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Research Article

Genotoxicity Study with Special Reference to Comet Test in the Blood Cells of Workers Exposed to Sewage Water

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Awareness among sewage workers to occupational exposure is growing slowly in many developing countries. Lead (Pb) and cadmium (Cd) are present in sewage water and workers are exposed to these metals as a result of unprotected handling. These heavy metals exposures are responsible for DNA damage and lowering blood total iron (Fe) concentration. Zinc (Zn) is an element for promoting metallothionine expression and binds the free Cd. The total suspended solids (TSS), total dissolved solids (TDS), Pb, and Cd were estimated in sewage water. The whole blood Zn and Fe concentration and Pd and Cd were also estimated. Genotoxicity as indicated by DNA damage was studied by comet assay. It was observed that there were significant differences ($P < 0.001$) of Pb and Cd concentration in blood for the sewage workers when compared with control population. DNA damage was also observed to be significantly ($P < 0.001$) higher in the exposed groups but their blood Fe concentration was significantly lower, which may be the reason for their tendency for retention of blood Cd and make them more susceptible. This study also indicated that aged workers had higher blood Zn concentrations as compared to the younger (working < 20 years) workers. This may indicate a possible adaptive response. The present study proposes that younger (working < 20 years) group is more susceptible as compared to aged group (working > 20 years).

1. Introduction

Municipal wastewater has already been reported as genotoxic [1–3] and has been frequently found to be mixed with liquid waste from small scale industries [4]. This multiple effluent mixture has deleterious somatic effect [5] and also has adverse genetic effect [6]. People who are contaminated with this wastewater are at a higher risk of cancer development [7]. In recent years, detection and assessment of occupational health impact and different health hazards have become an area of growing importance, in which it is important to know whether workers have adopted adequate safety measures during the period of working [8]. Apart from organic pollutants, sewage water has also been reported to be contaminated with different metals especially Pb and Cd. In urban area, the large number of local automobile repair shops has led to increased Pb contamination in sewage water [9]. Cd compounds have also been considered mutagenic and *in vitro* studies in human cells have shown increased damage in chromosomes as well as DNA strand breaks [10]. Cd exposure also reportedly causes anemia in human. Blood Fe level is an important indicator for anemia in people subjected to heavy metal exposure [11]. Regular smoking has been shown to be one of the important reasons for increased level of Pb and Cd in the blood of workers who are professionally exposed to Pb polluted waters. These workers have also been reported as suffering from increased skin ailments [12].

Generally Pb and Cd can enter the body both through skin and via ingestion and there is a chance of exposure to both these metals during work and because of inadequate safety measures. It has been reported that Zn-Cd interaction plays a major role in maintaining the body's oxidative stress and Zn acts as efficient antioxidant against the reactive oxygen species generated by Cd toxicity [13].

The present study aims to determine heavy metals in sewage water and blood samples as well as genotoxicity study indicated by DNA damage in human subjects exposed to sewage water.

2. Materials and Methods

2.1. Selection of Study Area, Sample Collection, and Analysis of Wastewater and Blood Sample. Selected study area was southern part of Kolkata (India). This area was selected based on efficient drainage clearance and conservancy work being carried out by an active workforce. Water samples were collected from sewage (two different outlets in a single drain where drainage workers were actively engaged) in a sealed container. These were stored at 4°C for subsequent analysis as detailed below.

2.1.1. Estimation of Total Dissolved Solids (TDS), Total Suspended Solids (TSS), and Heavy Metals in Wastewater and Human Blood. TDS and TSS of sewage water handled by drainage workers and simple domestic water used by control population were analyzed according to APHA 22nd Edition, 2540 C and 2540 D.

Water samples were digested using nitric acid and perchloric acid mixture (1 : 3) [14] and heavy metals, Pb, and Cd were estimated in graphite furnace atomic absorption spectrophotometer (Perkin-Elmer; Model Analyst 200) for both drain water and normal water.

Subjects were Indian adult men, who had been living for at least 5 years in south Kolkata region and working strictly on drainage work. Candidates (drainage workers of Kolkata) and control population (living in the same area but never exposed to such polluted and toxic water) were asked to answer questions in order to verify any possible additional exposure through ingestion of food, smoking, use of medicine, and so forth. This information was utilized in the evaluations of the final data. Among 200 workers, 57 exposed male workers were selected for the experiment who were engaged in drainage work, had no addiction to either alcohol or tobacco, used hygienic toilets (corporation toilet), drank water supplied by the corporation, and also had a regular monthly income but randomly handled sewage water every day and also ate their food without properly washing their hands especially during working hours. After ethical committee, clearance blood was drawn (5 mL) from each subject under proper medical supervision and care. Blood samples, thus obtained, were kept in a citrate (3.8%) container and stored in 4°C refrigerators for further experiment. Similarly, venous blood (5 mL) was taken from 57 healthy males of the control population as described above.

Blood sample (1 mL) was digested by nitric acid perchloric acid mixture (1 : 3) using the method of Nkolika et al., 2009 [14, 15]. Concentration of Pb and Cd was analyzed by atomic absorption spectrophotometer (Perkin-Elmer; Model Analyst 200) and the detection limit was 0.001 ppm. The stored samples were again digested as per the above mentioned procedure to determine the reproducibility of the metal concentrations.

2.1.2. DNA Damage Study in Human Peripheral Blood by Comet Assay. Alkaline single cell gel electrophoresis (comet assay) was performed using modification of the method of Tice et al. (2000) [16]. Fresh peripheral blood (10 μL) was diluted with phosphate buffer saline (1 mL). After dilution, it was (100 μL) mixed with 20% low melting agarose (100 μL). A part of this solution (75 μL) was layered on 1% low melting agarose (LMP agarose). After preparation, the slides were immersed in lysis solution and refrigerated at 4°C for 4 hours. From this lysis solution (pH > 12), the slides (Blue Star) were placed in alkaline solution (pH ≈ 11), for electrophoresis slides were kept for 15 minutes for salt equilibration. After this, electrophoresis was continued for 30 minutes at 30 V and 330 Ma at 4°C in dark. For neutralization, slides were then coated with tris buffer (0.4 M, pH 7.5) for 10 minutes and this step was repeated. The cells were stained with ethidium bromide (80 μL, 20 μg/mL water) for 5 minutes and dipped in distilled water for few seconds. The slides were dried and covered with a cover slip. Comet images were analyzed using a fluorescence microscope (Leica) equipped with CCD camera. One hundred cells were examined randomly for each sample. The tail moment of DNA was measured by using comet score software (TriTEk Corporation).

Blood samples (2 mL) were lyophilized at −80°C. These dried blood samples were powdered using mortar and pestle. Powdered sample (150 mg) was taken and compressed to form a pellet of 13 mm diameter, with a table top pelletizer using 100 kg/cm^2 pressure. These pellets were subjected to energy dispersive X-ray fluorescence (EDXRF) technique [17]. The concentration of Fe and Zn in the blood samples was measured by EDXRF (Xenemetrix former Jordon Valley, Israel); detection limit for Zn and Fe is 5 ppm.

Statistical analysis was done using ANOVA (one way), at 5% level of significance and for correlation coefficient, we studied the Pearson correlation coefficient (IBM SPSS, Version 20). For comparing the differences between two groups, 95% confidence intervals were used.

3. Results

Water from two different spots (in the same area, from two different outlets) as represented in (Table 1) was found to be of inferior quality than control tap water samples as handled by control population. TDS and TSS of sewage water were found to be >20 times higher than control tap water samples.

Table 1: Levels of TDS, TSS, Pb, and Cd in sewage water compared with nonsewage water from different sources.

Location and water samples	TDS (mg/L)	TSS (mg/L)	Pb (mg/L)	Cd (mg/L)
Waste drain and sewage water-1	3804	210.8	2.86	1.89
Waste drain and sewage water-2	3804	219	2.02	3.0
M ± SD	3804	214.9 ± 5.79	2.44 ± 0.59	2.44 ± 0.78
Local tap and general fresh domestic water 1	135.6	8.0	Nil	Nil
Local tap and general fresh domestic water 2	135.6	8.0	Nil	Nil
M ± SD	135.6	8.0	nil	Nil

Table 2: Levels of blood Pb, Cd, Fe, Zn, and TM of comet assay.

Factors	Control population $n = 57$	Affected group (workers) $n = 57$
Blood Pb ± SD (mg/L)	0.132 ± 0.093	0.386 ± 0.24
Blood Cd ± SD (mg/L)	0.0032 ± 0.004	0.016 ± 0.01
Blood Fe ± SD (mg/L)	1998.52 ± 52.66	1835 ± 147.92
Blood Zn ± SD (mg/L)	31.5 ± 1.80	29.43 ± 4.43
DNA damage study (TM), M ± SD	1.37 ± 0.37	4.35 ± 1.47

On comparison, TDS and TSS of sewage water quality were also inferior to the standard recommended sewage water discharge quality. Concentration of Pb in sewage water was considerably higher, that is, 2.44 ± 0.59 ppm, than maximum permissible limit of Pb of 1.0 ppm for wastewater as recommended by Central Pollution Control Board, pollution control acts, rules, and notifications issued there under 4th edition pp. 358-359, New Delhi, CPCB, Ministry of Environment and Forests, 897 pp. Concentration of Cd was also found to be higher, that is, 2.44 ± 0.78 ppm, than maximum permissible limit of cadmium, that is, 1.0 ppm, in waste water according to the previous standards. In case of control (corporation supplied tap water) samples, Pb and Cd concentration were found to be below the detection limit of measurement (0.001 ppm).

In this study, overall mean Pb concentration as measured from blood samples of the 57 drainage workers was found to be 0.386 ppm (SD = 0.24), (Table 2). The 57 drainage workers were subdivided into two groups, that is, workers who had been working <20 years (36 workers) and workers who had been working >20 years (21 workers). The mean Pb concentration for first group was found to be 0.318 ppm (SD = 0.10) while, for the second group, it was found to be 0.470 ppm (SD = 0.37), which was significantly ($P < 0.05$) higher than that for the first group of workers. In case of the control population, mean Pb concentration was found to be 0.132 ppm (SD = 0.093), which was significantly ($P < 0.001$) lower than the Pb concentration in blood of workers working >20 years and also of workers working < 20 years. The degree of freedom for F statistics used in the ANOVA test for equality of treatment means was (2,111). The critical F value and experimental F values were 3.078 and 31.369, respectively.

It was found that, in the control population, 49% of males tested below detectable limit for Cd in blood. The rest of the controls had Cd levels of 0.003 ppm (SD = 0.004). All the workers were above detection limit (0.001 ppm), and the mean Cd concentration in workers was 0.0160 ppm (SD = 0.01). There was a significant difference ($P < 0.001$) between the control population and the occupationally exposed groups. Further in the two groups divided on the basis of the number of years in service less than or greater than 20 years, mean Cd concentration was 0.0141 ppm (SD = 0.004) and, in workers working >20 years, the mean was 0.02 ppm (SD = 0.01). Thus significant difference was observed ($P < 0.001$) between these two groups. The degree of freedom for the F statistics used in the ANOVA test for equality of treatment means was (2,111), critical F value was 3.078, and experimental F value was 99.67.

Based on the comet assay, a significant increase ($P < 0.001$) in DNA damage was observed in workers with high-level of Pb and Cd concentration in blood as compared to the control population (Table 3 and Figure 1). Mean tail moment (TM) value of DNA damage for control population was 1.37 (SD = 0.37) while, for workers working < 20 years, the mean TM value was 3.57 ± 0.84 and, for workers working >20 years, mean TM value was 5.69 (SD = 1.37). There was a significant difference ($P < 0.001$) of the intensity of DNA damage between control and workers and also between the younger workers (working < 20 years) and aged workers (working > 20 years). The degree of freedom for the F statistics used in this ANOVA test for equality of treatment means was (2,111); critical F value and experimental F value were 3.078 and 246.94, respectively.

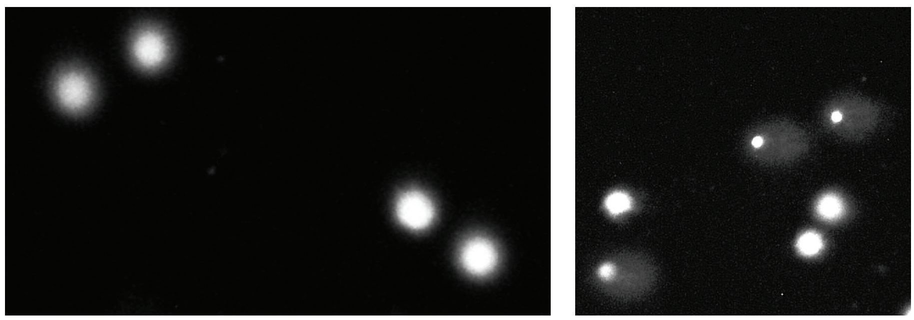
There was a significant difference ($P < 0.001$) among the whole blood Fe concentration in the control group and the workers working < 20 years and working >20 years (Table 3). Though there is no significant difference between the control population and the total of 57 workers (Table 2), in case of control population, mean Fe concentration was 1998.52 ppm (SD = 52.66) and mean blood Fe concentration of worker was 1835 ppm (SD = 147.92). Among the workers working <20 years, mean blood Fe concentration was 1927.97 ppm (SD = 74) and, for the >20 years group, the mean Fe concentration was 1677.28 ppm (SD = 99.94). Degree of freedom for the F statistics used in the ANOVA test for equality of treatment means was (2,111), critical F value is 3.078, and experimental F value was 161.

For the workers working >20 years had the highest blood Zn concentration, it was 34.27 ppm (SD = 2.74) compared to

Table 3: Effects of years of exposure on blood Pb, Cd, Fe, and Zn level and DNA damage (TM = tail moment).

Factors	Control population (no exposure) $n = 57$	Workers, working <20 years, $n = 36$	Workers, working >20 years, $n = 21$
Blood Pb ± SD (mg/L)**	0.132 ± 0.093^{c}	0.318 ± 0.12^{b}	0.47 ± 0.36^{a}
Blood Cd ± SD (mg/L)**	0.0032 ± 0.004^{c}	0.0141 ± 0.004^{b}	0.02 ± 0.01^{a}
Blood Fe ± SD (mg/L)**	1998.52 ± 52.66^{c}	1927.12 ± 74.04^{b}	1677.28 ± 99.94^{a}
Blood Zn ± SD (mg/L)**	31.5 ± 1.8^{c}	26.61 ± 2.23^{b}	34.27 ± 2.74^{a}
DNA damage study (TM), M ± SD**	1.37 ± 0.37^{c}	3.57 ± 0.84^{b}	5.69 ± 1.37^{a}

**Values with different alphabets are statistically significant.



(a) (b)

Figure 1: Photomicrograph (400x) of comet formation in peripheral blood of human after exposure to sewage water samples. (a) Undamaged cells (no comet formation) and (b) DNA damaged cell (formation of comet, prominent head and tail).

the control population (31.5 ppm, SD = 1.8) and the workers working < 20 years had the lowest blood Zn level (26.61 ppm, SD = 2.23). There was a significant difference ($P < 0.001$) of whole blood Zn concentration between these two working groups (Table 3). The degree of freedom for F statistics used in the ANOVA test for equality of treatment means was (2,111) and critical F value is 3.078 and experimental F value was 98.215.

The 95% confidence intervals for the difference of the mean for each above mentioned factor and each pair of groups (group I-control, group II-workers working < 20 years, and group III-workers working > 20 years) have been given in Table 4. Since none of the 95% confidence intervals in this mentioned table contains the point zero (0), it can be inferred at 95% level of significance that the mean level of each pair of groups for each parameter was significantly different.

From the above data, it may be observed that, in case of the control population, the correlation coefficient of comet scoring and blood Pb concentration were not statistically significant ($r = 0.105$) and P value was 0.435. However, comet scoring and blood Cd concentration were found to be significant ($r = 0.453$) and the P value was 0. In case of workers (working < 20 years), correlation coefficient of comet scoring and blood Pb concentration were highly significant ($r = 0.471$) and P was 0.004. It was followed by Cd and correlation coefficient was significant too ($r = 0.338$) and P was 0.044. In case of workers working >20 years, blood Pb concentration and comet assay scoring were not significant ($r = 0.239$) while correlation coefficient of Cd and comet assay scoring were shown to be negatively significant ($r = -0.93$).

It was further found that there was no significant difference between the initial reading of elemental profile and the 2nd reading for each group and for each element (Table 5).

4. Discussion

The present study involves the interpretation of genotoxicity results with special reference to DNA damage by the presence of the chemical contaminants, Pb and Cd, in sewage water when compared with normal tap water (Tables 2 and 3 and Figure 1).

Generally, formations of comets indicate primary DNA lesion leading to genetic damage in any cell [18]. In case of sewage water, TDS and TSS were considerably higher than in the normal tap water, which was consumed by control population. This data was significant because heavy metals, especially Cd, are known to adsorb solid particles in waste and these are attached to the skin during working periods. As the isoelectric point of skin is negatively charged, heavy metals can get attached to skin and micro amounts can penetrate skin as suggested by some workers [19]. They may also be ingested as due to lack of awareness; the workers take their food or sometimes rinse their mouth without properly washing their hands.

It is noteworthy to mention here that though tap water samples handled by control population have heavy metal below detection limit of 0.001 ppm and control population selected was nonaddicted, had no history of routine drug

Table 4: 95% confidence intervals.

Factors	Control and workers working <20 years	Workers, working <20 years and workers, working >20 years,	Control and workers working >20 years
Pb	(−0.262, −0.109)	(−0.250, −0.053)	(−0.42, −0.24)
Cd	(−0.013, −0.008)	(−0.008, −0.004)	(−0.019, −0.103)
Fe	(41.626, 101.173)	(211.438, 288.241)	(285.531, 356.940)
Zn	(3.985, 5.794)	(−8.826, −6.493)	(−3.854, −1.685)
TM	(−2.531, −1.868)	(−2.547, −1.692)	(−4.717, −3.922)

Table 5: Further measurement of elements.

Factor	Control initial (n = 57) 1st reading	Control (n = 57) 2nd reading	Workers, working <20 years, n = 36, 1st reading	Workers, working <20 years, 2nd reading	Workers, working >20 years, n = 21, 1st reading	Workers, working >20 years, 2nd reading
Pb (M ± SD) (mg/L)	0.132 ± 0.093	0.133 ± 0.092	0.318 ± 0.12	0.319 ± 0.123	0.47 ± 0.36	0.4780 ± 0.366
Cd (M ± SD) (mg/L)	0.0032 ± 0.004	0.00325 ± 0.004	0.0141 ± 0.004	0.0143 ± 0.005	0.02 ± 0.01	0.021 ± 0.0087
Fe (M ± SD) (mg/L)	1998.52 ± 52.66	1998.57 ± 52.56	1927.12 ± 74.04	1928 ± 74	1677.28 ± 99.94	1677.47 ± 99.86
Zn (M ± SD) (mg/L)	31.5 ± 1.80	31.44 ± 1.811	26.61 ± 2.23	26.65 ± 2.24	34.27 ± 2.74	34.29 ± 2.71

There is no significant difference between the 1st reading and the repeated 2nd reading of the same population group for any concern element.
Limit of detection for Pb and Cd is 0.001 ppm (mg/L).
Limit of detection for Fe and Zn is 5 ppm (mg/L).

intake and occupational hazards, Pb concentration in their blood samples was far above the detectable limit. This could be due to the fact that control population samples taken were from urban areas residing of south Kolkata where air pollution was found to be higher [20]. Previous Studies have already pointed out that, in South East Asian countries, nonoccupational exposure to Pb is high due to dietary habit and due to air pollution [21]. During blood collection as part of work, thorough counselling was performed. The control populations were free of any kind of extra xenotoxic compounds through food. They were not exposed to any extra automobile exhaust as all were strictly from residential areas and far from highways. The higher concentration of Pb in blood samples of drainage workers who did not have the history of an addiction was still higher than that of the control population and could be due to the fact that the drainage or sewage water sample contained higher Pb concentration which could attach and penetrate through the skin [22], which may also enter the body through oral routes when food is ingested without proper hand washing.

The present study has shown high Cd concentration in the blood of workers possibly because of their working exposure. This is possible as the water they handled is contaminated by Cd and, during their working hours, they took food without washing their hands. In case of control population (49%), though it was significantly less than in the exposed workers, Cd concentration was found to be above the detection limit. The cause of this was unclear though ingestion through food or air is a possibility [14].

In case of the exposed aged workers (workers working > 20 years), blood Fe level was significantly lower than that of the control population (Table 3). This may be owing to the high concentration of Pb and Cd level in the blood. Workers, working >20 years, had minimum Fe concentration compared to the workers with less degree of occupational exposure. It has been reported earlier that persons have less Fe capture capacity when the Cd levels are high. The higher blood Cd concentration of the workers, especially for the older group, may thus be related to the lower Fe concentrations in blood. So, this less Fe concentration may be due to their high Cd concentration [23].

In case of the older working group (working > 20 years), the correlation coefficient of Cd with comet assay TM showed negative correlation. A probable reason for this result may be linked to their high blood Zn concentration. Since Cd and Zn belong to the group IIB transition elements and show similarities in chemical and environmental properties, Cd and Zn (IIB transition elements) have a similar electronic configuration and valence state, possessing equal affinities for sulphur, nitrogen, and oxygen ligands. Further these two elements have a metal-metal interaction. It has already been established that the antioxidant properties of Zn may counteract Cd toxicity. In the present study, it was observed that older workers (working > 20 years) had higher Zn concentration in blood (Table 3). The detoxification and/or bonding of Cd by Zn may be predominant in this group of population. This may account for the negative correlation with the DNA breakdown as evident from the results of the comet assay. In the case of aged workers, the condition may be considered as a balancing factor. On one hand, due to lower blood Fe level, they can accumulate more Cd and, on the other hand, due to the high blood Zn concentration, the detoxification of Cd was high. This situation leads them to a very interesting position for further study as it put many research scopes for future assessment of health status of sewage workers, especially for aged groups.

The DNA integrity of an organism is fundamental to its survival and the DNA is susceptible to stresses induced by exposure to genotoxic contaminants [24]. Raw wastewater is a complex mixture of organic and inorganic compounds that also include hazardous substances [25]. This recent finding supports the reports of other genotoxins such as polyaromatic hydrocarbons (PAH) that induce DNA lesions in the living organisms [26]. It is assumed that there is a chance of genotoxicity by the presence of Pb and Cd among the sewage workers.

Owing to increased awareness and stringent Government regulations during the past decade, the risk of health problems for conservancy workers due to occupational exposure has decreased significantly. However, continuous monitoring is essential to achieve continued wellbeing of municipal conservancy workers, especially for the workers with continuous and long term association with conservancy work. In this context, our work supports the need for awareness with regard to the heavy metals with a potential for genotoxicity and presents a simple approach to detect the consequences of such exposure.

5. Conclusion

Sewage workers provide an essential service in the protection of public and environmental health. However, they are exposed to varied mixtures of chemicals; some of which are known or suspected to be genotoxins or carcinogens. This genotoxicity study helps to know the health hazards of sewage workers. Due to occupational exposure, sewage workers have high blood Pb and Cd level that may be responsible for DNA damage. Further they have altered Fe and Zn concentrations in their blood. More susceptible groups are younger (i.e., working < 20 years) compared to aged groups (i.e., working > 20 years). The younger groups have lower blood Zn levels in comparison to aged workers. Thus an effective counterbalance to Cd is poor. This would lead to a more vulnerable situation with regard to DNA damage. This is an initial report, and further work on detailed health status including xenotoxicity study is being carried out.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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