

A Study of Prolactin as a Diabetogenic Factor in Type I & Type II Diabetic Patients

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Abstract: ***Aims & Objectives:** 1) To study the serum levels of prolactin in 30 normal healthy persons & 30 Type I & 30 Type II Diabetes Mellitus (DM) patients. 2) To assess prolactin as a most suitable parameter for prognosis & treatment of DM patients. **Materials & Methods:** The present study was carried out in Dept. of Biochemistry, GMC, Nagpur from May 2003 to May 2005. A total of 60 patients diagnosed as having DM aged between 15 – 80 years were selected from the diabetic OPD of GMC, Nagpur under Dept. of Medicine & 30 normal healthy volunteers aged 15 – 80 years were selected as control after satisfying all inclusion & exclusion criteria & willing to give consent. The subjects were divided into three Groups as Group I (30, control), Group II (30, Type I DM patients) & Group III (30, Type II DM patients). Serum PRL levels was estimated using ELISA LISA kit supplied by transasia, Mumbai. **Results:** The results presented in the table indicates that PRL levels in Type I & Type II DM in males & females are not significantly changed as compared to controls. **Discussion:** Lowering PRL failed to improve GTT or alter insulin release. It was concluded that PRL has no significant effect on glucose disposal.*

Keywords: ELISA LISA kit

1. Introduction

The first systematic description was written by the Arelaeus of cappadosis in Asia minor, probably in the 1st century AD, the disease as “A melting down of flesh into the urine”. The discovery by Van Mering & Minikowaski in 1889 that pancreatotomy causes a metabolic disorder called DM is the result of insulin deficiency. It is characterized by either the absence of insulin that is NIDDM – Type I or which is insensitive to the insulin that is IDDM or Type II¹. Prolactin (PRL) or Leuteotrophic hormone (LTH) is secreted by anterior pituitary gland. It is secreted by lactotrophα cells of anterior pituitary. It is a monomeric simple protein with MW 23000 daltons. It contains 199 amino acids with 3 –s-s-linkages. It has sequence homology with growth hormone. It has mammatropic action as it stimulates mammary growth & secretion of milk. It has lactogenic action as it synthesizes milk protein such as Lactalbumin & Casein after parturition. Estrogen, thyroid hormone & glucocorticoids, increases the number of prolactin receptor on mammary cell membrane. Prolactin has general metabolic action in hypophysectomised animals. Under suitable circumstances prolactin has been shown to be calorogenic, diabetogenic, promotes protein synthesis & increase the rate of chondroitin sulphate formation in cartilage².

2. Materials & Methods

The present study was carried out in Department of biochemistry, GMC, Nagpur from May 2003 to May 2005. A total of 60 patients diagnosed as having DM aged between 15-80 years were selected from diabetic OPD of GMC, Nagpur under Dept. of Medicine & 30 normal healthy volunteers aged between 15-80 years were selected as controls. Inclusion criteria: Patients attending diabetic OPD of GMC, Nagpur, willing to enter study, with no h/o chronic

diseases like HT, TB etc & no h/o alcohol, smoking were included in the present study. A 5 ml of fasting venous blood was withdrawn from each control & patient using a disposable syringe & needle under all aseptic precautions. The blood obtained was collected in a sterile bulb & allowed to clot at RT for at least 20 minutes. After this serum was separated by centrifugation. The serum thus obtained was used for estimation of prolactin without further delay. All the water used in the estimations was distilled & deionized & all the reagents used were of analytical grade. The subjects were divided into 3 groups as group I (30, Control), Group II (30, Type I DM patients) & Group III (30, Type II DM patients). Serum prolactin levels was estimated using ELISA³ LISA kit supplied by transasia Mumbai in which a immobilized monoclonal Ab to prolactin on the microwells was used. A purified polyclonal prolactin antiserum conjugated to the enzyme HRP is used to detect prolactin. In the assay procedure the prolactin standard & patient serum are added along with prolactin antibody HRP conjugate AB coated wells. Reaction between the two Abs & native prolactin Ag forms a sandwich complex that binds with the coated wells. After equilibrium is attained, the Ab bound fraction is separated from unbound Ag by decantation & aspiration in washing step. The activity of the enzyme is quantitated by reaction with an enzyme substrate 3, 3', 5, 5'TMB (Tetramethylbenzidine) which is hydrolyzed to a coloured end product. The intensity of the colour produced is measured spectrophotometrically at 450 nm. The enzyme activity in the Ab bound fraction as measured by the intensity of the colour development is directly proportional to the native Ag conc. The conc. of the prolactin is interpolated from the standard curve. The concentration of prolactin in a given specimen, determined with assays from different manufacturers can vary due to differences in assay method & reagent specifications. Procedure: Appropriate numbers of microwell strips were removed from the bag & following procedure was followed. 1) 25 µl of standards/

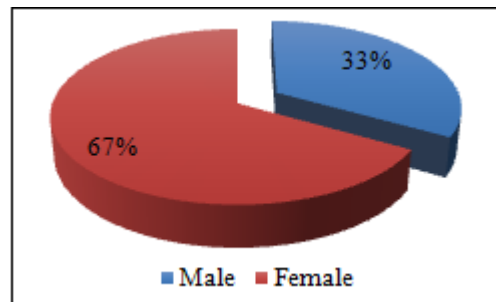
control/ test samples were added in the appropriate wells. 2) 100 µl of prolactin conjugate was added to each well & mixed well for 10 seconds. Bubble formation avoided. The wells were sealed with parafilm. Incubated at 37°C in water bath for 30 minutes. 3) Cover seal removed & discarded. Supernatant were aspirated from all wells. Wells were washed five times with distilled water. 4) 100 µl of TMB substrate solution was added to each well & mixed for 10 seconds & incubated for 30 minutes at room temperature. 5) 100 µl of stop solution was added to each well. 6) Reading was taken at 450 nm. Normal prolactin levels in males 0.94 – 11.94 ng/ml & in females 8.39 – 20.15 ng/ml was taken.

Statistical Analysis: Data was analyzed on statistical software Intercoastal stata version 7. Continuous variables are presented as Mean \pm SD (Standard Deviation). Comparison between variables was done by using student – t-test . Analysis of variance (ANOVA) was used to see significant difference between variables. Categorical variables are represented in percentages. Categorical data was analyzed by using Chi-square test & $p < 0.05$ was considered as statistically significant.

3. Observations & Results

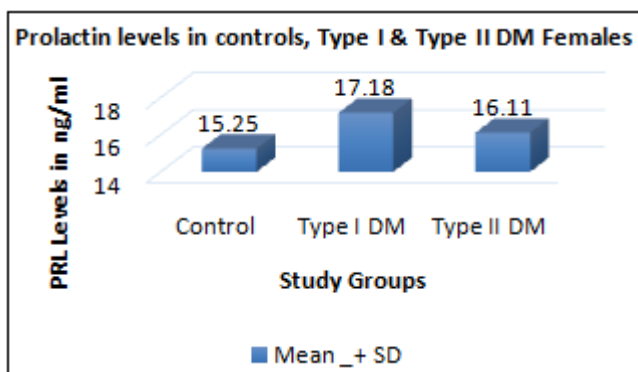
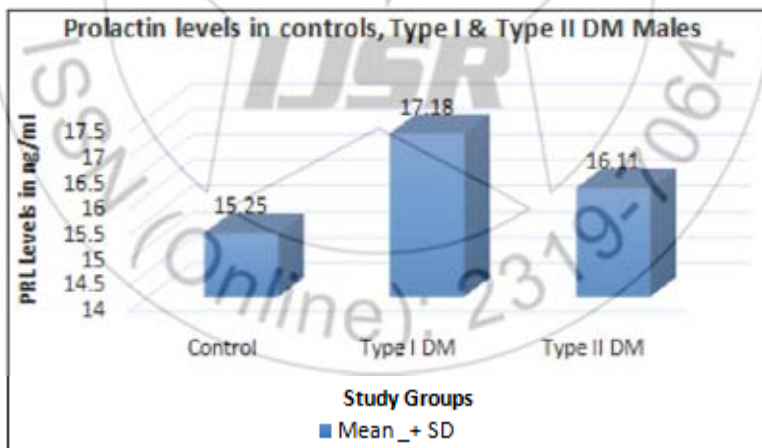
Distribution of study subjects according to sex is shown in table I

Subjects	Control	%	Type I DM	%	Type II DM	%
Male	10	33.33	10	33.33	10	33.33
Female	20	66.66	20	66.66	20	66.66
Total	30	100	30	100	30	100



Serum prolactin levels (ng/ml)

		Mean \pm SD	Range of values	p-value
Males	Control	6.525 \pm 1.28	2.219-10.819	
	Type I DM	8.90 \pm 2.10	5.82-10.92	$p > 0.05$ (NS)
	Type II DM	7.82 \pm 3.18	3.89-10.89	$p > 0.05$ (NS)
Females	Control	15.25 \pm 6.37	9.397-18.15	
	Type I DM	17.18 \pm 5.86	10.89-22.42	$p > 0.05$ (NS)
	Type II DM	16.11 \pm 4.29	12.71 – 20.82	$p > 0.05$ (NS)
Normal Range	Males	0.94 – 11.94 ng/ml		
	Females	8.39 – 20.15ng/ml		



Results: The results presented in the above table indicate that PRL levels in Type I & Type II DM in males & females are not significant as compared to controls.

4. Discussion

We observed that there was not significant change in PRL level in Type I & Type II DM both in males & females compared to controls.

Hanssen K F et al⁴ studied the levels of PRL in patients with DKA & correlate between serum sodium & serum PRL concentration. They found out that serum PRL was

increased in those patients after correction of ketoacidosis, the PRL level was decreased.

insulin secretion in patients with prolactinomas
HormMetas Res 1981; 13: 667-9.

Our findings are similar to those of BratuschMarrain P et al⁵ who found out nonsignificant increase in DM. Hence they reported that PRL is not involved in severe endocrine & metabolic disturbances in DM.

In contrast, A Inanmanesh et al⁶ observed reduced serum PRL concentration in men with poorly controlled IDDM. Whereas Hanssen K F et al found increased level of PRL in DKA patients, after correction of ketoacidosis PRL level was found to be restored.

Zukowski E et al⁷ compared serum PRL level in patients with or without diabetic nephropathy. They found out that PRL concentration was not significantly altered in patients without nephropathy.

Milasinovic I et al⁸ found out increased secretion of PRL in sera of mothers with glucose intolerance than in case of mothers with normal pregnancy. It was due to presence of gestosis.

Kart Z E et al⁹ carried out GTT in six patients with PRL secretion pituitary adenoma. The testing was performed in each individual in untreated stage & thereafter PRL was reduced by bromocriptine treatment after 3 months.

Lowering PRL failed to improve GTT or alter insulin release. It was concluded that PRL has no significant effect on glucose disposal.

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