

Research Article

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Evaluation of Environmental Influence of Arsenic Compounds on Patients Suffering From Type 2 Diabetes

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Abstrac

Background: Diabetes Mellitus type 2 is a metabolic ailment. It is a condition when insulin is produced by our body but, it is not used properly by us. The number of diabetic patients is increasing in the whole world. The problem of obesity is also very closely related to it, which itself is expanding. The individuals diagnosed with type 2 Diabetes Mellitus have high chance of microvascular problems (like nephropathy). They are also at the verge of facing macrovascular ailments (like cardiovascular comorbidities). These issues are usually because of hyperglycemia and specific mechanisms of the insulin resistance (metabolic) disorder. Environmental factors and genetic features result in different pathophysiological issues. Arsenic is the chemical that act as diabetogenic agent as it effect the functioning of pancreatic beta cells and also cause insulin resistance through several ways like oxidative stress by the production of free radicals in the body and also due to the disturbance of protein hormone that disrupt insulin sensitivity.

Methods: Initially data through Questionnaire and samples of 100 diabetic patients and 100 normal healthy persons were collected. Then different parameters including AST, ALP, AST, Creatinine, Bilirubin, Urea, and HBA1c were estimated. Then the main parameter (Arsenic) was evaluated. Then Statistical analysis was done on collected data of both groups.

Results: All the parameters including glucose level was higher in diabetic patients. By comparing values of two groups statistically, it was observed that arsenic levels in diabetic group are far higher than the control group and the value of diabetic group is exceeding than the standard value.

Conclusion: So, arsenic level was higher in diabetic persons and working as diabetic agent. There should be no residence near rice paddies or any arsenic rich area. We should promote the usage of PPE "personal protective equipment" in order to avoid arsenic exposure.

Keywords: Diabetes Mellitus, Insulin Resistance, Biochemical Parameters, Arsenic, Statistical Analysis.

Introduction

The metabolic disorders categorized under hyperglycemia which occur because of insulin secretion problems, insensitivity to it, or both are termed as diabetes." The permanent harm, dysfunction, and failure of multiple human body parts, like eyes, kidneys, heart, nerves and blood vessels could be caused by persistent hyperglycemia [1]. There are many chemicals in the environment that effect sensitivity of insulin and effect beta cells then trigger type 2 diabe-

tes. POPs, dioxins, BPA and arsenic are chemicals that play their role to make a person diabetic and arsenic is the chemical of great interest. Persistent organic pollutants are mostly known as POPs, includes a great variety of chemicals that are may be anthropogenic or may be accidentally released in any industrial process [2].

It was observed that individuals with exposed levels of TCDD higher than 15ppb had greater fasting and post-prandial levels of

insulin after glucose consumption as compared to minute quantity of TCDD, analyzing that high TCDD levels are responsible for insulin resistance [3].

In environment arsenic has been found in both organic and inorganic forms with different valence numbers and oxidation states. As arsenic react readily with sulphur containing compounds and produce reactive oxygen species in the body. In this way oxidative stress increases in the body because free radicals interfere with the activity of natural anti-oxidants of the body. Natural anti-oxidants of the body are glutathione reductase, glutathione S- transferase and many others [4]. So, simply when free radicals produce in the body and increase oxidative stress then the capacity of anti-oxidants to work decrease in the plasma [5]. This study is intended to evaluate the effect of environmental pollution on type 2 diabetes. People who are poisonous with arsenic have more vulnerability to diabetes. Present study is designed to assess the effect of potential environmental pollutant on type 2 diabetes.

Material and Methods

Sample Survey and Sample Collection: In the present study first of all questionnaire of the sample size of 100 was circulated among patients suffering from type 2 diabetes and healthy individuals. Blood samples of diabetic patients were collected from Sheikh Zaid hospital, Lahore. 100 confirmed diabetic patients and 100 controls were selected. Samples were collected from diabetic patients and healthy individuals according to the law of blood collection and then stored at -80°C for further analysis.

Inclusion Criteria

- All Ethnic Groups
- Only type 2 diabetic patients
- Should Have Diabetic Symptoms
- Have Target Blood Pressure

Exclusion Criteria:

- Pregnant Women
- Known Infectious or Contagious Diseases
- Alcohol and Drug Addiction
- Psychiatric Patients

Biochemical Parameters:

A complete detail of "patient's past and present history, socio-economical history and different biochemical parameters like HBA1c, Creatinine, BUN, ALP, ALT, AST were recorded in this study. These parameters of diabetic patients were recorded at Sheikh Zaid Hospital, Lahore.

Assessment of Arsenic Level:

Arsenic is detected in the serum sample by using atomic absorption spectrometry technique. By using this technique arsenic level was detected in all the samples and the study completed.

Protein Assessment:

The measurement of protein was done by method of Bradford by Kruger, 1994. The attachment of protein molecules to Coomassie dye under acidity is the principle of this method. This outcomes in a shade shift from the reddish/brown appearance of the dye with absorbance maximum of 465nm to the blue appearance of the dye with absorbance maximum of 610 nm. The amount of Coomassie dye ligands attach to each protein molecule is almost proportional to the amount of positive charges present on the protein.

Statistical Analysis:

Analysis of the observations and data acquired through experimentation is the most critical constituent of scientific and social research. Data of sample survey was analyzed through the IBM SPSS Statistics (version 23). After analyzing data result and information were plotted in the form of graphs. Graphs represented all the information gathered from diabetic patients regarding to their disease. Then Microsoft Excel 2010 was used to analyze the results obtained after experimentation. Results obtained of all the parameters were analyzed th the help of excel.

Results

Ethical Approval:

"Ethical approval was received from Ethics committee of School of Biological Sciences, University of The Punjab Lahore, Pakistan.

Composition of Groups:

Total sample size was n=200 which include both experimental and control group in it. The sample were collected by keeping vision on inclusion and exclusion criteria. Two main groups were developed in order to analyze the study in detail:

Group 1: Control group containing healthy persons

Group 2: Diabetic patients including male and female both

Diabetes and Physical Inactivity:

Results showed that 38 % male and 41 % female follow exercise. Rest of the patients did not follow any kind of exercise. The ratio of individuals which are doing physical activity have further divided into two categories. One category include the kind of exercise by their own will and the other category comprises of following their doctor or physician regarding to their physical exercise. So, 61% males and 58% females are doing exercise prescribed by their doctor as shown in figure 1a

Diabetes and Increasing Trend of Hoteling:

25% males and 30% females were eating outside of their houses in restaurants. Out of which 33% males and 27% female were frequently going out for meals. Other than hoteling there was another aspect regarding to meal which is skipping of meal. 43% diabetic males and exactly the same amount 43% females were skipping their meals as shown in figure 1b.

Nutrient intake by Diabetic Patients

There were only 31% patients which were taking fruits on daily basis as a part of their diet. In the similar manner only 29% diabetic individuals were taking milk regularly and rest of the patients were neither taking milk nor eating fruits. The percentage of diabetic individuals who are taking tea on regular basis is 40% so it is very much greater as compared to individuals taking milk and fruits as shown in figure 1c.

Weight Gain or Loss in Diabetic Patients

In the first category diabetic patients were never found fluctuation in their sugar level and only 21% of them were suffering from weight gain and only 14% were facing weight loss. Then in the second category people fall who were sometimes facing fluctuation in their sugar levels, and 19% and 15% of them were facing weight gain weight loss respectively. Then the third category includes the people, who have high fluctuation in their sugar levels, 18% of them were suffering from weight gain and 15% were facing weight loss. So, weight gain was greater as compared to weight loss in diabetic individuals as 21% were gaining weight and only 15% were losing weight as shown in figure 1d.

Interference of Diabetes in Daily Life

There were 5.91% of diabetic male and 19% of females feel hindrance in their daily activities and routine tasks. 6.3% male and 19.29% females have issues in dealing with their hobbies and in recreational activities. 6.3% males and 18.9% females have more sensitive and emotional behavior. They get scary in dealing with their problems and start to react when tangled in situation. 6.3% males and 18.5% females have problem in doing household chores s shown in figure 1e.

Smoking and its Link with Other Problems

Active and passive both type of smoking contribute to diabetic complications. 28.2% of Active smokers and 26.3% passive smokers are suffering from skin problems. 23% active and 19% passive smokers are dealing with dental problems. 28% active smokers and 26% of passive diabetic smokers have wound healing problem. 20% active smokers and 24% passive smokers are facing drastic weight loss as shown in figure 1f.

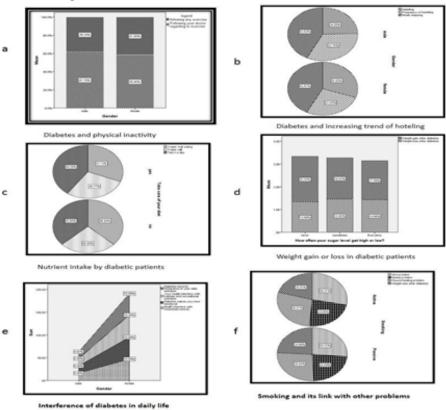


Figure 1.

Exposure to Chemicals by Different Age Groups

Individuals of different age groups have different exposure rate to chemicals. Mostly people who are consuming different chemicals through any source have greater risk of having diabetes as shown in figure 2a.

Type of Water Consume by Individuals

Most of the people have good personal hygiene, they are consuming filter water and avoid to drink tap water. There are only

9.78% people who are drinking tap water but mostly people rely on filter water. There are only 8% people who have dumping sites near their houses. Mostly people take good care of themselves to avoid from toxic exposure as shown in figure 2b.

Estimation of Biochemical Parameters:

The mean values of all the biochemical parameters for both Diabetic and control group were summarized in Table 1.

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Table 1: Average Values of Different Parameters of Control and Diabetic Patients.

Sr No.	Parameters	Diabetic	Control
1	Fasting Blood Sugar (mg/dl)	195.1*** ± 4.09	93.9 ± 8.68
2	HBA1c (%)	7.20* ± 1.00 %	$5.32 \pm 1.17\%$
3	Creatinine (mg/dl)	$2.64* \pm 3.43$	0.96 ± 0.09
4	BUN (mg/dl)	$35.6** \pm 3.37 \text{ mg/dl}$	7.9 ± 1.93
5	ALP (U/L)	$135.2*** \pm 3.60$	70.4 ± 17.86
6	ALT (U/L)	77.7*** ± 3.32	45.5 ± 5.85
7	AST (U/L)	76.9*** ± 3.51	32.1 ± 5.14
8	Arsenic (ng/ml)	56.67** ± 36.2	6.82 ± 6.05

Blood Sugar Fasting (mg/dl)

A test to determine how much glucose (sugar) is in a blood sample after an overnight fast. Results was compared by the diabetic and control group. The mean value for diabetic and control group are $195.1^{***}\pm4.09$ mg/dl and 93.9 ± 8.68 mg/dl respectively. The comparison is elaborated in figure 2c which showed a great difference between the two groups. The glucose level in the diabetic individuals was higher than the control group and also higher than the standard value.

HBA1c (%)

Blood HbA1c levels are reflective of how well diabetes is controlled. Results were compared by the diabetic and control group. The mean value of HbA1c for diabetic and control group were $7.20^* \pm 1.00$ % and 5.32 ± 1.17 % respectively. Furthermore, the comparison is elaborated in figure 2d. The normal value of HBA1c in the human body is 4-6%. So, the figure showed that the HBA1c level in the diabetic individuals was higher than the control group and also higher than the normal or standard value.

Creatinine Estimation (mg/dl)

Creatinine produced from the natural lysis of muscle tissues. It is measured to test the function of kidney. Results are compared by the diabetic and control group as shown in figure 2e. The value of diabetic group and control group are $2.64^*\pm3.43$ mg/dl and 0.96 ± 0.09 mg/dl respectively. So creatinine level in the diabetic individuals was higher than the control group and also higher than the normal or standard value.

BUN (mg/dl)

Urea nitrogen is a waste product that's created in the liver when the body breaks down proteins. The mean value of diabetic and control groups are $35.6^{**}\pm3.37$ mg/dl and 7.9 ± 1.93 mg/dl respectively. Furthermore, the comparison is elaborated in figure 2f. So, the figure showed that the BUN level in the diabetic individuals was higher than the control group and also higher than the normal or standard value.

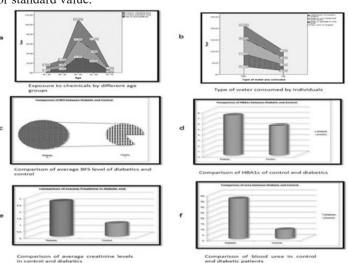


Figure: 2

Liver Function Tests

Liver function test (LFT) includes ALP (alkaline phosphate), ALT (alanine aminotransferase) and AST (aspartate aminotransferase). These tests are conducted to test the proper functioning of liver.

ALP: The mean value of diabetic group and control group for estimation of ALP was 135.2 \pm 3.60 U/L and 70.4 \pm 17.86 U/L respectively.

ALT: The mean value of diabetic group and control group for estimation of ALT was 77.7 \pm 3.32 U/L and 45.5 \pm 5.85 U/L respectively.

AST: In the same way AST was measured. The value of diabetic and control group for estimation of AST was 76.9 ± 3.51 U/L and 32.1 ± 5.14 U/L respectively.

All the results were statistically significant. Furthermore, the comparison is elaborated in figure 3a, 3b and 3c respectively. Results were statistically significant.

Arsenic Estimation (ng/ml)

The mean value of arsenic for diabetic and control group are $56.67** \pm 36.2$ ng/ml and 6.82 ± 6.05 ng/ml respectively. Further-

more, the comparison in figure 3d showed that there was a great difference between the two groups. The normal value of arsenic in the human body is below 10 ng/ml. But diabetic group showed the average value much higher than normal that is 56.67 ng/ml, which is highly significant.

Protein Estimation in Serum by Bradford

Bradford test depend on the binding of the coomassie brilliant blue stain to polypeptide chains of proteins as, the amount of the dye bounded will be as much as the protein's concentration in that sample. BSA standards were prepared with a concentration having range of 0.2 mg/ml to 1 mg/ml giving an OD range from 0.6 to 1.6 as shown in Table 2. Graph showed the standard protein estimation by Bradford was shown in figure 3e. Graph of protein estimation in serum samples was shown in figure 3f.

Table 2: Values of Standard Protein Estimation by Bradford Method

BSA mg/ml	O.D of BSA	Serum sample (per 50ul)	Optical density of samples	Serum proteins (ug/50ul)	Serum proteins (per 1ul)
0.2 mg/ml	0.662	1ul	1.43	1.069	50.696
0.3 mg/ml	0.714	1ul	1.52	1.078	53.989
0.4 mg/ml	0.881	1ul	1.43	1.038	50.769
0.5 mg/ml	0.921	1ul	1.54	1.086	54.892
0.6 mg/ml	1.212	1ul	1.59	1.128	55.876
0.8 mg/ml	1.467	1ul	1.42	0.969	50.991
0.9 mg/ml	1.541	1ul	1.49	1.138	54.834
1 mg/ml	1.698	1ul	1.59	1.156	56.276

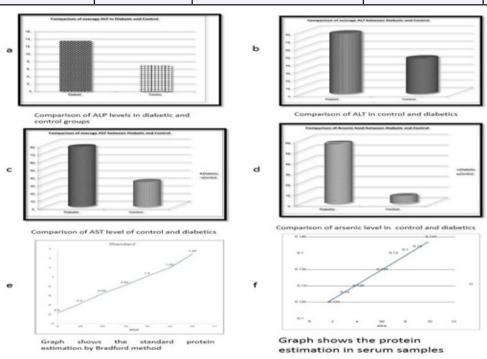


Figure 3

Discussion

Pancreatic beta cells are responsible for glucose maintenance in the body, a diabetogenic agent or endocrine disrupter may cause interference with the functioning of pancreatic beta cells and will trigger type 2 diabetes. POPs are classified as endocrine disrupters and cause disruption and interference activities with estrogen receptors and act as a diabetogenic agent [6]. Diabetes is a fourth foremost cause of death in most of the advanced countries where Pakistan is at 7thposition in the grade of countries with diabetes mellitus and it is said that it will move to the 4thposition if the condition continuous [7, 8].

In this research work total 200 subjects were selected, 100 were control and 100 were based on their diabetes status. Two groups were studied, first group was healthy normal (control) and the second group was diabetic female and male patients of Lahore. Comparison of different physical and biochemical parameters between diabetic population and non-diabetic normal healthy control population were done to check the difference between them in Lahore [9-11].

Conclusion

Arsenic level was higher in diabetic persons and working as diabetic agent. Other biochemical parameters was also higher in diabetic patients. There should be no residence near rice paddies or any arsenic rich area. We should promote the usage of PPE "personal protective equipment" in order to avoid arsenic exposure. In future, assessment of the levels of these levels appeared to be helpful in not only early diagnosis but also in prognosis of diabetes mellitus.

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