Relationship between Urinary Arsenic Level and Urinary Iodine, Radioiodine Uptake, TSH, FT_4 Levels: Research is Based on Experimental and Control Groups

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ABSTRACT: In Bangladesh drinking water is heavily contaminated with arsenic. An estimated 50 million people are at risk from drinking of arsenic contaminated water in Bangladesh and West Bengal in India. Millions of people in many districts of Bangladesh are drinking ground water with arsenic concentrations above acceptable levels (0.05 mg/l) and many of them have already been diagnosed with poisoning symptoms. That is why, in this research an attempt has been made to find the relation between arsenic levels and urinary iodine, radioiodine uptake, thyroid stimulating hormone (TSH) and free thyroxin (FT_4) levels in Bangladeshi population. Another purpose has also been made to find the impacts of arsenic level on simple diffuse goiters. This is a case-control analytic study. The study was carried out at the Institute of Nuclear Medicine and Ultrasound, in collaboration with the thyroid out patient department of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh. From our analytical results it has been found that, only the variable radioiodine uptake at 24 hours play significant role for reducing the arsenic level. Also, it has been found that iodine deficiency is not only the factor of causing simple diffuse goiter, but arsenic level is also one of the most important factors of causing simple diffuse goiter in Bangladeshi population.

Keywords: Simple Diffuse Goiter, Urinary Arsenic, Urinary Iodine, Thyroid Stimulating Hormone, Free Thyroxin, Radioiodine uptake

INTRODUCTION

In chronic arsenic poisoning, the long-term retention of arsenic is most apparent in hair and skin, squamous epithelium of the upper gastrointestinal tract (oral cavity, esophagus, and the esophageal part of the stomach mucosa), the epididymis, thyroid, lens and skeleton.[1] The mean concentration of arsenic in thyroid gland determined by neutron activation analysis and reported is 0.04 mg/kg of thyroid gland in patients with chronic arsenicosis.[2] In a study it has been found that in rat the thyroid tissue concentration of arsenic increased after a period of steady, intake of arsenic.[3] In another study it was observed that thyroid hormones through a mechanism of unknown at present inhibit arsenic accumulation in liver and kidney.[4] There are some postulated mechanisms of arsenic affecting the thyroid gland. One of those is arsenic reduces thyroid uptake of iodine. It was found that high levels of arsenic led to iodine deficiency which causes goiters.[5]

In respect of arsenic problem in Bangladeshi population some researchers have done several studies but no one conducted any experiment in order to find the relationships between urinary arsenic level and urinary iodine, radioiodine uptake, TSH and FT_4 levels.[6-13] That is why, in this research the principal purpose has been made to find the relationships between urinary arsenic levels and urinary iodine, radioiodine uptake, TSH and FT_4 levels. Another purpose has also been made to find the impacts of arsenic level on simple diffuse goiters. This research

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Dhaka Univ. J. Pharm. Sci. **10**(1): 13-20, 2011 (June)

is based on experimental group with simple diffuse goiter and control group. In this study urinary arsenic concentration of patients of simple diffuse goiter are considered for measurement to assess the body arsenic status. Urinary arsenic is considered for measurement because arsenic in urine, hair and nail has been used as an indicator for monitoring the exposure of victim to arsenic and urinary arsenic is generally reported as the most reliable indicator of recent exposure to arsenic.[14]

MATERIALS AND METHODS

Type of study. This was a case-control analytical study. In this study, we used the variables urinary iodine, urinary arsenic, TSH, FT_4 and radioiodine uptake for both experimental group with simple diffuse goiter and control group.

Place and period of the study. The study was carried out in the Institute of Nuclear Medicine and Ultrasound, in collaboration with the thyroid out patient department of Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh.

Subjects. A total of eighty five (85) patients were included in the study. Among the 85 subjects a sample of forty five (45) patients was considered for experiment with simple diffuse goiter after diagnosis and another forty (40) patients were considered for control group without any obvious thyroid disorder. All of them were apparently healthy. Among the 45 patients thirty eight (38) were female and seven (7) were male; and in control group twenty nine (29) were female and eleven (11) were male.

Selection criteria. Subjects were selected under the following criterion:

Inclusion criteria. (1) Clinically diagnosed case of simple diffuse goiter, (2) Biochemically euthyroid, (3) Patients between the age of 5 and 25 years.

Exclusion criteria. (1) Patient having nodular goiter, (2) Patient with thyroid dysfunction, (3) Pregnancy, lactation and patients taking drugs like steroid, lithium, iodine containing drug and contrast agent.

Study procedure. Relevant information were recorded on a "Questionnaire" from all patients. After taking the history each patient underwent careful physical and thyroid examination. All these findings were recorded in a data sheet.

Sampling. Blood samples were collected from the subjects with all aseptic preparation in clean and dry test tubes for the measurement of (i) Serum thyroid stimulating hormone (TSH), (ii) Serum free thyroxin (FT_4), (iii) Thyroid autoantibodies-, (iv) Antithyroperoxidase antibody (Anti TPO Ab), (v) Antithyroglbulin antibody (Anti Tg Ab). Urine samples were collected in clean dry and metal free containers for the determination of urinary iodine and urinary arsenic.

Collection of blood. Under aseptic precaution 5 ml of venous blood was collected from the patient and control by disposable syringe.

Collection of Urine. Early morning urine was collected in metal freed plastic container. The collected urine was then stored at 4ºC for estimation of urinary iodine and urinary arsenic.

Analytical method

Estimation of serum TSH and FT_4. Serum human TSH and FT_4 measurement was based on RIA and IRMA. Estimation of serum Anti TPO & Anti Tg Ab measurement was based on microparticle enzyme immunoassay (MEIA) principle.[15]

Urinary iodine determination. Urinary iodine was measured by Method–B.[16] Urine was diagnosed with chloric acid solution. Iodide is the catalyst in the reduction of ceric ammonium sulfate (yellow) to cerous form (colorless), and is detected by rate of color disappearance.[17]

Urinary arsenic determination. Urine samples were diluted with matrix modifier using a standard protocol. The determination of total content of arsenic in urine was done by GFAAS systems.[18] Calibration curve was used before each cycle of measurement using standard supplied by Wako Inc.

Statistical analysis. In this study the χ^2-test was done to determine the homogeneity between two groups. The statistical significance of differences

between the values of several variables was assessed by t-test and Mann Whiteney U-test. In order to measure the degree of relationship of several variables with arsenic level for both experimental and control groups, here the Speraman's Rank Correlation coefficients were calculated and to test whether the Speraman's Rank Correlation coefficients are statistically significant or not here we applied the *t*-test. Regression analyses were done by taking appropriate dependent and independent variables. A two-tailed *p*-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

From Figure 1 , it has been found that maximum patients had above the normal level of arsenic (05ppb-50ppb) for experimental group, but for control group maximum patients were within the normal level, which indicated the differences between experimental and control groups. From the estimated results in Table (1), it has been found that urinary arsenic is positively related with urinary iodine level for both experimental and control groups. It is positively associated with TSH level for experimental group but negatively associated for control group, with FT_4 it is negatively associated for both experimental and control groups; it is also negatively correlated with radioiodine uptake levels at 2 hours and 24 hours for both experimental and control groups. From the *t*-test results, it has been found that the relationships of urinary arsenic level with urinary iodine, TSH , FT_4 and radiiodine uptake levels at 2 hours were not statistically significant for both experimental and control groups, but the relationship with radioiodine uptake level at 24 hours for experimental group was statististically significant at any level of significance, but for control group it was statistically significant at 10% level of significance.

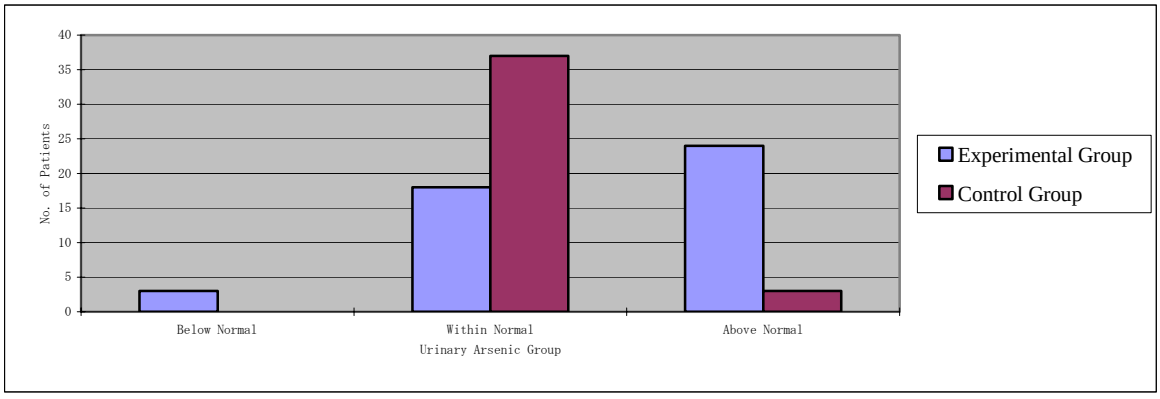

Figure 1. Urinary arsenic level with number. of patients for both experimental and control groups

From Table 2 it has been found that 6.67% patients fall below normal level, 40% patients fall within normal level and 53.33% patients fall above normal level of arsenic for the experimental group but for control group 92.5% patients fall within normal level and 7.5% patients fall above normal level of arsenic for the control group. This indicates that there is a significant difference between experimental and control groups in respect to arsenic level due to the problem of simple diffuse goiter. From the χ^2-test in Table 2, it has been found that the experimental and control groups are not homogeneous in respect to arsenic level. Regarding radioiodine uptake level at 2 hours, it has been found that 42.22% patients fall below the normal level, 57.78% patients fall within normal level and there is no patient above normal of radioiodine uptake in experimental group, and for control groups it has been found that 100% patients fall within normal levels, which indicates the differentials between experimental and control groups in terms of radioiodine uptake level at 2 hours. Also from the

Table 1. Spearman's rank correlation coefficients between different pairs of variables for experimental and control groups

Urine Arsenic Level	Parameters	(Mean ± SD)	Spearman's rank correlation coefficient	*t*-Test (*p*-value)
	Experimental Group			
	Between Arsenic and Urinary Iodine	357.95 ± 151.89	0.1676	1.114720 (0.27116228)
	Between Arsenic and RAIU 2 Hours	3.89 ± 1.72	-0.0149	-0.097422 (0.92284399)
Medain Value 85.35	Between Arsenic and RAIU 24 Hours	11.84 ± 5.17	-0.4531	-3.3329 (0.00177501)
	Between Arsenic and TSH (µlU/L	2.38 ± 1.22	0.1738	1.157172 (0.25359189)
	Between Arsenic and FT_4 (ng/dl)	12.63 ± 2.33	-0.1105	-0.7289 (0.47002043)
Urine Arsenic Level	Control Group			
	Between Arsenic and Urinary Iodine	397.85 ± 58.25	0.1361	0.846960 (0.40232008)
	Between Arsenic and RAIU at 2 Hours	5.40 ± 1.256	-0.1163	-0.721962 (0.47473710)
Median Value 36.98	Between Arsenic and RAIU at 24 Hours	12.95 ± 2.396	-0.2763	-1.772310 (0.08436095)
	Between Arsenic and TSH((µlU/L)	1.98 ± 1.16	-0.0273	-0.168051 (0.86743411)
	Between Arsenic and FT_4 (ng/dl)	1.98 ± 1.16	-0.0488	-0.301062 (0.76500882)

Note : The numbers inside the parentheses are the *p*-values of the *t*-test.

Table 2. Groups of Arsenic, Radioiodine uptake levels with the number of Patients and the χ^2-test for Homogeneity

Sample	Urinary Arsenic Groups, Normal Range is (05ppb – 50ppb)				χ^2-test for homogeneity
	No. of patients	Below normal	Within normal	Above normal	
Experimental	No. of patients	3	18	24	25.691751
Group	No. of patients as %	6.67%	40%	53.33%	(0.00000264)
Control	No. of patients	0	37	3	
Group	No. of patients as %	0	92.5%	7.5%	
Sample	Radioiodine Uptake Groups at 2 Hours , Normal Range (4%-10%)				χ^2-test for Homogeneity
	No. of patients	Below Normal	Within Normal	Above Normal	
Experimental	No. of patients	19	26	0	21.750842
Group	No. of patients as %	42.22%	57.78%	0	(0.00001892)
Control Group	No. of patients	0	40	0	
	No. of patients as %	0	100%	0	
Sample	Radioiodine uptake Groups at 24 Hours, Normal Range (10%-25%)				χ^2-test for Homogeneity
	No. of patients	Below Normal	Within Normal	Above Normal	
Experimental	No. of patients	14	31	0	11.928108
Group	No. of patients as %	31.11%	68.89%	0%	(0.0025694)
Control Group	No. of patients	1	39	0	
	No. of patients as %	2.5%	97.5%	0%	

	Urinary Iodine Groups							χ^2-test for Homogeneity
	No. of patients	Severe iodine deficiency	Moderate iodine deficiency	Mild iodine deficiency	Optimal	More than adequate	Excessive	
Experimental Group	No. of patients	0	3	2	3	4	33	
	No. of patients as %	0%	6.67%	4.44%	6.67%	8.89%	73.33%	8.105357 (0.150523)
Control Group	No. of patients	0	0	0	0	3	37	
	No. of patients s %	0%	0%	0%	0	7.5%	92.5	

Table 3. Mann-Whitney U-test results for comparison of urinary arsenic, urinary iodine, TSH, FT_4 and radioiodine uptake levels at 2 hours and 24 hours, between experimental and control groups

Study subjects	Mann-Whitney U-test				
	Median value	Range	U-test value	E(U)	Normal test (p-value)
	Urine Arsenic Level (ppb)				
Experimental Group (n =45)	85.35	1.50-275.5	1236	900	2.9583
Control Group (n =40)	36	18-70			(0.003093)
Study Subjects	Urinary Iodine Concentration Level (µg/L)				
Experimental Group (n =45)	443.70	45.05-480.4	1021.5	900	1.069749
Control Group (n =40)	413	240-474			(0.28473211)
Study Subjects	Radioiodine Uptake Levels at 2 Hours				
Experimental Group (n =45)	4	1-15	514	900	-3.398540
Control Group (n =40)	5	4-8			(0.0006774)
Study Subjects	Radioiodine Uptake Levels at 24 Hours				
Experimental Group (n =45)	13	2-44	824	900	-0.66914
Control Group (n =40)	13	8-18			(0.503404)
Study Subjects	TSH((µg/L))				
Experimental Group (n =45)	2.17	0.38-5.07	717.5	900	-1.606823
Control Group (n =40)	1.75	0.26-4.80			(0.10809322)
Study Subjects	FT_4 (ng/dl)				
Experimental Group (n =45)	12.3	9.50- 18.62	662	900	-2.095473
Control Group (n =40)	12.7	10.40-16.10			(0.03612895)

Note : The numbers in the parentheses are the *p*-values of the *t*-test

Table 4. Parameter Estimates of the Regression Equation for both Experimental and Control Groups

Parameters	Experimental group				
	Parameters value	Standard error	t-test	p-value	R^2
α_0	169.9883026	64.0918155	2.65226	0.01150166	
α_1	-0.0219976	0.0696214	-0.31596	0.75371771	
α_2	-1.9973025	6.2882471	-0.31762	0.75246404	67.9714%
α_3	-4.3805897	1.9281385	-2.27193	0.02868515	
α_4	6.9287725	8.3628173	0.82852	0.41241721	
α_5	-2.8751893	4.2276390	-0.68009	0.50046280	
Parameters	Control group				
	Parameters value	Standard error	t-test	p-value	R^2
α_0	35.70168394	27.47363003	1.29949	0.20251861	
α_1	0.04587977	0.05296514	0.86623	0.39243813	
α_2	-0.12841871	1.60331266	-0.08010	0.93663062	92.8855%
α_3	-1.38404344	0.87672563	-1.57865	0.12367608	
α_4	0.59147085	2.62667154	0.22518	0.82318813	
α_5	-0.03539730	1.28690939	-0.02751	0.97821723	

χ^2-test results it has been found that these two samples are not homogeneous in terms of radioiodine uptake level at 2 hours. Same conclusion can be drawn in terms of radioiodine uptake level at 24 hours. From the χ^2-test value it has been found that the two samples are homogeneous in terms of urinary iodine level, which indicates that iodine is only the not factor of causing simple diffuse goiters but arsenic is also another important factor of causing simple diffuse goiter. Thus, it can be concluded that arsenic level plays significant role of causing simple diffuse goiter in Bangladesh population. From the estimated results in Table 3, it has been found that the median and range of urinary arsenic level, urinary iodine levels TSH level and FT_4 level for experimental and control groups are significantly different. The median values of radioiodine uptake level at 2 hours and 24 hours for experimental and

control groups are not significantly different but their range are different. From Mann-Whitney U-test results or results of the standard normal test, it can be concluded that the arsenic level, FT_4 and radioiodine uptake levels at 2 hours between experimental and control groups are significantly different but the urinary iodine level, TSH levels and radioiodine uptake levels at 24 hours between experimental and control groups are not statistically different. Thus, from the Mann-Whitney U-test results it can also be concluded that the iodine deficiency is not the only factor of causing simple diffuse goiter, but arsenic level is also another most important factor of causing simple diffuse goiter in Bangladeshi population.

Regression analysis. In order to find the impacts of urinary iodine, radioiodine uptake, thyroid stimulating hormone (THS) and free thyroxin (FT_4) on urinary arsenic levels, we considered the following regression equation;

$$Arsenic_i = \alpha_0 + \alpha_1 Iodine_i + \alpha_2 RIU2_i + \alpha_3 RIU24_i + \alpha_4 TSH_i + \alpha_5 FT_{4_i} + \varepsilon_i \; ; \quad (i=1, 2,\ldots, n) \qquad (1)$$

where,

Arsenic: indicates the level of arsenic, Iodnine: indicates level of iodine; RIU2: indicates serum radioiodine uptake at 2 hours; RIU24: indicates radioiodine uptake at 24 hours, TSH: indicates serum thyroid stimulating hormone, FT_4 indicates the serum free thyroxin; ε is the random error term; the subscript i indicates the ith observation and n is the total number of observations; fFor the experimental group n = 45 and for the control group n =40; α_0 is the regression constant; α_i (i=1, 2, 3, 4 and 5) are the regression coefficients by which we can measure the impacts of these variables on arsenic levels. The estimated results are given in Table 4. From the results in Table 4, it has been found that variable iodine level has negative impact on arsenic level for experimental group, but for the control group it has positive impact. But the impacts are not statistically significant. It has also been found that the radioiodine uptake levels at 2 hours and 24 hours and the variable serum FT_4 has negative impacts on arsenic level for both the experimental and control group. Their impacts are not statistically significant except the variable radioiodine uptake level at 24 hours. The impact of radioiodine uptake level at 24 fours is statistically significant for the experimental group but for the control group it is not statistically significant. The serum TSH has positive impact on arsenic level for both experiment and control groups but not statistically significant. Thus from the estimated regression equation, it can be concluded that the variable radioiodine uptake level at 24 hours plays the significant role for reducing the arsenic level.

CONCLUSION

From the χ^2-test results, it has been found that the experimental and control groups are not homogeneous in respect of arsenic level and radioiodine uptake levels at 2 hours and 24 hours, but they are homogeneous in respect of urinary iodine level, which indicates that iodine is not the only factor of causing simple diffuse goiters, arsenic is also another important factor of causing simple diffuse goiter in Bangladeshi population. Thus, it can be concluded that, the level of arsenic plays a significant role of causing simple diffuse goiter. From the Mann-Whitney U-test results or value of the standard normal test in Table (3), it can be concluded that the arsenic level, FT_4 and radioiodine uptake level at 2 hours between experimental and control groups are significantly different but the urinary iodine level, TSH level and radioiodine uptake level at 24 hours between experimental and control groups are not statistically different. So, from this test it can also be concluded that iodine deficiency is not the only factor of causing simple diffue goiter but arsenic level is also an important factor on causing simple diffuse goiter.

From the estimated regression results in Table 4, it has been found that the variable iodine level has negative impact on arsenic level for experimental group, but for the control group it has positive impact. But these impacts are not statistically significant. Also it has been found that the radioiodine uptake levels at 2 hours and 24 hours and the variable serum FT_4 have negative impacts on arsenic level for both the experimental and control group. Their impacts are

not statistically significant except the variable radioiodine uptake level at 24 hours. The impact of radioiodine uptake level at 24 fours is statistically significant for the experimental group but for the control group it is not statistically significant. The serum TSH has positive impact on arsenic level for both experiment and control groups but not statistically significant. Thus from this research it has been found that the radioiodine uptake level at 24 hours play the significant role of reducing the arsenic levels for both experimental and control groups. It has also been found that the two groups are not homogeneous in terms of arsenic level, radioiodine uptake levels at 2 hours and 24 hours, but homogeneous in terms of urinary iodine level. Thus finally it can be concluded that the radiiodine uptake level at 24 hours plays the significant role for reducing urinary arsenic level.

CONCLUSION

From the findings of χ^2 and Mann-Whitney tests it can be concluded that iodine deficiency is not the only factor of causing simple diffue goiter but arsenic level is also one of the important factor of causing simple diffuse goiter in Bangladeshi population. From the estimated regression results in Table 4, it is found that the variable iodine level has negative impact on arsenic level for experimental group, but for the control group it has positive impact. But these impacts are not statistically significant. Also it is found that the radioiodine uptake levels at 2 hours and 24 hours and the variable serum FT_4 have negative impacts on arsenic level for both the experimental and control group. Their impacts are not statistically significant except the variable radioiodine uptake level at 24 hours. The impact of radioiodine uptake level at 24 fours is statistically significant for the experimental group but for the control group it is not statistically significant. The serum TSH has positive impact on arsenic level for both experiment and control groups but not statistically significant. Thus from this research it has been found that the radioiodine uptake level at 24 hours play the significant role of reducing the arsenic levels for both experimental and control groups. It has also been found that the two groups are not homogeneous in terms of arsenic level, radioiodine uptake levels at 2 hours and 24 hours, but homogeneous in terms of urinary iodine level. Thus finally it can be concluded that the radiiodine uptake level at 24 hours plays the significant role for reducing urinary arsenic level.

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