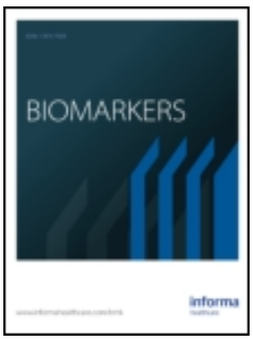

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Serum levels of the extracellular domain of the epidermal growth factor receptor in individuals exposed to arsenic in drinking water in Bangladesh

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Abstract
Epidermal growth factor receptor-dependent mechanisms have been implicated in growth signal transduction pathways that contribute to cancer development, including dermal carcinogenesis. Detection of the extracellular domain of the epidermal growth factor receptor (EGFR ECD) in serum has been suggested as a potential biomarker for monitoring this effect *in vivo.* Arsenic is a known human carcinogen, producing skin and other malignancies in populations exposed through their drinking water. One such exposed population, which we have been studying for a number of years, is in Bangladesh. The purpose of this study was to examine the EGFR ECD as a potential biomarker of arsenic exposure and/or effect in this population. Levels of the EGFR ECD were determined by enzyme-linked immunosorbent assay in the serum samples from 574 individuals with a range of arsenic exposures from drinking water in the Araihazar area of Bangladesh. In multiple regression analysis, serum EGFR ECD was found to be positively associated with three different measures of arsenic exposure (well water arsenic, urinary arsenic and a cumulative arsenic index) at statistically significant levels ($p \leq 0.034$), and this association was strongest among the individuals with arsenic-induced skin lesions ($p \leq 0.002$). When the study subjects were stratified in tertiles of serum EGFR ECD levels, the risk of skin lesions increased progressively for each increase in all three arsenic measures (also stratified in tertiles) and this increasing risk became more pronounced among subjects within the highest tertile of EGFR ECD levels. These results suggest that serum EGFR ECD levels may be a potential biomarker of effect of arsenic exposure and may indicate those exposed individuals at greatest risk for the development of arsenic-induced skin lesions.

Keywords: *Biomarker of effect, epidermal growth factor receptor (EGFR), arsenic, skin lesions*

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Introduction

Inorganic arsenic, a known human carcinogen, is associated with cancers of the skin, urinary bladder, liver, spleen and lung (Tapio & Grosche 2006). Drinking water sources in certain regions of the world, including many areas of Bangladesh, contain high levels of arsenic (Tapio & Grosche 2006). Nearly half of the estimated 10 million

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tube wells in Bangladesh yield arsenic-contaminated water, exposing between 35 and 77 million people (Smith et al. 2000, MacDonald 2001).

For the past 6 years, we have been engaged in a multidisciplinary investigation of this problem in the Araihazar area of Bangladesh involving geochemical, hydrological, biomedical, epidemiological, sociological and interventional studies (Ahsan et al. 2006). In these investigations to date, we have focused primarily on the relationship between exposure and the occurrence of arsenic-induced skin lesions, which in addition to basal and squamous cell cancers include characteristic hyper- and hypopigmentary changes and hyperkeratoses that are considered to be premalignant conditions. We have employed various measures of arsenic exposure in this study population, including well water arsenic levels, urinary arsenic levels, and a cumulative arsenic index. Details of the diagnosis of skin lesions, collection of data and serum samples, and determination of the arsenic exposure measures have been described previously (Ahsan et al. 2006). However, in addition to such measures of exposure, it would be useful to identify other biomarkers of effect of arsenic exposure in order to better determine the risk for subsequent development of arsenic-related health effects, including skin cancer, in these exposed populations.

In the present study, we hypothesised that the epidermal growth factor receptor (EGFR) could be a potential biomarker of effect from arsenic exposure based on several lines of evidence. First, arsenic exposure has been shown in animal models to cause increased expression of transforming growth factor-alpha (TGFα), one of the stimulatory ligands of EGFR (Germolec et al. 1998). Prolonged exposure to TGFα of cells in culture, including epidermal and melanocytic skin cell lines, has been shown to produce an apparent up-regulation of EGFR, which could contribute to an autocrine growth effect and uncontrolled proliferation (McCulloch et al. 1998, Gordon-Thomas et al. 2005). In other studies, tumor cells in culture and in animal models that over-express EGFR have been found to release increased amounts of its ligand-binding, extracellular domain (ECD) into the culture medium or blood, respectively (Brandt-Rauf 1995). Also, in individuals with tumors that are capable of over-expressing EFGR, increased levels of the EGFR ECD have been detected in their serum (Choi et al. 2000, Oh et al. 2000). Thus, it is possible that exposure of individuals to arsenic could cause an increase in TGFα expression, which could result in an up-regulation of EGFR, with a concomitant increase in the levels of the EGFR ECD in their blood. In fact, in our previous studies of arsenic-exposed individuals in Bangladesh and Taiwan, we demonstrated that urinary or plasma levels of TGFα increased with increasing exposure to arsenic (Do et al. 2001, Hsu et al. 2006). Furthermore, in studies of other cohorts exposed to carcinogens, we have observed concomitant elevations of both TGFα and the EGFR ECD in their serum prior to the development of malignancies (Partanen et al. 1994a, 1995). Therefore, in the present study, we determined the levels of the EGFR ECD in the serum of a subset of our arsenic-exposed cohort in Bangladesh to examine its relationship to arsenic exposure and the presence of premalignant, arsenic-induced skin lesions.

Materials and methods

The overall Health Effects of Arsenic Longitudinal Study (HEALS) is being conducted in the Araihazar thana in the Narayangonj district, 25 km southeast of Dhaka, among 11 746 men and women. Details of the study methodologies for

HEALS have been described previously (Ahsan et al. 2006). During the baseline recruitment of the cohort, among the data collection procedures, all participants underwent a thorough physical examination for identification of any arsenic-induced skin lesions, as well as a venipuncture for collection of blood samples. A total of 714 patients with such skin lesions have been diagnosed among the 11 746 cohort members. All patients with skin lesions and a random sample of approximately 10% of the unaffected cohort members ($n = 1100$) were included in a sub-study examining the association of skin lesions in relation to urinary arsenic metabolite patterns, including unmetabolised inorganic arsenic and the mono-methylated and dimethylated metabolites. In April 2003, 335 cases of skin lesions and 239 individuals without skin lesions were randomly selected from this sub-study population for the current study, based on the availability of suitable serum samples and relevant demographic data (age, gender, body mass index) and exposure information (well water arsenic levels, urinary arsenic levels, cumulative arsenic index) at that time.

Briefly, arsenic exposure measures were determined as follows. Water arsenic concentrations of tube wells at each subject's home were determined during a survey of all wells in the study region (van Geen et al. 2003), where the samples were shipped to Columbia's Lamont-Doherty Earth Observatory for the analysis. These samples were analysed by graphite furnace atomic absorption spectrometry methods with a detection limit of 5 $\mu g\ l^{-1}$ (van Geen et al. 2002). Those samples found to have less than 5 $\mu g\ l^{-1}$ were subsequently re-analysed by inductively coupled plasma mass spectrometry with a detection limit of 0.1 $\mu g\ l^{-1}$ (Cheng et al. 2004). The cumulative arsenic index was calculated by multiplying water arsenic concentration by the estimated amount of water drunk per year times the number of years the well had been in use by each study participant (Ahsan et al. 2006). Daily water consumption was estimated by multiplying the number of glasses of water consumed per day times the volume of a typical glass. If the participant had changed wells and the arsenic concentration was known for the previous well, this information was also taken into account. The median duration of exposure information for our participants was 9 years. Urine samples were collected in acid-washed plastic tubes and shipped on dry ice to the Columbia Trace Metals Core Laboratory for analysis. Total urinary arsenic concentrations were determined by graphite furnace atomic absorption spectrometry, as previously described (Nixon et al. 1991). All measurements are expressed as micrograms of arsenic per gram of creatinine, which was measured using a colorimetric Sigma Diagnostics Kit (Sigma, St. Louis, MO, USA). In addition, urinary arsenic metabolites were speciated using a method adapted from Vela and Heitkemper (2004). This method employs high-performance liquid chromatography separation of arsenobetaine, arsenocholine, arsenate, arsenite, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), followed by detection by inductively coupled plasma mass spectrometry. The percentages of inorganic arsenic (AsIII + AsV), MMA and DMA were calculated after subtracting the arsenobetaine and arsenocholine from the total urinary arsenic.

Serum samples collected by routine venipuncture techniques were bar-coded to minimise sample-handling errors and shipped frozen to the Columbia Biomarkers Core Laboratory for storage before further analysis. Serum samples were analysed blinded to participant status for levels of the EGFR ECD via a sandwich ELISA that utilises a mouse monoclonal capture antibody and a rabbit polyclonal detector antibody for the EGFR ECD as described previously (Partanen et al. 1994a). For the

assay, microtiter wells were pre-coated with the mouse monoclonal antibody at a concentration of 3 μg/well. Diluted standards and serum samples (1:50 in PBS, pH 7.4, containing 0.1% sodium azide, 1% BSA and 10% normal mouse serum) were added as 100 μl aliquots to the microtiter wells for incubation overnight at room temperature. After exhaustive washing, 100 μl of the rabbit antiserum was added to each well and incubated for 30 min at room temperature. Then, 100 μl of goat anti-rabbit IgG conjugated to horseradish peroxidase was added to each well and incubated for 30 min at room temperature. After exhaustive washing, the wells were incubated with 100 μl of *o*-phenylenediamine substrate solution in the dark at room temperature for 1 h, and the color was measured at 490 nm using a microplate reader. A standard curve was generated from the absorbance of serial dilutions in duplicate containing purified standard from A431 cells. This assay does not cross-react with similar antigens, such as c-erbB-2. With serum samples spiked with known quantities of standard, this assay gave an average recovery of 71.0–94.6%. Intra-assay variability ranged from 5.8–8.1%, and interassay variability ranged from 6.0–14.6%. The concentration of EGFR ECD in samples (assayed in duplicate) was determined by interpolation of sample absorbance from the standard curve.

The data were first analysed for differences in the mean levels of EGFR ECD by arsenic exposure measures (well water arsenic levels, urinary arsenic levels, cumulative arsenic index) as well as other possible determinants (age, gender, body mass index (BMI), skin lesions) using ANOVA and Spearman rank order correlation. Then, arsenic exposure measures and levels of EGFR ECD were log-transformed to normalise the distributions, and multiple linear regression analyses were used to further examine the relationship between EGFR ECD and the other variables including each type of arsenic exposure measure. To determine whether particular arsenic species might be responsible for the effect on serum EGFR ECD levels, the association with various measures of arsenic metabolites were examined using linear regression models, including percentages of urinary inorganic arsenic (AsIII + AsV), monomethyl arsenic (MMA) and dimethyl arsenic (DMA), as well as their ratios as indices of the efficiency of each methylation step, namely a primary methylation index (PMI = MMA/(AsIII + AsV)) and a secondary methylation index (SMI = DMA/MMA). Finally, the adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association of skin lesions with each of the exposure measures was determined by multiple linear regression stratified by EGFR ECD levels in tertiles.

Results

In the univariate analysis, serum levels of EGFR ECD were found to be statistically significantly associated with characteristics of the study population, such as age, gender, BMI and skin lesions, as well as one measure of arsenic exposure, namely well water arsenic levels, as shown in Table I. While levels of EGFR ECD were also associated with the two other measures of arsenic exposure, urinary arsenic levels and cumulative arsenic index, these associations were not statistically significant.

In multivariate analysis by each measure of arsenic exposure separately, levels of EGFR ECD remained negatively associated with age and skin lesions and positively associated with BMI but were no longer associated with gender, as shown in Table II. Also, levels of EGFR ECD were now significantly associated with all three measures of

Table I. Mean serum epidermal growth factor receptor extracellular domain (EGFR ECD) levels by age, gender, body mass index (BMI), skin lesion status, and measures of arsenic (As) exposure.

	n (total *n* = 572)	EGFR ECD ng ml^{-1}, mean (SD)	*p* value for ANOVA	Spearman correlation, value (*p* value)
Age (years)				
19–35	205	43.2 (9.7)	<0.01	−0.21 (<0.01)
36–45	190	41.7 (10.0)		
46–70	177	38.5 (8.9)		
Gender				
Male	231	42.7 (9.9)	<0.01	
Female	341	40.3 (9.6)		
BMI				
<18.1	188	40.2 (8.8)	<0.01	0.17 (<0.01)
18.1–20.5	187	40.0 (10.0)		
>20.5	194	43.6 (10.0)		
Skin lesions				
No	238	43.1 (10.1)	<0.01	
Yes	334	40.0 (9.3)		
Well water As (μg l^{-1})				
0.1–43	192	40.6 (10.2)	0.03	0.11 (0.01)
44–144	188	40.4 (9.9)		
145–768	192	42.8 (9.0)		
Urine As (μg g Cr^{-1})				
21.1–162.7	187	40.6 (9.5)	0.08	0.07 (0.10)
162.8–339.0	188	41.0 (10.3)		
>339.1	193	42.4 (9.4)		
Unknown	4			
Cumulative As (mg)				
0.1–315	181	40.1 (10.1)	0.20	0.08 (0.08)
316–1313	181	41.9 (10.5)		
>1313	185	41.5 (8.8)		
Unknown	25			

arsenic exposure. Furthermore, when the study population was stratified by presence or absence of arsenic-induced skin lesions, the significance of the association between levels of EGFR ECD and measures of arsenic exposure was found to be attributable primarily to the group with lesions, as shown in Table II, despite the persistent negative association between EGFR ECD levels and the presence of skin lesions overall.

When the urine samples were analysed for arsenic metabolites, for the overall study group, DMA constituted 69.7 ± 8.8%, MMA constituted 13.4 ± 5.0% and (AsIII + AsV) constituted 16.0 ± 7.3% of the excreted arsenic. The corresponding figures for those with skin lesions were DMA 69.1 ± 8.5%, MMA 14.3 ± 4.9% and (AsIII + AsV) 15.8 ± 6.4%, and for those without skin lesions were DMA 70.6 ± 9.2%, MMA 12.2 ± 4.9% and (AsIII + AsV) 16.3 ± 8.5%. As shown in Table III, for the overall study group, no statistically significant associations were found between any of the measures of arsenic metabolism (MMA%, DMA%, PMI, SMI) and serum EGFR ECD levels; when stratified by skin lesion status, two statistically significant associations were noted between the percentage of urinary arsenic as DMA and serum EGFR ECD

Table II. Associations between log of serum epidermal growth factor receptor extracellular domain (EGFR ECD) levels and log of measures of arsenic exposure for all study subjects and subjects with or without skin lesions.

	All subjects		Subjects with lesions		Subjects without lesions	
	Parameter	*p* Value estimate	Parameter	*p* Value estimate	Parameter	*p* Value estimate
Intercept	3.594	<0.001	3.404	<0.001	3.594	<0.001
Age	−0.004	0.001	−0.004	0.001	−0.002	0.276
Male	0.008	0.742	0.023	0.462	−0.002	0.543
BMI	0.011	<0.001	0.015	0.004	0.009	0.020
Log well water As	0.015	0.008	0.034	<0.001	0.008	0.637
Skin lesions	−0.064	0.006				
Intercept	3.556	<0.001	3.389	<0.001	3.586	<0.001
Age	−0.004	0.001	−0.005	0.001	−0.002	0.297
Male	0.005	0.827	0.020	0.549	−0.027	0.471
BMI	0.012	0.001	0.015	0.004	0.011	0.028
Log cumulative As	0.013	0.017	0.025	0.002	0.002	0.814
Skin lesions	−0.064	0.008				
Intercept	3.490	<0.001	3.204	<0.001	3.677	<0.001
Age	−0.004	0.001	−0.005	0.001	−0.002	0.269
Male	0.003	0.888	0.019	0.560	−0.026	0.464
BMI	0.012	0.000	0.017	0.001	0.009	0.019
Log urine As	0.026	0.034	0.055	0.001	−0.009	0.618
Skin lesions	−0.055	0.019				

As, arsenic.

levels and between the percentage of urinary inorganic arsenic and serum EGFR ECD only in the group without skin lesions.

In an attempt to resolve some of the inconsistencies in the data noted above and to further explore the interaction among arsenic exposure, EGFR and skin lesions, the risk of occurrence of skin lesions with increasing arsenic exposure (for each measure of exposure stratified in tertiles) was determined stratified by tertiles of serum EGFR ECD levels and adjusted for age, gender and BMI, as shown in Table IV. In the low tertile of EGFR ECD levels, the adjusted OR for skin lesions increased from 1.0 in the low-exposure groups to 1.0–1.3 in the medium-exposure groups to 1.6–2.1 in the high-exposure groups, depending on the particular measure of arsenic exposure, but these ORs were not statistically significant. In the medium tertile EGFR ECD stratum, the adjusted OR for skin lesions increased from 1.0 in the low-exposure groups to 3.3–4.4 in the medium-exposure groups to 4.0–6.7 in the high-exposure groups, depending on the particular measure of arsenic exposure, and were statistically significant in all cases. In the high tertile EGFR ECD stratum, the adjusted OR for skin lesions increased from 1.0 in the low-exposure groups to 3.3–8.1 in the medium-exposure groups to 4.9–24.0 in the high-exposure groups, depending on the particular measure of arsenic exposure, and were highly statistically significant in all cases. Although the p value for interaction of arsenic exposure and serum EGFR ECD levels with the occurrence of skin lesions was not statistically significant in any case because of the relatively small numbers in each tertile, for the high EGFR ECD stratum and the well water arsenic level measure of exposure, the term approached significance ($p = 0.08$).

Table III. Association between urinary arsenic (As) metabolite indices and log of serum epidermal growth factor receptor extracellular domain (EGFR ECD) levels.

	All subjects			Subjects with lesions			Subjects without lesions		
	Parameter estimate	SE	*p* Value	Parameter estimate	SE	*p* Value	Parameter estimate	SE	*p* Value
PMI	0.024	0.020	0.218	−0.008	0.027	0.781	0.051	0.028	0.072
SMI	0.000	0.003	0.978	−0.009	0.005	0.088	0.006	0.004	0.188
DMA%	0.000	0.001	0.841	−0.003	0.002	0.081	0.004	0.002	0.028
MMA%	0.002	0.002	0.443	0.003	0.003	0.256	−0.001	0.004	0.854
(AsIII +AsV)%				0.003	0.002	0.140	−0.005	0.002	0.012

Discussion

These results suggest that arsenic exposure may up-regulate EGFR leading to increased circulating levels of the EGFR ECD, as hypothesised based on the previous literature. As noted, it is known that EGFR is the receptor for TGFα through which it stimulates the cascade of growth signal transduction in cells; furthermore, prolonged exposure of epithelial cells to TGFα apparently up-regulates EGFR, and arsenic exposure can increase the expression of TGFα. For example, in animal studies, after application of a phorbol ester tumor promoter, a marked increase in the number of

Table IV. Association between risk of skin lesions and measures of arsenic (As) exposure by tertiles of serum epidermal growth factor receptor extracellular domain (EGFR ECD) levels.*

	Low EGFR		Medium EGFR		High EGFR	
	Lesions/ no lesions	OR (95% CI)	Lesions/ no lesions	OR (95% CI)	Lesions/ no lesions	OR (95% CI)
Well water As (μg l^{-1})						
0.1–43	45/25	1.0	25/37	1.0	15/44	1.0
44–144	45/25	1.0 (0.4–2.2)	44/24	3.0 (1.4–6.6)	32/17	7.8 (2.9–20.0)
145–768	37/12	2.1 (0.8–5.3)	41/17	4.0 (1.7–9.2)	48/36	4.9 (2.1–11.5)
p for interaction				0.22		0.08
Urine As (μg g Cr^{-1})						
21.1–162.7	46/29	1.0	19/32	1.0	17/43	1.0
162.7–339.0	38/18	1.0 (0.4–2.2)	45/28	4.1 (1.8–9.6)	29/29	3.3 (1.3–8.2)
>339.0	40/15	1.8 (0.7–4.2)	45/18	6.7 (2.6–16.8)	49/25	10.2 (3.9–26.7)
p for interaction				0.41		0.62
Cumulative As (mg)						
<314.8	43/25	1.0	22/41	1.0	8/41	1.0
315.6–1313.6	36/20	1.3 (0.6–3.1)	42/20	4.4 (2.0–9.9)	34/28	8.1 (2.7–24.0)
>1313.6	45/14	1.6 (0.7–3.9)	42/14	5.4 (2.3–12.9)	49/20	24.0 (7.6–75.8)
p for interaction				0.65		0.62

*Adjusted for age, gender and BMI

skin papillomas occurred in transgenic mice carrying the v-Ha-*ras* oncogene that received 0.2% sodium arsenite in their drinking water as compared with control drinking water. Increases in growth factors, including TGFα identified by mRNA expression and immunohistochemical staining, were found in the epidermis of the treated transgenic mice within 10 weeks of arsenic exposure even at clinically normal sites (Germolec et al. 1998). Therefore, arsenic exposure through its effect on TGFα and EFGR expression may promote the production of premalignant skin lesions.

To our knowledge, this is the first report linking EGFR expression with arsenic exposure or arsenic-induced skin lesions, but aspects of this study are consistent with other reports in the literature. For example, we have previously noted an inverse relationship between serum EGFR ECD levels and age, of a similar magnitude as reported here, in several other populations (Partanen et al. 1994b, Hemminki et al. 1999). As noted above, increased levels of serum EGFR ECD have been detected in individuals with types of cancers that over-express EGFR (Choi et al. 2000, Oh et al. 2000), and individuals at risk for cancer from carcinogen exposures have been found to have increased levels of serum EGFR ECD years prior to their clinical diagnosis of malignancy (Partanen et al. 1994a, 1994b). For example, asbestos exposure is known to up-regulate EGFR expression in cells in culture (Faux et al. 2000). Therefore, we examined the EGFR ECD levels in banked serum samples of asbestosis cases who subsequently developed cancer, matched asbestosis controls who did not go on to develop cancer, and matched non-asbestos-exposed individuals without cancer; the mean serum level of EGFR ECD in the individuals who developed cancer was significantly elevated in comparison to the level in either control group, and serum levels were elevated as much as 10 years prior to the clinical diagnosis of cancer, suggesting that this was an early biomarker of cancer risk (Partanen et al. 1994a, 1994b). If the same holds true for the present arsenic-exposed cohort, one would predict that the individuals with elevated EGFR ECD are the ones most likely to develop subsequent skin cancer. The fact that the relationship between arsenic and EGFR ECD in this cohort was strongest in the group with skin lesions, which are considered to be premalignant conditions, is consistent with that premise.

Until recently, arsenic metabolism via methylation has generally been considered a detoxification mechanism, based primarily on acute toxicity studies (Nriagu 1994). However, recent evidence suggests that methylated species, particularly the mono-methylated AsIII species may actually be more toxic than inorganic arsenic (Styblo et al. 2002). This prompted us to examine the relationship with EGFR and various species of arsenic in urine in addition to total urinary arsenic. In general, the results are inconclusive. As noted, for the overall study population no associations between arsenic species or indices of methylation ability and EGFR ECD approached statistical significance. Among individuals with skin lesions, DMA percentage was negatively associated with EGFR at a borderline level of significance ($p = 0.081$). That is, individuals who eliminated a higher proportion of arsenic in urine as the dimethylated species had a tendency toward lower EGFR ECD levels. This would be expected if incompletely methylated arsenic species were primarily responsible for inducing the EGFR effect and contributing to the development of skin lesions. On the other hand, among individuals without skin lesions, the proportion of urinary arsenic eliminated as DMA was positively associated with EGFR ECD levels to a significant degree ($p = 0.028$); thus, good 'complete methylators' had a tendency toward higher EGFR ECD levels without the development of skin lesions. Also, in this sub-group,

inorganic arsenic (AsIII + AsV) percentage was negatively associated with EGFR ECD levels to a significant degree ($p = 0.012$); in other words, 'poor methylators' had a tendency toward lower EGFR ECD levels. However, neither of these latter observations is consistent with the observation among individuals with skin lesions or with the overall association among total arsenic exposure measures, EGFR ECD levels and skin lesions. Therefore, the significance of arsenic metabolism for EGFR ECD expression remains uncertain at this time and will require further study before any definitive conclusions can be drawn. These apparent inconsistencies in arsenic metabolism patterns between individuals with and without skin lesions, as well as the observation of an overall negative association between EGFR ECD levels and the presence of skin lesions, may be at least partially attributable to individuals without skin lesions currently who are committed to development of skin lesions in the future due to their high expression of EGFR. In addition, since our study included prevalent skin lesions detected during baseline cohort recruitment and many of these lesions may have been long-standing, EGFR ECD measures based on blood samples collected from those with prevalent skin lesions may not truly reflect associations with pre-diagnostic serum levels. These issues may be resolved over time both with continued follow-up of the cohort and with future prospective studies based on newly diagnosed incident cases of skin lesions.

Overall, these results suggest that serum EGFR ECD levels may be a potentially useful biomarker of effect of arsenic exposure. Furthermore, it may be helpful in identifying individuals in exposed populations who are at greatest risk for the development of arsenic-induced skin lesions, including cancer, in the future, and who could thus be targeted for more aggressive preventive interventions.

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