

## ARTICLE

# MEASURING THE AMOUNT OF TRANS TRANS-MUCONIC ACID (TTMA) IN CORRELATION WITH THE CD4+ AND CD8+ LYMPHOCYTES AND THEIR RATIOS CAUSED BY BENZENE EXPOSURE, AND ASSOCIATED OCCUPATIONAL HEALTH HAZARDS

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## ABSTRACT

Workers working in a benzene filling station face a risk of benzene exposure. Benzene is a chemical which its exposure has been proven to have adverse effects to the bone marrow which is an organ responsible for the production of blood components. Testing for the exposure workers in this case study was done through the measurement of the amount of trans-trans-muconic acid in correlation with the CD4+ and the CD8+ lymphocyte cells in the body. Six stations around the Medan City were chosen for analysis. Urine samples were collected for testing after the work shifts of the workers and analyzed at the Medan Sumatra Hospital. The collected data was analyzed using SPSS. A total of 43 participants were included in the study, they constituted both males and females of whom 79% were below the age of 20 and 30. The level of ttMA, lymphocytes, CD4+ cells and CD8+ cells were 21,37,2 and 23 respectively. The correlation of the ttMA and the lymphocytes, CD4+ cells and the CD8+ cells showed that there was a high probability that Benzene was the major cause of all the irregularities of the ratios of these components of blood. Therefore, the study concludes that the exposure of workers at the benzene filling stations to benzene may be the leading cause of the health risks hazards that they may face during the course of duty.

## INTRODUCTION

Working in Benzene filling station comes with a fair share of its health risks. Benzene has been long recognized a carcinogen and its exposure to individuals even at low concentrations may lead to leukemia (Duarte-Davidson *et al* 2001). The WHO and the ILO are working in collaboration to identify and eliminate the workplace hazards that may affect the health of the workers which includes benzene ("WHO | Occupational health", 2017;"International Labour Standards on Occupational Safety and Health", 2017). Exposure of workers to Benzene may lead to adverse long term health effects especially on the blood and organs that manufacture blood. When these organs are affected, there may be a decrease in the formation of red blood cells that may lead to anemia or even cause leukemia. However, it is important to note that, these health effects greatly depend on the amount and period of exposure to Benzene ("CDC | Facts About Benzene", 2017) .There are three small cohort studies that give a close relationship between benzene and Leukemia and the dose-response relationship (Wong, 1987; Bond, 1986; Rinsky, 1987). These studies have shown that workers that are exposed to high levels of benzene have an equally high chance of getting leukemia. This gives proof of a relationship between benzene exposure and leukemia. In the body, benzene undergoes a chain of complex biotransformation that lead to the formation of toxic and carcinogenic end products(Snyder & Hedli ,1996). The common biomarker that is normally used for ascertaining the presence of benzene in the body is the trans, trans-muconic acid (ttMA). The analysis of ttMA is done using urine samples from individuals (Zhang *et al*, 2011; Wiwanikit *et al* 2001). Testing of lymphocytes for the presence of Benzene is carried out through the examination of the CD4+ and the CD8+ T cells. Normally, after an exposure of an individual to high levels of Benzene, a decrease in count of the CD4+ and the CD8+ T cells is expected since Benzene affect the production of blood components(Lan *et al*, 2004; Li & Yin, 2006).

This research paper seeks to find the occupational hazards that the workers in a Benzene filling station by measuring the amount of ttMA in their urine as well as the amount of the CD4+ and the CD8+ T cells in the blood and find their ratios.

## MATERIALS AND METHODS

Urine samples of the employees were taken at the end of shift and taken to the laboratory for analysis of the benzene metabolite; ttMA. Inspection of lymphocytes on the other hand, involved taking of the blood samples of the participants and examination of them at the Medan Sumatra University Hospital. The examination of CD4 + and CD8 + T cells was done on the blood samples obtained at the Prodia Jakarta laboratory.

### KEY WORDS

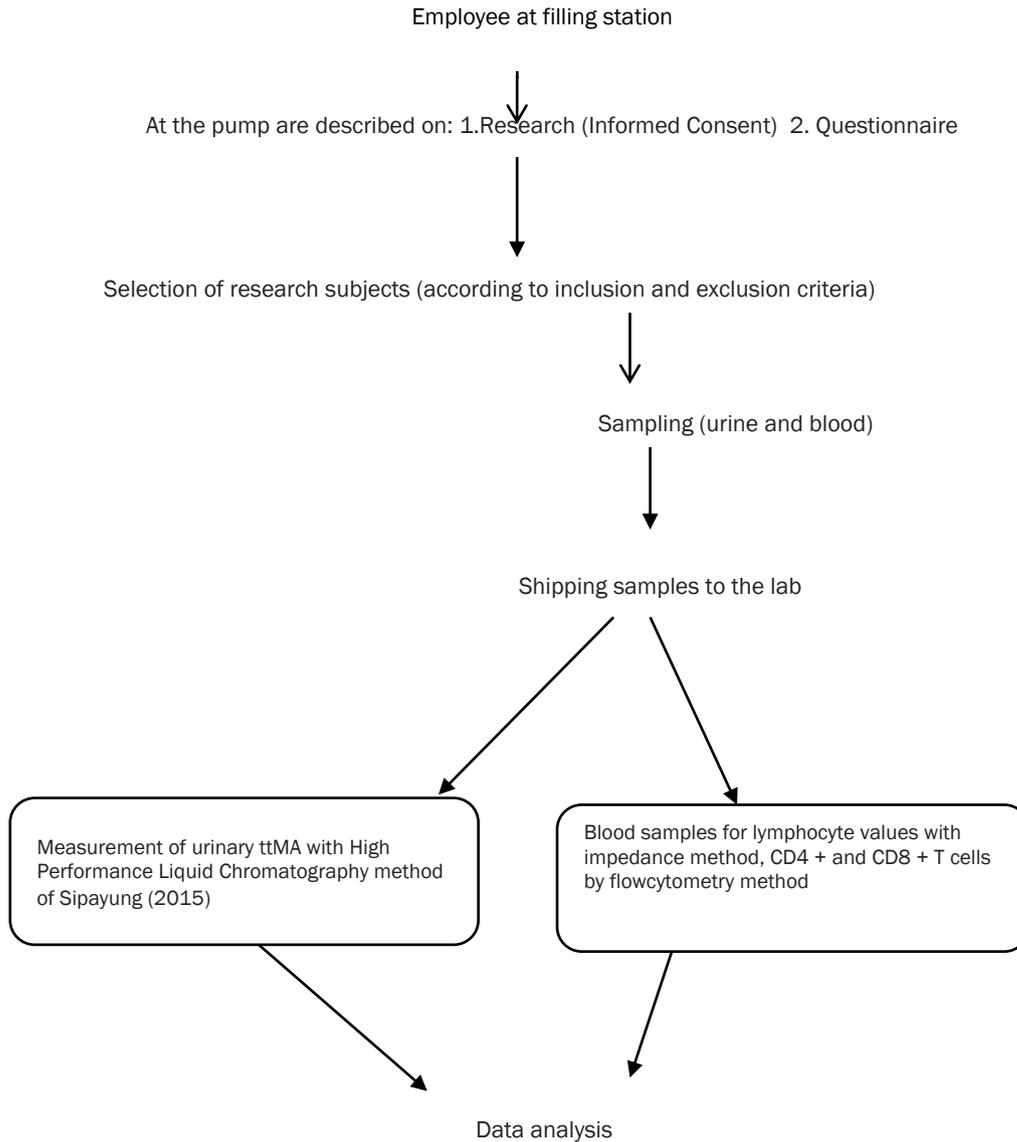
Benzene, occupational hazards, exposure, health risk

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**Research Schematic framework of the implementation of the research**



**RESULTS**

Six stations in Medan city were selected for the study. Questionnaires were given to those who met the inclusion criteria and their results obtained.

**Data research**

The data analysis of the study included both univariate and bivariate analysis.

**Univariate analysis**

Univariate analysis involved giving an overview of the character of the participants, value ttMA, lymphocytes, CD4 + T cells, CD8 + T cells, the ratio of CD4 / CD8 T cells obtained.

**Character Research Subjects**

Characters include the age, sex, duration of work, smoking status, and job roles. Data that was obtained is shown below [Table 1];

**Table 1:** Characteristics of subjects

Attribute	CHARACTER	RESULT	
		N	%
Age	< 20 YEAR	5	11,6
	20 – 30 YEAR	34	79
	≥ 30 YEAR	4	9,4
Gender	Man	25	58,2
	Women	18	41,8
Employment duration	≤ 1 year	13	30
	> 1 year	30	70
Smoking status	Smoker	11	25,5
	Non smoker	32	74,5
Job role	Administration	8	18,6
	Petrol filling operator	35	81,4

**Table 2:** statistical picture laboratory measurements, which includes the value ttMA,

Measurement	Mean	Median	SD	Lowest average	Highest average
ttMA (µg/gCr)	587	75	1326,5	32	6825
Lymphocyte (10 <sup>3</sup> /mm <sup>3</sup> )	3,1	3,1	0,6	2,1	4,4
CD4 <sup>+</sup> (cell/uL)	899	894	245	454	1654
CD8 <sup>+</sup> (cell/uL)	917	838	309	401	1748
CD4/CD8 Ratio	1,1	0,93	0,4	0,57	1,94

lymphocytes, CD4 + T cells, CD8 + T cells, CD4 / CD8 ratio of T cells at the gas station employee. Source: ttMA reference value of Sipayung (2016); Lymphocytes, CD4 +, CD8 +, and the ratio of CD4 / CD8 of laboratory measurements in 2015, n = 43 subjects.

**Table 3:** Picture of normality laboratory measurements on the employees of gas stations in medan city

MEASUREMENT	Normal Value		Above normal		Below normal	
	n	%	N	%	n	%
ttMA (µg/gCr)	34	79	9	21	-	-
Lymphocyte (10 <sup>3</sup> /mm <sup>3</sup> )	27	63	16	37	-	-
CD4 <sup>+</sup> (cell/uL)	42	98	1	2	-	-
CD8 <sup>+</sup> (cell/uL)	33	77	10	23	-	-
Ratio CD4/CD8	40	93	-	-	3	7

Source: Value ttMA referral of Sipayung (2016)

**Bivariate analysis**

This type of analysis is used to show the correlation between different sets of data. The Table-4 shows the correlation of benzene exposure through the measurement of the value ttMA lymphocytes, CD4 + and CD8 + T cells, and the ratio of CD4 / CD8 T cells at the gas station.

Table 4: correlation of benzene exposure

Correlation with ttMA	N	R	p-value
Lymphocytes	43	0,133	0,219
CD4 <sup>+</sup>	43	0,183	0,086
CD8 <sup>+</sup>	43	0,223	0,036
Ratio CD4/CD8	43	-0,138	0,198

## CONCLUSION

Based on the above findings, it can be concluded that exposure to benzene is associated with varying levels of lymphocytes, CD4 and CD8 cells together with alteration in their ratio. Most of these parameters are above normal in the blood samples obtained from the participants and thus points towards the deregulation in the hematopoietic system and thus we can make a conclusion that indeed high levels of benzene in the body are associated with harmful health risk such as lymphocytic leukemia, myeloid leukemia among others.

## RECOMMENDATIONS

Employees working in benzene producing industries or gas filling stations should be routinely rotated to different department and regularly monitored to prevent the buildup of benzene to more harmful levels. Companies also ought to put in measure that will prevent emission of benzene into the environment. Governments have a role to play by coming up with legislations that will curb these emissions.

### CONFLICT OF INTEREST

All authors declare no conflict of interest in the current research.

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## REFERENCES

- [1] Adgate JL., Goldstein BD, McKenzie L M. [2014] Potential public health hazards, exposures and health effects from unconventional natural gas development. *Environmental science & technology*, 48(15), 8307-8320.
- [2] Bond GG, McLaren EA, Baldwin C L., Cook R R. [1986] An update of mortality among chemical workers exposed to benzene. *British journal of industrial medicine*, 43(10):685-691.
- [3] CDC | Facts About Benzene. [2017] [emergency.cdc.gov](https://emergency.cdc.gov/agent/benzene/basics/facts.asp). Retrieved 16 January 2017, from <https://emergency.cdc.gov/agent/benzene/basics/facts.asp>
- [4] D'Andrea M A, Reddy GK. [2015] Adverse health effects of benzene exposure among children following a flaring incident at the British Petroleum Refinery in Texas City. *Clinical pediatrics*, 0009922815594358.
- [5] Duarte-Davidson R, Courage C, Rushton L., Levy L. [2001] Benzene in the environment: an assessment of the potential risks to the health of the population. *Occupational and environmental medicine*, 58(1): 2-13.
- [6] *Exposure to Benzene: A Major Public Health Concern*. [2010] (1st ed.). Geneva, Switzerland. Retrieved from <http://www.who.int/ipcs/features/benzene.pdf>
- [7] *International Labour Standards on Occupational Safety and Health*. [2017] [ilo.org](http://ilo.org). Retrieved 16 January 2017, from <http://ilo.org/global/standards/subjects-covered-by-international-labour-standards/occupational-safety-and-health/lang-en/index.htm>
- [8] Lan Q, Zhang L., Li G, Vermeulen R, Weinberg RS, Dosemeci M, Kopp W. [2004] *Hematotoxicity in workers exposed to low levels of benzene*. *Science*, 306(5702): 1774-1776.
- [9] Li G, Yin S. [2006] *Progress of epidemiological and molecular epidemiological studies on benzene in China*. *Annals of the New York Academy of Sciences*, 1076(1):800-809.
- [10] Rinsky RA, Smith AB, Hornung R, Filloon TG, Young R. J, Okun, AH, Landrigan PJ. [1987] Benzene and leukemia. *New England journal of medicine*, 316(17): 1044-1050.
- [11] Sastroasmoro S Ismael, [2011] *Dasar-dasar Metodologi Penelitian Klinis*. Jakarta: Sagung Seto.
- [12] Sipayung LP. [2016] Korelasi Paparan Benzene Melalui Pemeriksaan Kadar trans, trans-Muconic Acid (ttMA) Dalam Urin Dengan Gambaran Complete Blood Count (CBC) pada Karyawan di Stasiun Pengisian bahan Bakar Umum (SPBU) PT. Pertamina Medan.
- [13] Snyder R, Hedli CC. [December 01, 1996] *An overview of benzene metabolism*. *Environmental Health Perspectives*, 104:1165-1171.
- [14] WHO | Occupational health. (2017). *Who.int*. Retrieved 16 January 2017, from [http://www.who.int/topics/occupational\\_health/en/](http://www.who.int/topics/occupational_health/en/)

- [15] Wiwanitkit V, Suwansaksri J, Nasuan P. [2001]*Urine trans, trans-muconic acid as a biomarker for benzene exposure in gas station attendants in Bangkok, Thailand*. Annals of Clinical & Laboratory Science, 31(4):399-401.
- [16] Wong O. [1987] An industry wide mortality study of chemical workers occupationally exposed to benzene. II. Dose response analyses. British journal of industrial medicine, 44(6): 382-395.
- [17] Zhang L., Ye FL., Chen T, Mei Y, Song SZ. [2011]*Trans, Trans-Muconic Acid as a Biomarker of Occupational Exposure to High-Level Benzene in China*. Journal of Occupational and Environmental Medicine, 53(10):1194-1198.