SERUM CONCENTRATION OF THYROID HORMONES AND THYROID STIMULATING HORMONE IN ALLOXAN-INDUCED TYPE 1 DIABETIC WISTAR ALBINO RATS

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ABSTRACT

Background: Literature has reported thyroid functional abnormalities in diabetes mellitus. The objectives of this study were to determine and compare the serum concentrations of T3, T4 and TSH in alloxan-induced type 1 diabetic and control Wistar albino rats.

Materials & Methods: It was an experimental animal study on 20 Wistar albino rats, extending over a period of eight weeks. Alloxan, a diabetogenic agent, was used to produce animal models of type 1 diabetes. Animals were divided equally into two groups: control and diabetic. The animals in the diabetic group were injected intraperitoneally with 150 mg/kg body weight of 10% alloxan to induce diabetes. After 72 hours, diabetes was confirmed with glucometer (glucose >350mg/dL). During the course of experiment, one rat in control group and 2 rats in diabetic group died. Blood was collected for estimation of serum concentrations of thyroid hormones, thyroid stimulating hormone at the end of experimental period. Serum T3, T4, and TSH were measured using ELISA kits.

Results: At the end of eight weeks, the mean concentration of serum T3 was 0.69 ± 0.29 ng/ml and 0.44 ± 0.02 ng/ml in control and diabetic groups, respectively. The mean concentration of T4 was 3.78 ± 1.16 μg/dL and 2.24 ± 0.86 μg/dL in control and diabetic groups respectively. The mean concentration of TSH was 0.77 ± 0.20 μU/ml and 1.41 ± 0.23 μU/ml in control and diabetic groups respectively. The mean serum concentrations of T3 (p = .0025) and T4 (p = <.00001) were significantly lower in diabetic and that of TSH (p = <.00001) were significantly higher in diabetic than control group.

Conclusion: This study concludes that the serum concentrations of both T3 and T4 are significantly lower and that of TSH is significantly higher in alloxan-induced type 1 diabetic as compared to control group in Wistar albino rats.

KEY WORDS: Alloxan; Diabetes Mellitus Type 1; Diabetes Mellitus Type 2; Thyroid Gland; Thyroid Hormones; Thyroid Diseases; Thyroid Stimulating Hormone.

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1. INTRODUCTION

1.1 Background: Diabetes mellitus (DM) is a syndrome of persistent hyperglycemia due to relative or absolute deficiency of insulin leading to its related complications.1 Diabetes mellitus may be categorized into type 1 and type 2. In type 1 the cause is an absolute deficiency of insulin secretion. In type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response.2 Thyroid diseases and DM are common endocrine abnormalities in clinical practice. Thyroid disorders and DM mutually influence each other.3 Both T3 and T4 contribute to the normal control of glucose metabolism and functions of the pancreas and also diabetes interferes with the functional capability of thyroid gland. Several studies have reported thyroid functional abnormalities in diabetics.
Serum concentration of thyroid hormones and thyroid stimulating hormone in alloxan-induced type 1 diabetic and control Wistar albino rats

Perros, et al. reported a prevalence of 13.4% thyroid disorders in 1,310 adult diabetic patients during annual screening for thyroid disorders; highest in type 1 diabetic females as 31.4% and lowest in type 2 diabetic males as 6.9%. Furthermore, a prevalence of 12.3% of thyroid dysfunction was found among 1,092 type 2 diabetic patients in Greece. Akber, et al. reported 16% patients with type 2 diabetes to have thyroid dysfunction from Saudi Arabia. Radaideh, et al. reported thyroid dysfunction in 12.5% of type 2 DM patients (n=908) in year 2000 from Amman, Jordan.

Type 1 DM is associated with thyroid dysfunctions which are the most frequently occurring autoimmune disorders. Kordonouri, et al. reported thyroid autoimmunity in 15 of 335 patients (4.5%) at type 1 diabetes onset. Radetti, et al. from Bolzano, Italy reported 3.5% (55) cases out of 1420 children with type 1 DM having Hashimoto’s thyroiditis. Out of 216 patients with type 1 diabetes, 22 (10.0%) had significantly elevated titres of anti-TPO, 19 (8.7%) of anti-TG and 13 (5.9%) of both autoantibodies. Initial screening of children and adolescents with type 1 diabetes, showed 15.4% of patients having raised anti-TPO and 14.4% raised anti-TG. Thyroglobulin autoantibodies were positive in 33% of the diabetics and thyroid peroxidase autoantibodies were positive in 38%. Ghawil, et al. from Tripoli, Libya has shown that 23.4% of type 1 DM children had positive anti-microsomal peroxidase antibodies (TPO-Ab) and 7.8% had positive anti-thyroglobulin (TG-Ab) antibodies; whereas 6.9% of the patients were positive for both TPO-Ab and TG-Ab.

So, all these studies show that there is interdependent relationship between type 1 DM and thyroid disorders. In Pakistan, the available literature regarding the levels of thyroid hormones and thyroid stimulating hormone in type 1 diabetes is very meager and, at best, inconclusive.

1.2 Research Objectives (ROs):
RO 1-3: To determine the serum concentrations of T3, T4 and TSH in alloxan-induced type 1 diabetic and control Wistar albino rats.
RO 4-6: To compare the serum concentrations of T3, T4 and TSH in alloxan-induced type 1 diabetic and control Wistar albino rats.

1.3 Research (Null) Hypotheses (RHs):
H1: There is statistically no significant difference in the serum concentrations of T3 in alloxan-induced type 1 diabetic and control Wistar albino rats (RO3).
H2: There is statistically no significant difference in the serum concentrations of T4 in type 1 alloxan-induced diabetic and control Wistar albino rats (RO4).
H3: There is statistically no significant difference in the serum concentrations of TSH in alloxan-induced type 1 diabetic and control Wistar albino rats (RO5).

2. MATERIALS & METHODS

2.1 Design, Duration & Setting: This experimental animal study was carried out at the Department of Physiology, Postgraduate Medical Institute, Lahore, Pakistan from 16th January 2011 to 15th March 2011. The protocol was approved by Advanced Studies and Research Board of University of Health Sciences, Lahore, Pakistan. As there was intervention but no randomization, so it was a non-randomized quasi experimental design. Experimental group included alloxan-induced type 1 diabetic while control group included non-diabetic Wistar albino rats.

2.2 Conduct of Procedure & Intervention: Twenty healthy Wistar albino rats, including 10 males and 10 females, were obtained from National Institute of Health, Islamabad, Pakistan. The age of the rats was 12 weeks and their average weight was 210 grams. Numbers were allocated as 1 to 10 to males and 11 to 20 to females. Experimental group was allocated S.No. 1 to 5 male & S.No. 11 to 15 female rats, while control group was allocated S.No. 6 to 10 male & S.No. 15 to 20 female rats.

The rats were acclimatized for two weeks before starting the experiment. Experimental and control groups were kept in separate iron cages under optimum temperature (24±2°C) and hygienic conditions with observation of light and dark cycles. These were given water ad libitum. Ordinary/commercially available rats food was given twice/day at 8 am and 8 pm @ 12 grams/rat/feed.

Alloxan was used to induce type 1 diabetes mellitus in rats of diabetic group. A single dose of alloxan monohydrate (10%) powder was given in a dose of 150 mg/kg body weight, dissolved in 3 ml of 0.9% NaCl and given as intra-peritoneal injection. Control group rats received the same volume of 0.9% NaCl as intra-peritoneal injection. Three days later, DM was confirmed using fasting blood glucose (FBG) by getting a drop of blood from the tails of rats and checking it by glucometer. A rat was labeled to be diabetic if its FBG was more than 130 mg/dL. The minimum level of FBG of each of the 10 rats was >300mg/dL. During the course of experiment, one female rat in control group and one male and one female rat in diabetic group died. So we were left with nine rats in experimental and eight rats in control group.

2.3 Blood Sample Collection: At the end of eight weeks experimental period, blood was collected from the rats. Samples were collected by cardiac puncture using 5 ml sterile syringes after giving deep ether anesthesia in the morning. Before collecting the blood samples, the animals were kept fasted overnight. From each rat, approximately 5 ml blood was collected and transferred to test tube for estimation of serum concentration of T3, T4 and TSH. After centrifuging the blood, serum was separated. Until the time of measurement, serum was stored...
at -20°C for hormones estimation. Enzyme-linked immunosorbent assay (ELISA) Kits (BioCheck, Inc., South San Francisco, CA, USA) were used to measure serum concentrations of thyroid hormones and thyroid stimulating hormone.

2.4 Data Collection & Data Analysis Plan: Serum concentrations of $T_3$, $T_4$ and TSH were three research variables. The data type for these variables was ratio (numeric). All these were described by mean, minimum, maximum, range and standard deviation, for each group separately for the sample. Estimated parameters for population were given as confidence intervals (CI) of mean at 95% confidence level (CL).

Each research variable was compared between the two groups through independent-samples t-test. Sample size, mean & SD for each group, mean difference, CI of mean difference, degree of freedom, t-value and significance (p-value) were given for each test separately. Data was analyzed by SPSS v.17 (SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1 Descriptive Statistics & Estimation of Parameters:
The serum concentration of $T_3$ and $T_4$ and TSH are given for sample and as CI for population. The mean concentration of serum $T_3$ and $T_4$ are lower for diabetic group than control group rats. The mean concentration of serum TSH is higher for diabetic group than control group rats. (Tables 1)

3.2 Hypotheses Testing:

$H_0$: Here the mean serum concentration of $T_3$ in diabetic rats was compared to control group animals through independent-samples t-test at alpha .05. As p-value was less than .05, hence $H_0$ was proved to be false and rejected, showing the difference to be statistically significant. In simple words, the concentration of serum $T_3$ was found lower in diabetic rats than control group animals. (Table 2)

$H_0$: Here the mean serum concentration of $T_4$ in diabetic rats was compared to control group animals through independent-samples t-test at alpha .05. As p-value was less than .05, hence $H_0$ was proved to be false and rejected, showing the difference to be statistically significant. In simple words, the concentration of serum $T_4$ was found lower in diabetic rats than control group animals. (Table 3)

### Table 1: Descriptive statistics & estimation of parameters of thyroid hormones & thyroid stimulating hormone in diabetic (n=9) and control group (n=8) Wistar albino rats

| Variables | Group   | Sample Statistics                  | 95% CI of Mean |
|-----------|---------|------------------------------------|----------------|
|           |         | Mean     | Min    | Max    | Range | SD   | Lower | Upper |
| $T_3$ (ng/ml) | Control | 0.6889   | 0.50   | 1.00   | 0.50  | 0.1900 | 0.5428 | 0.8350 |
|           | Diabetic | 0.4375   | 0.40   | 0.50   | 0.10  | 0.0517 | 0.3942 | 0.4808 |
| $T_4$ (μg/dl)  | Control | 3.7778   | 3.10   | 4.30   | 1.20  | 0.3800 | 3.4856 | 4.0699 |
|           | Diabetic | 2.2375   | 1.70   | 2.70   | 1.00  | 0.3502 | 1.9447 | 2.5303 |
| TSH (μU/ml)    | Control | 0.7667   | 0.50   | 1.00   | 0.50  | 0.1581 | 0.6451 | 0.8882 |
|           | Diabetic | 1.4125   | 1.10   | 1.60   | 0.50  | 0.1807 | 1.2614 | 1.5636 |

### Table 2: Comparison of mean serum concentrations of $T_3$ in alloxan-induced diabetic versus control group Wistar albino rats

| Groups  | n | Mean     | SD  | Mean difference | 95% CI of difference | t-value | d.f. | p-value (2-tailed) |
|---------|---|----------|-----|-----------------|-----------------------|---------|-----|-------------------|
|         |   |          |     |                 | Lower                 | Upper   |     |                   |
| Control | 9 | 0.6889   | 0.1900 | 0.2513          | .1030                 | .3997   | 3.613 | .0025             |
| Diabetic| 8 | 0.4375   | 0.0517 | Independent-samples t-test | H$_0$ rejected at $\alpha$ 0.05 |

n = Sample size, SD = Standard deviation, d.f. = Degree of freedom

### Table 3: Comparison of mean serum concentrations of $T_4$ in alloxan-induced diabetic versus control group Wistar albino rats

| Groups  | n | Mean     | SD  | Mean difference | 95% CI of difference | t-value | d.f. | p-value (2-tailed) |
|---------|---|----------|-----|-----------------|-----------------------|---------|-----|-------------------|
|         |   |          |     |                 | Lower                 | Upper   |     |                   |
| Control | 9 | 3.7778   | 0.3800 | 1.5403          | 1.1607                | 1.9198  | 8.650 | .00001            |
| Diabetic| 8 | 2.2375   | 0.3502 | Independent-samples t-test | H$_0$ rejected at $\alpha$ 0.05 |

n = Sample size, SD = Standard deviation, d.f. = Degree of freedom
Serum concentration of thyroid hormones and thyroid stimulating hormone in alloxan-induced type...  

### Table 4: Comparison of mean serum concentrations of TSH in alloxan-induced diabetic versus control group Wistar albino rats

| Groups   | n | Mean   | SD    | Mean difference | 95% CI of difference | t-value | d.f. | p-value (2-tailed) |
|----------|---|--------|-------|-----------------|----------------------|---------|------|-------------------|
| Control  | 9 | 0.7667 | 0.1581| -0.6458         | -0.8209 -0.4707      | -7.862  | 15   | <.00001           |
| Diabetic | 8 | 1.4125 | 0.1807| Independent-samples t-test | H₀,3 rejected at α 0.05 |

n = Sample size, SD = Standard deviation, d.f. = Degree of freedom

H₃: Here the mean serum concentration of TSH in diabetic rats was compared to control group animals through independent-samples t-test at alpha .05. As p-value was less than .05, hence H₃ was proved to be false and rejected, showing the difference to be statistically significant. In simple words, the concentration of serum TSH was found higher in diabetic rats than control group animals. (Table 4)

**4. DISCUSSION**

Insulin and thyroid hormones show inter-dependence for normal cellular metabolism so that both can mutually influence each other. Therefore, thyroid dysfunctions can have a major effect on normal control of glucose metabolism, and untreated thyroid disorders can have a great impact on the management of diabetic patients. It has been shown that metabolic alterations caused by DM, or insulin lack itself, can directly affect some functions of thyroid gland.

In this study, we analyzed the serum concentrations of thyroid hormones and thyroid stimulating hormone in type 1 DM. Our study showed that the serum concentrations of both T₃ and T₄ were lower and that of TSH were higher than control group.

**4.1 Thyroid Hormones (T₃ & T₄):**

Similar to our findings are reported by Monajemzadeh, et al. from Ahwaz, Iran for the period from March 2005 to August 2008, including type 1 diabetics (n₁=75) and non-diabetic controls (n₂=105). Serum concentrations of T₃ and T₄ were significantly lower in diabetic patients as compared to controls.

Similar findings are by Volzke et al., from Greifswald, Germany who showed that type 1 diabetic subjects (n₁=224) had lower serum T₃ levels as compared to normal subjects (n₂=3481), both groups aged 20-69 years.

Similar to our results are by Rodgers, et al. from London, Ontario, Canada, showed that serum T₄ level of streptozotocin-induced type 1 diabetic male Sprague-Dawley rats was lower than controls in the first week and was sustained through the seventh week.

Similar to our findings are from Messina, Italy by Bernasconi, et al. who revealed that the levels of serum T₄ were lower in youngsters non-ketoacidotic type 1 diabetic patients than in controls.

Contrary to our findings are from Lahore, Pakistan by Ditta et al., including 9-50 years type 1 diabetics (n₁=50) and age & sex matched healthy controls (n₂=26). The serum concentration of T₄ was comparable in both the groups.

**4.2 Thyroid Stimulating Hormone (TSH):**

Similar to our findings are reported by Monajemzadeh, et al. from Ahwaz, Iran for the period from March 2005 to August 2008, including type 1 diabetics (n₁=75) and non-diabetic controls (n₂=105). Serum concentrations of TSH were significantly higher in diabetic patients as compared to controls.

Similar to our findings are from Lahore, Pakistan by Ditta et al., including 9-50 years type 1 diabetics (n₁=50) and age & sex matched healthy controls (n₂=26). The serum concentration of TSH was higher in diabetic versus healthy controls.

**CONCLUSION**

This study concludes that the serum concentrations of both T₃ and T₄ are significantly lower and that of TSH is significantly higher in alloxan-induced type 1 diabetic as compared to control group in Wistar albino rats. So, a timely systematic approach to thyroid testing is recommended for early diagnosis of thyroid disorders in diabetic patients.

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