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High Frequency of Micronuclei in Buccal Mucosa of Women Residing Near a Sewage Disposal Drain in Amritsar

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INTRODUCTION

Water resources, the world over, are depleting as well as being contaminated by industrial pollutants, sewage waste, pesticides and fertilizers.

In India, 4000 cubic km of water is received annually from precipitation. Only half of it is left on land for use and rest is lost through evaporation and plant transpiration. India has 1100 cu. Km of utilizable surface water sources and nearly 900 cu km of groundwater (Pachauri, 1999). The surface water has a continuous exchange with atmosphere of dissolved atmospheric gases and can also get polluted by water run-off, industrial discharge and municipal waste.

The groundwater sources like water springs, deep or shallow wells or aquifers are the major source of drinking water in rural India. The groundwater was assumed to be impervious to pollution as soil would bind the chemicals and cleanse the water as it percolated through. But uncontrolled extraction of water without commensurate recharge, leaching of pollutants into aquifers from industrial and municipal solid wastes, pesticides and fertilizers have resulted in pollution of groundwater. A higher proportion of dissolved constituents have been found in groundwater than in surface water due to greater interaction of groundwater with various minerals in geologic strata (Tyagi et al., 2000, p174-181). In India, few municipalities and cities have a proper water treatment and sewage disposal arrangement but villages in rural India lack these arrangements. The domestic waste water is collected and discharged into various water sources and land, impairing the quality of life.

There are documented reports of contaminated water adversely affecting human health (Botkin, 1995; Chhatwal, 1996). In India, ground-water contamination by metals and having high bacteriological load has been reported in twenty two industrialized zones (TERI Report, 2000) From North India, reports from the Industrial town of Ludhiana, Punjab have indicated a high intake of heavy metals in people using ground water (Chhatwal, 1996; Sangha et at., 2000, p387-389; Ahuja et al., 2000, p351-354).This has caused increased incidence of cardiovascular disease, anaemia and reproductive failure.

The present study was conducted at Amritsar (Punjab), a city in North India located between 31^0N and 75^0E. The study aimed at assessing the genotoxic effect of exposure to polluted ground water.

Amritsar has many small scale industries notably the textile processing woollen cloth dyeing, electroplating, iron foundries, Pulp and Paper mills, Milk products, glass and plastic ware. The city has open and underground sewerage system. The effluents of industries are discharged into two main drains, the Gumtala drain (carrying paper mill and textile processing mill effluents), and the Verka drain (carrying milk plant, iron foundries and woollen dyeing mill effluents). These drains together fall into a main drain, the Tung Dhab drain. It is 20 km long, has catchment area of 80.63 sq. miles, capacity of 31 cusecs, bed width of 45ft (at out fall) and 4ft (at starting point). The drain also receives sewage water of Amritsar city. The main municipal sewage disposal site is located 7.5 km from city Railway terminal, 1 km from the University campus and 0.2 km from a village, Mahal, located to the west of Amritsar city. Mahal is spread along the right bank of Tung Dhab drain covering a total area of 30,000 sq. m. It is inhabited by 6002 people. Most of them are from low socio-economic class, only 27% males are regular earners and only 4% females work.

The village was selected for sampling for the present study, because the villagers depend on groundwater from shallow hand pumps (depth 60-90 ft) for domestic purpose and even use the water of Tung Dhab drain directly for irrigating their fields. The village has no sewerage network. The villagers mainly defecate in open, only some have pit- latrines in their houses. The rain and sewerage water flows through open drains and terminates at either the village pond or in agricultural fields around the village. The extracted ground water in the village is rusty in colour, bitter in taste and stains the utensil it is kept in. It is likely the ground water is being contaminated by field run-off, domestic waste and pit effluents and seepage from Tung Dhab drain as similar contaminations have been reported in previous studies at other sites (Paliwal, 1991,p36-60; Botkin et al., 1995; Jain et al., 2000, p641-648; Pandey et al., 2000, p405-411).

The water analysis for some physico-chemical parameters was carried out of water samples for Tung-Dhab drain and hand-pumps of village Mahal. It showed an increased concentration of heavy metal like Zn, Pb, Cd, Cu, Cr, increased alkalinity and hardness and high bacterial load. The water analysis findings are given in table 1 as per four studies done between 1994 and 2001 (Mahajan et al, 1994,p818-820; Dhillon et al, 1997,p613-616; Singh 2000, Dhami et al, 2001,p369-373). As heavy metal have long half life, there could be accumulation of heavy metals in the people using contaminated water over a long period of time (Bolognesi et at., 1999, p287-292).

The present study was conducted to find any genotoxic effect of a consistent exposure to polluted water, having higher concentration of heavy metals, in people living near a sewage disposal drain.

MATERIAL AND METHOD

The subject were selected by Random sampling. The study group consisted of 150 women residents of village Mahal. These included 62 women who had been living there since birth and 88 women who migrated to Mahal, from areas within 100 km of Amritsar city, after their marriage to a male resident of Mahal. These women used ground water for all their household purposes and being housewives had a continuous exposure to the groundwater of the village. They were non-alcoholic, non-smokers and did not take any other intoxicants.

The control sample consisted of 100 women selected from Amritsar city. These women had municipal tap water for drinking and household purpose and did not reside near a sewage disposal drain or industrial unit.

The subject and controls were in age group 15-40 years and had same socio-economic status. Healthy women, who were not taking any medication for atleast two years before date of sampling were included in the study.

The buccal mucosol micro-nucleus assay was selected as a non-invasive method for studying genotoxicity. Mircronuclei reflect cytogenetic anomalies in dividing cells of basal epithelial layers and survive during passage of cells from basal layers to surface of mucosa of oral cavity.

The sample was collected between May to July, 2001. The individuals were made to rinse their mouth with water. The buccal mucosal cells were scrapped from inner side of cheek with a sterilized spatula. First scrapping was discarded. The buccal cells taken from deeper, second scrapping were collected in sterilized containers

Table 1: Water analysis report of ground water of Mahal

S. No.	*Parameter*	*Range in ground water sample of Mahal*	*WHO standard for drinking water*
1.	MPN/100ml of water	11-28	1
2.	pH (mg/l)	3.73-7.38	9.2
3.	Hardness (mg/l)	262-406	500
4.	Chloride (mg/l)	36-140	600
5.	Alkalinity (mg/l)	390-530	-
6.	Sodium (mg/l)	105-125	No guideline value set
7.	Acidity (mg/l)	30-116	-
8.	TDS (mg/l)	102-124	1000
9.	Conductivity (μmhos/cm)	155-187	-
10.	Heavy metal concentration in mg/ml		Concentration in mg/l
(a)	Zn	0.36	3.00
(b)	Cd	0.015	0.003
(c)	Pb	0.040	0.010
(d)	Cr	0.060	0.050
(e)	Ni	0.095	0.020
(f)	Mn	0.370	0.500
(g)	Cu	0.20	1.00

having 8 ml phosphate buffered saline (PBS). The standard protocol (Stich et al., 1983, p93-105) was followed with slight modifications. The cell sample was washed thrice in PBS by centrifuging each time at 1200 rpm for 10 min. From the cell pellet obtained, smear preparation were made on pre-cleaned slides. The slides were fixed in absolute Methanol for 20 min followed by staining in 0.25% May-Grunwald's stain for 8 min. The slides were then rinsed in distilled water and counterstained with 1% Giemsa for 5 min.

Each slide was coded and scored blind. For each subject, 500 cells were scored. The scanning was done at low power and the presence of micronuclei was confirmed at 100x under oil immersion. The student's 't'-test was used for statistical analysis.

RESULT

The MN/cell frequency was 0.0101 ± 0.006 in the women resident in Mahal. In the control subject, the MN/cell frequency was 0.0013 ± 0.0001 (Table 2). The resident of Mahal had significantly much higher frequency of micronucleated cells. A comparison was made between MN/cell in different age groups of subjects and controls (Table 3). The MN frequency was significantly higher in all women residents of Mahal as compared to control women for all age groups. There was a non-significant increase in MN frequency with increasing age of subjects.

To assess the impact of a prolonged stay in the village, hence a chronic exposure to the same ground water, the MN frequency with reference to duration of stay was calculated (Table 4). The women who had migrated to Mahal after their marriage and had a shorter stay in village (upto 5 years,) had significantly lower MN frequency (0.0042 ± 0.0039) , as compared to women living in the village since their birth (40-45) years, (0.0172 ± 0.0049). There was nearly a linear increase in the MN frequency with increasing duration of stay in the village. Women with more than 10 years of stay in the village had significantly higher frequency than women with shorter stay. The women who had a similar duration of stay in the village (15-25 years) irrespective of being a migrant or living in village since birth had non-significantly different MN frequency (Table 5).

DISCUSSION

The results showed an increased frequency of Micronucleated Buccal mucosa cells in women residents of Mahal village. The increase was positively correlated with the duration of stay in the village. The MN frequency was high in all the women who had been living in the village for more than ten years whether it was since birth or having migrated to village in their youth

Table 2: MN/Cell frequency of the study group and control group

S. No.	*Group*	*Number of subjects*	*MN/Cell Frequency and S.D.*	*Statistical analysis by 't' test*
1.	Residents of Village Mahal	150	0.0101 ± 0.006	Significant (at 5% level)
2	Control from Amritsar City	100	0.0013 ± 0.0001	

Table 3: Comparison of MN/Cell frequency with reference to age of subjects and controls

S. No.	*Age group (years)*	*Number of Subjects*	*Number of Controls*	*MN/Cell frequency*	*Statistical Analysis*
1.	15-20	41		0.0107 ± 0.006	Significant difference between subjects and controls in all age groups.
			25	0.0012± 0.0004	
2.	20-25	20		0.0071 ± 0.004	
			12	0.0016 ± 0.0001	
3.	25-30	23		0.009 ± 0.003	
			15	0.0021 ± 0.003	
4.	30-35	28		0.01178± 0.008	
			19	0.0061 ± 0.0012	
5.	35-40	16		0.00756± 0.005	
			12	0.001 ± 0.0001	
6.	40-45	22		0.0128 ± 0.004	
			17	0.0039 ± 0.0006	

Table 4: Comparison of MN/ Cell frequency with reference to duration of stay in the village

S. No.	*Range of duration of stay in village Mahal (in years)*	*Number of subjects*	*MN/Cell frequency and S. D.*	*Statistical analysis*
1.	0-5	9	0.0042 ± 0.0039	Significant difference at 5% level between group 1 and 2, 1 and 9, 1 and 8 Non significant difference between group 2 and 3, 3 and 4, 4 and 5
2.	5-10	17	0.0092 ± 0.0034	
3.	10-15	37	0.0085 ± 0.0043	
4.	15-20	56	0.0105 ± 0.0065	
5.	20-25	20	0.0106 ± 0.0077	
6.	25-30	2	0.012 ± 0	
7.	30-35	1	0.012 ± 0	
8.	35-40	3	0.0093 ± 0.0023	
9.	40-45	5	0.0172 ± 0.0049	

Table 5: Comparison of MN /Cell Frequency with reference to similar duration of stay

S. No.	*Duration of stay (in yrs.)*	*Category*	*Age group (in yrs)*	*No.of subject*	*MN/Cell ± S.D.*	*Statistical analysis*
1.	15-20 .	Born in village	15-20	41	0.0107 ± 0.006	Non Significant differences in group 1and 2 between both categories
		Migrant	29-40	15	0.0105 ± 0.005	
2.	20-25 .	Born in village	20-25	9	0.0911 ± 0.004	
		Migrant	20-41	11	0.0106 ± 0.008	

after marriage.

These village women did not have any direct exposure to any industrial pollutant as there was no industrial unit in immediate vicinity of the village. These women also did not directly handle any agricultural chemicals. They were only using the polluted ground water for drinking and household purposes. As the Tung Dhab drain carries foul smelling, murky, polluted water with effluents from various industries, it is probable that some pollutants have seeped into the village aquifer by lateral and vertical flow in and around the Tung Dhab drain. The consumption of contaminated water has been reported to induce DNA damage.

As even minute quantities of heavy metals can induce genetic damage (Berces et al., 1993) the consistent use of heavy metal contaminated water by residents of Mahal might have resulted in increased DNA damage in the basal epithelial layer. An elevated DNA damage has been reported due to consumption of water contaminated by Arsenic (Warner et al., 1994, p583-590), Chlorohydroxy furanone found in chlorine treated water (Holme et al., 1999, p145-153; Curiex et al., 1999, p457-462), Fluorine (Li et al., 1995, p1468-1474), heavy metal like As, Cd, He, Se, Pb, Hg, Te, TI (Berces et al.,1993; Moore et al., 1997, p31-36; Migloire et al., 1999, p279-294). A high concentration of heavy metal like, Pb, Ni, Fe, Cu and Co has been reported in girls of age group 7-9 years using sewage contaminated water from the industrial town of Ludhiana, Punjab (Sangha and Kaur, 2000, p387-389).

The appraisal of the literature has not revealed any direct or indirect study from view point of genetic damage on the effect of residing near waste water drain and using the underground water resources therein. Our preliminary study has indicated a genotoxic effect caused by the groundwater being contaminated due to the wastewater drain. The long term use of such water can have a deleterious effect on the health of the residents as heavy metals are stable and persistent environmental contaminants. A chronic exposure to sublethal doses can lead to long-term adverse effects. Therefore the population residing near sewage disposal sites need to be educated about the effect of these contaminants on their health.

KEYWORDS Urban Fringe. Polluted Groundwater. Heavy Metals. Genotoxic Effect.

ABSTRACT The study was conducted at a village, Mahal, an urban fringe of Amritsar city in North India, located adjacent to main sewage disposal drain, the Tung Dhab drain, of Amritsar. The drain carries largely untreated industrial effluents and Municipal Sewage. The water of Tung Dhab drain and groundwater of village Mahal is hard, more alkaline, has very high bacteriological contents and higher concentrations of heavy metals like Cr, Pb, Ni, and Cd, and comparatively lower level of Zn, Mn and Cu. As the villagers depend entirely on groundwater from shallow hand pumps for their water requirement, the genotoxic effect, if any, of a consistent exposure

to polluted water was assessed using buccal mucosal MN assay. 150 women randomly selected from village Mahal had significantly high MN/cell frequency (0.0101±0.006) as compared to 100 age matched controls (0.0013±0.0001) selected from Amritsar city. The MN frequency was higher in women having longer duration (25-45 years) of stay in the village than women having a shorter duration (5-10 years) of stay in the village. The results indicate a DNA damage being caused by a chronic exposure to polluted water having heavy metals.

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