

# Comparison of Urinary Iodine, Urinary Arsenic, Radioiodine Uptake, Thyroid Stimulating Hormone (TSH) and Free Thyroxin (FT<sub>4</sub>) Levels, Between Experimental Group with Simple Diffuse Goiter and Control Group

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**ABSTRACT:** In Bangladesh some researchers have done several studies to find the correlation of simple diffuse goiter with arsenic level but no one conducted any study to find the differences of urinary iodine, urinary arsenic, radioiodine uptake, the thyroid stimulating hormone (TSH) and free thyroxin (FT<sub>4</sub>) levels between goitrous and non goitrous. That is why, in this research an attempt has been made to compare the urinary iodine, urinary arsenic, radioiodine uptake, TSH and FT<sub>4</sub> levels between experimental group with simple diffuse goiter and healthy control group. In this study another purpose has also been made to find the associations between different pairs of variables for both experimental and control group. In this study we have tried to find the impacts of arsenic level on simple diffuse goiter. This is a case-control analytical study. The study is carried out in the Institute of Nuclear Medicine and Ultrasound, in collaboration with the thyroid out patient department of Bangabandhu Sheikh Mujib Medical University. A total of eighty five (85) subjects are included in the study. Among the 85 subjects, a sample of forty five is considered for experimental group with simple diffuse goiter and another sample of forty is considered for healthy control group without any thyroid disease. From the experimental results it has been found that, the arsenic levels, FT<sub>4</sub> and radioiodine uptake levels at 2 hours between the experimental and control groups are significantly different but the urinary iodine levels, TSH levels and radioiodine uptake levels at 24 hours between the experimental and control groups are not statistically different. From the experimental results it has also been found that there is significant differentiation between experimental and controls groups in respect of association between different pairs of variables. In this study, another significant finding is that iodine deficiency is not only the factor of causing simple diffuse goiter, but arsenic level is also one of the most important factor of causing simple diffuse goiter.

**Key words:** Iodine deficiency, Simple diffuse goiter, Urinary arsenic, Statistical analysis, Association, Experimental and Control groups

## INTRODUCTION

Thyroid disorder is one of the major health hazards in many countries including Bangladesh.<sup>1</sup> Bangladesh is situated in the zone of iodine deficiency area in the belt of the Brahmaputra River. So goiter and iodine deficiency are very common in our part.<sup>2</sup> Goiter may be classified into many ways. It

may be toxic or nontoxic according to patients toxic status, diffuse or nodular according to its consistency, endemic and sporadic epidemiologically. Simple diffuse goiter (SDG) usually presents between the ages of 15-25 years. Female and adolescents are usually sufferer and often during pregnancy. Simple diffuse goiter is also termed as endemic goiter as it usually occurs in large numbers of certain population. The great arc of the Himalayas from Pakistan across India, Nepal, Northern Thailand, Vietnam and

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Indonesia are one of the most highly endemic regions of the world.<sup>3</sup> Regarding the word endemic, this has been defined as a prevalence rate of more than 10%.<sup>4,5</sup> There is now a tendency to decrease this figure from 10% to 5%. Therefore, more areas will be classified as having a problem of endemic goiter.<sup>6</sup> According to 1999 national survey in 80 areas in Bangladesh, conducted on children of 5-11 years and adults of 15-44 years of 20,978 subjects, total goiter rate (TGR) was 17.85.<sup>7</sup> In 1993 national survey nearly half (47%) of the population had symptoms of goiter.<sup>7</sup> Total goiter rate (TGR) is declining due to availability of iodized salt in the market. The main cause of endemic goiter is the iodine deficiency.<sup>6,8</sup> Other causes are genetic, iodine excess, goitrogens and some drugs like PAS, thiocyanate and so on.<sup>8</sup> Poverty is also another factor of causing of endemic goiter. The arguments supporting iodine deficiency as the cause of endemic goiter. Several other goitrogens have been described in various plants as well as in the drinking water.<sup>6</sup> Iodine in large concentration is itself a goitrogen. "Iodide goiter" has been described in Japan in a coastal area where the inhabitants consumed large quantities of sea-plants with high iodine content<sup>9</sup> and recently in China due to a high iodine concentration in the drinking water.<sup>8</sup>

In Bangladesh drinking water is heavily contaminated with arsenic. Arsenic is an important environmental contaminant being number one in the EPA (environmental protection agency) superfund list. An estimated 50 million people are at risk from drinking arsenic contaminated water in Bangladesh and West Bengal, India.<sup>10</sup> A large number of populations in many districts of Bangladesh are drinking ground water with arsenic concentrations for above acceptable levels (0.05 mg/L) and many of them have already been diagnosed with poisoning symptoms, even though much of the at-risk population has not yet been assessed for arsenic related health problems.

So there might be a correlation of body arsenic status with goiter. Arsenic is a direct antagonist of selenium and uses up selenium in the process of detoxifying arsenic. On the other hand selenium is

critical for converting thyroxin ( $T_4$ ) to triiodothyronin ( $T_3$ ). So arsenic can contribute to hypothyroidism by using up selenium.<sup>10</sup>

According to Dhaka Community Hospital.<sup>11</sup> diabetes mellitus and goiter have also been reported in association with prolonged ingestion of arsenic through drinking water. The above studies showed a definite correlation between arsenic and goiter formation. Iodine is an indispensable component of the thyroid hormones, comprising 65% of  $T_4$ 's by weight, and 58% of  $T_3$ . Iodine in food and drink is the source of this micronutrient for hormone production. About 120-150  $\mu\text{g}$  of iodine is needed daily to maintain the body physiology. However in iodine deficient zone, it may be lower than 10 $\mu\text{g}$  daily intake. Radioiodine uptake test is done for evaluation of thyroid disorders. As thyroid is a dynamic gland, iodine uptake is changed with change of iodine intake or thyroid physiology. There is an inverse relationship between the daily intake of iodine and the radioiodine uptake. The measurement of the ability of the thyroid gland to accumulate radioactive iodine has become an established diagnostic procedure.<sup>12</sup>

The overall total goiter rate (TGR) is very high (17.8%) in spite of using iodized salt as a prophylaxis for iodine deficiency for many years.<sup>7</sup> Bangladesh is also an endemic area of chronic arsenic poisoning.<sup>10</sup> There are some studies regarding correlation with chronic arsenic poisoning and goiter in Bangladesh, Taiwan,<sup>13</sup> and other countries of the world.<sup>9</sup> In Japan and in China it has been found the correlation of goiter with iodine excess and/or arsenic.<sup>14</sup> High level of arsenic affects the thyroid gland by reducing the uptake of iodine. In our country some studies were done to find out the correlation of simple diffuse goiter with iodine excess and or arsenic but no experiment has been done to find the differences of the urinary iodine, urinary arsenic, radioiodine uptake, TSH and  $FT_4$  levels between goitrous and non goitrous.

That is why in this research the principal purpose has been made to compare the urinary iodine, urinary arsenic, radioiodine uptake, TSH and  $FT_4$  levels

between experimental group with simple diffuse goiter and control group. Another purpose has also been made to find the association between different pairs of variables for both experimental and control group. In this research we have tried to find the impacts of arsenic levels on simple diffuse goiters. 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 the most reliable indicator for monitoring the exposure of victim to arsenic.<sup>15</sup>

## MATERIALS AND METHODS

**Type of the Study.** This is a case-control analytical study. Urinary iodine, urinary arsenic, radioiodine uptake, TSH and FT<sub>4</sub> levels of an experimental group (patients group) with simple diffuse goiter are compared with the urinary iodine, urinary arsenic, radioiodine uptake, TSH and FT<sub>4</sub> levels of healthy 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. This study was conducted in 2005.

**Subjects.** A total of eighty five (85) patients were included in the study. Among the 85 subjects a sample of forty five (45) patients is considered for experiment with simple diffuse goiter after diagnoses and another sample of forty (40) patients were considered for control group without any obvious thyroid disease all of them 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 respectively. All the subjects in the study were informed and explained clearly about the nature of the study and included in the study only after they had given their written consent.

**Selection Criteria.** Subjects are 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.** A Questionnaire is made to collect the relevant information of the patients. All the relevant information were recorded in Questionnaire from all patients. After taking the history each patient underwent careful physical examination and thyroid examination, all the findings are 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<sub>4</sub>), (iii) Thyroid autoantibodies-, (iv) Antithyroperoxidase antibody (Anti TPO Ab), (v) Antithyroglobulin 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 all 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 4°C for later estimation of urinary iodine and urinary arsenic.

## Analytical Method

**Estimation of serum TSH and FT<sub>4</sub>.** Serum human TSH and FT<sub>4</sub> measurement was based on RIA and IRMA. Estimation of serum Anti TPO and Anti Tg Ab measurement was based on microparticle enzyme immunoassay (MEIA) principle.

**Urinary iodine determination.** Urinary iodine was measured by Method-B.<sup>16</sup> Urine is digested 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 (Sandell- Kolthof reaction).

**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. Calibration curve was used before each cycle of measurement using standard supplied by Waco Inc.

**RESULTS AND DISCUSSION**

From figure 1, it has been found that maximum patients fall in the above normal levels of urinary arsenic for experimental group, but for control group maximum patients fall within the normal level, which

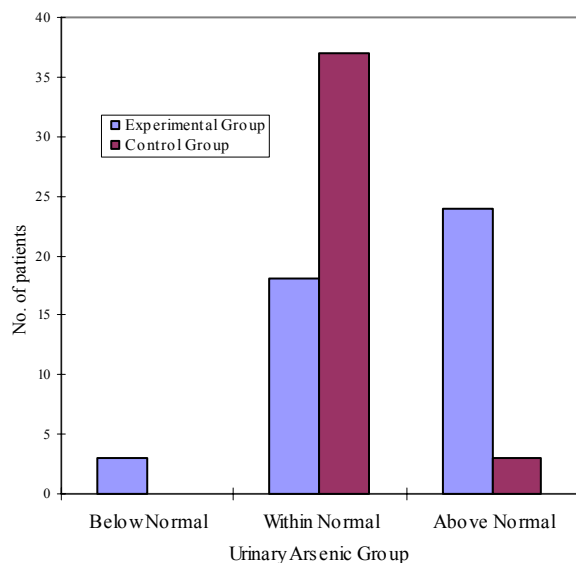


Figure 1. Urinary arsenic groups with the number of patients for both experimental and control groups

indicates the differences between experimental and control groups. From figure 2, we see that maximum patients contain iodine more than optimal level i.e. greater than 300 µg/L for both experimental and control groups. From figures 3 and 4, it has been found that the maximum patients fall within the normal range of radioiodine uptake levels at 2 hours and 24 hours respectively. Therefore, it can be said

that in respect to iodine, and radioiodine uptake levels there is no significant differences between the experimental and control groups. For further investigation, we will use different types of statistical techniques to compare the urinary arsenic, urinary iodine, radioiodine uptakes, TSH and FT<sub>4</sub> levels between experimental and control groups.

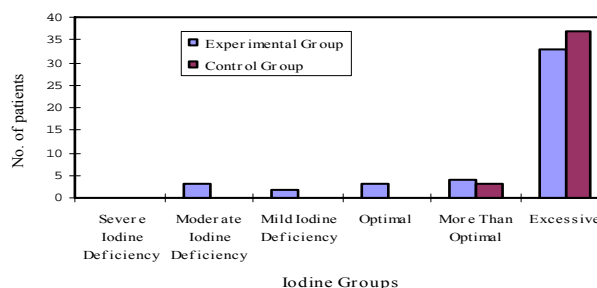


Figure 2. Urinary iodine groups with the number of patients for both experimental and control groups

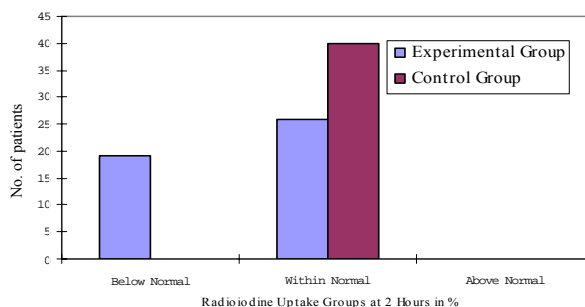


Figure 3. Radioiodine uptake groups at 2 hours with the number of patients for both experimental and control groups

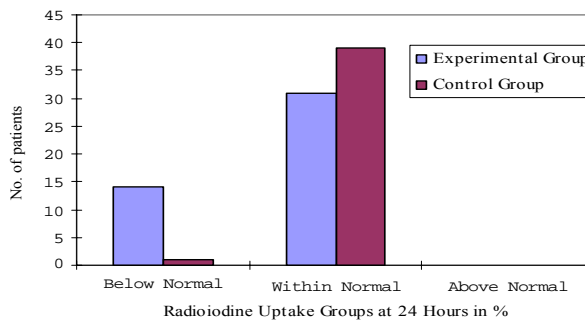


Figure 4. Radioiodine uptake Groups at 24 hours with the number of patients for both experimental and control groups

**Statistical Methods.** The different statistical analyses have been done to compare the arsenic, iodine, radioiodine uptake, TSH and FT<sub>4</sub> levels between experimental and control groups. For testing

homogeneity of experimental and control groups for the variables urinary arsenic, urinary iodine and radioiodine uptake at 2 hours and 24 hours we applied the chi-square test. Also to compare the urinary arsenic, urinary iodine, radioiodine uptake, TSH and FT<sub>4</sub> levels between experimental and control groups here we applied the Mann Whitney U-test. The Spearman's rank correlation test is

applied to test whether the associations between different pairs of variables are statistically significant or not. The statistical techniques with their results are discussed below.

To know the no. of patients that fall in different age groups for both experimental and control groups the obtaining results are reported in Table 1.

**Table 1. Age and sex distribution with the No. of patients for both experimental and control groups**

Age in years	Study subjects			
	Control group (n = 40)	Experimental group (n = 45)	% of the Patients	t-test for equality of two mean age (p-value)
(Mean ± SD)	19.875 ± 6.517	17.044 ± 4.4515	0%	
(Mean ± 2SD)	19.875 ± 13.034	17.044 ± 8.903	At least 75%	2.309859
(Mean ± 3SD)	19.875 ± 19.551	17.044 ± 13.3545	At least 89%	(0.02398481)
Sex	Male (11)	Male (7)		
	Female (29)	Female (38)		

From the statistical analysis, it has been found that for the experimental group, the mean age of patients is 19.88 years, and for control group the mean age is 17.04 years. In case of control group, there is no patient between age 13.36 and 26.40, at least 75% patients falls between age 6.84 and 32.92, at least 89% patients fall between age 0.3240 and 39.4260. In case of experimental group, 0% patient fall between age 12.59 and 21.49, at least 75% patients fall between age 3.46 and 22.26 and at least 89% patients fall between age 3.6895 and 30.3985. From the t-test results it has been found that the mean ages of the two groups are statistically different at 5% level of significance.

In order to know the no. of patients that fall in different arsenic groups, radioiodine uptake groups and iodine groups for both experimental and control groups, the obtaining results are reported in the Table 2. Also, to know whether the experimental and control groups are homogeneous or not we applied the  $\chi^2$ -test for these variables. The estimated results of the  $\chi^2$ -test with the p-value are reported in Table 2.

From Table 2 it has been found that 6.67% patients contain below normal level, 40% patients contain normal level and 53.33% patients contain above normal levels of arsenic for the experimental group, and there is no patient below normal level, 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 terms of arsenic levels. From the  $\chi^2$ -test, it has been found that the experimental and control groups are not homogeneous in respect of arsenic levels. Regarding radioiodine uptake levels 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 levels at 2 hours. Also from the  $\chi^2$ -test results it has been found that these two samples are not homogeneous in terms of radioiodine uptake levels at 2 hours same conclusion can be drawn in terms of radioiodine uptake levels at 24 hours. From the chi-square test results it has been found that the two samples are homogeneous in terms of urinary iodine levels, which indicates that iodine is not only the factor of causing simple diffuse goiters, arsenic is also another important factor of causing simple diffuse goiter. Thus it can be concluded that, the level of arsenic plays significant role of causing simple diffuse goiter.

In order to compare the arsenic level, iodine level, radioiodine uptake level at 2 hours and 24 hours, TSH level and FT<sub>4</sub> level between experimental and control groups, we applied the Mann Whitney U test. The test results are reported in Table 3.

**Table 2. No. of Patients Fall in Different Arsenic, Radioiodine Uptake and Iodine Groups and the  $\chi^2$ -test for Homogeneity**

Sample	Urinary Arsenic Groups, Normal Range is (05ppb – 50ppb)						$\chi^2$ -test for homogeneity	
	No. of Patients	Below normal	Within normal	Above normal				
Experimental group	No. of Patients	3	18	24			25.691751 (0.00000264)	
	No. of Patients as %	6.67%	40%	53.33%				
Control group	No. of Patients	0	37	3			21.750842 (0.00001892)	
	No. of Patients as %	0	92.5%	7.5%				
Sample	Radioiodine Uptake Groups at 2 Hours , Normal Range (4%-10%)						$\chi^2$ -test for homogeneity	
	No. of Patients	Below normal	Within normal	Above normal				
Experimental group	No. of Patients	19	26	0			11.928108 (0.0025694)	
	No. of Patients as %	42.22%	57.78%	0%				
Control group	No. of Patients	0	40	0			11.928108 (0.0025694)	
	No. of Patients as %	0	100%	0%				
Sample	Radioiodine Uptake Groups at 24 Hours , Normal Range (10%-25%)						$\chi^2$ -test for homogeneity	
	No. of Patients	Below normal	Within normal	Above normal				
Experimental group	No. of Patients	14	31	0			8.105357 (0.15052)	
	No. of Patients as %	31.11%	68.89%	0%				
Control group	No. of Patients	1	39	0			8.105357 (0.15052)	
	No. of Patients as %	2.5%	97.5%	0%				
	Urinary Iodine Groups							$\chi^2$ -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	8.105357 (0.15052)
	No. of patients as %	0%	6.67%	4.44%	6.67%	8.89%	73.33%	
Control group	No. of patients	0	0	0	0	3	37	8.105357 (0.15052)
	No. of patients as %	0%	0%	0%	0%	7.5%	92.5	

**Table 3. Mann-Whitney U-Test for Comparison of Urinary Arsenic, Urinary Iodine, TSH, FT<sub>4</sub> and Radioiodine Uptake Levels, Between Experimental and Control Groups**

	Mann-whitney U-test					
	Median value	Range	U -Test value	E(U)	Lower and upper critical values of U	Normal test (p-value)
<b>Urine arsenic level (ppb)</b>						
Experimental group (n =45)	85.35	1.50-275.5	1236	900	Lower Limit (677.39) Upper Limit (1122.61)	2.9583 (0.003093)
Control Group (n=40)	36	18-70				
<b>Urinary iodine concentration level (µg/l)</b>						
Experimental group (n =45)	443.70	45.05-480.4	1021.5	900	Lower (677.39) Upper (1122.61)	1.069749 (0.28473211)
Control Group (n=40)	413	240-474				
<b>TSH (µg/l)</b>						
Experimental group (n =45)	2.17	0.38-5.07	717.5	900	Lower (677.39) Upper(1122.61)	-1.606823 (0.10809322)
Control Group (n=40)	1.75	0.26-4.80				
<b>FT<sub>4</sub> (ng/dl)</b>						
Experimental group (n =45)	12.3	9.50- 18.62	662	900	Lower (677.39) Upper(1122.61)	-2.095473 (0.03612895)
Control Group (n =40)	12.7	10.40-16.10				
<b>Radioiodine Uptake Levels at 2 Hours</b>						
Experimental group (n =45)	4	1-15	514	900	Lower Limit (677.39) Upper Limit (1122.61)	-3.398540 (0.0006774)
Control Group (n =40)	5	4-8				
<b>Radioiodine Uptake Levels at 24 Hours</b>						
Experimental group (n=45)	13	2-44	824	900	Lower Limit (677.39) Upper Limit (1122.61)	-0.66914 (0.503404)
Control Group (n=40)	13	8-18				

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

The median values, range, and the Mann Whitney U-test results with their lower and upper limits, and the normal test results with the p-value for all the variables are reported in the Table 3. From the estimated results, it has been found that the median and the range of urinary arsenic level, urinary iodine level, TSH level and FT<sub>4</sub> level for experimental and control groups are significantly different. The median values of radioiodine uptake levels at 2 hour and 24 hour 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<sub>4</sub> and radioiodine uptakes levels 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.

Also to know the relationship between different pairs of variables for both experiment and control groups we calculated the Spearman's rank correlation coefficient which are reported in Tables 4 and 5.

**Table 4. Correlation between different pairs of variables for experimental group**

Correlation of Urine Iodine with TSH, FT <sub>4</sub> , and Radioiodine Uptake Level		
	Spearman's Rank Correlation (P)	t-Test Statistic (p-value)
Correlation Between Urinary Iodine and Urine Arsenic	0.1676	1.114720 (0.27116228)
Correlation Between Urinary Iodine and TSH	0.1296	0.8574 (0.39599387)
Correlation Between Urinary Iodine and FT <sub>4</sub>	-0.1068	-0.704045 (0.48520032)
Correlation Between Urinary Iodine and RAIU at 2 Hours	-0.3685	-2.59916 (0.01275292)
Correlation Between Urinary Iodine and RAIU at 24 Hours	-0.0545	-0.3582 (0.72193747)
Correlation of Urine Arsenic with TSH, FT <sub>4</sub> , and Radioiodine Uptake Level		
Correlation Between Urinary Arsenic and TSH	0.1738	1.157172 (0.25359189)
Correlation Between Urinary Arsenic and FT <sub>4</sub>	-0.1105	-0.7289 (0.47002043)
Correlation Between Urinary Arsenic and RAIU at 2 Hours	-0.0149	-0.097422 (0.92284399)
Correlation Between Urinary Arsenic and RAIU at 24 Hours	-0.4531	-3.3329 (0.00177501)

**Table 5. Correlation between different pairs of variables for control group**

Correlation of Urine Iodine with TSH, FT <sub>4</sub> , and Radioiodine Uptake Level		
	Spearman's Rank Correlation (P)	t-Test Statistic (p-value)
Correlation Between Urinary Iodine and Urine Arsenic	0.1361	0.846960 (0.40232008)
Correlation Between Urinary Iodine and TSH	-0.7000	-6.043128 (0.000000)
Correlation Between Urinary Iodine and FT <sub>4</sub>	0.1457	0.907746 (0.36973333)
Correlation Between Urinary Iodine and RAIU at 2 Hours	-0.0663	-0.409743 (0.68429541)
Correlation Between Urinary Iodine and RAIU at 24 Hours	0.0945	0.584939 (0.56204622)
Correlation of Urine Arsenic with TSH, FT <sub>4</sub> , and Radioiodine Uptake Level		
Correlation Between Urinary Arsenic and TSH	-0.0273	-0.168051 (0.86743411)
Correlation Between Urinary Arsenic and FT <sub>4</sub>	0.0488	(0.301062) (0.76500882)
Correlation Between Urinary Arsenic and RAIU at 2 Hours	-0.1163	-0.721962 (0.47473710)
Correlation Between Urinary Arsenic and RAIU at 24 Hours	-0.2763	-1.772310 (0.08436095)

From the estimated results in Table 4, it has been found that urinary iodine is positively associated with urinary arsenic and TSH levels and negatively associated with FT<sub>4</sub> and radioiodine uptake levels at 2 hours and 24 hours for experimental group. Only the association between iodine level and radioiodine uptake at 2 hours is statistically significant. Also, it has been found that urinary arsenic is positively related with TSH level and negatively related with FT<sub>4</sub> and radioiodine uptake levels. But these associations are not statistically significant. Thus it can be said, if TSH level is increased the arsenic level also will be increased, if FT<sub>4</sub> and radioiodine uptake levels are increased the arsenic level will be decreased.

From the estimated results in Table 5 it has been found that urinary iodine is positively associated with urinary arsenic FT<sub>4</sub> levels and radioiodine uptake level at 24 hours and negatively associated with TSH and radioiodine uptake level at 2 hours. Only the association between iodine level and TSH level is statistically significant. Also, it has been found that urinary arsenic is positively related with FT<sub>4</sub> level and negatively associated with TSH and radioiodine uptake level. But these associations are not statistically significant. Thus from the estimated results it can be concluded that if TSH and radioiodine uptake levels are increased arsenic level will be decreased and if FT<sub>4</sub> level is increased the arsenic level will be increased.

Thus it can be concluded that there is a significant differentiation between experimental and control groups in terms of association between different pairs of variables.

## CONCLUSION

For the experimental group it has been found that the maximum patients fall at the age group of 15-20 years but for the control group maximum patients falls at the age group of 20-25 year, which indicates the differences between these two groups. From the test result it has been found that the mean ages of the two groups are significantly different. From the

graphical presentation it has also been found that for the experimental group, maximum patients contain the arsenic level more than 50 (ppb) but for the control group most of the patients contain arsenic levels within normal range, which implies significant differences between experimental and control groups. From figure 2, it has been found that maximum patients contain more than optimal level of iodine i.e. greater than 300 (µg/L) for both experimental and control groups, so there is no difference between the experimental and control groups in respect of containing iodine level. From figures 3 and 4, it has been found that the maximum patients falls within the normal range of radioiodine uptake levels at 2 hours and 24 hours respectively. Therefore, it can be said that in respect to iodine, and radioiodine uptake levels there is no significant differences between the experimental and control groups. For further investigation, we applied different statistical techniques to compare the urinary arsenic, urinary iodine, radioiodine uptakes, TSH and FT<sub>4</sub> levels between an experimental and control groups.

From the estimated results it has been found that the median urinary arsenic level of SDG patients is 85.35 ppb and 36 ppb in control group. Control group has iodine level below the recommended value of 50 ppb for Bangladesh, whereas the urinary arsenic level in simple diffuse goiter patients is far above the recommended level. So, it can be said that high level of urinary arsenic is one of the important factor of causing simple diffuse goiter. From the  $\chi^2$ -test, it has been found that the experimental and control groups are not homogeneous in respect of arsenic level and radioiodine uptake level at 2 hours and 24 hours also it has been found that the two samples are homogeneous in terms of urinary iodine level, which indicates that iodine deficiency is not only the factor of causing the simple diffuse goiter, but arsenic level is also another important factor of causing simple diffuse goiters.

From the estimated results of the Mann Whitney U-test that are reported in Table 3, it has been found that the median and range of urinary arsenic level, urinary iodine level, TSH level and FT<sub>4</sub> 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 in terms of their range. From the Mann-Whitney U-test results or results of the standard normal test, it can be concluded that the arsenic level, FT<sub>4</sub> and radioiodine uptakes 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. Thus from this result it can be concluded that iodine deficiency is not only the factor of causing simple diffuse goiter but arsenic level is also another important factor of causing simple diffuse goiter.

From the Spearman's rank correlation coefficients it has been found that urinary iodine level is positively associated with urinary arsenic level for both experimental and control groups. This implies that arsenic level does not cause the iodine deficiency. In case of experimental group it has been found that urinary iodine level is positively associated with TSH level and negatively associated with FT<sub>4</sub> and radioiodine uptake level at 2 hours and 24 hours. Only the association between iodine level and radioiodine uptake at 2 hours is statistically significant. Also, it has been found that urinary arsenic is positively associated with TSH level and negatively associated with FT<sub>4</sub> and radioiodine uptake level. But these associations are not statistically significant. But for control group it has been found that urinary iodine is positively associated with FT<sub>4</sub> levels and radioiodine uptake level at 24 hours and negatively associated with TSH and radioiodine uptake level at 2 hours. Only the association between iodine level and TSH level is statistically significant. Also, it has been found that urinary arsenic is positively associated with FT<sub>4</sub> level and negatively associated with TSH and radioiodine uptake level. But these associations are not statistically significant.

Finally, from the Spearman's rank correlation coefficients it can be concluded that, if TSH level is

increased the arsenic level also will be increased, if FT<sub>4</sub> and radioiodine uptake levels are increased the arsenic level will be decreased in case of experimental group, but for control group if TSH and radioiodine uptake levels are increased arsenic level also will be decreased and if FT<sub>4</sub> level is increased the arsenic level will be increased.

From the estimated results it can be concluded that, the arsenic, FT<sub>4</sub> and radioiodine uptakes levels at 2 hours between the experimental and control groups are significantly different but the urinary iodine level, TSH level and radioiodine uptake level at 24 hours between the experimental and control groups are not statistically different. In this study both control and experimental groups are found iodine sufficient. Thus it can be said that iodine is not only the factor of causing simple diffuse goiter, but arsenic levels is also one of the important factor of causing simple diffuse goiter. Also from this study, we found that arsenic level does not cause the iodine deficiency.

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