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RESEARCH PAPER

Effect of Arsenic Exposure on Human Telomerase Reverse Transcriptase (*hTERT*) Gene Expression and Telomere Length in Cardiovascular Disease Susceptibility

Mohammad Al-Forkan[1], Md. Omar Hasan Chowdhury[1], Rahee Hasan Chowdhury[1], Fahmida Binta Wali[1], Amit Datta[1], Md. Nezam Uddin[2], Md. Jibran Alam[1], Laila Khaleda[1]

[1]Department of Genetic Engineering and Biotechnology, Faculty of Biological Sciences, University of Chittagong, Chittagong, Bangladesh; [2]Department of Medicine, Marine City Medical College, Chittagong, Bangladesh

Abstract

Background: The deleterious impact of arsenic (As) contaminated groundwater on human health has been reported worldwide. Epidemiological studies have identified adverse association of arsenic exposure with the risk of cardiovascular diseases (CVD). Telomere dysfunction is emerging as an important factor underlying the pathogenesis of various cardiovascular complications.

Objective: The aim of the study was to investigate the effect of arsenic exposure on human telomerase reverse transcriptase (*hTERT*) gene expression and telomere length in arsenic-exposed cardiovascular disease patients of Bangladesh.

Methods: A total of 53 CVD patients from known As-affected and unaffected areas of Bangladesh and subjected to open heart surgery were recruited. Nail samples were collected and analysed for arsenic content as a biomarker of chronic exposure. RNA and DNA extracted from blood samples were used for *hTERT* expression analysis and telomere length measurement respectively, using real-time polymerase chain reaction.

Results: The patients from known As-affected areas (As-exposed patients group) showed approximately 9.7 fold higher expression of *hTERT* gene and approximately 1.4 fold higher telomere length than the patients from known As-unaffected areas (As-unexposed patients group). We found significant association of both *hTERT* expression (r= 0.407, *p*= 0.001) and telomere length (r=0.437, *p*=0.003) with as concentration in nail samples. Of the total study population, the coronary artery disease (CAD) patients in particular showed approximately 3.4 fold higher expression of *hTERT* gene and approximately 1.5 fold higher telomere length than the non-CAD patients group.

Conclusion: Our findings suggest that chronic arsenic exposure is positively associated with increased *hTERT* expression and telomere length in As-exposed CVD patients of Bangladesh and that this association in turn can influence the cardiovascular outcomes of prolonged arsenic exposure. We also suggest that As-induced CVD possibly adopts a mechanism that is different from that of As-independent CVD. Findings of this study will pave the way to unfold the mechanism behind As-induced CVD through more in-depth research.

Keywords: Arsenic, Cardiovascular disease, Telomere, hTERT

Introduction

Arsenic is considered to be one of the world's most hazardous chemicals.[1] It is a ubiquitous element found in the earth's crust that can enter in groundwater from anthropogenic or natural sources.[2] Increased concentration of arsenic in groundwater causes severe health problems for human. Over 200 million people all over the world are afflicted with arsenic-induced diseases.[3] Bangladesh and West Bengal are the worst affected areas. Arsenic has been listed by the International Cancer Research Agency as a category 1 human carcinogen, evidence of other health impacts including cardiovascular effects is yet to be studied extensively.[4]

Arsenic affects our body's different organs, e.g. liver, heart and kidney.[5] Arsenic exposure has been correlated to cancer, stroke, diabetes and lower chronic respiratory disorders.[6] Evidences from epidemiological

***Correspondence:** Mohammad Al-Forkan, Department of Genetic Engineering and Biotechnology, Faculty of Biological Sciences, University of Chittagong, Chittagong, Bangladesh*
e-mail: alforkangeb@cu.ac.bd
ORCID: 0000-0002-4284-2447

studies suggest that ingested inorganic arsenic (iAs) can adversely affect the cardiovascular system.[7, 8]

Human telomeres, formed by tandem repeats (TTAGGG) located at the end of the chromosome, play an important role in the maintenance of genomic stability and cellular life span.[9] The telomeres are shortened during each cell division due to "the end replication problem".[10] The principal protein that is responsible for telomere maintenance, human telomerase, consists of two major components: human telomerase RNA (hTR)- providing a template for the human telomeric repeat synthesis, and human telomerase reverse transcriptase (hTERT)- playing a catalytic role in the replication of linear DNA ends.[11] The strong carcinogenicity of inorganic arsenic is possibly connected to its interaction with the telomere length.[9] The mechanism behind arsenic-induced cardiac toxicity is still unclear.

There are some evidence that arsenic alters the telomeres: arsenic increased telomere attrition, chromosomal rearrangements, and apoptotic cell death in mouse embryos with short telomere growth.[12] On the other hand, *in vitro* studies showed that arsenic increased the activity of TERT growth.[13] In addition, TERT expression in people exposed to arsenic by drinking water (1"1000 ìg/L) in Inner Mongolia was highly correlated with both water and nail arsenic concentrations and the severity of hyperkeratosis, a common arsenic-related skin lesion growth.[14]

Inorganic arsenic is well known as a clastogenic compound. Telomerase expression and telomere length are associated with cell death triggered by iAs^{III}, through the development of reactive oxygen species as well as with effects induced by iAs^{III} on cell differentiation processes and cell growth rate.[15]

Chronic arsenic exposure has also been implicated in increased *hTERT* gene expression.[14] In a research carried out in Bangladesh, elevated hTERT mRNA expression was reported to be associated with As exposure which in turn modified the risk of cardiovascular diseases.[16] A positive correlation between high arsenic exposure and increased telomere length was observed among Bangladeshi arsenic exposed population.[17] Studies of human cord blood cells and human cell lines *in vitro* have shown that arsenic exposure can both increase and decrease telomerase activity and telomere length.[18] These apparently contrasting effects may be related to the arsenic dose. The combined effect of arsenic exposure, *hTERT* gene expression, and telomere length on As-induced CVD is still unexplored.

The aim of this study was to investigate the effect of chronic arsenic exposure on hTERT mRNA expression and telomere length in connection with modifying CVD susceptibility. We compared hTERT mRNA expression levels and telomere lengths between As-exposed and As-unexposed CVD patients groups for determining the extent to which these parameters are affected by prolonged exposure to arsenic and whether these effects modify the cardiovascular outcomes of chronic arsenic exposure.

Materials and Methods

Study subjects and sample collection: In this cross-sectional study, a total of 53 CVD patients were recruited who underwent open heart surgery at the department of cardiac surgery, Chittagong Medical College and Hospital, and National Heart Foundation Hospital & Research Institute, Dhaka, Bangladesh. Pre-operative nail and peripheral blood samples were obtained from each subject. For demographic and clinical information, questionnaires were provided to all participants. Ethical permission was taken from the Ethical Review Committee, Chittagong Medical College and Hospital. Each subject was informed about the study and written consents were taken.

Arsenic exposure assessment: Nail samples were analysed for As content by Hydride Generation Atomic Absorption Spectrophotometry (HG-AAS) at the International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b) using a Shimadzu AA-7000 Atomic Absorption Spectrophotometer (Japan).

DNA and RNA extraction from blood samples: Three mL of peripheral venous blood was collected from each patient for nucleic acid extraction. DNA was extracted by using standard phenol-chloroform method. Total RNA was extracted following TRIzol™ Reagent user guide.[19] Extracted RNA and DNA were stored at -80°C and -20°C, respectively.

Quantitative real-time polymerase chain reaction (qPCR) for analysing hTERT expression: The first strand cDNA synthesis was performed using the GoScriptTM Reverse Transcription Method (Promega, USA) with a total reaction volume of 30µl. The cDNA sample was amplified by the GoTaq® qPCR Master Mix Systems (Promega, USA) using the following primers: *hTERT*-2164S (5´-GCCTGAGCTGTA CTTTGTCAA-3´and *hTERT*-2620A (5´-CGCAAA

CAGCTTGTTCTCCATGTC-3´). The cycling conditions were 95°C for 300s, followed by 45 cycles of 95°C for 30s, 58°C for 10s, and 72°C for 40s. The *hTERT* mRNA levels were normalised to â-actin mRNA levels. As negative control, no template control (NTC) was used. Relative changes in *hTERT* expression was analysed by $2^{-\Delta\Delta C}$T method from qRT-PCR experiments using the expression of â-actin gene as the reference. The sample used as control was denoted as calibrator sample (from CVD patients from As-unaffected areas) and the samples from CVD patients from As affected areas were denoted as test sample. The ratio of the target gene expression was calculated in the test sample over the calibrator sample. This ratio is the expression fold change or relative quantification of gene expression. All the reactions were done in triplicates and average Ct (cycle threshold) values were taken for analysis.

Quantitative real-time polymerase chain reaction (qPCR) for telomere length measurement: Two master mixes of PCR reagents were prepared, one with the telomere (T) primer pair, the other with the single copy gene (S) primer pair. Thirty microliters of T master mix was added to each sample well and standard curve well of the first plate and 30 µl of S master mix was added to each sample well and standard curve well of the second plate. For each individual in whom the T/S ratio was assayed, three identical 20 µl aliquots of the DNA sample (35 ng/aliquot) were added to plate 1 and another three aliquots were added to the same well positions in plate 2. For each standard curve, one reference DNA sample was diluted serially in TE buffer by <"1.68-fold per dilution to produce five concentrations of DNA ranging from 0.63 to 5ng/µl, which were then distributed in 20 µl aliquots to the standard curve wells on each plate.

The composition of T and S PCRs were identical except for the oligonucleotide primers. The final telomere primer concentrations were: tel 1, 270 nM; tel 2, 900 nM. The final 36B4 (single copy gene) primer concentrations were: 36B4u, 300 nM; 36B4d, 500 nM. The primer sequences (written 52 '!32) were: tel 1, GGTTTTTGA GGGTGAGGGTGAGGGTGAGGGTGAGGGT; tel 2, TCCCGACTATCCCTA TCCCTATCCCTATCCCTATCCCTA; 36B4u, CAGCAAGTGGGAAGGTGTAAT CC; 36B4d, CCCATTCTATCATCAACGGGTACAA. The thermal cycling profile for both amplicons began with a 95°C incubation for 10 min to activate the AmpliTaq Gold DNA polymerase. For telomere PCR, there followed 18 cycles of 95°C for 15 s, 54°C for 2 min. For 36B4 (single copy gene) PCR, there followed 30 cycles of 95°C for 15 s, 58°C for 1 min.

Statistical analyses: For data analysis, the data was transformed by taking the log ($base_{10}$) to reduce the skewness evident in hTERT. The relationship between continuous dependent variables (hTERT mRNA expression) and independent predictor variables was evaluated using simple linear regression model and Spearmen correlation coefficients. Continuous variables were expressed as "Mean ± Standard Errors of Mean (SEM)" and categorical variables as percentages. Microsoft Excel, SPSS and RStudio were used to conduct statistical analysis. All reported *p* values are two-sided and values less than 0.05 were considered statistically significant. Same process was followed for telomere length measurement.

Results

A total of 53 CVD patients who underwent open heart surgery were recruited for this study. Of them 34 patients were from known As-affected areas (As-exposed group) and 19 patients were from known As-unaffected areas (As-unexposed group) of Bangladesh. The mean age of the patients from As-affected areas was found slightly higher (51.6±6.9 years vs. 46.4±11.3 years) than that of the patients from As-unaffected areas. But no significant difference (p=0.273) was found for the age of the two patient groups. The association of the occurrence of coronary artery disease (CAD) with arsenic exposure was found significant (p=0.014). Significantly higher As concentration was found in the nail samples of the patients from As-affected areas (391.2±264.7 ppb vs. 183.9±91.9 ppb) than in the patients from As-unaffected areas (table I).

Table I: Distribution of study population characteristics (n=53)

Variables	CVD Patients from As -affected areas (n=34)	CVD Patients from As-unaffected areas (n=19)	P value
Age (years)	51.6±6.9	46.4±11.3	0.273
Cases of Coronary Artery Disease (CAD)	27(79.4%)	8(42.1%)	0.014
Nail As conc. (ppb)	391.2±264.7	183.9±91.9	0.038

The values are shown as Mean±SEM (except where indicated otherwise).
The *p* values in 'bold' are significant (significance level is $p< 0.05$)

Relation of age with hTERT expression and telomere length: Higher *hTERT* mRNA levels and telomere length were found in patients with increasing age (figure 1). Statistical significance was found for the association of age with *hTERT* mRNA expression (p=0.005) (figure 1 A) but not with telomere length (p=0.525) (figure 1 B).

Relative expression analysis of hTERT gene and measurement of telomere length in patient groups: Relative expression of *hTERT* gene in As-exposed patients group compared to the As-unexposed patients group was calculated as fold changes by $ÄÄC_T$ method. The patients from As-affected areas showed approximately 9.7 fold higher (normalized with reference gene, *actb*) expression of *hTERT* gene than the patients from As-unaffected areas (figure 2).

Measurement of relative telomere length in As-exposed patients group compared to the As-unexposed patients group was also calculated as fold changes by $ÄÄC_T$ method. The patients from As-affected areas showed approximately 1.4 fold higher (normalized with reference gene, *rplpo*) telomere length than the patients from As-unaffected areas (figure 2).

Relation of As-exposure with hTERT expression and telomere length: The increase in the relative *hTERT* mRNA expression level and telomere length among study subjects were significantly associated with the concentrations of nail (figure 3) Spearman coefficient, r =0.407 and P=0.001 represents the significant association of *hTERT* expression with nail as concentration figure 3 (A). Similar significant association was also found for the telomere length with the concentration of nail as (r=0.437, p=0.003) (figure 3 B).

Relative hTERT mRNA expression levels and telomere length in patients with Coronary Artery disease (CAD): Among our total study subjects (n =53), 35 patients were diagnosed with CAD. Other 18 patients were diagnosed with various types of cardiovascular diseases other than CAD. Average expression of *hTERT* gene (Figure: 4) and telomere length (Figure: 4) in CAD patients group compared to the non-CAD patients group were calculated as fold changes by $ÄÄC_T$ method. The CAD patients showed approximately 3.4 fold higher (normalized with reference gene, *actb*) expression of *hTERT* gene than the non-CAD patients group. The CAD patients also showed approximately 1.5 fold higher (normalized with reference gene, *rpolp*) telomere length than the non-CAD patients group (figure 4).

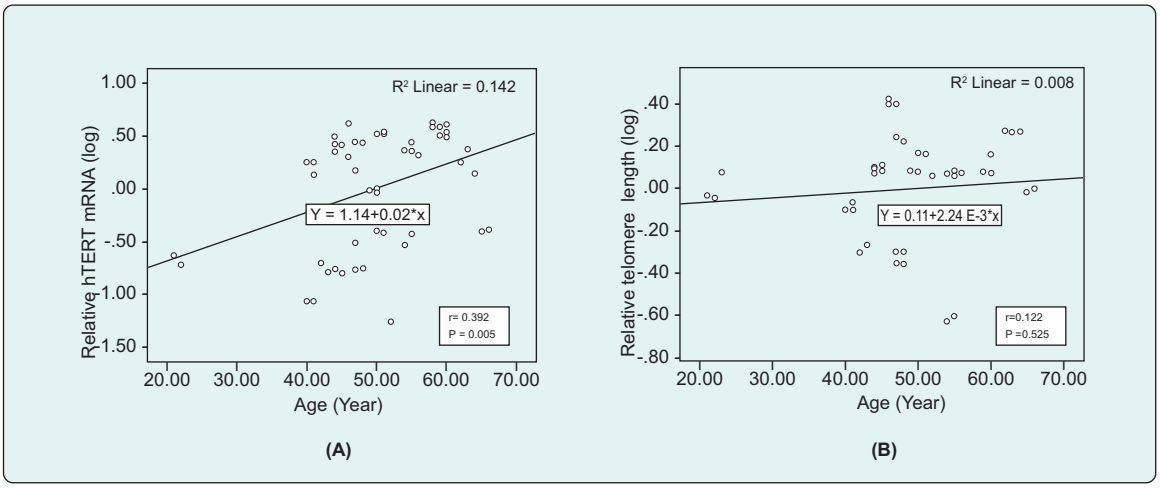

Figure 1: Association of relative *hTERT* mRNA expression levels (A) and telomere length (B) with Age. Here, *hTERT* expression levels and telomere length are shown as log transformed value. In figure (A), the trend line indicates significant association of higher *hTERT* mRNA levels with the higher Age (Simple Linear regression model: n = 53, r=0.392, p=0.005). In figure (B), the trend line indicates the association of higher telomere length with the higher age (r=0.122, p=0.525). Spearman correlation coefficient, r= 0.392 (A) and r=0.122 (B) indicate very weak correlation with age.

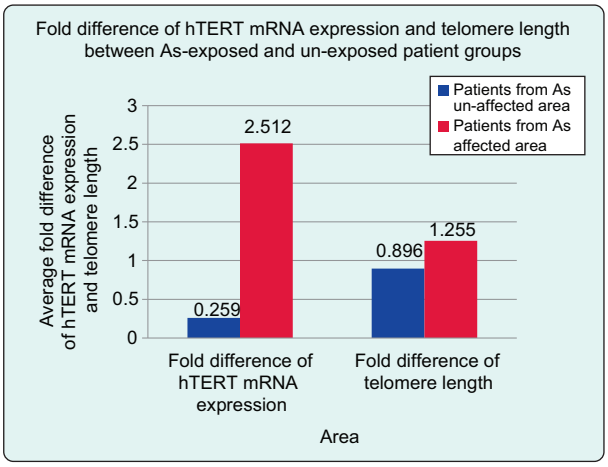

Figure 2: Average fold changes in *hTERT* gene expression and telomere length in the patients from As-affected areas in comparison with the patients from As-unaffected areas.

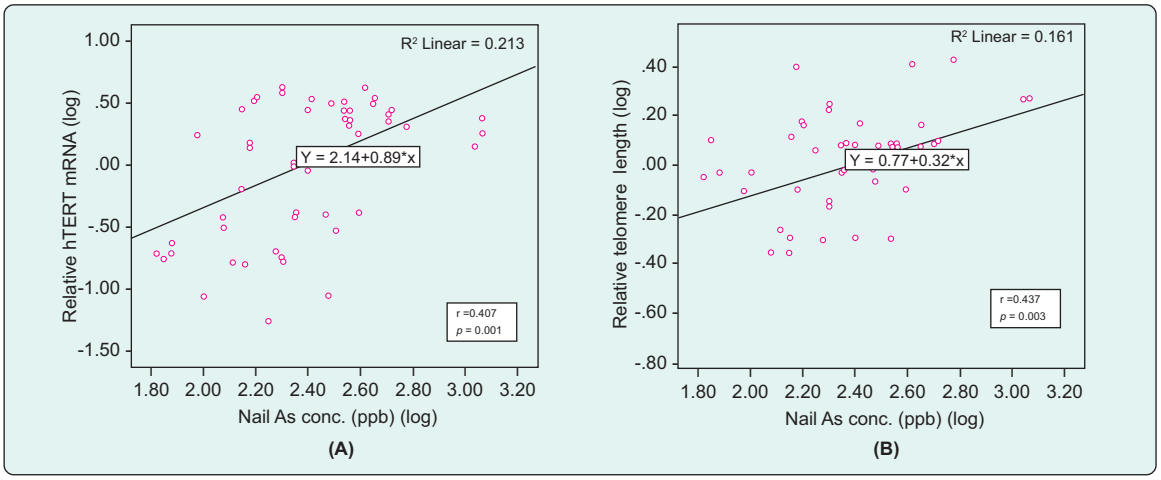

Figure 3: Association of (A) *hTERT* mRNA and (B) telomere length with nail as concentration. The trend lines in both (A) and (B) graphs indicate the significant association of higher *hTERT* mRNA levels (log) and telomere length (log) with the higher as concentration in nail samples.

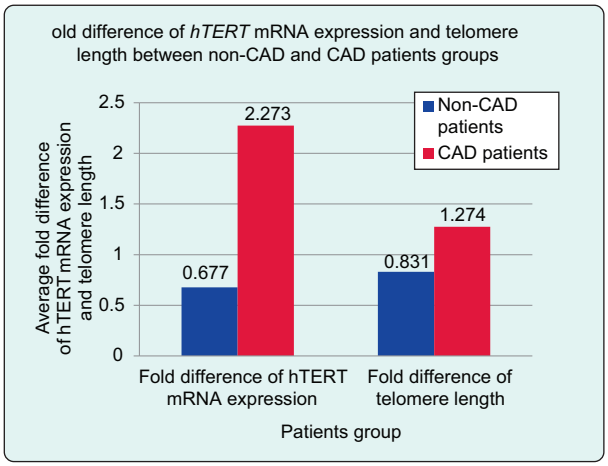

Figure 4: The average fold changes of *hTERT* mRNA expression and telomere length in the CAD patients in comparison with the non-CAD patients.

Discussion

Cardiovascular diseases are the leading cause of mortality worldwide and chronic arsenic exposure has been implicated in various subclinical and clinical outcomes of cardiovascular system including carotid atherosclerosis, hypertension, coronary artery disease etc.[20] The association between arsenic exposure and CVDs is an area of increasing research interest. Independently of age, telomeres may be associated with the initiation of CVDs and telomerase activity seems to be a key element in maintaining telomere integrity.[21] Arsenic may play a role on telomere length through up-regulation of telomerase (TERT) activity.[17] In our study, we observed a positive correlation of chronic As exposure with hTERT mRNA expression and telomere length through a comparative study of

As-exposed and As-unexposed CVD patients, suggesting their possible combined role in As-induced CVD pathogenesis.

Nail arsenic concentration is considered as a viable biomarker for the assessment of chronic arsenic exposure.[14,22,23] We found significantly higher As concentration in the nail samples of CVD patients from As-affected areas compared to patients from As-unaffected areas, clearly indicating long-term exposure.

Chronic arsenic exposure has been shown to increase *hTERT* gene expression in blood cells. A positive association has been found between *hTERT* expression and concentrations of arsenic in the nails.[14] hTERT mRNA expression in peripheral blood cells is positively correlated with As exposure in case of As-induced CVD.[16] In our study, we found significant association of *hTERT* expression with nail As concentration (r=0.407 and *p*=0.001) (figure 3 A). We also observed approximately 9.7 fold higher *hTERT* expression in the As-exposed CVD patients than in the As-unexposed patients. Our results seem to be supportive of previous findings.[14, 16] However, *hTERT* expression seems to behave the other way in case of CVDs not related to arsenic exposure since lower telomerase activity has been reported in hypertensive humans.[24] Our finding shows contradiction between As-induced and As-independent cardiovascular conditions.

Arsenic exposure has been shown to be associated with longer telomeres in peripheral blood.[9,17,25] We found a significant association between telomere length and concentration of nail As (r=0.437, *p*=0.003) (figure: 3 B). The As-exposed CVD patients showed approximately 1.4 fold higher telomere length than the As-unexposed patients. Studies have shown that CAD patients, not related to arsenic exposure, have shorter telomeres in circulating blood cells compared with control subjects.[26, 27] Another study showed that hypertensive patients had shorter telomeres than healthy subjects.[28] Those findings clearly suggested that the presence of shortened telomeres is a major anomaly in atherosclerotic coronary diseases, which is clearly not the case with our study subjects.

However, in comparison to the circulating leukocyte telomere shortening that is usually associated with cardiovascular risk, long telomere length and telomerase activation have been observed in leukocytes isolated directly from human unstable coronary artery plaques[29-31] suggesting a possible role in the early phases of instability. In our study, the association between occurrence of CAD and arsenic exposure was significant. A positive association has previously been reported between *hTERT* expression and CAD.[16] We report approximately 3.4 fold higher expression of *hTERT* gene and 1.5 fold higher telomere length in CAD patients group than the non-CAD patients group.

Our results suggest that As-induced CVD pathogenesis may possibly adopt a mechanism somewhat different from that of non-As-induced CVDs. To our knowledge, this is the first attempt of investigating the coordinated effects of chronic arsenic exposure, *hTERT* expression levels and telomere length in As-induced CVD susceptibility.

Conclusion

The most intriguing part of our observation is the possibility that CVDs associated with As exposure may have a different underlying mechanism from that of As-independent CVDs. We observed elevated *hTERT* expression and increased telomere length in CVD patients with increasing exposure to arsenic. Moreover, CAD patients showed higher *hTERT* expression and telomere length than the non-CAD patients. Further research is required to use telomerase activity and telomere length as a biomarker to predict CVD risk associated with As exposure which in turn may lead to novel diagnostic and therapeutic approaches to combating cardiovascular diseases more effectively.

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Conflict of Interest: The authors have no conflict of interest to disclose

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References

1. Epa US. National primary drinking water regulations: arsenic and clarifications to compliance and new source contaminants monitoring. Federal Register. 2001;66:69-76.
2. Shankar S, Shanker U. Arsenic contamination of groundwater: a review of sources, prevalence, health risks, and strategies for mitigation. The Scientific World Journal. 2014;2014.
DOI: 10.1155/2014/304524
3. Naujokas MF, Anderson B, Ahsan H, Aposhian HV, Graziano JH, Thompson C, Suk WA. The broad scope of health effects from chronic arsenic exposure: update on a worldwide public health problem. Environmental Health Perspectives. 2013;121:295-302.
DOI: 10.1289/ehp.1205875
4. Chen Y, Graziano JH, Parvez F, Liu M, Slavkovich V, Kalra T, Argos M, Islam T, Ahmed A, Rakibuz-Zaman M, Hasan R. Arsenic exposure from drinking water and mortality from cardiovascular disease in Bangladesh: prospective cohort study. BMJ. 2011 ;342:d2431.
DOI: 10.1136/bmj.d2431
5. Wang GS, Cooper TA. Splicing in disease: disruption of the splicing code and the decoding machinery. Nature Reviews Genetics. 2007;8:749-61.
DOI: 10.1038/nrg2164
6. Kingston RL, Hall S, Sioris L. Clinical observations and medical outcome in 149 cases of arsenate ant killer ingestion. Journal of Toxicology: Clinical Toxicology. 1993;31:581-91.
DOI: 10.3109/15563659309025763
7. Navas-Acien A, Sharrett AR, Silbergeld EK, Schwartz BS, Nachman KE, Burke TA, Guallar E. Arsenic exposure and cardiovascular disease: a systematic review of the epidemiologic evidence. American Journal of Epidemiology. 2005;162:1037-49.
DOI: 10.1093/aje/kwi330
8. States JC, Srivastava S, Chen Y, Barchowsky A. Arsenic and cardiovascular disease. Toxicological Sciences. 2009;107:312-23.
DOI: 10.1093/toxsci/kfn236
9. Li H, Engstrom K, Vahter M, Broberg K. Arsenic exposure through drinking water is associated with longer telomeres in peripheral blood. Chemical Research in Toxicology. 2012;25:2333-9.
DOI: 10.1021%2Ftx300222t
10. Blackburn EH. Structure and function of telomeres. Nature. 1991;350:569-73.
DOI: 10.1038/350569a0
11. Metzger R, Vallbohmer D, Mueller-Tidow C, Higashi H, Bollschweiler E, Warnecke-Eberz U, Brabender J, Baldus SE, Xi H, Berdel WE, Serve H. Increased human telomerase reverse transcriptase (hTERT) mRNA expression but not telomerase activity is related to survival in curatively resected non-small cell lung cancer. Anticancer Research. 2009;29:1157-62.
PMID: 19414359
12. Liu L, Trimarchi JR, Navarro P, Blasco MA, Keefe DL. Oxidative stress contributes to arsenic-induced telomere attrition, chromosome instability, and apoptosis. Journal of Biological Chemistry. 2003;278:31998-2004.
DOI: 10.1074/jbc.m303553200
13. Zhang TC, Schmitt MT, Mumford JL. Effects of arsenic on telomerase and telomeres in relation to cell proliferation and apoptosis in human keratinocytes and leukemia cells in vitro. Carcinogenesis. 2003;24:1811-7.
DOI: 10.1093/carcin/bgg141
14. Mo J, Xia Y, Ning Z, Wade TJ, Mumford JL. Elevated human telomerase reverse transcriptase gene expression in blood cells associated with chronic arsenic exposure in Inner Mongolia, China. Environmental Health Perspectives. 2009;117:354-60.
DOI: 10.1289%2Fehp.11532
15. Ferrario D, Collotta A, Carfi M, Bowe G, Vahter M, Hartung T, Gribaldo L. Arsenic induces telomerase expression and maintains telomere length in human cord blood cells. Toxicology. 2009 16;260:132-41.
DOI: 10.1016/j.tox.2009.03.019
16. Khaleda L, Al-Forkan M, Wali FB, Alam MJ, Datta A, Shawon II, Hosain N, Rahman MZ. Effect of arsenic exposure on human telomerase reverse transcriptase (hTERT) gene expression: Risk of cardiovascular diseases. Bangladesh Medical Res Coun Bull. 2019:15;45:3-10.
DOI: 10.3329/bmrcb.v45i1.41802
17. Gao J, Roy S, Tong L, Argos M, Jasmine F, Rahaman R, Rakibuz-Zaman M, Parvez F, Ahmed A, Hore SK, Sarwar G. Arsenic exposure, telomere length, and expression of telomere-related genes among Bangladeshi individuals. Environmental Research. 2015;136:462-9.
DOI: 10.1016/j.envres.2014.09.040
18. Bhattacharyya J, Mihara K, Bhattacharjee D, Mukherjee M. Telomere length as a potential biomarker of coronary artery disease. The Indian Journal of Medical Research. 201;145:730.
DOI: 10.4103%2F0971-5916.216974
19. Scientific TF. TRIzol Reagent User Guide-Pub. No. MAN0001271-Rev. A. 0. User Guide. 2016;15596018:1-6.
20. Wang CH, Hsiao CK, Chen CL, Hsu LI, Chiou HY, Chen SY, Hsueh YM, Wu MM, Chen CJ. A review of the epidemiologic literature on the role of environmental arsenic exposure and cardiovascular diseases. Toxicology and Applied Pharmacology. 2007;222:315-26.
DOI: 10.1016/j.taap.2006.12.022
21. Saliques S, Zeller M, Lorin J, Lorgis L, Teyssier JR, Cottin Y, Rochette L, Vergely C. Telomere length and cardiovascular disease. Archives of Cardiovascular Diseases. 2010;103:454-9.
DOI: 10.1016/j.acvd.2010.08.002
22. Chakraborti, D., Rahman, M.M., Mukherjee, A., Alauddin, M., Hassan, M., Dutta, R.N., Pati, S., Mukherjee, S.C., Roy, S., Quamruzzman, Q. and Rahman, M., 2015. Groundwater arsenic contamination in Bangladesh—21 Years of research. Journal of Trace Elements in Medicine and Biology, 31, pp.237-248.
DOI: 10.1016/j.jtemb.2015.01.003

23. Button M, Jenkin GR, Harrington CF, Watts MJ. Human toenails as a biomarker of exposure to elevated environmental arsenic. Journal of Environmental Monitoring. 2009;11:610-7.
DOI: 10.1039/b817097e

24. Imanishi T, Moriwaki C, Hano T, Nishio I. Endothelial progenitor cell senescence is accelerated in both experimental hypertensive rats and patients with essential hypertension. Journal of hypertension. 2005;23:1831-7.
DOI: 10.1097/01.hjh.0000183524.73746.1b

25. Ameer SS, Xu Y, Engström K, Li H, Tallving P, Nermell B, Boemo A, Parada LA, Peñaloza LG, Concha G, Harari F. Exposure to inorganic arsenic is associated with increased mitochondrial DNA copy number and longer telomere length in peripheral blood. Frontiers in Cell and Developmental Biology. 2016;4:87.
DOI: 10.3389/fcell.2016.00087

26. Brouilette S, Singh RK, Thompson JR, Goodall AH, Samani NJ. White cell telomere length and risk of premature myocardial infarction. Arteriosclerosis, thrombosis, and Vascular Biology. 2003;23:842-6.
DOI: 10.1161/01.atv.0000067426.96344.32

27. Samani NJ, Boultby R, Butler R, Thompson JR, Goodall AH. Telomere shortening in atherosclerosis. The Lancet. 2001;358:472-3.
DOI: 10.1016/s0140-6736(01)05633-1

28. Lung FW, Ku CS, Kao WT. Telomere length may be associated with hypertension. Journal of Human Hypertension. 2008;22:230-2.
DOI: 10.1038/sj.jhh.1002314

29. Gizard F, Heywood EB, Findeisen HM, Zhao Y, Jones KL, Cudejko C, Post GR, Staels B, Bruemmer D. Telomerase activation in atherosclerosis and induction of telomerase reverse transcriptase expression by inflammatory stimuli in macrophages. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011;31:245-52.
DOI: 10.1161/atvbaha.110.219808

30. Huzen J, Peeters W, de Boer RA, Moll FL, Wong LS, Codd V, de Kleijn DP, de Smet BJ, van Veldhuisen DJ, Samani NJ, van Gilst WH. Circulating leukocyte and carotid atherosclerotic plaque telomere length: interrelation, association with plaque characteristics, and restenosis after endarterectomy. Arteriosclerosis, Thrombosis, and Vascular Biology. 2011;31:1219-25.
DOI: 10.1161/atvbaha.110.217158

31. Narducci ML, Grasselli A, Biasucci LM, Farsetti A, Mulè A, Liuzzo G, La Torre G, Niccoli G, Mongiardo R, Pontecorvi A, Crea F. High telomerase activity in neutrophils from unstable coronary plaques. Journal of the American College of Cardiology. 2007;50:2369-74.
DOI: 10.1016/j.jacc.2007.08.048