

### Evaluation of the Relationship between Pro-inflammatory Cytokines and Health Hazards in Workers Involved in Hazardous Waste Sites at Karnataka, India

Ravibabu Kalahasthi<sup>\*a</sup>, Adithya Pradyonna<sup>a</sup>, Pavithra Narendran<sup>a</sup>, Rajmohan Hirehal Raghavendra Rao<sup>a</sup>

<sup>a</sup> Regional Occupational Health Centre (Southern), Nirmal Bhavan Complex, Indian Council of Medical Research, Poojanhalli, Kannamangala (Post), Bangalore-562110, India.

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##### \*Corresponding author:

Ravi Babu K. (PhD)

Tel: +91 080 28467904

Fax: +91 080 28477102

E-mail addresses:

kalahasthi20012002@yahoo.co.in

#### ABSTRACT

**Background:** Most of the studies have reported higher occupational health hazards on hazardous waste workers (HWW). However reports regarding the association between health hazards and pro-inflammatory cytokines in HWW are scanty. The present study was undertaken to evaluate the relationship between occupational health hazards and serum pro-inflammatory cytokines in HWW.

**Methods:** One hundred two subjects working in hazardous waste areas in different parts of Bangalore at Karnataka, India were enrolled into the study in 2009. Using questionnaire the respiratory morbidity and other work related problems in HWW was carried. The levels of pro-inflammatory cytokines in study subjects were determined by using Enzyme-linked-immunosorbent assay kits (Pierce Biotechnology, USA). The differences of serum pro-inflammatory cytokines levels between the groups were computed by using non-parametric Mann-Whitney U test. SPSS 10.0 for windows version of statistical software was used in the analysis.

**Results:** The morbidity conditions such as respiratory (34%), musculo-skeletal (24.5%), past illnesses (12.7%), dermatological (11.8%), gastrointestinal (9.8%), injuries due to contact with sharp objects & heavy equipments (7.8%) and nose and eye (4.9%) problems were observed in HWW. The levels of serum pro-inflammatory cytokines such as IL-8 ( $P=0.041$ ) & TNF- $\alpha$  ( $P=0.040$ ) significantly increased in landfill area workers and subjects having respiratory symptoms.

**Conclusion:** The levels of serum pro-inflammatory cytokines IL-8 & TNF- $\alpha$  were significantly associated with subjects having respiratory symptoms & further supporting that they are inflammatory markers in respiratory symptoms.

#### Introduction

Hazardous Waste (HW) is generated from main sources viz. industrial and household. This waste is disposed by different methods i.e. recycling and landfill (waste disposal on land). The job process of waste disposal areas involves i.e. waste collectors, waste sorters and workers at landfill [1]. In India the amount of HW generated is of the order of 7.243 million tons per annum (TPA) and out of this 1.4 million tons are recycled, 0.4 million tons are incinerated and the rest is disposed by landfill. There are 454 waste generating units in

Bangalore at Karnataka, India which generated 1.023 million tons per annum and out of the 0.47 million tons are recycled, 0.33 million tons incinerated and the rest is disposed by landfill. The processes of waste disposal areas are covered under section of 6, 8 & 25 of environment (protection) act of hazardous waste management 1989. According to a report published during 2000 by the Ministry of Environment and Forests, Govt. of India, much of the HW disposal is done by landfill [2].

The composition of waste stream at Columbia landfill in Missouri have reported 41% paper, 21% organic, 16% plastic, 6% metal, 3% glass and 13% other waste<sup>[3]</sup>. The handling and disposal of waste cause environmental pollution, which create breeding grounds for pathogenic organisms and spreading of infectious diseases<sup>[4]</sup>. Occupational health hazards associated with waste disposal area are infections to the skin, blood, eyes and intestines<sup>[5]</sup>. The workers involved in waste disposal areas have reported higher level of health complaints such as respiratory, dermatological and musculo-skeletal effects<sup>[6-12]</sup>. Other health hazards reported as increased chromosomal aberrations<sup>[13]</sup> and occurrence of stillbirths and congenital malformations in general population residing near the landfill<sup>[14]</sup>. The waste disposal areas (collection and disposal) have reported higher levels of respirable dust, microbial component like endotoxins, (1→3)-β-D-glucan, viable fungi and its spores and fungal extra-cellular polysaccharides<sup>[15-19]</sup>. A significant association was noticed between upper airways inflammation with nasal lavage concentration of interleukin-8 and microbial components of endotoxin & (1→3)-β-D-glucan in waste disposal areas<sup>[20-22]</sup>. The cause of inflammation to respiratory system was related to higher levels of neutrophil counts and production of pro-inflammatory cytokines<sup>[23]</sup>. The level of pro-inflammatory cytokines in nasal lavage is an indicator to upper airways inflammation but not on lower airways inflammation<sup>[24]</sup>. The levels of serum pro-inflammatory cytokines showed more detectability compared with the levels in nasal lavage and induced sputum of workers exposed to molds<sup>[25]</sup>. Most of the occupational exposure studies have reported higher levels of serum pro-inflammatory cytokines in firefighters exposed to wood smoke<sup>[26]</sup>, polypropylene flock exposure<sup>[27]</sup>, asbestos<sup>[28,29]</sup>, dust from tunnel construction<sup>[30]</sup>, sawmill<sup>[31]</sup>, flax<sup>[32]</sup>, cotton<sup>[33]</sup>, grain<sup>[34]</sup>, house painters<sup>[35]</sup> & coke-oven workers<sup>[36]</sup>.

No reports are available in the literature regarding the association between occupational health hazards and serum levels of pro-inflammatory cytokines among workers involved in waste disposal area. The pro-inflammatory cytokines i.e., Interleukin-1 (IL-1) is a family of biological active mononuclear cell-derived pro-

teins, which are involved in inflammatory and immune responses<sup>[37]</sup>. Interleukin-6 (IL-6) is a multifunctional cytokine, which regulates immune response by synthesizing acute phase reaction proteins<sup>[38]</sup>. Interleukin-8 (IL-8) is a neutrophil-activating peptide-1, which stimulates the ability of neutrophils and T-lymphocytes to invade injured or inflamed tissue<sup>[39]</sup>. The exogenous concentration of endotoxin, IL-1 & TNF-α induce the secretion of IL-8<sup>[40]</sup>.

The present study was undertaken to evaluate the relationship between occupational health hazards and serum pro-inflammatory cytokines among workers involved in hazardous waste disposal area.

## Materials and Methods

The sample size was estimated based on the target populations, which were 138 people. A confidence level of 95% and a confidence interval of 5% were assumed. The Worst-case percentage of 50% was considered. The sample frame was calculated as 102 adults. The survey was performed in study subjects working in hazardous waste areas of Bangalore at Karnataka, India during the year 2009. These subjects were categorized into three groups. The first group consisted of 36 subjects occupied in landfill disposal area, the second group consisted of 26 subjects engaged in compost process and the third group consisted of 40 office workers with no exposure of biological hazards from waste disposal area as control group. Ethical committee of center approved the study. Informed consent was obtained from each of study participants.

### Medical examination

The study subjects were interviewed with the American Thoracic Society (ATS) standard questionnaire. The other work related symptoms were collected through a questionnaire developed by Ray et al<sup>[7]</sup>.

### Blood Collection

Five ml of whole blood was collected from each subject in test tubes and centrifuged at 3000 rpm for 10 min at 4° C and serum was used for the determination of pro-inflammatory cytokines.

### Pro-inflammatory cytokine

The concentrations of interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8) & tumor

necrosis factor  $\alpha$  (TNF- $\alpha$ ) were determined in the serum samples of workers involved in hazardous waste management area with enzyme-linked immunosorbent assay (ELISA). The absorbance of standards and samples were measured by using Thermo Scientific Multiskan EX-reader (USA) at 450 nm. Taking the mean of the duplicate readings and subtracting the absorbance of the average zero standard measured the absorbance of each standards and samples. Plot the standard curve with standard concentration on the X-axis and absorbance on the Y-axis. The concentration of each sample was calculated by using standard curve. The levels of serum pro-inflammatory cytokines were expressed as Pg/ml.

**Statistical Analysis:** The levels of serum pro-inflammatory cytokines were expressed as geometric mean (GM). The levels of serum pro-inflammatory cytokines were found in highly skewed, a logarithmic transformation (Log10) was performed to obtain a normal distribution. The differences of serum pro-inflammatory cytokines between groups were computed by using non-parametric Mann Whitney-U test.  $P < 0.05$  was considered as significant. SPSS 10.0 for windows version of statistical software was used in the analysis.

## Results

The demographic details of mean age, experience, body mass index (BMI) and frequency distribution of life style confounding factors (food habits, smoking, alcohol consumption and chewing of tobacco products) of study subjects is shown in Table 1. The morbidity responses of solid waste workers occupied in landfill area, compost and administrative sections were reported. The morbidity condition identified were respiratory problems (34%), followed by musculo-skeletal problems (24.5%), past illnesses (12.7%), dermatological (11.8%), gastrointestinal (9.8%), injuries due to contact of sharp objects and heavy equipments (7.8%) and nose and eye (4.9%). Among respiratory problems breathlessness (14.7%) was found to be higher followed by cough (11.8%), phlegm (11.8%) and wheezing (11.8%), (Table 2).

The geometric mean levels of serum pro-inflammatory cytokines in subjects according to

job category are presented in Fig-1. The levels of serum interleukin -8 (IL-8) ( $P = 0.041$ ) and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) ( $P = 0.040$ ) increased significantly in landfill workers as compared to administrative section of workers. The compost plant workers showed no significant increases as compared to administrative workers.

The levels of serum pro-inflammatory cytokines in subjects according to morbidly conditions in hazardous waste management area are reported in Table 3. The levels of serum interleukin -8 (IL-8) and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) were significantly associated with workers who had respiratory complaints ( $P < 0.05$ ). The levels of serum pro-inflammatory cytokines according to lifestyle confounding factors indicated that smoking history was associated with increased levels of serum IL-6 & IL-8, chewing of tobacco products and alcohol consumption was also significantly associated with serum IL-8 ( $P < 0.05$ ). The other lifestyle confounding factors such as BMI & consumption of coffee/tea did not show any significant association with serum pro-inflammatory cytokines levels (Table 4).

**Table 1:** Demographic Detail of Waste disposal area workers

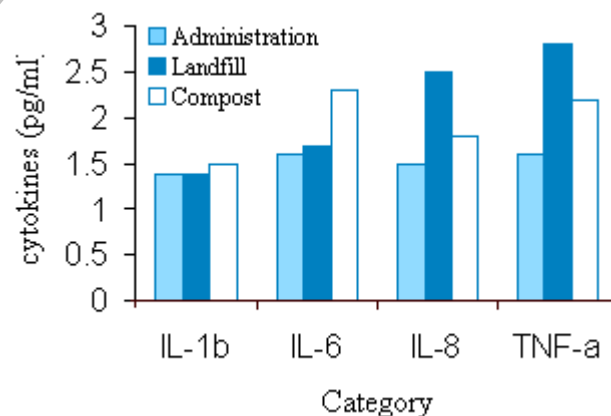
Variables	Subjects (n=102) N (%)
Age (yr)	29.1 $\pm$ 9.0 <sup>a</sup>
Experience (yr)	5.7 $\pm$ 3.5
Body mass index (kg/m <sup>2</sup> )	21.8 $\pm$ 3.3
Consumption of food	
Vegetarian	8 <sup>b</sup> (7.8)
Non- Vegetarian	94 (92.2)
Consumption of coffee/tea	
Tea	51 (50.0)
Coffee	6 (5.9)
Both	28 (27.5)
None	17 (16.6)
Smoking History	
Smokers	36 (35.3)
Non- smokers	66 (64.7)
Alcohol consumption	
Regular	3 (02.95)
Occasional	48 (47.05)
Never	51 (50.00)
Chewing of tobacco	
Yes	22(21.6)
No	80(78.4)

<sup>a</sup> Mean & Standard deviation

<sup>b</sup> Number & percentage of subjects

**Table 2:** Details of morbidity in waste disposal workers (n=102)

	Respiratory	Yes	N (%)
	Cough	12 <sup>a</sup> (11.8)	90 (88.2)
	Phlegm	12 (11.8)	90 (88.2)
	Cough & Phlegm	05 (4.9)	97 (95.1)
1	Wheezing	12 (11.8)	90 (88.2)
	Breathlessness	15 (14.7)	87 (85.3)
	Chest cold & chest illness	3 (3.0)	99 (97.0)
	<b>Combined respiratory</b>	35 (34.3)	67 (65.7)
	<b>Past illness</b>		
	Attack of bronchitis	03 (2.94)	99 (97.05)
	Asthma	03 (2.94)	99 (97.05)
2	Chest illness	04 (3.92)	98 (96.08)
	Heart trouble	03 (2.94)	99 (97.05)
	<b>Combined past illness</b>	13 (12.7)	89 (87.30)
	<b>Musculo-skeletal</b>		
	Weakness of arms & legs	16 (15.70)	86 (84.30)
3	Back pain	16 (15.70)	86 (84.30)
	<b>Combined MS disorders</b>	25 (24.50)	77 (75.50)
4	<b>Injuries</b>	8 (7.84)	94 (82.16)
	<b>Gastrointestinal problems</b>		
	Nausea	1 (00.98)	101 (99.02)
	Loss of appetite	3 (02.95)	99 (97.05)
5	General weakness	1 (00.98)	101 (99.02)
	Burning in gastric region	5 (04.90)	97 (95.10)
	<b>Combined G.I</b>	10 (9.80)	92 (91.20)
	<b>Skin related problems</b>		
	Skin allergies	6 (05.88)	96 (94.12)
6	Redness & itching of arms	1 (00.98)	101 (99.02)
	Skin lesions	6 (05.88)	96 (94.12)
	<b>Combined skin</b>	12 (11.8)	90 (88.2)
	<b>Nose &amp; eyes problems</b>		
	Burning of eyes	3 (2.94)	99 (97.05)
7	Redness & watering of eyes	1 (0.98)	101 (99.02)
	Irritation of nose	2 (1.96)	100 (98.04)
	<b>Combined nose &amp; eye</b>	5 (4.90)	95 (95.10)

**Figure 1:** Geometric mean levels of pro-inflammatory cytokines according to job category

**Table 3:** Geometric mean levels of serum pro-inflammatory cytokines in subjects according to morbidity

Complaints	N	%	IL-1 $\beta$ (Pg/ml)	IL-6 (Pg/ml)	IL-8 (Pg/ml)	TNF- $\alpha$ (Pg/ml)
Respiratory						
Yes	35	34	1.44	1.9	2.7*	3.1*
No	67	66	1.40	1.6	1.7	1.8
Musculoskeletal						
Yes	25	25	1.8*	2.0	2.0	2.1
No	77	75	1.3	1.7	1.9	2.2
Past illness						
Yes	13	13	1.5	1.9	2.0	3.5*
No	89	87	1.4	1.7	2.0	2.0
Gastrointestinal						
Yes	13	13	1.2	1.4	1.3	1.9
No	89	87	1.5	1.8	2.0	2.2
Skin problem						
Yes	12	12	1.4	1.8	2.0	1.8
No	90	88	1.4	1.8	2.0	2.2
Nose/eye						
Yes	05	05	1.00	1.2	3.2	3.4
No	97	95	1.44	1.8	1.9	2.1

\* $P < 0.05$ **Table 4:** Geometric mean levels of serum pro-inflammatory cytokines in subjects according to life style factors

Life style factors	n=102	%	IL-1 $\beta$ (Pg/ml)	IL-6 (Pg/ml)	IL-8 (Pg/ml)	TNF- $\alpha$ (Pg/ml)
Smoking						
Yes	36	35	1.2	1.9*	2.6*	2.0
No	66	65	1.5	1.5	1.7	2.2
Body mass index (Kg/m <sup>2</sup> )						
>18- 24.9	82	80	1.4	1.8	2.0	2.2
25-29.9	20	20	1.5	1.7	2.0	2.2
Type of food						
Vegetarian	08	08	2.2	2.0	2.6	2.2
Non-vegetarian	94	92	1.4	1.7	1.9	2.2
Alcohol consumption						
Yes	51	50	1.4	1.7	2.3*	2.1
No	51	50	1.5	1.8	1.7	2.3
Consumption of coffee/tea						
Yes	85	83.3	1.4	1.7	1.9	2.2
No	17	16.7	1.5	2.1	2.5	2.3
Consumption tobacco chewing						
Yes	22	22	1.5	1.9	3.0*	3.0
No	80	78	1.4	1.7	1.8	2.0

\* $P < 0.05$ 

## Discussion

The present study assessed the relationship between occupational health hazards and serum pro-inflammatory cytokines in workers engaged in hazardous waste area. The occupational health hazards reported as respiratory followed by musculo-skeletal, dermatological, gastrointestinal, injuries and nose and eye problems. The highest occupational health complaint was found as respiratory system. Bunker et al [41] reported 24% respiratory, 13.8% skin and 7% of gastro-

intestinal complaints in hazardous waste workers (HWW) who had 3 yr of mean duration of exposure. The present study has reported higher health complaints in HWW because they had 5 yr of mean duration of exposure. Vrijheld et al [42] have reported health complaints such as headaches, sleepiness, respiratory, psychological, gastrointestinal, cancers, adverse pregnancy outcomes, abnormalities in liver and renal function of workers exposed from landfill area. Goo-

rah et al<sup>[43]</sup> have reported respiratory symptoms such as bronchitis 26.1%, COPD 15.1% & asthma 13%, 26.9% of skin irritation, 19.9% nasal irritation, 22.9% eye irritation and gastrointestinal problems (5.25% diarrhea, 11.1% constipation, 10.3% abdominal pain, 14.9% vomiting and nausea) in landfill area workers from Mare chicose in Mauritius with 30 yr of exposure. Most of the studies indicated that the workers involved in these hazardous waste industries are exposed to high levels of microorganisms causes higher prevalence of respiratory symptoms and airways inflammation<sup>[44, 22]</sup>. The present study also indicated higher level of respiratory symptoms as compared to other health hazards.

The waste disposal areas (collection and disposal) have reported higher levels of respirable dust, microbial component like endotoxins, (1→3) -β -D-glucan, viable fungi and its spores and fungal extra-cellular polysaccharides<sup>[15-19]</sup>. The respiratory symptoms and airways inflammation due to higher levels of neutrophil counts and production of pro-inflammatory cytokines<sup>[23]</sup>.

Wouters et al<sup>[22]</sup> have reported the association between nasal lavage concentrations of IL-8 and respiratory symptoms in workers exposed to biological hazards (endotoxin, (1→3) -β -D-glucan, viable fungi and it spores and fungal extra cellular polysaccharides) from landfill area. The levels of nasal lavage interleukin-8 considered as upper airways inflammation responses but not on lower airways inflammation responses. Heldal et al<sup>[23]</sup> have reported higher levels of neutrophils and interleukin-8 in induced sputum of workers exposed to bio-aerosols (endotoxin & (1→3) -β -D-glucan) during waste collection. These investigations were characterized as inflammatory responses in the lower airways Reponen et al<sup>[25]</sup> have assessed the inflammatory mediators in nasal lavage, induced sputum and serum samples of workers exposed to molds. Highest detectability of inflammatory mediators was found in serum as compared to induced sputum and nasal lavage. The levels of serum inflammatory cytokines could be utilized as the clinical and prognostic tests for evaluation of the disease status. Most of occupational exposure studies have assessed the pro-inflammatory cytokines levels in serum. So the present study assessed serum pro-

inflammatory cytokines among workers involved in hazardous waste sites and found significant higher levels of serum IL-8 & TNF-α in landfill area and subjects who had respiratory symptoms. The other studies also found an increased serum concentration of IL-8 and TNF in chemical exposure with bronchitis<sup>[45]</sup>, polypropylene flock exposure with decreased diffusion capacity<sup>[27]</sup> and house painters with organic solvent exposure<sup>[35]</sup>. Increased IL-8 and TNF could be used as inflammatory responses in respiratory symptoms.

## Conclusion

The levels of serum pro-inflammatory cytokines IL-8 & TNF- α were significantly associated with subjects who had respiratory symptoms & further supporting that they are inflammatory markers in respiratory symptoms.

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