RESEARCH ARTICLE

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Ground Water Arsenic Causes Premature Senescence in Population from Gangetic Basin, India

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Abstract

Groundwater arsenic is the main public health concern in the Indo-Bangladesh Gangetic basin. Much work has been done on the carcinogenic effect of arsenic. Compared to that other cellular effects, cellular senescence in the human system was not studied. Replicative senescence that occurs by the gradual shortening of telomeres, and other cellular changes, is characteristic of human somatic cells. Premature senescence, characterized by increased beta-galactosidase activity; abnormal decrease in telomere length may also be the effect of arsenic. The objective of this study was to know whether premature senescence in the human cellular system can be induced by arsenic. Human cases were selected from the arsenic-affected district (Murshidabad), and controls were taken from the unaffected district (East Midnapore) of West Bengal. Senescence-associated beta-galactosidase in cell and telomere length measured by Southern blotting by DIG-labelled chemiluminescence method was conducted. The result indicated that arsenic causes premature cellular senescence in the human system.

Keywords: Arsenic, SA-beta-Gal, Senescence, Telomere, Terminal Restriction Fragment

1. Introduction

Arsenic is a major human carcinogen, which has caused severe health hazards in many countries across the world[1]. The Gangetic basin, including both India and Bangladesh, are reported to be suffering from much higher level of arsenic in ground water. The concentration are detected alarmingly more than the permissive level recommended by US EPA[2]. Arsenic is already known as potent carcinogen and major reason for other multi-systemic health issues[3]. However, there is a dearth of information and knowledge about effects of arsenic at cellular level, such as premature cellular senescence.

Telomeres, the terminal part of the chromosomes, play an important function in cellular ageing, cell cycle, and cell death process. It also maintains cellular integrity and ensures chromosomal stability. Thus, if any environmental or internal factors affect the chromosomal ends, it will have a direct influence on cellular health and integrity. The length of the Terminal Restriction Fragment (TRF) of chromosomes with (TTAGGG) repeats acts as a signal for healthy chromosomes or an apoptotic signal in mammalian cells. The inherent process of DNA replication causes a sequential loss of telomeric end in normal human cells. Once a chromosome reaches a critical length of TRF, it signals for either senescence or apoptosis as a natural course[4-8]. However, if this process is abnormally enhanced under stress or some external factors, cells senesce too early to be recognized as normal. This study aimed to decipher whether arsenic is affecting chromosomal ends and other senescence-related gene expression patterns inducing premature or rapid cellular senescence in the human population or not.

2. Materials and Methods

2.1 Study Design and Sample Collection from Arsenic Affected and Unaffected Districts

Murshidabad, one of the most affected districts of West Bengal, is well documented for its high arsenic level in ground water was selected from where blood samples of cases were collected[3]. East Midnapore was selected as unaffected district to recruit control individuals. A questionnaire and consent form approved by Institutional Ethical Committee was recorded for each individual. The questionnaire included information on age, gender, drug use, socio-economic status, source and amount of drinking water. The cases and controls were well

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matched in accord to socio-demographic characteristics and ethnicity. This Pilot Study was conducted where 21 cases and 21 controls were recruited as study subjects. A Table showing age, gender and actual TRF Length of each study subject was added for better comparison (Table 1) Furthermore, the total study subjects were divided into three major age groups (15≤35, 35≤50 and 50≤65) stratifying the population to compare the TRF length in different age levels.

2.2 Isolation of Peripheral Blood Leucocytes

Five ml of blood was mixed with an equal amount of PBS. Then the mixture was poured onto five ml of histopaque and centrifuged at 1000rpm for 45 mins. For proper lymphocyte separation, the pellet was washed several times with PBS.

2.3 Cell Viability Assay by Trypan Blue Exclusion Method

Random sampling from cases and controls was collected to study the Trypan Blue assay. Lymphocyte suspension of 10^6 cells/ml concentration was prepared and mixed with 1:1 dilution using 0.4% trypan blue solution[9]. The mixture was loaded on a hemocytometer and was kept for 1-2 minutes. Then, stained dead cells and a total number of cells were counted. Unstained cells represent the percentage of viable cells.

2.4 Staining for Senescence Associated-Beta-Gal

Lymphocytes from cases and controls were stained with Senescence Associated-beta-galactoside to study their enzymatic activity. Cells fixed on the slide were incubated in a buffer and treated as per the protocol mentioned elsewhere[8]. These slides were incubated overnight at 37°C. Slides from both cases and controls were stained with eosin as a background stain and were studied under the microscope. Enzymatic activity of SA-beta galactosidase results in blue-green precipitate in the cells. Those cells showing blue-green colours were counted[8].

2.5 Genomic DNA Isolation by Phenol-Chloroform Method

Genomic DNA was isolated from the peripheral blood leukocytes according to the standard Protease K digestion followed by phenol-chloroform extraction procedure[9]. Purified DNA was spooled from the aqueous phase and partially dried in a vacuum. The isolated DNA was dissolved in 200 to 500 µL of TE (pH 7.2) buffer, checked for purity (260/280nm ratio) and quantified (OD at 160nm) spetrophotometrically[9].

2.6 Telomere Length Assay

Purified Genomic DNA (5 µg) was treated with restriction enzymes *Hin*fI and *Rsa*I to get terminal TRF[9]. Later, Southern Blot was carried out using DIG-labelled TTAGGG (Roche, USA). Hybridisation was carried out at 42°C for 3 hrs in a hybridisation buffer followed by three 5 min washes with 1 mcg SSC/0.1% SDS, and then three 15 min washes with 0.1 mcg SSC/0.1% SDS[9]. Hybridisation signals were detected by chemiluminescence and quantified by computer-assisted scanning densitometry using the public domain NIH Image software (version 1.62, available at http://rsb.info.nih.gov/nih-image). The mean TRF lengths were calculated by integrating the signal intensity over the entire TRF distribution as a function of TRF length using the formula TRF length=∑(ODi) / ∑ (ODi/Li), where, ODi and Li are the signal intensity and TRF length respectively at position i on the autoradiogram.

3. Results and Discussion

The Senescence Associated beta gal expression in lymphocytes showed that cases were having more senescent cells (by the presence of greenish blue coloured cells) compared to the control population indicating a higher level of cellular senescence due to arsenic exposure. When this study was stratified into three sub groups of ages the same results were reflected (data not shown). However, an overall difference in cell count in cases and controls suggested the effect of arsenic in cases causing more senescent cells (Figure 1).

Telomere length assay showed that telomeric reduction is more rapid in cases compared to control group, which again corroborated with previous data that arsenic damages chromosomal ends ensuing premature cellular senescence (Figure 2). The total study samples were divided in three age groups (15≤35, 35≤50, and 50≤65), stratifying the population. Among the three age groups, the result was reflected in middle aged and older groups more prominently (Table 1, Figure 3).

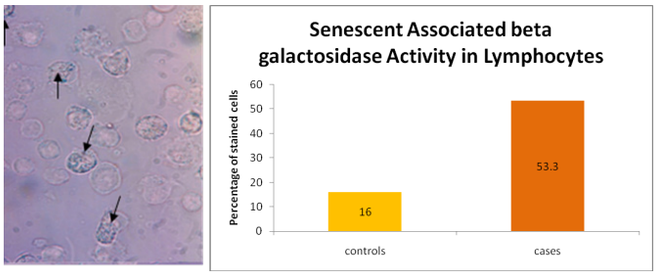


Figure 1. Frequency of senescence associated beta gal-stained cells.

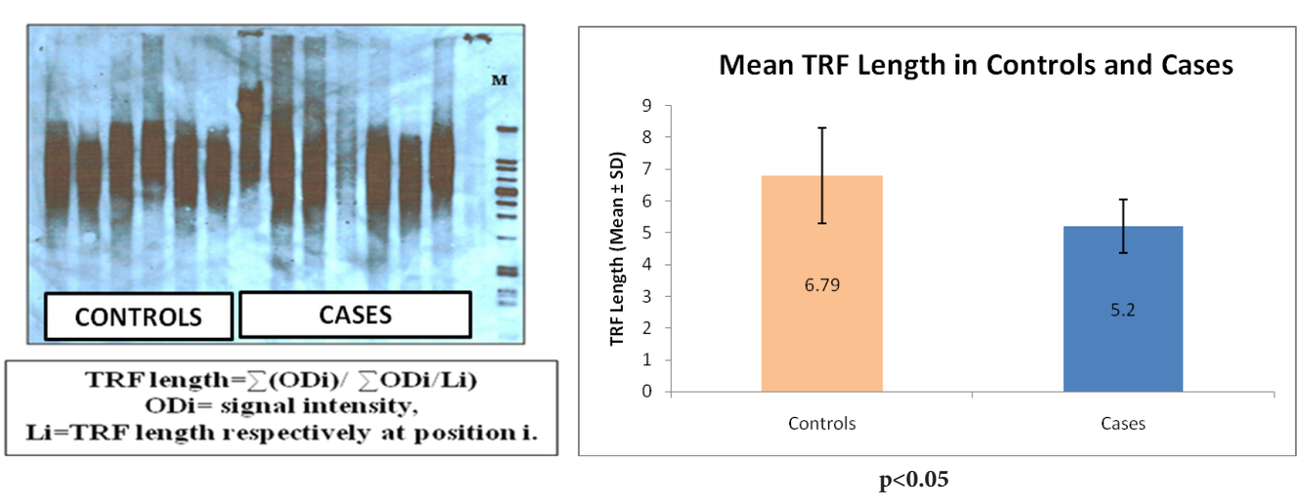
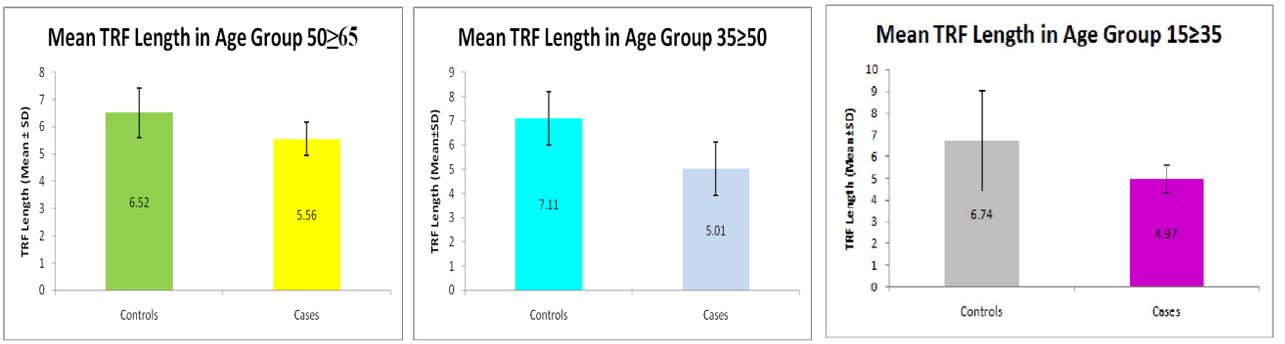


Figure 2. Representative Chemiluminescene data and graph showing the difference in average length of Telomric end in controls and cases.

Table 1. Table showing TRF length (Mean ± SD) in different age groups

Controls	Actual TRF Length	Cases	Actual TRF Length
Age Group 50-65	(Mean ± SD) 6.52±0.91	Age Group 50-65	(Mean ± SD) 5.56±0.61
62M	6.98	50F	6.49
60M	7.74	50F	5.96
60F	6.19	55M	5.75
60F	6.17	60M	4.68
60M	5.68	60F	5.398
66M	7.5	65M	5.64
62F	5.35	73F	4.987
Age Group 35-50	(Mean ± SD) 7.11±1.1	Age Group 35-50	(Mean ± SD) 5.01±1.1
36M	6.98	40F	6.024
43M	8.83	38F	5.44
41F	8.27	42F	3.6
45M	6.85	47M	4.67
35F	5.995	43F	4.3
36F	6.95	48M	6.022
45F	5.9	43M	4
		38M	6.5
Age Group 15-35	(Mean ± SD) 6.74±2.3	Age Group 15-35	(Mean ± SD) 4.97±0.66
27M	6.84	14F	3.79
28M	6.8297	16M	5.5
19F	3.39	16M	4.72
18M	6.29	24M	5.3
27F	6.37669	24F	5.54
32M	11.579	21M	4.989
26F	5.88		
TRF Length (Mean±SD)	6.79±1.5		5.2±0.83



p<0.05 p<0.05 p<0.05

Figure 3. The mean TRF length in cases and controls among different age groups.

This also shows that chronic exposure to arsenic in the long-term basis damages the telomeric end of chromosomes and cellular integrity. The less prominent effect in younger group suggested that long term ingestion of arsenic infested drinking water is required to demonstrate the marked difference on chromosomal ends. This pilot study consisting 21 cases and 21 controls were conducted to decipher any trend of cellular senescence induced by arsenic. Now, once the trend is prominent, we have a scope to increase the population size to check on epidemiological standard. A more detailed study can also be done to discover the actual molecular procedure involved in the process. At the end, this study showed that arsenic is damaging the telomeres and ensuing early senescence at cellular stage. The effect is more prominent in middle and older age group showing chronic or long-term exposure is the key issue in the overall degradation in cellular integrity.

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