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Biomonitoring of Serum Markers as a Measurement of Occupational Exposure in Personnel at Landfill Site

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Abstract

Improper handling and management of municipal solid wastes can produce adverse health effects on waste pickers through biological, chemical, physical, mechanical or psychological means. They are vulnerable to various occupational health hazards such as respiratory disorders, gastrointestinal diseases, skin diseases, eye infections and musculoskeletal disorders. Hence, we aimed to conduct biomonitoring and health risk assessment on women rag pickers. This study included 250 women waste pickers: 160 exposed working on the Pirana dumping site and 90 control subjects from around the residential areas of Ahmedabad city of Gujarat (India). C-reactive protein, Alkaline phosphatase, Alanine transaminase and Aspartate transaminase were significantly high in serum samples of exposed subjects suggesting gallbladder inflammation, acute hepatitis A and B or oxidative stress. After comparing the lipid profile of exposed and control subjects, we found a significant decrease in cholesterol, high-density lipoprotein cholesterol and triglyceride frequency which is a sign of malnutrition or hyperthyroidism. Random blood sugar, globulin and total serum protein were reduced significantly. In contrast, a significant increase was found in albumin, phosphorus, creatinine, urea and uric acid levels of exposed individuals, which indicates potential disorders of the liver and kidney. Direct exposure to solid waste, agrochemicals, heavy metals, toxic industrial and hospital waste, and toxic air having high concentrations of polyaromatic hydrocarbons and bioaerosols, unhealthy eating and sanitary habits, and negligence towards health were major contributing factors to ill effects on their liver and kidney. We suggest effective education, and communication programs for preventive and protective measures to address problems of rag pickers. The present study emphasizes the need for regular monitoring of contamination among the rag-picking population and areas around the Pirana landfill site for better information on exposure to rag pickers.

Keywords: Biomarkers, Inflammation, Occupational Hazard, Polyaromatic Hydrocarbons, Serum Biochemistry, Solid Waste

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1. Introduction

Annually, about 40-55 million tons/year of Municipal Solid Waste (MSW) is generated in India and is projected to be 270 million tons by 2047[1]. Landfills create a clear threat to human health and to our environment stemming from the hazardous and contaminated air emissions released from the landfill biodegradation, which is produced through bacterial decomposition, volatilization and chemical reactions. Ahmedabad is the 7th largest city in India and generates about 4000 metric tons of waste daily which is mostly dumped at the Pirana dumping site[2]. Waste pickers collect, sort and sell all kinds of material that can be recycled from the landfills in the city and so are directly exposed to health risk factors. Occupational risks include chemical hazards, skin infections, musculoskeletal damage, mechanical trauma, emotional vulnerabilities, and environmental contamination[3]. Waste management exposes many of the workers to municipal solid wastes indirectly by subjecting them to ingestion and inhalation of contaminated water, soil, food, and air which affects vital organs such as the lungs, liver and kidney[4]. Pesticide derivatives are also major toxic compounds present at the landfill site as large amounts of organic farm waste are dumped there too. Evidence continues to accumulate that chronic pesticide exposure is associated with impaired health, including biochemistry markers, carcinogenesis, neurotoxicity, reproductive and development effects, and immunological effects[5]. However, the mechanisms by which these chemicals can contribute to chronic events are largely unknown.

According to the Central Pollution Control Board (CPCB), every year in India itself, 8 million tons of plastic products are consumed[6]. Plastic being resilient, non-reactive and mostly non-biodegradable in nature, stays in landfills for a longer period and is hazardous since dangerous chemicals like styrene are drained out and contaminate the underground water bodies[7]. With industrialization, humans are often exposed to contamination by metals in environmental and occupational settings. Moreover, heavy metals present in landfill soil are drained out mainly by leaching into the water which adversely affects the underground water around landfill sites[8,9]. Waste pickers are commonly exposed to Polycyclic Aromatic Hydrocarbons (PAH) mixtures through the inhalation of polluted air, burning waste smoke and cigarette smoke[10,11]. The toxicity of PAHs also appears to be related to their biotransformation into reactive metabolites, resulting in the generation of Reactive Oxygen Species (ROS) which are capable of inducing lipid and protein oxidation and the depletion of endogenous antioxidants within body[12].

Serum biomarker measurement is a routine objective tool that aids in early diagnosis and predicts the severity and prognosis of inflammation in occupational workers. Serum enzymes studied in the present study such as Alkaline Phosphatase (ALP), Alanine Transaminase (ALT/SGPT), and Aspartate Transaminase (AST/SGOT) are considered to be important serum markers for investigating liver disorders, detecting inflammation, and avoiding damage to the liver of occupationally exposed individuals in concern. Occupational exposure, obesity, medications, viral infections, genetic disorders, heavy alcohol consumption, etc. can damage the liver[13]. Many studies including our previous study[14-16] have reported the presence of musculoskeletal disorders in waste pickers due to carrying heavy loads over longer distances. Measurement of uric acid levels can be useful to identify musculoskeletal disorders, kidney stones and kidney failure. The adverse effects of occupational exposure on the kidney and liver may be triggered by changes in the enzyme production and redox system[17]. The measurement of serum biochemical parameters can be useful to identify the health status and target organs affected by exposure to toxicants. Therefore, realizing the need for a thorough investigation, the present study was undertaken for the first time in Gujarat in which the potential hazards faced by these waste pickers were analyzed using their blood samples for serum biochemistry analysis.

2. Materials and Methods

2.1 Study Population and Sample Collection

This study has been approved by the Institutional Ethical Committee (IEC) (No: GU/IEC/03/2018) of Gujarat University and women rag pickers were enrolled after taking informed written consent. A total number of 250 female individuals including, exposed women (n = 160) who were working at the Pirana dumping site of Ahmedabad city for minimum of 3 years, and those working near Gujarat University residential areas were recruited as controls (n = 90) from the similar socio-economic background and were age-matched. None of the control individuals were recently exposed to any

other suspected genotoxic agents or visited the dump site. Women who had any disease/infection/pregnancy were excluded from both groups. Medical examination was performed by a doctor and specific points were noted in the proforma for demographic analysis. A blood sample (4 mL) was collected by technical staff with no potential risk using serum-separating vacutainers and was tested for biochemical parameters. The serum was separated after centrifugation at 5000 rpm and stored at 4^0 C until tested.

2.2 Serum Biochemistry Analysis

The serum biochemistry biomarkers such as C – reactive protein (CRP, mg/L) (CRPAQ2-01), Serum Glutamic Oxaloacetic Transaminase (SGOT, U/L) (GOTAQ1-01), Aspartate Aminotransferase (SGPT, U/L) (GPTAQ1-01), Alkaline Phosphatase (ALP, U/L) (ALPAQ1-01), Direct Bilirubin (DBIL, mg/dl) (BIDAQ1-01), Total Bilirubin (TBIL, mg/dl) (BITAQ1-01), Cholesterol (mg/dl) (CHOAQ1-01), (HDL-C, mg/dl) (HDLAQ1-01), Triglyceride (mg/dl) (TRGAQ1-01), Random blood sugar (mg/dl) (GLUAQ1-01), Albumin (mg/dl) (ALBAQ1-01), Globulin (g/dl) (GLBAQ1-01), Total protein (TP, mg/dl) (TPRAQ1-01), Phosphorus (mg/dl) (PHOAQ1-01), Creatinine (mg/dl) (CREAQ1-01), Urea (mg/dl) (UREAQ1-01) and Uric Acid (UA, mg/dl) (URCAQ1-01) were analyzed using automated clinical chemistry analyzer (AutoQuant-100) and kits were obtained from Meril Diagnostics. Reference ranges are according to standard operating procedures at the scientific laboratory of the National Institute of Occupational Health.

Table 1. Demographic data: "control" and "exposed"

Categories	Control (n= 90)	Exposed (n =160)
Age distribution (in years) (ns)		
18 – 30	19 (21%)	50 (31%)
31 – 50	46 (51%)	81 (51%)
50 – 70	25 (28%)	29 (18%)
Body Mass Index (BMI) (***)		
Underweight: <18.5	4 (4%)	25 (15%)
Normal: 18.5-24.9	9 (10%)	84 (52%)
Overweight: 25-29.9	22 (25%)	39 (24%)
Obesity: ≥ 30	55 (61%)	12 (9%)
Addiction - Chewing habits (ns)		
Chewer	64 (72%)	129 (80%)
Non-chewer	26 (28%)	31 (20%)

Results are expressed as the Frequency (%). $^{ns}p > 0.05$, $^{***}p < 0.001$

2.3 Statistical Analysis

Results are expressed as Mean ± Standard Error (SE). Demographic results were analyzed using the Chi-Square test and serum biochemical parameters using *Student's t – test* and data was confirmed using Graph-pad Prism - 7software. Values having $p < 0.05$ are considered significant.

3. Results

Table 1 shows various demographic parameters of rag pickers. The age distribution among control and exposed subjects was non-significant as both groups were age-matched and most of them belonged to the middle age group (31-50 years). The frequency of chewing tobacco/pan masala in exposed (80%) as compared to Control subjects (72%) was found to be non-significant. On comparing Body Mass Index (BMI) ($p < 0.001$) it was observed that only 4% of the control subjects were underweight, whereas 15% of exposed subjects were in this category. Table 2 includes liver profile tests done on rag pickers and results were compared with given reference ranges. C-reactive protein was found to be present in significantly high ($p < 0.001$) frequency in exposed subjects as in controls. Similarly, other vital liver enzymes such as SGOT, SGPT, ALP, DBIL and TBIL were found to be increased significantly ($p < 0.001$) in

Table 2. Comparison of liver enzymes present among control and exposed subjects

Sr. No.	Variables	Control (n=90)	Exposed (n=160)	Reference range
1.	CRP (mg/L)	9.61±0.42	26.14±0.58***	< 10 mg/L
2.	SGOT (U/L)	21.52±0.59	85.04±1.56***	0-37 U/L
3.	SGPT (U/L)	46.58±0.73	101±1.34***	7-56 U/L
4.	ALP(U/L)	71.98±1.72	144.11±1.71***	38-94 U/L
5.	DBIL (mg/dl)	0.24±0.18	10.10±0.62***	0-0.3 mg/dl
6.	TBIL (mg/dl)	0.43±0.17	18.40±0.40***	0-1 mg/dl

Results are expressed as the mean value ± SEM. $^{***}p < 0.001$

serum samples of exposed subjects when compared with control subjects. The lipid profile of exposed and control subjects revealed a significant reduction ($p < 0.001$) in cholesterol, HDL-C and triglyceride frequency in exposed as compared to controls (Table 3). Other biomarkers such as random blood sugar, albumin and total serum protein (TP) were reduced significantly ($p < 0.001$) whereas a significant increase ($p < 0.001$) was found in albumin, phosphorus, creatinine, urea and Uric Acid (UA) levels (Table-4).

Table 3. Comparison of lipid profile among control and exposed subjects

Sr. No.	Variables	Control (n=90)	Exposed (n=160)	Reference range
1.	Cholesterol (mg/dl)	245.09±1.96	174.76 ±1.27***	150-250 mg/dl
2.	HDL-C (mg/dl)	40.58±0.86	30.49 ±0.47***	≤50 mg/dl
3.	Triglyceride (mg/dl)	86.56±1.7	25.75 ±0.64***	65-190 mg/dl

Results are expressed as the mean value ± SEM. ***p < 0.001

Table 4. Comparison of different biochemical markers among control and exposed subjects

Sr. No.	Variables	Control (n=90)	Exposed (n=160)	Reference range
1.	Random blood sugar (mg/dl)	136.02±1.94	126.24±1.89***	≤ 140 mg/dl
2.	Albumin (mg/dl)	4.59±0.53	13.68±0.38***	3.7-5.3 mg/dl
3.	Globulin (g/dl)	3.64±0.18	0.66±0.03***	2.0-3.5 g/dl
4.	Total protein (mg/dl)	7.36±0.21	3.86±0.14***	6.6-8.3 mg/dl
5.	Phosphorus (mg/dl)	4.66±0.91	12.84±0.27***	2.4-4.1 mg/dl
6.	Creatinine (mg/dl)	0.72±0.65	11.48±0.36***	0.7-1.4 mg/dl
7.	Urea (mg/dl)	11.94±0.48	35.33±0.53***	6-24 mg/dl
8.	Uric Acid (mg/dl)	4.44±0.94	11.17±0.31***	2.4-6 mg/dl

Results are expressed as the mean value ± SEM. ***p < 0.001

4. Discussion

Household waste is one of the major components of MSW responsible for the growth and multiplication of molds and fungi which produce secondary metabolites, known as mycotoxins that exert toxicity against animals and humans[18]. Since the recyclable dry wastes can be contaminated by fungal substrates in many processing sites, the workers can be exposed to aflatoxins by ingestion, contact, and inhalation (airborne source) [4]. The waste collection process is usually manual and involves the release of bioaerosol that may cause environmental pollution and severe airway inflammation[19]. Increased proinflammatory cytokines, usually indicated by excess CRP in the blood, are a major underlying cause of systemic inflammation[20]. Inhalation exposure represents an additional source of risk in such occupational workplaces that must be addressed, assessed, and as far as possible, prevented. The liver is the main target organ for aflatoxin toxicity and carcinogenicity[21,22]. Aflatoxin is one of the most hazardous mycotoxins that workers are exposed to in the waste occupational workplaces[23]. These natural substances have been classified as Group 1 human carcinogens by the International Agency for Research on Cancer (IARC) based on the epidemiological and laboratory results for carcinogenicity[24]. The aflatoxin toxicity may be responsible for the increase in liver markers in the exposed individuals. Also, the mean CRP of the exposed group was even higher than the reference range which directly indicates liver diseases such as jaundice. The characterization of suitable biomarkers can greatly advance the assessment of aflatoxin exposures and related health hazards for humans[25] and over the last decades, there has also a growing body of interest and recognition of their potential impact in various occupational settings[26,27].

Landfill operations generate air pollutants such as Particulate Matters (PM) and Landfill Gases (LFG) including bioaerosols and may originate from the waste or may be generated during its decomposition which may induce lung cancer[28,29]. Chronic exposure to bioaerosols and Particulate Matters (PM 2.5 and PM 10) damages the respiratory and cardiovascular systems which can increase the level of CRP in serum[30,31]. Reduction in the level of serum total protein, synthesized mainly in the liver, can be observed due to liver damage, reduced absorption, and protein loss and thus may be an indication of the deteriorating health status of women. Similar outcomes

were observed in a study by Öner *et al*[17]., where serum protein loss was observed in fishes affected by heavy metal toxicity.

Metal contamination in underground water is seen as a potential threat for humans who are exposed to significant amounts of heavy metals as a consequence of industrial, agricultural, and anthropogenic activities. Heavy metals at high concentrations can cause hazardous effects on the metabolic, physiological, and biochemical systems of occupational workers[32]. According to Singh *et al*[33]., heavy metal concentration was found to be high in the underground water of the Pirana landfill site. Also, the rag pickers use underground water for drinking purposes which is highly contaminated with heavy metals such as lead and arsenic and thus directly face heavy metal toxicity. As a result, serum biochemistry was influenced, predicting the failure of vital organs in future. A study by Ahmad *et al*[34]., found a significant reduction in serum total protein and cholesterol in automobile workshop personnel who were exposed to lead. Protein loss in lead toxicity might decrease the level of specific proteins such as albumin, hormones, metal binding proteins, drug binding proteins, enzymes etc. and thereby disturb the homeostasis and rate of metabolic activities[35]. On absorption, arsenic is stored in the liver, kidney, heart, and lungs. A lower amount of arsenic is observed in muscles and neuronal tissues[36]. The accumulation of arsenic in these tissues is associated with many disorders including cancer, diabetes, hepatotoxicity, neurotoxicity, and cardiac dysfunction[37,38].

Our study revealed that a significantly high number of rag pickers eat leftover food at dumping sites which may be highly contaminated with microbes as dumpsites have a considerable level of microbial contamination because whenever waste is dumped on land, soil microbes especially anaerobic fungi and bacteria inhabit the waste and extract nutrients by carrying out decomposition[39]. This can directly affect the liver leading to an increase in liver biomarkers and can even cause jaundice. According to Burkowska *et al*[40]., dumpsites could have microbial contamination in the atmosphere from a few hundred meters up to one kilometre apart even if they are well protected. A previous study has reported that damage to the liver observed due to a marked increase in enzymes could result in circulatory failure with 'shock' and hypoxia and myocardial infarction[41]. Chronic exposure to toxic air leads to renal, hepatic, blood and genetic complications among humans and animals including cancers, renal adenoma, elevation of liver enzymes, Blood Urea Nitrogen (BUN), creatinine and potassium as well as hypochloremia and hyponatremia among lab animals[42]. In addition, workers exposed to toxic fumes containing heavy metals and PM 2.543 have reported elevated liver enzymes, proteinuria and fatty liver changes. Similarly, inhaling a small amount of toxic landfill air can lead to nasal and throat irritation, headache, vertigo, nausea, vomiting, dizziness and respiratory problems. Some of the dermal effects of toxic air exposure are rash, erythema, swelling and allergic (hypersensitivity) reactions[44]. Such allergic reactions or inflammatory responses may lead to an increase in oxidative stress through an increase in free radicals in cellular systems[16]. Moreover, free radicals following tobacco consumption can damage hepatocytes and renal parenchyma. In addition, smoking/tobacco which is majorly used by rag pickers can lead to liver cancer and chronic renal disorders potentially increasing the liver enzymes. Some of the elements of the smoke can prevent intracellular junction formation resulting in tissue destruction leading to a decrease in serum protein[45]. The exposed group of the present study reported higher than normal albumin levels which may be a sign of dehydration, caused by severe diarrhoea or other conditions. The significant reduction found in the mean frequency of cholesterol, HDLC and triglycerides may be due to the prevalence of low BMI and malnutrition among exposed subjects. The renal function parameters revealed the presence of kidney disease or hypoparathyroidism in the exposed individuals. It may also suggest the presence of autoimmune diseases, bacterial infection of the kidneys, blocked urinary tract etc. We propose that the exposed subjects may be suffering from kidney disease or preeclampsia-like conditions as they reported significantly high urea and uric acid in their serum samples.

A study by Odonkor and Mahami[46] revealed that high SGOT and SGPT can lead to numerous diseases such as tuberculosis and Severe Acute Respiratory Syndrome (SARS) which have been linked with poor air quality caused by bioaerosols wreaking the greatest havoc socioeconomically. It has previously been suggested that the physiological differences between men and women play a main role in their response to occupational hazards, and women are at higher health risks than men in many occupations[47]. Tunsaringkarn *et al*[48]., reported an association between BMI and liver function so that the liver enzymes were significantly different between subjects with different BMIs. Our previous study[16] reported low BMI in exposed rag pickers as compared to

controls. Therefore, it is necessary to address the changes in renal/hepatic indices according to the body mass index. Previous research found significant associations between age, work history and liver enzyme elevation[49].

A study by Lee *et al*[50]., shows the power of the metabolomics approach in investigating the molecular signature of lifestyle-related habits that aggravate environmental or occupational exposures. They demonstrated that smoking tobacco is associated with concentration variations in amino acids, ether lipid and glycerophospholipid metabolism at an 'omics' level. Thus, the tobacco chewing addiction in women could have a confounding impact on abnormal values of biochemical markers. However, since the addiction to chewing was non-significant between the exposed and control women, it cannot be the cause of significant differences in the serum biochemistry between exposed and control women of the present study.

Globally, environmental pollution by plastic waste majorly found at landfill sites has become a severe ecological and social problem. Landfill sites are regarded as sources of Volatile Compounds (VOCs) such as styrene emitted into the atmosphere. A study by Kumar *et al*[51]., on plastic degrading microorganisms from Proteobacteria phylum present at the Pirana landfill of Ahmedabad found high emission of styrene into the air which is a known xenobiotic and carcinogenic compound and can be easily absorbed by the body. Polycyclic Aromatic Hydrocarbons (PAHs) are also one of the major components along with methane which are found to be present in the volatile fumes on the dump site of Pirana[33]. The study of Kuppusamy *et al*[52]., stated that there was an increase in oxidative stress levels and inflammation in the workers who are occupationally exposed to Polycyclic Aromatic Hydrocarbons (PAHs). A Study by Shaikh *et al*[53]., on Workers Occupationally exposed to Automobile Fuels revealed that high oxidative stress was observed in Petrol pump workers and Traffic police personnel. In the present study too, the rag pickers are exposed to fumes at dump sites which contain PAHs as pollutants. Styrene, a kind of VOC, has a strong, unpleasant smell and is irritating to the eyes and mucous membranes[54]. Long-term exposure to VOCs, such as benzene, toluene, xylenes, and benzene chloride, may harm human health[55], which might pose non-carcinogenic or carcinogenic risks to the surrounding people[56]. A study by Hasani *et al*[57]., found high hepatic injuries in occupational workers exposed to styrene with high levels of liver enzymes (SGOT and SGPT). In the present study too, high liver biomarkers in exposed individuals may be a result of styrene exposure which is present at the Pirana landfill site. The present study also emphasizes the need for regular monitoring of waste pickers and abstaining from jobs for short periods to reduce load on their health.

5. Conclusion

It appears that occupational exposure in women rag pickers can lead to adverse effects on renal and liver functions. According to our search, this is the first study on the renal-hepatic effects of MSW and toxic air coming out of it among women rag pickers. The serum biochemistry could be used as a sensitive tool to assess the occupational impact on waste pickers and also would be beneficial in determining their baseline health and physiology. For this purpose, our results may provide useful data for further investigations; nevertheless, it should be noted that, during advanced research, appropriate biomarkers must be selected due to the variable responses to different pollutants/heavy metals present in landfill sites. It is of great importance to assess air quality in vicinities or towns close to dumpsites, to constantly ascertain biological and physical health risks, and to inform stakeholders, policymakers, and the general population. Therefore, there is a dire need for targeted interventions for the holistic development of rag pickers.

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7. Data Availability Statement

Data is available on reasonable request from the authors.

8. References

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