among those who consumed alcohol compared to non-consumers. Regardless of exercise habits, no substantial disparities in DNA damage were identified within the study groups.

These results suggest that within the studied population of gas station attendants, the examined lifestyle factors—tobacco and alcohol consumption, as well as physical exercise—may not be major determinants of the observed DNA damage. This unexpected

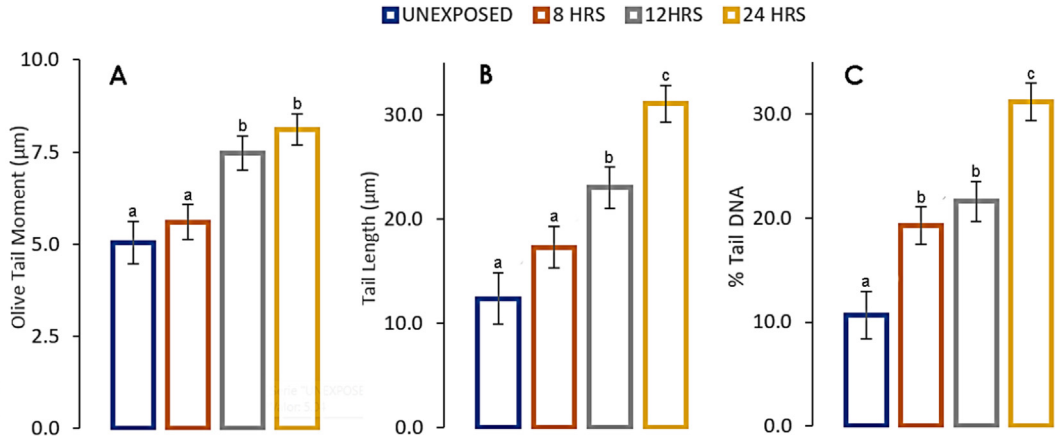

Fig. 1. Genotoxic impact of length of working day on DNA damage. Histograms showing (A) OTM, (B) TL and (C) percentage of tail DNA by length of working day among unexposed and exposed groups. Bars indicate standard error of the mean. Statistical analysis by Student's test (*t*-test). Distinct lowercase letters denote a statistically significant difference ($p < 0.05$) comparing each group exposed with unexposed group.

Table 4
DNA damage in relation to length of service and biological age

	OTM R2	TL R2	% tail DNA R2
Seniority in the job	0.255*	0.241***	0.182**
Biological age	0.0012	0.003	0.001

Linear regression $*p < 0.001$, $**p < 0.005$ and $***p < 0.0005$.

lack of significant associations prompts a reevaluation of the conventional understanding of these factors' direct impact on genetic integrity in the context of occupational exposure to gasoline components.

Consistent with previous studies [18,19] which found no direct effect of smoking on DNA damage, our results suggest that occupational exposure to gasoline components may overshadow individual lifestyle effects in this specific occupational setting. The absence of statistically significant differences in DNA damage based on tobacco and alcohol consumption, as well as physical exercise, emphasizes the need for a more nuanced understanding of interactions influencing genetic integrity in the context of occupational exposures.

4.4. Impact of length of working day

The analysis of DNA damage parameters (OTM, TL, and % Tail DNA) about the duration of the length of the working day provided valuable insights into the impact of varying work shifts on the genetic integrity of gas station attendants (Fig. 1).

Notably, statistically significant differences were observed in OTM and TL between work shifts of 12 and 24 hours compared to the unexposed group, while the 8-hour shift did not exhibit significant differences in these parameters. This suggests that the longer work shifts, specifically 12 and 24 hours, are associated with a heightened level of DNA damage, as evidenced by the increased OTM and TL, reinforcing the notion that extended exposure to gasoline components may contribute to genotoxic effects.

Moreover, the % Tail DNA, a critical indicator of genetic damage, demonstrated statistically significant differences across all three work shift durations (8, 12, and 24 hours) compared to the unexposed group. This increase in % Tail DNA indicates a cumulative effect of occupational exposure, regardless of work shift duration.

These findings are consistent with Xiong et al. (2016) who conducted a retrospective study evaluating the long-term effect of low concentrations of BTEXs on DNA fragmentation, which reduces the antioxidant ability and increases the risk of DNA damage in gasoline station attendants.

These findings align with the primary objective of our study, which aimed to evaluate the influence of different work shift durations on DNA damage in gas station attendants. The observed differences underscore the importance of considering the duration of work shifts as a crucial factor in assessing the genotoxic impact of occupational exposure to gasoline.

It is plausible that the longer duration of exposure during extended work shifts allows for a more sustained contact with VOCs present in gasoline, leading to a higher magnitude of DNA damage. This emphasizes the need for targeted interventions and occupational safety measures, particularly for individuals engaged in longer work shifts.

In conclusion, the significant differences observed in OTM, TL, and % Tail DNA across different work shift lengths highlight the intricate relationship between occupational exposure to gasoline components and genetic damage. These findings provide valuable information to understand how the length of the working day influences genotoxic damage, emphasizing the importance of personalized safety measures in the gas station work environment.

4.5. Correlation between DNA damage and biological age and job seniority

The correlation analysis between DNA damage and biological age did not reveal statistically significant differences. However, a statistically significant, albeit modest, correlation was observed between DNA damage and job seniority among gas station attendants (see Table 4). This novel finding establishes, for the first time, a connection between occupational longevity and DNA damage in this specific work environment.

Also suggests that other unmeasured factors, such as individual variation, genetics, or additional environmental influences, may contribute significantly to the observed DNA damage. It is crucial to interpret these results with caution and recognize the inherent limitations of our study. Despite the significant correlation observed, it may not fully reflect the complexity of the relationship between job seniority and DNA damage.

Variables not considered in our research could potentially influence the results. Additionally, the modest correlation underscores the multifactorial nature of DNA damage, highlighting the need for additional research to explore factors not addressed in this study. However, it is of paramount importance to consider job seniority as a determinant in the occupational health of gas station attendants. This discovery could have substantial implications for the implementation of occupational health measures and long-term prevention strategies. The aim is to safeguard the genetic integrity of workers throughout their years of service, emphasizing the need to consider occupational aging factors when designing specific interventions for this population.

Conflicts of interest

The authors declare that they have no conflicts of interest concerning this article.

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