

COMPARISON OF THE ANC OF BTEX EXPOSURE BETWEEN OUTDOOR AND INDOOR GASOLINE STATIONS, RAYONG PROVINCE

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Abstract

The purpose of this cross-sectional study is to compare cases of Absolute Neutrophil Counts (ANC) from organic solvent exposure among employees working indoors and outdoors in gasoline stations, Rayong province. A hundred employees working indoors and the other hundred operating outdoors gathered a total of 200 workers from eight gas stations. The study instruments were interviews and containers for urine and blood sample collection. The study results found employees worked for an average of 2.44 ± 4.06 years, 6.31 ± 0.47 days per week, and 9.05 ± 1.56 h. per day. ANC analysis in the laboratory revealed that the median was 4,484.00 cells/mm³ (interquartile range: IQR=2,128.00 cells/mm³). There was no difference in ANC exposure between workers who worked outdoors and those who worked indoors. When compared to other factors, gasoline station locations, personal safety behavior, and t, t-MA, and mHA exposure all had statistically significant effects on ANC exposure ($p < 0.05$). As a result, even if laborers do not work in the fuel dispenser areas, they should use personal protective equipment in stations to reduce benzene and ethylbenzene exposure, which helps avoid body cell inflammation and prevents serious health effects in the future.

Keywords: Organic solvents, Absolute neutrophil counts, Gasoline stations

Introduction

Various chemical substances, including hydrocarbons in both saturated and unsaturated forms, were discovered in gasoline. There are over 500 different ingredients, the majority of which are organic solvents such as alkanes, alkenes, and aromatics (Ahmadi *et al.*, 2019). Furthermore, BTEX (Benzene, Toluene, Ethylbenzene, and Xylene) was reported as the most toxic organic solvent to human health (Azari *et al.*, 2012). Especially Benzene was a crucial chemical hazard (Ekpenyong and Asuquo, 2017). Previous research

discovered that BTEX was contaminated in gas station environments (Xiong *et al.*, 2016; Chaiklieng *et al.*, 2019) and employee bodies (Tunsaringkarn *et al.*, 2013; Werder *et al.*, 2020). As a result, workers in gas stations, including filling attendants who worked both indoors and outdoors, were affected. Also, convenience store employees were exposed to toxic solvents, damaging their health (Geraldino *et al.*, 2020). Furthermore, a study on the difference in BTEX concentrations between outdoor and indoor in Tehran, Iran, was found to

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have no difference and BTEX could impact the exposed individual's health (Hadei *et al.*, 2018).

Gas station employees are exposed to BTEX vapours through inhalation at work (Geraldino *et al.*, 2020). A portion of BTEX is exhaled in its original form (Parent compound), while the majority of it is dispersed in the blood system and then metabolized by the liver to have water-soluble properties that are easier to excrete with urine. This toxicokinetic process, which involved reactive oxygen species, oxidative oxygen and oxidation, resulted in structural alteration of BTEX (Ekpenyong and Asuquo, 2017). However, the American Conference of Governmental Industrial Hygienists (ACGIH) organization suggested evaluating BTEX exposure in the form of trans, trans-muconic acid (t, t-MA), Toluene in urine (TU), Methylhippuric acid (mHA), and Mandelic acid (MA) (ACGIH, 2021), as well as in the metabolism process, which benzene causes inflammation of the body. A previous study discovered that BTEX, an inflammation indicator, and the benzene in the blood correlated with inflammatory substances such as Cytokeratin18, Interleukin 1beta, Interleukin-6, and 8, but toluene in the blood did not (Werder *et al.*, 2020).

The main effects of BTEX exposure were on the blood and nervous systems, but they were frequently found to be strongly correlated with long-term exposure at high concentrations (Hazrati *et al.*, 2016). In addition, the BTEX substances contained benzene, which causes leukemia (IARC, 2021), however, the studies of the effects of low BTEX concentrations on blood cells used various methods. Low levels of benzene, for example, were discovered not to impact the blood system or bone marrow function for blood production (Shimada *et al.*, 2012; Moro *et al.*, 2015). However, some results of studies were contradictory (Jabbar and Ali, 2020). It was unclear that blood cells should be used as a biomarker of effect, and blood cells that were likely to be a sensitive response to bodies, for instance, neutrophils and lymphocytes (Rothman *et al.*, 1996; Jabbar and Ali, 2020). When the body receives toxin exposure, inflammation occurs. Neutrophil increased and decreased in lymphocytes (Zahorec, 2001).

Absolute neutrophil counts (ANC) was the number of neutrophils in total white blood cells in which neutrophil type white blood cells were most sensitive to foreign substances entering the body. This is because ANC was the first response mechanism capable of phagocytes (Hughes *et al.*, 2002), high ANC levels in the body could indicate infections or toxins that cause inflammation. On the other hand, if found in small quantities, the body will easily be at risk of an

infection (Jabbar and Ali, 2020). In the past, ANC was used in studies as an indication of inflammation. For example, Watanabe *et al.* (2019) concluded that ANC was the best predictor of water duct cancer inflammation recurrent. The study of aerosol exposed workers in Norway found that neutrophil contents were related to the amounts of Interleukin-1beta (Fell *et al.*, 2010).

In addition to toxins or other foreign substances, internal factors such as age, body mass index, physical and mental illness (Gialluisi *et al.*, 2020), or personal health behavior factors such as drinking alcoholic beverages and smoking, among others, may cause body inflammation. Wannanethee *et al.* (2010) discovered that smoking and alcohol consumption had a stimulating impact on the liver enzyme function (Gamma-glutamyl transferase). Also being an inflammatory factor, these factors could contribute to the accumulation of ANC in the body.

The gas station workers were exposed to BTEX, which was toxic and could cause inflammation in the body. The ANC was a sensitive response biomarker. Based on previous literature reviews, the ANC study for carcinogenic inflammation studies BTEX exposure (Yoon *et al.*, 2012; Watanabe *et al.*, 2019) has shown no reports of BTEX exposure in humans. The ANC study in mice, conducted by Wang *et al.* (2012) found that different concentrations of benzene, toluene, and xylene caused different oxidative stress reactions in each group of mice, along with different inflammatory responses in white blood cell parameters. Human studies, on the other hand, required additional information to be clarified. There was insufficient academic evidence regarding the human body mechanisms for low concentrations of BTEX substance toxicity responses (Costa *et al.*, 2016). The objective of this study was to compare absolute neutrophil counts (ANC) from exposure to organic solvents during outdoor and indoor worker operations in gas stations in Rayong province to use as a sensitive response marker for inflammation and potentially for further screening of health effects among BTEX exposure in working groups as well.

Materials and Methods

Population and sample

According to a study conducted by Chaiklieng and Nantanuch (2015), the sample size was determined using a calculation formula for known populations. The average number of employees in each gas station was seven. From the statistics of the gas station in the Eastern Economic Corridor (EEC), 100 gasoline stations (Thai Department of

Energy Business, 2019), the variance was determined based on the dependent variable, the hematopoietic results of a study by Moro *et al.* (2015), 95% CI of 1.96, and an error of 0.05, when calculated. There was a sample of no less than 96.68 workers per group. There were two sample groups. The first group consisted of 100 outdoor filling attendants, and the second group consisted of 100 indoor staff, including those working in offices, convenience stores, coffee shops, and food vendors, for a total of 200 participants. The cluster sampling method was used in the samples of those who volunteered for the entire station because the operations in each station were similar. Inclusion criteria: Working in a gas station for at least three months, having no underlying blood diseases (such as leukemia, aplastic anemia, hemophilia, thalassemia and anemia etc), and willingness to participate voluntarily in research. Exclusion criteria: Work absence or medical conditions such as fever, trauma, and antibiotics were taken on the day of sample collection.

Research tools

The researchers divided study tools into three parts: interview-questionnaire, urine, and blood sampling device, details as follows. 1) Interview-questionnaire: the 14 questions covered three parts: 1.1) General information, including gender, age, weight, height, physical and mental illness during the past year, and h. of sleep per day. 1.2) Health behavior data consisted of smoking habits, alcohol consumption 1.3) Historical work data, consisting of years worked in gas stations, number of hours per day, frequency of days per week, personal protective equipment behaviors, and personal hygiene. Answers were optional and filling words. Interviews through a content validity assessment by 3 occupational medicine and occupational health and safety specialists, obtained the Index of item objective congruence (IOC) greater than 0.5 in all questions. The personal protective equipment behaviors and personal hygiene through a reliability test were 0.804 and 0.729, respectively. 2) Urine sample collection equipment: plastic glass, polyethylene urine collection container, volume 50 ml. 3) Blood sample collection equipment: tourniquet, dry cotton swabs, alcohol, needles, 3 ml. EDTA (Ethylenediaminetetraacetic acid) blood tubes, transpose, and infectious waste bags. Blood and urine samples were kept in refrigeration equipment, including foam cases and ice packs.

Collection and Interpretation of Results

1) Interview-questionnaire: The researchers and the assistant team collected data through interviews in which the research assistant has passed

the training to communicate the issues of the interview with the same understanding, on the day of the sampling, using the interview place around the service station, prepared by the station owner. Interviewing took approximately 10-15 minutes per worker. For the interpretation of personal protective equipment behaviors such as wearing masks, gloves, and long sleeves among others, each question got 1 point for practice and 0 for non-practice. The results were then interpreted in a clustered manner, that is, if 1 or more points were scored, they were considered in action, and 0 points were considered invalid for personal hygiene behaviors, questions such as eating in the work area, washing hands before eating, etc. were graded and interpreted as well.

2) Urine sample: the researcher gave a device for collecting samples to employees during work, clarified that urine samples were collected at the end of the shift, by mid-stream urine, placed in a plastic glass, then poured at least 50 ml. of polyethylene, immediately put in a cooler box, and then sent for laboratory analysis, whereby the laboratory reported the results of the analysis in the metabolized form of BTEX, which was quantitative data, and was conducted into two groups, using the median cut point, divided into two groups: Groups were less than or equal to the median and group were greater than the median.

3) Blood samples: using the blood collection site at the same appointment as the interview, blood draw by a medical technician or professional nurse in the amount of 3 ml, put in an EDTA tube, put in a cold storage box immediately, and sent for laboratory analysis. The interpretation was calculated using the formula for ANC from the formula $[(\% \text{ Neutrophils} + \% \text{ Band cells}) \times \% \text{ total white blood cells}] / 100$, the resulting units were cells/mm^3 (Brandt *et al.*, 1990).

Laboratory analysis

The urine and blood samples were delivered to the laboratory the same day, within 4 h. of sampling, in ice packs cooled foam boxes, maintained below 4°C. In urine samples, metabolize of the four BTEX substances were t, t-MA, TU, mHA, and MA. The quantity was analyzed by using column type C18, mobile phase, containing water/acetonitrile at a ratio of 50:50 by volume. (50:50v/v), flow rate of 1.0 mL/min at 37.0°C, sample solvent by methanol, and ultraviolet detection at 254 nm wavelength (PerkinElmer, 2013). Blood samples were analyzed for total white blood cells count, % neutrophils and % band cells, analyzed by automated techniques, flow cytometry, fluorescent stain flow cell separation method, using Sysmex XN 500 Japan instrument (Department of

Medical Science, 2017, Laboratory quality certified by the International Organization for Standardization (ISO) 15189:2012, No. 4247/63 and MOPH standard of the Ministry of Public Health, Thailand.

Human research ethics

The researchers requested permission from Burapha University to publicize the study and collect the research sample, and they clarified the research project with the individuals directly, and the respondents must only participate voluntarily. Burapha University approved this

research considering human research ethics under the certificate number 019/2563.

Data analysis

The data were analyzed by SPSS 21.0. The analytical statistics were divided into 2 parts: descriptive statistics and inferential statistics which were 1) Descriptive statistics: 1.1) calculated the number, percentage in qualitative variables such as gender, physical and mental illness, smoking cigarettes, the use of alcoholic beverages, and personal protective equipment behaviors. 1.2) calculated the mean and standard deviation in

Table 1. General information and work history among petrol attendant stations (n=200)

General information and work history	Outdoor (n=100)		Indoor (n=100)		Total (n=200)	
	n	%	n	%	n	%
Gender						
Male	44	44.0	19	19.0	63	31.5
Female	56	56.0	81	81.0	137	68.5
	$X^2 = 14.483, p\text{-value} < 0.001^*$					
Age (Years) (Mean±SD)	27.78±9.56		32.72±11.81		30.25±11.02	
	$t = -3.246, p\text{-value} < 0.001^*$					
Body mass index (Kg/m ²) (Mean±SD)	22.54±4.90		24.73±5.40		23.63±5.25	
	$t = 1.713, p\text{-value} = 0.088$					
Physical health in the past year						
Healthy	66	66.0	61	61.0	127	63.5
Illness	34	3.0	39	39.0	73	36.5
	$X^2 = 1.697, p\text{-value} = 0.428$					
Mental health in the past year						
Healthy	85	85.0	74	74.0	159	79.5
Illness	15	15.0	26	26.0	41	20.5
	$X^2 = 3.864, p\text{-value} = 0.145$					
Rest period (Hours) (Mean±SD)	7.50±1.41		7.48±1.33		7.49±1.38	
	$t = 0.070, p\text{-value} = 0.944$					
Smoking						
No	56	56.0	76	76.0	132	66.0
Yes	44	44.0	24	24.0	68	34.0
	$X^2 = 8.913, p\text{-value} = 0.003^*$					
Alcohol drinking						
No	51	51.0	58	58.0	109	54.5
Yes	49	49.0	42	42.0	91	45.5
	$X^2 = 0.988, p\text{-value} = 0.320$					
Years of working experience (Mean±SD)	1.61±2.84		3.81±5.26		2.44±4.06	
	$t = -2.927, p\text{-value} = 0.004^*$					
Working hours per day (Hours) (Mean±SD)	9.09±1.53		8.98±1.62		9.05±1.56	
	$t = 0.428, p\text{-value} = 0.673$					
Working days per week (Days) (Mean±SD)	6.32±0.48		6.30±0.46		6.31±0.47	
	$t = 0.253, p\text{-value} = 0.800$					

Table 1. General information and work history among petrol attendant stations (n=200) (Continue)

General information and work history	Outdoor (n=100)		Indoor (n=100)		Total (n=200)	
	n	%	n	%	n	%
Personal protective equipment behavior						
No	64	64.0	75	75.0	139	69.5
Yes	36	36.0	25	25.0	61	30.5
	$X^2 = 2.854, p\text{-value} = 0.091$					
Practice personal hygiene						
No	10	10.0	32	32.0	42	21.0
Yes	90	90.0	68	68.0	158	79.0
	$X^2 = 14.587, p\text{-value} < 0.001^*$					
Gas stations area						
Urban	62	62.0	75	75.0	137	68.5
Rural	38	38.0	25	25.0	63	31.5
	$X^2 = 3.337, p\text{-value} = 0.068$					

* $p < 0.05$

the quantitative variable in the case of the normal distribution when tested Kolmogorov-Smirnov tests such as age, body mass index, hours of restful sleep, and frequency of duration of work. 1.3) Calculated quartile, median, minimum, and maximum used in quantitative data in the case of abnormal distribution such as urinary BTEX and ANC exposure. And 2) Inferential statistics: 2.1) Chi-square test, comparing occupational groups with qualitative data, and 2.2) Independent sample t-test, comparing occupational groups with quantitative data, and hypothesis testing for general information, work history and BTEX exposure to ANC.

Results and Discussion

General information and work history

According to the general data, the study found 68.5% of workers in gas stations were female with a mean age of 30.25 ± 11.02 years and most of them were obese. The average body mass index was 23.63 ± 5.25 kilograms per square meter (Kg/m^2). Physical and mental health conditions were good, 63.5% and 79.5% respectively. Regarding health behavior, respectively, 66.0% and 54.5% of non-smoking and non-alcoholic workers had a mean rest period of 7.49 ± 1.38 h. per day. For the work history, it was found that workers had an average of 2.44 ± 4.06 years of working experience in gas stations which worked almost every day, an average of 6.31 ± 0.47 days per week, and in one working day for an average of 9.05 ± 1.56 h. per day. Most of the workers did not have personal protective equipment behaviors from gasoline (69.5%), but did practice personal hygiene care (79.0%), and workers at gas stations located in urban areas had only 68.5%.

The comparative test of general data and work history between the working groups in the outdoor area and the indoor group were found factors statistically significant ($p < 0.05$) such as gender, age, smoking, work experience, and personal hygiene behavior (Table 1).

Marker of ANC

According to the results of the ANC laboratory analysis, workers in both outdoor and indoor

settings had a median of 4,484.00 cells/ mm^3 , with the lowest and highest values ranging from 476.00 to 8,856.00 cells/ mm^3 . When comparing the ANC similarity between outdoor and indoor (Table 2), the ANC comparison with various factors, two workers had an ANC of less than 1,000 cells/ mm^3 .

Comparison between factors and ANC

The comparative test discovered several factors that resulted in statistically significant differences ($p < 0.05$) in four variables for the ANC, including gas station area, personal protective equipment behavior, the t,t-MA, and mHA levels (Table 3) which were urban gas stations, workers who did not wear personal protective equipment, and urinary exposure to t, t-MA, and mHA that exceeded median values. These are the potential drivers of an increase in ANC.

This study focused on BTEX exposure at low concentrations with ANC, which had previously reported the first sensitive biomarker response from body inflammation (Hughes *et al.*, 2002; Watanabe *et al.*, 2002). Researchers analyzed gas attendants in gasoline stations utilizing BTEX analysis with a high-performance analysis technique used in many research studies (HPLC) (Costa *et al.*, 2016; Chaiklieng *et al.*, 2019; Geraldino *et al.*, 2020), and blood analysis with flow cytometry technique (Moro *et al.*, 2015). The following is the study conclusion and discussion of the discovered factors.

General information; the majority of outdoor laborers were female (56.0% on average 27.78 ± 9.56 years old, had a higher body mass index (BMI) than the standard level, a healthy body, and a healthy mentality, which was similar to a previous study in the Bangkok area (Tunsaringkarn *et al.*, 2012). It was discovered that the petrol attendants had an average age of 28.1 years old, a body mass index of 23.0 kilograms per square meter and that filling workers in Thailand were the early-age working group, with fewer aged workers. It could be because of the nature of work during rush hour, when laborers have to move their bodies quickly at all times, mostly in a hot environment. As a result, older workers were unable to tolerate this type of work. In addition, the job description as petrol attendant did not require many qualifications for job

Table 2. Marker of ANC among petrol attendant stations (n=200)

ANC	Outdoor (n=100)	Indoor (n=100)	Total (n=200)
Mean	4,736.39	4,531.97	4,634.18
(SD)	(1,736.62)	(1,393.40)	(1,573.77)
Median	4,422.00	4,507.00	4,484.00
(Min-Max)	(476.00-5,960.25)	(2,132.00-8,568.00)	(476.00-8,856.00)
Quartile 1	3,553.25	3,269.25	3,487.50
Quartile 2	4,422.00	4,507.00	4,484.00
Quartile 3	5,960.25	5,356.75	5,615.50
Interquartile range : IQR	2,407.00	2,087.50	2,128.00

t= 0.918, p-value = 0.360

Table 3. Comparison between several factors and ANC (n=200)

Factors	Absolute neutrophil count (Cells/mm ³)		t	p-value
	mean	SD		
Gender				
Male	4,782.55	1,757.50	0.904	0.367
Female	4,565.94	1,483.59		
Age (Years)				
< 30	4,412.05	1,406.30	-1.742	0.083
≥ 30	4,801.74	1,675.76		
Body mass index				
Normal	4,717.59	1,697.83	0.663	0.508
Overweight/Obesity	4,568.64	1,473.46		
Physical health in the past year				
Healthy	4,577.67	1,605.69	-0.733	0.465
Illness	4,748.20	1,527.23		
Mental health in the past year				
Healthy	4,558.83	1,532.83	-1.287	0.200
Illness	4,920.28	1,724.66		
Rest period (Hours per day)				
< 8	4,562.47	1,557.53	-0.697	0.486
≥ 8	4,718.35	1,597.03		
Smoking				
No	4,559.66	1,540.54	-0.933	0.352
Yes	4,778.82	1,638.24		
Alcohol drinking				
No	4,485.29	1,405.65	-1.440	0.152
Yes	4,812.51	1,745.37		
Gas stations area				
Urban	4,854.45	1,682.71	2.976	0.003*
Rural	4,155.15	1,182.41		
Operation area				
Outdoor	4,736.36	1,736.62	0.918	0.360
Indoor	4,531.97	1,393.39		
Years of working experience (Years)				
< 5	4,550.18	1,553.68	-1.259	0.210
≥ 5	4,867.15	1,620.34		
Working hours per day (Hours)				
≤ 8	4,494.08	1,539.38	-1.424	0.156
> 8	4,812.47	1,607.60		
Working days per week (Days)				
≤ 6	4,592.73	1,509.27	-0.333	0.740
Every day	4,667.41	1,629.67		
Personal protective equipment behavioral				
No	4,787.87	1,629.08	2.103	0.037*
Yes	4,283.96	1,389.67		
Practice personal hygiene				
No	4,886.19	1,508.12	1.169	0.244
Yes	4,567.18	1,588.66		

Table 3. Comparison between several factors and ANC (n=200) (Continue)

Factors	Absolute neutrophil count (Cells/mm ³)		t	p-value
	mean	SD		
t,t-MA (μg/g Cr)				
≤ 393.40	4,324.70	1,429.81	-2.830	0.005*
> 393.40	4,943.66	1,655.49		
mHA (g/g Cr)				
≤ 0.40	4,359.98	1,522.61	-2.548	0.012*
> 0.40	4,919.57	1,582.99		
MA (g/g Cr)				
≤ 0.06	4,638.46	1,616.33	0.039	0.969
> 0.06	4,629.72	1,536.51		

application, and there was no need for specialized skills and experience, which made it easy to apply for the job even if you were an early working-age person. Health behavior found workers had smoking habits and alcohol consumption of 34.0% and 45.5%, respectively. All of these actions increase the probability of chemical exposure in the body. The prior study of gasoline station workers in

Thailand found more of t, t-MA average in the smoking and nonsmoking groups of 2.19±2.32 and 0.24±0.33 mg/g Cr (Wiwanitkit *et al.*, 2005). Cigarettes were also a key source of benzene exposure. As a result, this was another reason to promote employee health behavior (Jalai *et al.*, 2017) to help reduce the risk behaviors of smoking and alcohol consumption as controllable factors.

Petrol attendants' work experiences, the filling attendants working outdoors had an average age of 1.61 ± 2.84 years old, a working day of 6.32 ± 0.48 days per week, and a workday of 9.09 ± 1.53 h. In some respects, this is consistent with previous research by Tunsaringkarn *et al.* (2012) had shown that fuel service workers had 2.9 ± 2.5 years of work experience, a working day of 6.4 ± 0.6 days per week, and a workday of 9.9 ± 1.8 h. Furthermore, because the research area was Thailand's Eastern Economic Corridor (EEC), the researchers may discover that the laborers were an inexperienced workforce. In the EEC region, the government has supported road and street construction, particularly for industry and tourism. As a result of this, the gas station business expanded. The data was collected at gasoline stations that had only been open for about a year. In addition, some stations have expanded their operations and hired more staff. As a result, petrol attendants may have less work experience than those in other cities.

The study discovered the median ANC indication was 4,484.00 cells/mm³, which was considered normal when compared to standard criteria (Brandt, 1990). However, the study discovered that two workers with ANC levels less than 1,000 cells/mm³ were at high risk of infection. The previous study, which only studied animals, never used the ANC indication as a parameter for BTEX exposure on the human body (Wang *et al.*, 2012), or some studies used the neutrophil parameter (Moro *et al.*, 2015; Jabbar and Ali, 2020). For example, AM Moro *et al.* (2015) studied blood cell types and the body's immune system and were able to detect changes early. The findings of the study on different types of white blood cells revealed that the difference in neutrophil percentage between the filling attendants and the control group was statistically significant ($p < 0.05$). However, the percentage of neutrophils is compared to the total white blood cell count with a baseline value of 100. However, the total number of white blood cells that differs, results in a varying number of ANC, even though the same amount of % neutrophil is included in the ANC combination with % Band cells. Therefore, the ANC indication was visible as a more sensitive response than the percentage of neutrophils. ANC has previously been applied in studies of cancer inflammation, infection, and chemotherapy (Watanabe *et al.*, 2019).

They discovered that BTEX and ANC exposure in the urine of gasoline workers was statistically significantly higher than the median of t, t-MA, and mHA ($p < 0.05$). Previous research in this field has been performed, but with an absolute lymphocyte count (ALC) marker, by researcher Rothman *et al.* (1996). The effects of benzene

exposure were examined using hydroquinone and an ALC marker, and the results were found to be highly correlated. As an outcome, Mistry *et al.* (2016) investigated gasoline attendants and discovered that they had statistically significant differences in ALC from the control group ($p < 0.05$). Only the study of percent neutrophil was an essential marker for neutrophil-type white blood cells (Koh *et al.*, 2015; Jabbar and Ali, 2020). The results of this study revealed that the median ANC amounts were higher in the t, t-MA, and mHA exposure groups than in the other groups. Moro *et al.* (2015) also discovered a rapidly increasing percentage of neutrophils in the petrol attendants' group. It has been reported that in the early stages of exposure to petroleum products, phagocytic cells in neutrophils respond to foreign matters (Hughes *et al.*, 2002; Okoro *et al.*, 2006). Long-term exposure, on the other hand, will result in a decrease in the number of neutrophils. This behavior may have an impact on workers' immune systems, making them more susceptible to infection in the future (Okoro *et al.*, 2006). According to this study, outdoor workers had shorter work durations than those in other studies (Average work durations of 1.61 ± 2.84 years). As a result, ANC changes resulted in an upward trend. Lower ANC may result from continuous exposure.

In addition to the study results based on the main hypothesis stated above, additional comparisons were made between the factors and the ANC. It was discovered that employees working in urban gas stations had higher ANC than employees working in rural stations and was statistically significant ($p < 0.05$). It could be stated that this factor increased the risk of BTEX exposure, resulting in increased ANC secretion. This is because there were more cars in the city and more traffic congestion than outside the city, and also high-rise buildings obscuring the view (Fandi *et al.*, 2020). Geraldino *et al.* (2020) discovered that petrol attendants working in urban stations had higher t, t-MA exposure than workers working in suburban stations in a similar study. The medians were 0.15 mg/g Cr and 0.07 mg/g Cr, respectively.

Furthermore, workers who did not wear personal protective equipment behavior had higher ANC than workers who did, which was statistically significant ($p < 0.05$). The study results found, only about one-third (36.0 percent) of workers wear personal protective equipment, which includes carbon masks, which the workers wore regularly and were effective at protecting them from chemical vapors. (a daily wearing frequency of more than 6 h.) 29.0 percent and 24.0 percent wear long sleeves to protect their skin from vapors or oil splashes. As a result, wearing personal protective equipment reduces ANC. Therefore, health officials

should increase their efforts to encourage workers to wear personal protective equipment. Wearing respiratory and skin protection, for example, has been shown to reduce benzene absorption into the bloodstream (Cezar-vaz *et al.*, 2012). These preventive measures were effective in lowering exposure. If precautions were taken, the exposure would be reduced, as would the body's inflammatory response. For research limitations, this study collected samples during the COVID-19 outbreak, which may have resulted in fewer cars on the streets than would be expected in a normal situation. The results may be less than the actual BTEX exposure. Furthermore, the BTEX exposure between outdoor workers and the indoor group was statistically insignificant, which did not go as predicted. In the comparison, there were no obvious differences in both outdoor and indoor work operations. The researchers chose a group of indoor workers who work at gas stations and a group of outdoor laborers who work in closed buildings near the indoor working group for this study. Therefore, to confirm the results more clearly. In the future, control group selection should be workers who do not work in the gasoline stations and are located in outer cities. In addition, the examiners should compare the similarities between the workforce that operates indoors and outdoors for further information. For the strength of this research, the quality of the field sampling was strictly controlled. The research assistant team at each station collected urine and blood samples and immediately kept them at a cool temperature before delivering them to the laboratory, where they met international standards. As a result, the results obtained a reliable analysis.

Conclusions

There was no difference in BTEX exposure between outdoor and indoor workers. The BTEX exposure and the ANC indicator were investigated in this study. Surprisingly, exposure to t, t-MA, and MHA was higher than the median, resulting in higher ANC than the less exposed group. In addition, there are other risk factors for ANC increase, including working in urban stations and not participating in personal protection. The findings of this study suggest that ANC can be used as an early marker in the detection of monitoring health. Furthermore, personal protective equipment is required to protect the individual's health.

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Conflict of Interest

No conflict of interest has been declared by the authors.

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