

# Study of Insulin Levels in Patients of Type 2 DM With and Without Nephropathy and Thyroid Disorders.

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Received: January 2017

Accepted: January 2017

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## ABSTRACT

**Background:** Thyroid disorders are established risk factor for insulin resistance. In this study we find out whether association exists between insulin resistance in type 2 diabetes patients with and without nephropathy and thyroid disorders. **Methods:** Serum T3 T4 TSH, fasting and postprandial blood sugar, HbA1c, serum insulin levels, serum creatinine, and urinary albumin creatinine ratio were estimated in 100 patients with type 2 DM. These patients were divided into two groups of 50 patients, Group 1 – type 2 DM without nephropathy and Group 2 - type 2 DM with nephropathy. **Results:** In our study we found a statistically significant correlation between TSH and serum insulin levels in patients with diabetic and diabetic nephropathy. Out of 100 patients thyroid dysfunction was more prevalent in diabetic nephropathy group as compared to diabetic without nephropathy group. P-value for thyroid dysfunction in diabetic nephropathy was statistically significant. In present study higher prevalence of thyroid dysfunction in women as compared to men. **Conclusion:** Routine assessment of thyroid hormone level in addition to other biochemical parameters in the early stage of diabetes and diabetes nephropathy will help in the management of those patients whose condition are difficult to manage.

**Keywords:** Thyroid function Tests (T3 T4 TSH). Type 2 Diabetes Mellitus, Insulin Levels.

## INTRODUCTION

The role of hyperthyroidism in diabetes was investigated in 1927, by Coller and Huggins proving the association of hyperthyroidism and worsening of diabetes. It was shown that surgical removal of parts of thyroid gland had an ameliorative effect on the restoration of glucose tolerance in hyperthyroid patients suffering from coexisting diabetes.<sup>[1]</sup> There is a deep underlying relation between diabetes mellitus and thyroid dysfunction.<sup>[2]</sup>

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A plethora of studies have evidenced an array of complex intertwining biochemical, genetic and hormonal malfunctions mirroring this pathophysiological association. 5' adenosine monophosphate-activated protein kinase (AMPK) is a central target for modulation of insulin sensitivity and feedback of thyroid hormones associated with appetite and energy expenditure.<sup>[3]</sup> Hypothyroidism (Hashimoto's thyroiditis) or thyroid over activity (Graves' disease) has been investigated to be associated with diabetes mellitus.

A meta-analysis reported a frequency of 11% in thyroid dysfunction in the patients of diabetes mellitus.<sup>[4]</sup> Autoimmunity has been implicated to be the major cause of thyroid-dysfunction associated diabetes mellitus.<sup>[5]</sup> Unmanaged pro diabetes, both type 1 and type 2, may induce a "low T3 state" characterized by low serum total and free T3 levels, increase in reverse T3 (rT3) but near normal serum T4 and TSH concentrations.<sup>[8]</sup> The relation between T2DM and thyroid dysfunction has been a less explored arena which may behold answers to various facts of metabolic syndrome including atherosclerosis, hypertension, and related cardiovascular disorders. T2DM owes its pathological origin to inappropriate secretion of insulin, due to defective islet cell function or beta cell mass. Continuous consumption of calories-rich meals, junk food and sedentary lifestyle has culminated into an epidemic of diabetes projected to afflict around 300 million people across the globe by 2020.<sup>[9]</sup> Defective insulin secretion leads to various metabolic aberrations in T2DM, spanning from hyperglycemia due to defective insulin-stimulated glucose uptake and upregulated hepatic glucose production, along with dyslipidaemia, which includes impaired homeostasis of fatty acids, triglycerides, and lipoproteins.<sup>[10]</sup>

Diabetes Mellitus is an important health problem affecting major population worldwide. It is characterized by absolute or relative deficiency in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. Diabetic Nephropathy, a major microvascular complication of type 2 DM, is an important cause of chronic kidney disease. It results from interaction between hemodynamic and metabolic factors<sup>[12]</sup>. Subclinical hypothyroidism is the most prevalent form of thyroid dysfunction in type 2 DM<sup>[13]</sup>. Nephropathy affects both hypothalamus-pituitary- thyroid axis and thyroid hormone peripheral metabolism<sup>[14]</sup>. Uraemia influences the function and size of thyroid<sup>[15]</sup>. Serum TSH concentrations are usually normal or elevated in nephropathy, but its response to its releasing hormone i.e. thyroid releasing hormone is generally low<sup>[15]</sup>. Thyroid hormones (TH) play an important role in regulating energy balance, metabolism of glucose, and lipids<sup>[16]</sup>. While TH oppose the action of insulin and stimulate the hepatic gluconeogenesis and glycogenolysis they up-regulate the expression of genes such as glucose transporter type-4 (GLUT-4) and phosphoglycerate kinase, involved in glucose transport and glycolysis, respectively, thus acting synergistically with insulin facilitating glucose disposal and utilization in peripheral tissues<sup>[17]</sup>.

The prevalence of thyroid disorders in patients with diabetes is significantly higher than that in the general population<sup>[21]</sup>, this indicates a possible interplay between thyroid status and insulin sensitivity. Insulin resistance will be calculated by using values of fasting serum insulin by the Carbiotech Insulin Elisa Kit. Complex interplay between thyroid function and IR has been implicated in diabetic dyslipidemia<sup>[21]</sup>.

In the light of the existing reports, we decided to evaluate the correlation between Insulin levels in diabetics with and without nephropathy and altered thyroid state.

## MATERIALS AND METHODS

The study includes one hundred patients of either sex in age group of 40-70 years diagnosed to be a case of type 2 DM attending OPD / admitted in emergency and medical wards of Guru Nanak Dev Hospital / Government Medical College, Amritsar. After obtaining informed consent the patients were subjected to detailed history, clinical examination and various laboratory test for thyroid hormones and fasting insulin levels, blood sugar fasting and postprandial, HbA1c levels, serum creatinine, and urinary albumin creatinine ratio.

### Inclusion Criteria

1. Age 40-70 years

2. Type 2 DM

### Exclusion Criteria:

1. Known case of thyroid disorder (hypothyroidism or hyperthyroidism).
2. Age less than 40years.
3. Age more than 70 years.
3. Patients on long term glucocorticoids, thyroxin or anti-thyroid drugs.
4. Other known autoimmune disorders.
5. Human immunodeficiency virus (HIV+ve) patients
6. Hepatitis B & Hepatitis C positive patient

### Collection & Processing Of Blood Sample:

Blood samples were collected from all the 100 subjects. Patients were kept on overnight fast for at least eight hours before blood collection. From each subject 5ml of venous blood sample was drawn by aseptic technique.

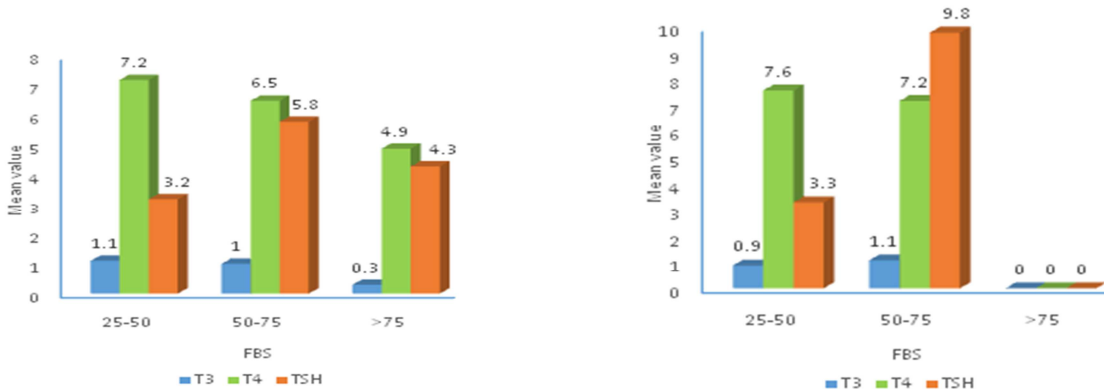
## RESULTS

Among 100 diabetic patients which were recruited in the study, out of 50 were without diabetic nephropathy and 50 with diabetic nephropathy. Mean age of patient with and without nephropathy was almost same i.e.  $56.86 \pm 9.68$  and  $56.94 \pm 8.51$  years respectively. Hypertension was more prevalent in diabetic nephropathy as compared to without diabetic nephropathy with significant p value=0.002 (significant). The mean value of T3 was  $1.09 \pm 0.45$  in group 1 and  $0.96 \pm 0.53$  in group 2. So T3 was decreased in group 2 as compared to group 1. The mean value of TSH was  $3.55 \pm 1.54$  in group 1 and  $4.46 \pm 4.22$  in group 2. So TSH was increased in group 2 as compared to group 1. TSH was found to be increased with increasing values of insulin in both the groups which suggest that correlation of insulin resistance with subclinical hypothyroidism (high levels of TSH). in diabetic patients both with and without diabetic nephropathy. TSH was found to be increasing trend with increased values of serum creatinine in diabetic nephropathy. TSH levels in type 2 diabetic nephropathy increases with increase in urinary albumin creatinine ratio, which shows that serum TSH level (subclinical hypothyroidism) was an independent risk factor of albuminuria. To investigate the association of thyroid dysfunction with diabetic nephropathy, the prevalence of thyroid dysfunction in group 1 was compared with that of group 2. Group 1 there were 44(88%) euthyroid patients, 2(4%) low T3 syndrome, 4(8%) subclinical hypothyroidism and no overt hypothyroidism patient. In group 2 there were 26(52%) euthyroid patients, 11(22%) low T3 syndrome, 12(24%) subclinical hypothyroidism and 1(2%) overt hypothyroidism patient respectively. P value was significant, so the prevalence of thyroid disorder was found to be higher in group 2. The prevalence of

thyroid dysfunction was found to be more in females as compared to males in both the groups.

**Table 1: Insulin Distribution.**

Insulin microIU/ml	n	Group I (n=50)			n	Group II (n=50)		
		T3	T4	TSH		T3	T4	TSH
25-50	43	1.1±.454	7.2±2.06	3.2±.84	41	0.9±.5	7.6±1.89	3.3±2.3
50-75	6	1.00± .378	6.5± 2.1	5.8± 3.2	9	1.1± .52	7.2± 1.08	9.8± 6.6
>75	1	0.3± .	4.9± .	4.3±	0	-	-	-
p-value		.194	.461	<.01		.267	.568	<.01



**Group 1: Correlation of Various Biochemical Parameters with Thyroid Function Test Abnormalities.**

**Group 2: Correlation of Various Biochemical Parameters with Thyroid Function Test Abnormalities.**

**Table 2: Thyroid Dysfunction.**

Thyroid Dysfunction	Group I (n=50)		Group II (n=50)		Total
	No.	%	No.	%	
Normal	44	88.0	26	52.0	70
Low T3 syndrome	2	4.0	11	22.0	13
Subclinical hypothyroidism	4	8.0	12	24.0	16
Overt hypothyroidism	-	-	1	2.0	1
Total	50	100.0	50	100.0	100

$\chi^2 = 15.859$ ;  $df = 3$ ;  $p = 0.001$ ; Significant

**Table 3: Correlation of Various Biochemical Parameters with Thyroid Function Test Abnormalities in Group I.**

Group 1	FBS	PPBG	HBA1c	Serum insulin level	T3	T4	TSH
Low T3 syndrome							
Male(n=0)							
• Range							
• Mean							
Female (n=2)							
• Range	134-312	336.-344	6.0-6.9	32.9-98	0.2-0.3	4.9-5.8	3.4-4.3
• Mean	223±125.8	340±5.65	6.45±0.636	65.45±46.03	0.25±.07	5.35±0 .63	3.85±0.636
Sub clinical hypothyroidism							
Male(n=1)							
• Range							
• Mean	210	249	7.5	56	0.5	4.8	8.7
Female (n=3)							
• Range	128-311	201-354	7.4-7.6	50.5-53.0	0.8-1.6	4.4-9.6	5.5-9.4
• Mean	209±93.07	287± 78.2	7.5 ± .10	51.36± 1.41	1.2± 0.40	7.5± 2.75	7.2± 1.99

We observe that in group 1, patients in the serum insulin range 25-30 microIU/ml were 43 in number with mean values of T3 (ng/ml), T4 (Microgm/dl) and TSH (MicroIU/ml) were 1.1(0.2-1.8), 7.2(4.4-10.8) and 3.2(0.9-9.4) respectively. Patients with serum insulin range 50-75 microIU/ml were 6 in number with mean values of T3, T4 and TSH were 1.00(0.5-1.8), 6.5(4.7-10.4) and 5.8(2.3-8.7) respectively.

Patients with serum insulin range >75 microIU/ml was 1 in number with mean values of T3, T4 and TSH were 0.3, 4.9 and 4.3 respectively. In group 2, patients in serum insulin range 25-50 were 41 in number with mean values of T3 (ng/ml), T4 (Microgm/dl) and TSH (MicroIU/ml) were 0.9 (0.1-1.9), 7.6(4.4-11) and 3.3(0.35-27) respectively. Patients with serum insulin range 50-75 microIU/ml

were 9 in number with mean values of T3 (ng/ml), T4 (Microgm/dl) and TSH (MicroIU/ml) were 1.1 (0.5-1.8), 7.2(5.8-11) and 9.8 (2.07-9.67)

respectively. No patient found in serum insulin levels >75microIU/ml. P value of TSH was significant in both the groups i.e. p <0.01.

**Table 4: Correlation of Various Biochemical Parameters with Thyroid Function Test Abnormalities in Group II.**

Group II	FBS	PPBG	HBA1c	Serum insulin level	S. creatinine	Urinary ACR	T3	T4	TSH
Low T3 syndrome									
Male(n=4)									
• Range	137-318	244-354	6.5-12.4	26.2-39.0	1.2-2.98	31-148	0.1-0.4	5.6-10.0	2.4-4.09
• Mean	225.5± 101	317.75± 50.45	8.75± 2.5	32.37± 5.23	2.12± 0.73	108.5± 53.2	0.25± 0.12	7.3± 1.97	3.055± 0.723
Female (n=7)									
• Range	153-321	204-345	6.2-8.2	29.6-37.6	1.8-3.8	43-468	0.1-0.4	5.8-9.2	0.86-3.19
• Mean	238± 70	299± 46.5	7.2± 0.73	32.75± 2.76	2.9± 0.74	209± 138.9	0.24± 0.09	7.64± 1.27	2.06± 0.81
Sub clinical hypothyroidism									
Male(n=3)									
• Range	143-300	245-321	7.1-8.8	46.62	1.93-2.5	51-186	1.3-1.5	6.5-9.3	8.35-9.98
• Mean	245.33± 88.692	289± 39.552	8.1± 0.88	55 ± 8.22	2.27± 0.30	138.3± 75.7	1.4± 0.1	7.66± 1.45	9.0± 0.85
Female (n=9)									
• Range	148-334	208-378	7.2-10.2	39-65.4	2-8.2	122-382	0.6-1.9	4.9-8.2	5.3-10
• Mean	260.4± 69.53	306± 50.46	8.0± 0.98	51.04± 7.88	3.25± 1.9	195± 85.5	1.18± 0.41	6.7± 1.07	7.9± 1.84
Over hypothyroidism									
Male(n=0)									
• Range									
• Mean									
Female (n=1)									
• Mean	150	228	7.9	53	6.4	225	0.1	6.4	27

We observe that in group 1 there were 44(88%) euthyroid patients, 2(4%) low T3 syndrome, 4(8%) subclinical hypothyroidism and no overt hypothyroidism patient.

In group 2 there were 26(52%) euthyroid patients, 11(22%) low T3 syndrome, 12(24%) subclinical hypothyroidism and 1(2%) overt hypothyroidism patient respectively. P value was significant.

## DISCUSSION

In our study 100 patients with type 2 DM attending Guru Nanak Dev hospital attached to GMC Amritsar were recruited. These patients were divided into two groups of 50 patients each. Group 1 consisted of patients of type 2 DM without nephropathy and group 2 consisted of patients of type 2 DM with nephropathy.

We observe that in group 1, patients in the serum insulin range 25-50 microIU/ml were 43 in number with mean values of T3 (ng/ml), T4 (Microgm/dl) and TSH (MicroIU/ml) were 1.1±0.45, 7.2±2.06 and 3.2±0.84 respectively. Patients with serum insulin range 50-75microIU/ml were 6 in number with mean values of T3, T4 and TSH were 1.00±0.37, 6.5±2.1 and 5.8±3.2 respectively. Patients with serum insulin range >75microIU/ml was 1 in number with mean values of T3, T4 and TSH were 0.3, 4.9 and 4.3 respectively.

In group 2, patients in serum insulin range 25-50 were 41 in number with mean values of T3 (ng/ml), T4 (Microgm/dl) and TSH (MicroIU/ml) were 0.9±0.5, 7.6±1.89 and 3.3±2.3 respectively. Patients with serum insulin range 50-75microIU/ml were 9 in number with mean values of T3 (ng/ml), T4 (Microgm/dl) and TSH (MicroIU/ml) were 1.1±0.52, 7.2±1.08 and 9.8±6.6 respectively. No patient found in serum insulin levels >75micro IU/ml. P value of TSH was significant in both the groups i.e. p <0.01. In a study done by BM Singh et al<sup>[22]</sup> in year 2010 found that TSH levels were positively correlated with serum insulin levels with significant P value <0.01. In one another study done by Rajeswari G. et al in year 2015 found that TSH levels were positively correlated with insulin in patients with subclinical hypothyroidism (SCH).<sup>[23]</sup> Their study indicated that insulin resistance was present not only in overt hypothyroidism but it was significantly present in subclinical hypothyroidism also.

In our study in group 1 there were 14(28%) males, 36(72%) females, mean age of patients was 56.86±9.689 and 21(42%) were hypertensive. In group 2 there were 16(32%) males, 34(68%) females, mean age of patients was 56.94±8.51 and 36(72%) patients were hypertensive. Similar study done by Furukawa et al found that mean age of

patients in group 1 was  $61.6 \pm 10.7$  years and 60% were male.

In group 2 mean ages of patients was  $61 \pm 11.5$  years and 72.4% were male. In present study we observed that in group 1 there were 21(42%) hypertensive and in group 2, 36(72%) patients were hypertensive. There were more hypertensive patients in group 2 as compare to group 1 with significant p value (0.002). There was no significant p-value found regarding age and sex (p-value 0.965, 0.0663 respectively.) Similar study done by Furukawa et. al<sup>[24]</sup> in year 2014 found that in group 1 there were 38.9% hypertensive while in group 2, 72.4% were hypertensive with significant p value regarding hypertension and age (p -value 0.001, 0 and 0.014 respectively) but insignificant p value regarding gender distribution (p =0.458). Result of above study similar to our study except age and gender distribution.

In present study mean value of T3 in group 1 was  $1.096 \pm 0.45$  ng/ml as compare to  $0.964 \pm 0.53$  ng/ml in group 2 indicating decreasing trend of T3 in group 2 which was comparable to study done by Srinidhi et al<sup>[25]</sup>.

The mean value of TSH in group 1 was  $3.55 \pm 1.54$  microIU/ml as compared to  $4.46 \pm 4.22$  microIU/ml in group 2 showing increasing trend with Type 2 DM nephropathy.

In present study in group 2 mean value of TSH level was  $4.4 \pm 4.2$  microIU/ml in 30-299 mg/g urinary albumin creatinine ratio(UACR) range while it was  $4.86 \pm 4.5$  microIU/ml in >300 mg/g UACR range, which shows the increasing trend of TSH with increase in UACR levels. Similarly Furukawa et. al<sup>[24]</sup> in year 2014, that study showed higher incidence of subclinical hypothyroidism (high levels of TSH) in type 2 diabetic nephropathy group as compared to type 2 diabetes without nephropathy group.

In present study in group 1 out of 50 patients, 2(4%) had low T3 syndrome, 4(8%) had subclinical hypothyroidism while in group 2, 11(22%) had low T3 syndrome, 12(24%) had subclinical hypothyroidism and 1(2%) had overt hypothyroidism. The overall P-value for thyroid dysfunction in diabetic nephropathy i.e. Group 2 was significant with p value =0.001.

Similarly a study done by Miulescu et. al<sup>[26]</sup> in 2014 year found that higher incidence of thyroid dysfunction in type 2 DM as compared to type 2 DM without nephropathy in the form of Low T3 syndrome (23.80% vs. 0.00%), subclinical hypothyroidism (23.80% VS 9.52%) and overt hypothyroidism (8.69% vs. 4.76%)

In a study done by Furukawa et al<sup>[24]</sup> in year 2014 the prevalence of subclinical hypothyroidism was 20.7% in diabetic nephropathy group while 8.7% in diabetic without nephropathy group. In another study done by Mansournia N. et. Al<sup>[27]</sup> in year 2016 showed incidence of subclinical hypothyroidism in

diabetic nephropathy was 29% as compare to 17% in without diabetic nephropathy group while in present study there were 24% patients in diabetic nephropathy group had subclinical hypothyroidism and 8% in type 2 diabetes without nephropathy group

### Summary

In our study 100 patients with type 2 DM attending Guru Nanak Dev hospital attached to GMC Amritsar were recruited. These patients were divided into two groups of 50 patients each. Group 1 consisted of patients of type 2 DM without nephropathy and group 2 consisted of patients of type 2 DM with nephropathy. Thyroid dysfunctions in the form of low T3 syndrome, subclinical hypothyroidism and overt hypothyroidism were more commonly found in type 2 DM nephropathy than type 2 DM without nephropathy. Among type 2 DM nephropathy patients 11(22%) had low T3 syndrome, 12(24%) had subclinical hypothyroidism and 1(2%) had overt hypothyroidism while in type 2 DM without nephropathy patients 2(4%) had low T3 syndrome and 4(8%) had subclinical hypothyroidism signifying that thyroid dysfunction was more prevalent in diabetic nephropathy group. In group 1 there were 14 male patients from which 13 normal, no low T3 syndrome, 1 subclinical hypothyroidism and no overt hypothyroidism patient and there were 36 female patients among them 31 were normal, 2 low T3 syndrome, 3 subclinical hypothyroidism and no overt hypothyroidism patient. In group 2 there were 16 male patients from which 9 normal, 4 low T3 syndrome, 3 subclinical hypothyroidism and no overt hypothyroidism patient. There were 34 female patients in group 2 from which 17 normal, 7 low T3 syndrome, 9 subclinical hypothyroidism and 1 overt hypothyroidism.

In present study thyroid dysfunctions were more prevalent in females in type 2 DM nephropathy patients as compared to type 2 DM without nephropathy patients. We observed that TSH levels were positively correlated with insulin levels with p value statistically significant (p<0.05). In present study serum TSH levels in type 2 diabetic nephropathy increases with increase in urinary albumin creatinine ratio. In our study hypertension has positive correlation (p<0.05) with type 2 diabetic nephropathy patients.

### CONCLUSION

As coronary artery disease continues to be prevalent in today society, it is imperative to ensure that all patients with MI are optimally treated for ongoing ischemia to prevent life-threatening complications like arrhythmias. Diligent monitoring for arrhythmias and instituting appropriate treatment can be life-saving.

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**How to cite this article:** Singh B, Aloona SP, Neki NS. Study of Insulin Levels in Patients of Type 2 Dm With and Without Nephropathy and Thyroid Disorders. *Ann. Int. Med. Den. Res*. 2017; 3(2):ME30-ME35.

**Source of Support:** Nil, **Conflict of Interest:** None declared