論文の内容の要旨

論文題目

Influence of gender and genetic polymorphisms on inorganic arsenic metabolism and oxidative stress among arsenic- exposed Bangladeshi population.

（無機ヒ素に曝露されたバングラデシュ集団における性と遺伝多型が

無機ヒ素代謝と酸化ストレスに及ぼす影響）

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ABSTRACT:

Background: Inorganic arsenic (iAs) is an environmental carcinogen to which millions of people are chronically exposed mainly via drinking water. After ingestion, it goes through a series of reduction and oxidative methylation steps in human body. Trivalent forms of arsenic such as monomethylarsonic acid (MMA) (III) and dimethylarsinic acide (DMA) (III) are considered as the most toxic forms among the metabolized arsenic species. Thus, metabolism of ingested arsenic plays an important role in determining arsenic toxicity, and previous studies reported that proportion of MMA in the total arsenic (%MMA) in urine is associated with a variety of diseases including cancer, diabetes, respiratory syndromes, skin lesions, and peripheral circulatory problems.

Substantial individual variation in the arsenic metabolism and toxicity has been recognized, although the causes of the variation remain to be elucidated. Along with other factors gender plays an important role in arsenic metabolism. Females have shown higher methylation efficiency than males in arsenic-exposed populations in various countries. Gender disparity has been also reported for many arsenic-exposure related diseases such as skin lesion, skin cancer, lung, bladder and kidney cancer and diabetes. The molecular and cellular mechanisms by which arsenic acts as a carcinogen have not been clearly elucidated, but lines of evidence suggest that

induction of oxidative stress through the generation of reactive oxygen species (ROS) is one of the important mechanisms. No preceding studies have examined the gender difference in arsenic-induced oxidative stress.

Genetic polymorphisms in arsenic-metabolizing enzymes and DNA repair enzymes are among other factors involved in the metabolism and toxicity of arsenic. Importantly, associations of genetic polymorphism with several diseases depend on sex reported in previous studies. However, the effect of gender and genetic polymorphism on arsenic-induced oxidative stress as well as gender effect on association of genetic polymorphism with arsenic metabolism in arsenic-exposed population has not been evaluated before.

Objectives: This dissertation evaluated the role of gender in arsenic-induced oxidative stress among Bangladeshi arsenic-exposed population. The impact of polymorphisms in arsenic metabolism-related genes as well as those in DNA repair-related genes on arsenic metabolism and oxidative stress, respectively were also evaluated.

Methods: The study protocol was approved by the Ethical Review Committee of the Graduate School of Medicine, University of Tokyo, Japan. Two hundred twenty three Bangladeshi participants aged 18 to 70 years from arsenic-exposed (water As >10ppb) area were participated in this cross-sectional study. Ninety-six males and 126 females giving informed consent were the participants in this study. Water of the tube wells that they were using, urine and peripheral blood samples were collected and analyzed.

Urinary and water arsenic levels were determined by inductively-coupled plasma mass spectrometry (ICP-MS) equipped with a direct reaction cell (Agilent7500ce, Agilent). Arsenic speciation was performed by a HPLC-ICP-MS system. Urinary 8-Hydroxy-2'-deoxyguanosine (8-OHdG) and urinary 15-F_{2t}-IsoP levels were determined with commercial ELISA kits.

Genotyping of studied SNPs were carried out by DigiTag2 multiplex PCR assay. Twenty-two SNPs of the following genes were studied: AS3MT, DNMT, MTHFR, GPX1, GSTO1, GSTO2, OGG1, APE1, XRCC1, XRCC3, ERCC5 and LIG4.

Pearson`s correlation, Student t test, Tukey-kramer post hoc, and multiple regression analysis used to evaluate the data. Data were analyzed using JMP statistical software (version-8).

Results: Urinary arsenic (u-As) had positive association with urinary oxidative stress markers- 8-OHdG ($r= 0.60$; $p<0.001$) and 15-F_{2t}-IsoP ($r= 0.46$; $p<0.001$). Arsenic-exposed female had significantly higher 15-F_{2t}-IsoP

and %DMA ($p<0.05$) than males. Gender difference in u-As and urinary 8-OHdG was not found in this study group.

Four AS3MT polymorphisms were significantly associated with As metabolism in this study population ($p \leq 0.01$). Exonic SNP of As3MT-rs11191439 significantly associated with arsenic metabolism. While three intronic AS3MT SNPs (rs3740393, rs3740390, and rs11191453) were significantly associated with arsenic metabolism in female group. None of intronic AS3MT polymorphisms affected arsenic methylation in arsenic-exposed male group. DNMT3b (rs2424913) SNP was associated with arsenic metabolism in females.

There were no significant differences in the urinary arsenic metabolites or oxidative stress markers within male or female subjects with different genotypes of MTHFR, GSTO1, GSTO2, OGG1, APE1, XRCC1, XRCC3, ERCC5 and LIG4. No DNA repair genes or arsenic metabolism related genes SNPs were associated with 8-OHdG concentrations in arsenic-exposed population. Three intronic AS3MT SNPs (rs3740393, rs3740390, and rs11191453) were significantly associated with 15-F_{2t}-IsoP in male group.

Discussion: This dissertation reported that arsenic induced oxidative stress in arsenic-exposed population. It also confirmed previous studies that with increase of u-As, 8-OHdG levels increase and males have higher %MMA ($p<0.01$), indicated less methylation efficiency of arsenic than females. Although females have better methylation ability, females have higher lipid peroxidation than males that indicated As-induced oxidative stress has not depended on arsenic methylation, so other mechanism may be involved. This finding provides certain evidence for women exposed to higher arsenic being at higher risk than men in term of oxidative stress, which may be a leading mechanism for gender disparity in arsenic-related health effects.

Gender stratified analyses showed exonic SNPs of AS3MT-rs11191439 (T/C) hetero type had significantly lower %MMA and higher secondary methylation index (D/M) than TT homo type, especially among arsenic-exposed males. This result indicated that male subjects with hetero type (T/C) carrier had better methylation capability than homotype carrier. While, heterotype of intronic SNPs of AS3MT (rs3740393, rs3740390, and rs11191453) females carrier has lower %MMA indicated better methylation than homotype female carrier. Heterotype of intronic SNPs of AS3MT (rs3740393, rs3740390, and rs11191453) has association higher 15-F_{2t}-IsoP in male group. Gender depended association of arsenic-metabolism related genes with arsenic metabolism and oxidative stress markers remain unknown.

This study confirmed previous studies that MTHFR, GSTO1 and GSTO2 SNPs has no association with As metabolism or oxidative stress. In contrast to previous studies this study reported that OGG1, APE1 and XRCC1 SNPs has no association with DNA repair. This gender depended association gene polymorphism with arsenic metabolism and oxidative stress may be another possible mechanism for gender disparity in arsenic-related health effects.

Conclusion: In conclusion, chronic arsenic exposure from drinking water may be related to induction of oxidative stress, as indicated by the increase in urinary 8-OHdG and 15-F_{2t}-IsoP levels. The present study also provides evidence that females have higher arsenic metabolism as well as higher As-induced oxidative stress 15-F_{2t}-IsoP levels, which may explain the difference in susceptibility to As-induced toxicity.

The present study identified four SNPs in AS3MT and one SNP in DNMT that may affect arsenic methylation in a Bangladeshi population. Hetero-type AS3MT (rs11191439) displayed a greater methylation index in an arsenic-exposed population, particularly in males. Intronic SNPs of AS3MT were associated with oxidative stress markers, particularly in males. Such a gender-specific association of As-metabolism gene polymorphism with altered u-As metabolites has never before been reported. Genotype associations with u-As metabolites as well as 15-F_{2t}-IsoP concentrations may depend on exposure level and gender. Further analyses of the association of these genes with arsenic metabolism may help to define the significance of these polymorphisms as a genetic marker of susceptibility to oxidative stress in arsenic-exposed populations.