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Urinary transforming growth factor-alpha in individuals exposed to arsenic in drinking water in Bangladesh

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Recent evidence suggests that the development of skin lesions from arsenic exposure may be mediated by increases in the expression of various growth factors, including transforming growth factor-alpha (TGFα). To investigate this association in humans, levels of total urinary arsenic and urinary TGFα were determined in 41 individuals with and without arsenic-associated skin lesions from Bangladesh who have chronic exposure to arsenic in their drinking water. After adjusting for age and sex, total urinary arsenic was found to be correlated with urinary TGFα ($R^2 = 0.37$; $p < 0.0001$), particularly in those individuals with arsenic-associated skin lesions ($R^2 = 0.70$; $p < 0.0001$). Stratification of the cohort into quartiles based on urinary TGFα levels demonstrated a trend of increasing odds ratios for the presence of arsenic-associated skin lesions with increasing urinary TGFα, although this was not significant ($p = 0.15$). These results suggest that urinary TGFα may be a useful biomarker for the epidermal effects of arsenic exposure.

Keywords: transforming growth factor-alpha, arsenic, drinking water, skin lesions, biomarker.

Introduction

Arsenic is a known human carcinogen, but the mechanisms by which arsenic produces cancer remain unclear (Nriagu 1994). Recent evidence suggests that dysregulation of growth factor expression, particularly transforming growth factor-α (TGFα), may play a role in arsenic carcinogenesis, especially in relation to the development of skin tumours (Germolec *et al*. 1996, 1998).

In skin, levels of expression of TGFα by keratinocytes is critical for maintaining homeostasis and the barrier integrity of the epidermis (Luger and Schwartz 1990). However, overexpression of TGFα can lead to various pathological processes, including neoplasia. For example, keratinocytes transfected with a constitutive TGFα transgene develop benign skin papillomas when grafted to nude mice (Finzi *et al*. 1988), and targeted overexpression of TGFα to the epidermis elicits hyperplasia, hyperkeratosis and spontaneous squamous cell carcinomas (Dominey *et al*. 1990). TGFα transgenic mice exhibit keratinocyte hyperproliferation and tumours of the pancreas, liver and mammary epithelium (Jhappan *et al*. 1990, Sandgren *et al*. 1990). Furthermore, TGFα appears to play a

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role in chemical-induced neoplastic development, including that of arsenic. In recent studies, after application of a phorbol ester tumour promoter, a marked increase in the number of skin papillomas occurred in transgenic mice carrying the v-Ha-*ras* oncogene that received 0.02% sodium arsenite in their drinking water as compared with control drinking water (Germolec *et al*. 1998). Increases in growth factors, including TGFα, identified by mRNA expression and immunohistochemical staining, were found in the epidermis of the treated transgenic mice within ten weeks of arsenic exposure even at clinically normal sites (Germolec *et al*. 1998). In addition, mRNA analysis of gene expression in samples of skin lesions obtained from humans chronically exposed to arsenic via their drinking water showed similar alterations in their growth factor expression, including TGFα (Germolec *et al*. 1998). Thus, elevated levels of TGFα might serve as a biomarker of effects associated with arsenic exposure.

TGFα is a potent, mitogenic polypeptide synthesized as a 160 amino acid, transmembrane precursor that undergoes sequential, external, proteolytic cleavage to a mature, active, acid- and heat-stable form of 5.5 kDa (Derynck 1988). Mature TGFα binds to the epidermal growth factor receptor and mediates tyrosine phosphorylation of the receptor which stimulates an intracellular cascade of growth signal transduction (Derynck 1988). Since TGFα is present in the extracellular environment, it is readily detectable by immunological methods in tissue culture medium and a variety of biological fluids. For example, elevated levels of TGFα have been detected in the urine or serum of patients with various malignancies, including the colo-rectum, liver, ovaries, breast, oesophagus, stomach, pancreas, lung and skin, and in individuals at high risk for cancer who subsequently developed malignancies (Ellis *et al*. 1990, Katoh *et al*. 1990, Chakrabarty *et al*. 1994, Moskal *et al*. 1995, Partanen *et al*. 1995). The purpose of the present study was to determine whether TGFα could be detected in the urine of individuals exposed to arsenic in their drinking water in Bangladesh, and to examine its relationship to urinary arsenic levels in residents with and without arsenic-associated skin lesions.

Materials and methods

As part of a pilot study of arsenic-related health effects in Bangladesh, several villages in two high arsenic districts were visited, and a convenience sample of residents was assembled. Potential villages with high arsenic ground water were identified with the help of the first comprehensive Bangladesh Government/British Geological Survey report (British Geological Survey 1998) in Sonargaon, a rural area immediately east of the capital Dhaka, and Laksham, a rural area approximately 60 miles south-east of Dhaka. With assistance from local leaders in each village, participants for the study were requested. A total of 47 individuals from six villages agreed to participate. Basic demographic information (age, sex) was collected, each participant was examined by a physician for the presence of arsenic-associated skin lesions (hyper/hypo-pigmentation, hyperkeratosis), and a urine sample was requested. Adequate urine samples were obtained from 41 participants. Urine samples were collected in 50 ml acid-washed plastic tubes, stored on ice and transported back to Columbia for analysis for total urinary arsenic concentrations and levels of urinary TGFα. In addition, the water from the nine wells that these individuals used as their primary source of drinking water was sampled and analysed for total arsenic and arsenite; total arsenic in the water ranged from < 10 μg l^{-1} to 1310 μg l^{-1} and was 87.5–99% arsenite (Ahsan *et al*. 2000).

Total urinary arsenic concentrations were determined by graphite furnace atomic absorption spectrometry methods with an Analyst 600 graphite furnace system, as previously described (Nixon *et al*. 1991). Urinary TGFα concentrations were determined with a sandwich ELISA which utilizes affinity purified goat polyclonal antibodies specific for mammalian TGFα according to the instructions of the manufacturer (Oncogene Research Products, Cambridge, MA), as previously described (Partanen *et al*. 1995). Urinary arsenic and TGFα concentrations were adjusted for urinary creatinine levels

analysed by a colorimetric assay according to the instructions of the manufacturer (Sigma Diagnostics, St Louis, MO).

Total urinary arsenic concentrations and urinary TGFα concentrations were log transformed to normalize their distributions. Associations between total urinary arsenic and TGFα concentrations were examined for all 41 individuals, and for individuals with and without arsenic-associated skin lesions, in a linear regression model with and without the inclusion of age and sex. The means of the urinary arsenic and TGFα concentrations for those individuals with and without arsenic-associated skin lesions were compared using the *t*-test. Samples were also stratified into approximately equal quartiles based on the urinary TGFα concentrations and the odds ratios and 95% confidence intervals for the presence of arsenic-associated skin lesions determined for each strata, after assigning an odds ratio of 1 to the lowest quartile, with and without adjustment for age and sex in a maximum likelihood model; the corresponding χ^2 for trend and p values were determined.

Results

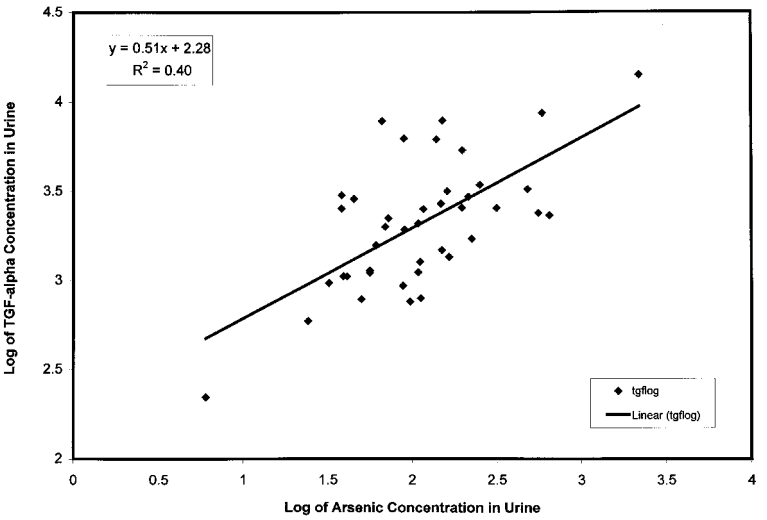
The study participants included 21 females and 20 males who ranged in age from 2 to 80 years old. Arsenic-associated skin lesions occurred in 18 of the subjects (11 females and 7 males) who ranged in age from 6 to 80 years old. Total urinary arsenic concentrations ranged from 6 to 454 μg g^{-1} creatinine, and urinary TGFα concentrations ranged from 232 to 14 165 ng g^{-1} creatinine.

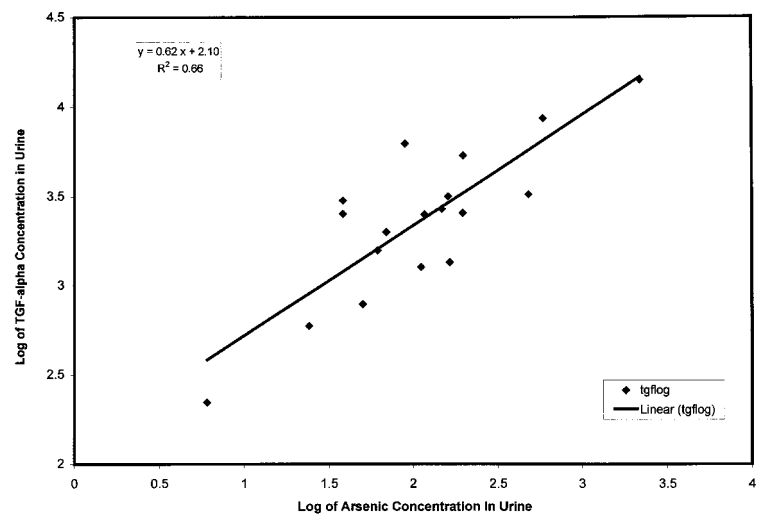
The relationships between the log-normalized urinary arsenic concentrations and TGFα concentrations are shown in figure 1 for the total group (1(a)) and for those with and without skin lesions (1(b) and 1(c), respectively). For the total group, the linear regression model shows a correlation between total urinary arsenic and urinary TGFα ($R^2 = 0.40$; $p < 0.0001$), which was the same for males and females or children and adults. Thus, when age and sex were included in the model, the correlation was unchanged ($R^2 = 0.37$; $p < 0.0001$). As can be seen in figure 1(b), most of this relationship is derived from individuals with arsenic-associated skin lesions. For this group, the linear regression model shows a stronger correlation between total urinary arsenic and urinary TGFα ($R^2 = 0.66$; $p < 0.0001$), which again was unchanged when age and sex were included in the model ($R^2 = 0.70$; $p < 0.0001$). Conversely, for those individuals without arsenic-associated skin lesions, the correlation between total urinary arsenic and urinary TGFα is relatively weak ($R^2 = 0.07$; $p > 0.05$), even after inclusion of age and sex ($R^2 = 0.16$; $p = 0.03$).

Although the mean urinary arsenic and TGFα concentrations were higher in those individuals with skin lesions than in those without (mean log urinary arsenic = 2.42 and 2.20, respectively; mean log urinary TGFα = 3.36 and 3.28, respectively), the differences were not significant ($p > 0.05$). Table 1 presents the results for the presence of arsenic-associated skin lesions stratified in quartiles by the urinary TGFα concentrations, where the lowest quartile is assigned an odds ratio of 1. After adjustment for age and sex, the odds ratio for the presence of skin lesions increases to 1.87 in the second stratum (log TGFα = 3.05–3.35), 2.31 in the third stratum (log TGFα = 3.35–3.48), and 3.89 in the fourth stratum (log TGF > 3.48). Due to the small numbers, however, the confidence intervals are wide, and the χ^2 for trend is not significant ($p = 0.15$).

Discussion

Although these results are preliminary and based on small numbers, they do suggest that: (a) total urinary arsenic concentrations are related to urinary TGFα concentrations, particularly in individuals with arsenic-associated skin lesions; and





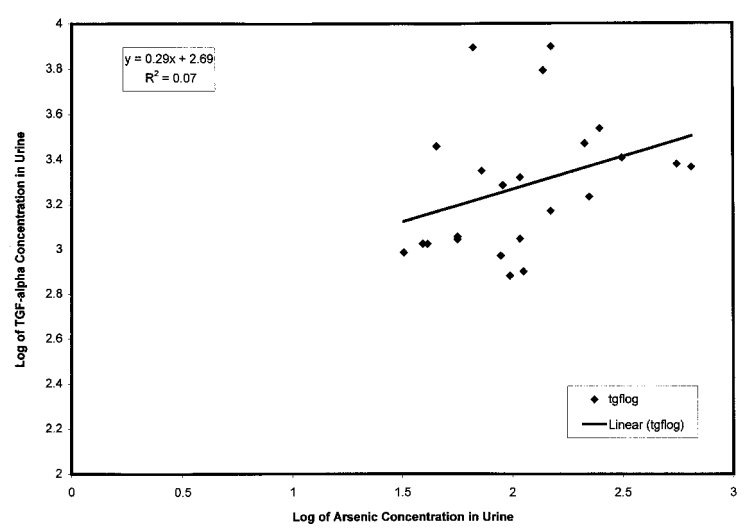


Figure 1. For legend see facing page.

Table 1. Relationship between urinary TGFα and presence of arsenic-associated skin lesions.

log TGFα (ng g^{-1} Cr)	Skin lesions		Adjusted odds ratio[a]	95% CI
	Absent	Present		
<3.05 (*n*=11)	8	3	1.00	
3.05-3.35 (*n*=10)	6	4	1.87	0.26–15.17
3.35-3.48 (*n*=10)	5	5	2.31	0.33–18.30
>3.48 (*n*=10)	4	6	3.89	0.55–33.94

[a]Adjusted for age and sex; χ^2 for trend is 2.3 (p=0.15).

(b) individuals with higher urinary TGFα concentrations are more likely to have arsenic-associated skin lesions. These findings are consistent with the known mitogenic properties of TGFα cited above and with the observations that targeted overexpression of TGFα can produce epidermal hyperplasia, hyperkeratosis and tumours (Dominey *et al*. 1990). Furthermore, these findings are consistent with animal data in which transgenic mice exposed to arsenic demonstrated increased epidermal TGFα expression and with human data in which skin lesions from individuals chronically exposed to arsenic in drinking water demonstrated increased TGFα expression (Germolec *et al*. 1998). Collectively, these findings provide suggestive evidence that urinary TGFα may be a convenient biomarker to monitor the mitogenic, epidermal effects of arsenic exposure. The fact that overexpression of TGFα could also be identified in the clinically normal skin of exposed transgenic mice (Germolec *et al*. 1998) and that elevated levels of circulating TGFα have been identified in humans who subsequently developed tumours (Partanen *et al*. 1995) suggests that urinary TGFα levels may even be useful in helping to predict which arsenic-exposed individuals are at risk for the development of skin lesions in the future.

Total urinary arsenic is known to include several chemical species, some of which are presumed not to contribute to its toxic effects. For example, organic species of arsenic derived from dietary exposures are believed to be non-toxic and excreted in the urine without undergoing metabolism (Nriagu 1994). Toxic, inorganic species are metabolized by methylation to less toxic species, both of which are excreted in the urine in widely varying amounts (Nriagu 1994). Human urine can therefore contain as many as six arsenic species including arsenate, arsenite, monomethyl arsonic acid and dimethyl arsinic acid (metabolites of inorganic arsenic), and arsenobetaine and arsenocholine (derived from dietary fish). Thus, one might anticipate that urinary TGFα would be more closely correlated with urinary inorganic arsenic than with total urinary arsenic, particularly in

Figure 1. Relationship between the log-normalized total urinary arsenic concentrations and the log-normalized urinary TGFα concentrations for all 41 arsenic-exposed individuals (a), for the 18 individuals with arsenic-associated skin lesions (b), and for the 23 individuals without arsenic-associated skin lesions (c).

individuals with skin lesions. Furthermore, if skin is the primary source of urinary TGFα identified, one might expect to find elevated levels of TGFα expression in the normal epidermis and/or skin lesions of these individuals. Studies are under way to examine these issues.

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