

Effects of Melatonin in Mild diabetics with Dyslipidaemia

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ABSTRACT Diabetes is spreading at an exponential rate in Indian sub-continent. Existing infrastructure of health in India is poor, particularly in its rural sectors. Of all varieties of diabetes, Type 2 diabetes is commoner and is about 80%. Different drugs, herbal medicines and nutraceuticals are used to control diabetes. Melatonin, the hormone of pineal gland is being considered as a nutraceutical by FDA. Many workers thought melatonin might have a role in diabetes control through central mechanisms. The studies lead to many controversies and counterclaims. Present study is intended to observe the effects of melatonin at a higher dosage per day. The effects in rural Indian diabetic population at that melatonin dosage in different blood parameters were observed and also change in endocrine secretions observed. Melatonin caused reduction in Serum Insulin, Serum Cortisol, Serum ACTH and Serum TSH levels while increase in Serum Gastrin level. Of the biochemical parameters, melatonin caused reductions in TLC, LDLC and FBS while increase in HDLC. It also caused reduction in neutrophil and increase in lymphocyte count in a diabetic with increase in faecal fat excretion.

INTRODUCTION

India is facing diabetic explosion (King et al., 1998). The exact causes of the diabetes and Insulin resistance are unknown. Both genetic and environmental factors are held responsible (Enase et al., 1992). In obesity, atherosclerosis, coronary vascular disease, hypertension and type 2 diabetes clinical and biochemical evaluations usually show insulin resistance (Figure-1, showing relationship of Insulin Resistance Syndrome with Type 2 diabetes, Obesity and Hyperlipidaemia, Hypertension and Atherosclerosis). As a result, β -cells of pancreas secrete more and more. Hyperinsulinaemia is a defense against insulin resistance and diabetes. Keeping in mind the role of insulin, the condition has profound repercussions on metabolism and protein synthesis. Over-expression of hypertension gene in insulin resistance leads to hypertension. Similarly who has inherited dyslipidaemia or atherosclerosis gene action of hyperinsulinaemia with insulin resistance may manifests as lipid abnormalities or as propensity for atheroma formation. Just as the degrees of insulin resistance and hyperinsulinaemia are multi-faceted, the degrees of expression of the various genes are variable. The phenotypic expression of the genetically predisposed individual depends on the degree of gene expression, insulin resistance and hyperinsulinaemia.

Both genetic tendencies as well as hyperinsulinaemia/insulin resistance have a role in the management of these disorders. A person with insulin resistance may not be resistant to all actions of insulin and increase in insulin values lead to more anabolic effects particularly on arterial smooth muscles, leading to proliferation of these cells and atherosclerosis. There is a positive correlation between systolic blood pressures and 2-hour post-prandial insulin concentrations in hypertensive obese individuals. Randle hypothesis suggests that insulin resistance is due to increased non-esterified fatty acids released from adipose tissues and these are the primary substrate for the muscles. They augment gluconeogenesis in liver and inhibit glucose uptake at the peripheral tissues. The defect in insulin resistance may be at post receptor level in impaired glucose tolerance stage and at the receptor and post-receptor level in frank type 2 diabetes (Mitra and Bhattacharya, 2006a, b).

Until the 1970s, it was widely believed that compared to the western world the prevalence of diabetes in India was low. But recent statistics now show that India has the world's largest diabetic population. The Chennai Urban Population study (1997) showed 12 per cent prevalence of diabetes in the Chennai population and The Chennai Urban Rural Epidemiology Study (CURES), which sampled 26,001 persons, recorded a prevalence of 16 % (Mohan et al., 2001;

Mohan et al., 2006). This rising trend in diabetes predicts a significant health burden. This problem started developing in the childhood in the urban areas more as children do not have any kind of physical exercise. The only things one do is to spend long hours on the computer or the cell phone or watch television (Mitra, 2006).

Dilman (1989) hypothesized hypothalamus as the grand biological clock, fails to synchronize adaptosis mechanisms as efficiently as it was in younger ages of life (failing rheostat mechanisms). The exact cause of such dysfunction being unknown, chronic stress is believed to play primordial roles. Insulin is anabolic in nature and its main role is to conserve energy. So in stress hypothalamus exerts suppressing role on insulin production. Synthesis of energy yielding hormones, which break down glucose and other substrates, are increased and feedbacks are suppressed. Chronic stresses lead to different sets of levels of control in hypothalamus and its efficiency to regulate as grand biological clock, though it does not produce any symptoms as the changes are within biological limits. Hypothalamic failure with age (within physiologic limits) may be a strong contributing factor of increased insulin level in blood.

The pineal gland has drawn attention of man for long and it was the Greek philosophers who considered it "seat of the soul". Descartes in the middle ages, furthered this concept and named it "espirits animaux" meaning psychic and somatic activating principle. In recent developments the pineal gland was found to play a pivotal role in fine-tuning and integrating various neural and endocrine functions. Melatonin is chemically identified as N-acetyl-5-methoxytryptamine and

plays an important role in sleeping disorders, depression, and immune system regulation. Melatonin appears to be the hormone that regulates biological rhythms and keeps the body clock in synchronicity with hypothalamus. Studies showed that melatonin could be safely given up to 75 mg per day (MacFarlane et al., 1991). Waldhauser et al. (1990) showed that in laboratory animals dose of 800 mg/kg did not cause death in mice and median lethal concentration could not be found because of the limited water solubility of melatonin did not allow a more concentrated solution.

METHODS

The study was done with 75g/day of melatonin spaced in five different doses of 15 g each and given to 10 patients of Type 2 diabetes (fasting serum insulin level 45 units) and 10 normal patients for one month. These 20 patients, residing at two different areas, aged between 45 and 52 years, were divided in 4 groups containing 5 patients each. They were kept under strict observations for four weeks to maintain uniformity of diet. Earlier, the patients with known renal and hepatic dysfunction and lipid lowering, anti-hypertensive and anti-diabetic therapies were excluded from the study. The patients underwent clinical and anthropometrical evaluations before the study and written consents from them obtained. Institute ethical committee was approached for approval.

Blood samples were taken by an indwelling catheter into ante-cubital vein and analyzed for fasting blood glucose (FBS), triglyceride (TG), total cholesterol (TLC), high density lipoprotein

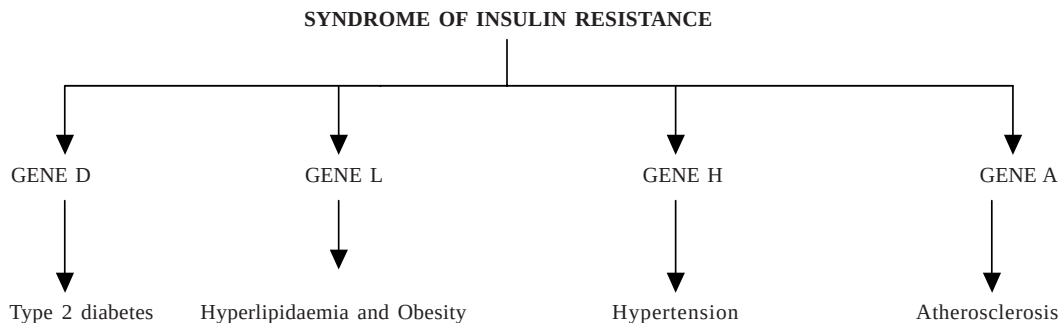


Fig.1. Relationship of Insulin Resistance Syndrome with Type 2 diabetes, Obesity and Hyperlipidaemia, Hypertension and Atherosclerosis

cholesterol (HDLc), low density lipoprotein cholesterol (LDLc), very low density lipoprotein cholesterol (VLDLc). The tests were done as per standard practice depicted by Boehringer Mannheim. Serum Insulin, Serum Gastrin, Serum Cortisol, Serum ACTH, Serum TSH and Serum HGH values and other bio-chemic parameters were measured by radioimmunoassay and as per standard methods.

RESULTS

The patients underwent clinical, anthropometrical evaluation before the study. The characteristics of the patients were:

Average age: 51.23 ± 8.29 years, (Mean $\pm$ SD)

Sex: Males: 10 Females: 10

Average weight: 73.36 ± 4.08 kg,

Body Mass Index (BMI) = Weight (kg) / Square of height (m^2) = 22.32 ± 2.23 .

At the end of the study it was found that in the volunteers the weight became 73.38 ± 3.23 kg and BMI was found to be 22.32 ± 3.12 , this variation may be due to non-identical conditions prevailing during measurements. Initial blood samples as analysed for plasma glucose and lipid profiles were shown in the table below. As the samples were drawn from different patients, having different socio-cultural backgrounds, considering the diversity of Indian population in intake of food, life-styles, socio-cultural believes etc, the variations in the initial readings of different blood parameters in different patients were noted. Clinical Parameters were also evaluated at the end of study. All the parameters remain as like and variations were insignificant.

The effects of melatonin on Serum Insulin values were estimated along with Serum Gastrin, Serum Cortisol, Serum ACTH, Serum TSH and Serum HGH at two weeks intervals purely on

economic reasons. Table 1 showed the changes in the values of TLC, HDLc, LDLc, VLDLc, TG and FBS with 75 g of melatonin/day. It was found that melatonin caused 4-6% reductions in TLC from the initial values of 233 ± 7 mg/dl to 215 ± 9 mg/dl ($p \leq 0.025$) and 7-9% reductions in LDLc values from 166 ± 5 mg/dl to 153 ± 7 mg/dl ($p \leq 0.05$). HDLc values were increased by 7-8 % from 38 ± 5 mg/dl to 41 ± 3 mg/dl ($p \leq 0.025$). FBS values were reduced by 5-6% from 160 ± 7 mg/dl to 152 ± 6 mg/dl ($p \leq 0.05$) while no statistically significant changes were observed in VLDLc or TG values. Table 2 (the effects of melatonin 75 g of melatonin/day, on Serum Insulin, Serum Gastrin, Serum Cortisol, Serum ACTH, Serum TSH, Serum HGH) showed Serum Insulin values (12 hour fasting) were reduced by 8-12% from 33 ± 3 μ U/ml to 30 ± 2 μ U/ml ($p \leq 0.075$) while Serum Gastrin values (at 8AM) were increased by 7-9% from 78 ± 8 pg/ml to 85 ± 5 pg/ml ($p \leq 0.05$). Serum Cortisol values (at 8AM) reduced by 15-18% from 26 ± 3 μ g/dl to 22 ± 3 μ g/dl ($p \leq 0.05$). Serum ACTH (at 8AM) was increased from 57 ± 6 pg/ml to 52 ± 4 pg/ml, an increase of 9-11% ($p \leq 0.05$). Serum TSH (at 8AM) was reduced from 5 ± 1 μ U/ml to 4.2 ± 1 mU/ml, a reduction of 15-18 % ($p \leq 0.025$) while total Serum T_3 (122 - 156 μ g/dl), FT_4 (1.2 - 1.8 ng/dl) and Serum total T_4 (7 - 10 μ g/dl) values remained normal. Serum HGH (at 8AM) values showed no changes and remained at 4 ± 2 ng/ml.

Other biochemical parameters like LFT (Serum Bilirubin 0.7 - 0.9 mg/dl, GGT 22 - 31 U/L, Total Protein 6.4 - 7.5 g/dl, Serum Albumin 4.2 - 4.5 g/dl, Serum Globulin 2.1 - 2.9 g/dl, Alkaline Phosphates 60 - 88 U/L, SGOT 22 - 28 U/L), Serum Uric Acid (4.2 - 6.5 mg/dl), Blood Urea (22 - 26 mg/dl) and Serum Creatinine (1.2 - 1.5 mg/dl), Serum Lipase (66 - 82 U/L) and Urinary 24 hours VMA (3.5 - 4.5 g/day) showed statistically no significant changes. Routine blood, urine and stool tests were done

Table 1: The effects of melatonin (75 g of melatonin/day)

Time (weeks)	TLC(mg/dl)	HDLc(mg/dl)	LDLc(mg/dl)	VLDLc(mg/dl)	FBS(mg/dl)	TG(mg/dl)
0	233 ± 7	38 ± 5	166 ± 5	29 ± 2	160 ± 7	148 ± 5
2 weeks	227 ± 6	39 ± 6	160 ± 9	29 ± 2	156 ± 5	148 ± 6
4 weeks	225 ± 9	41 ± 3	155 ± 7	29 ± 2	152 ± 6	145 ± 5

Table 2: The effects of melatonin (75 g of melatonin/day)

Time (weeks)	Serum Insulin	Serum Gastrin	Serum Cortisol	Serum ACTH	Serum TSH	Serum HGH
0 week	33 ± 3 μ U/ml	78 ± 8 pg/ml	26 ± 3 μ g/dl	57 ± 6 pg/ml	5 ± 1 μ U/ml	4 ± 2 ng/ml
2 weeks	32 ± 2 μ U/ml	82 ± 4 pg/ml	24 ± 2 μ g/dl	54 ± 5 pg/ml	4.5 ± 1 μ U/ml	4 ± 2 ng/ml
4 weeks	30 ± 2 μ U/ml	85 ± 5 pg/ml	22 ± 3 μ g/dl	52 ± 4 pg/ml	4.2 ± 1 μ U/ml	4 ± 2 ng/ml

and no changes were evident before and after the experiment except increase in lymphocyte count of about 10-12% ($p \leq 0.025$) and reduction in neutrophil count of about 4-6% ($p \leq 0.05$). Fat excretion in stool (5.5-6.4 g/day) was increased by 15-18% ($p \leq 0.075$).

DISCUSSION

Diet and lifestyle patterns are important in diabetes. In rural Midnapur, a district in Bengal of India, the incidence of diabetes is less compared to other parts of country. Interpopulation differences exist in both diet and socio-cultural factors within and outside the subcontinent. Type 2 prevalence rates are higher on the east coast of Andhra Pradesh, particularly Eluru and Tenali, where rice is traditionally consumed as only cereal. The dietary pattern, eating and methods of cooking vary in different parts of India (Ramaiya et al., 1990). Mitra and Bhattacharya (2005) found diet has a role to play in the incidence of Type 2 Diabetes and the condition improves with diet prescribed as per American Diabetes Association guidelines (Mitra and Bhattacharya, 2005 and 2006; American Diabetes Association, 1987). Table 3 (Prevalence of diabetes and impaired glucose tolerance (IGT) in different cities in India) shows the prevalence of Diabetes and impaired glucose tolerance (IGT) in different cities in India (Ramachandran et al., 2003).

UKPDS (United Kingdom Prospective Diabetic Study) showed micro vascular complications of diabetes could be controlled with strict control of blood sugar and blood pressure levels. Serum insulin level is a strong prognostic indicator of macro vascular complications. Resistance to the actions of insulin is strongly associated with the microvascular complications of diabetes. It is now clear that insulin also has direct actions in the microvasculature that influence the development

and progression of microvascular disease. In the healthy state, due to activation of opposing mediators like nitric oxide and endothelin-1 insulin appears to have only minor effects on vascular function. Diabetes and obesity, however, are associated with selective insulin resistance. Inactivation in the phosphatidylinositol-3-kinase signaling pathway leads to reduced synthesis of nitric oxide, impaired metabolic control and compensatory hyperinsulinemia. Insulin signaling via extracellular signal-regulated kinase dependent pathways is relatively unaffected in diabetes. This results abnormal vasoreactivity, angiogenesis, and other pathways implicated in microvascular complications and hypertension. In addition, preferential impairment of nonoxidative glucose metabolism leads to increased intracellular formation of advanced glycation end products, oxidative stress and activation of other pathogenic mediators. Nonetheless, insulin resistance remains an important marker of risk and a key target for intervention. Patients with a greater improvement in insulin sensitivity achieve better microvascular outcomes (Per-Henrik Groop et al., 2005).

The pineal body secretes sufficient melatonin up to the age 40 or so and then the secretion declines. Daily supplements are probably unnecessary prior to that age (Mitra and Bhattacharya, 2006 a,b). Children tend to excrete large amounts of this hormone, while older adults produce relatively little. But individual levels of melatonin vary widely. About 1% of the population naturally has quite low levels, while another 1% has levels 500 times above the average. Melatonin is intimately involved in synchronizing the body's hormone secretions, setting the brain's internal clock and generating circadian rhythms (daily biorhythms). These patterns particularly govern the release of hormones that regulate such body functions as reproduction and digestion. Significantly, melatonin also works around the clock to signal

Table 3: Prevalence of diabetes and impaired glucose tolerance (IGT) in different cities in India

City	n	Males:Females	Diabetes% (95% CI)	IGT% (95% CI)
Chennai	1668	708:960	13.5 (11.8-15.2)	16.8 ^{b,f} (14.6-19.0)
Bangalore	1359	638:721	12.4 ^b (10.5-14.3)	14.9 ^{b,f} (12.8-16.9)
Hyderabad	1427	685:742	16.6 (14.6-18.6)	29.8 ^f (26.9-32.8)
Calcutta	2378	1163:1215	11.7 ^{a,b} (10.4-13.0)	10.0 ^{a,b,d} (8.7-11.4)
Mumbai	2084	987:1097	9.3 ^{a,b,c} (7.7-10.1)	10.8 ^{a,b,d} (9.3-12.2)
New Delhi	2300	1107:1193	11.6 ^{a,b} (10.3-12.9)	8.6 ^{a,b,d,e,f} (7.4-9.7)

P<0.001:^a vs Chennai;^b vs Hyderabad;^c vs New Delhi;^d vs Bangalore;^e vs Calcutta; ^f vs Mumbai

the body, which is sensitive to light, cues, when to sleep and when to awaken. Typically, the pineal gland begins excreting melatonin around dusk, rapidly increases its output between 2 A.M. and 4 A.M., and then decreases its output with daylight (Mitra and Bhattacharya, 2006a, b).

Dilman (1989) pointed that hyperadaptoptosis and slackening of feedback mechanisms due to ageing and repetitive stress play a crucial role in the genesis of Insulin Resistance Syndrome. Overall slowing of endocrine regulations manifests hypothalamus slackening. Pineal gland regulates circadian rhythms and endocrine secretions are dependent on time. Hence pineal gland, possibly through hypothalamus, must have a role in endocrine regulations and triggering may be hormonal or neural. Melatonin is regulated by signals of light or darkness- the primitive responses of lower organisms to remain active or inactive. Hence this study was conducted to see the effects of different hormonal changes in response to melatonin particularly in a mild diabetic. Melatonin caused reductions in Serum Insulin, Serum Cortisol, Serum ACTH and Serum TSH levels while increase in Serum Gastrin level. Of the bio-chemical parameters, melatonin caused reductions in TLC, LDLC and FBS while increase in HDLC. It also caused reductions in neutrophil and increase in lymphocyte count in a diabetic with increase in faecal fat excretion.

CONCLUSION

India is facing diabetic explosion. The cause is unknown. Both nature and nurture play important role. Lack of health infrastructure in India particularly in its rural areas shows the importance of alternative directions in diabetic researches. Researches in diabetes is mainly oriented towards increase in insulin secretion, reduction of insulin resistance, increase in insulin sensitivity, reducing glucose absorption from gastro-intestinal system and increase in peripheral utilization of glucose. Insulin resistance syndrome is multi-factorial and some workers pointed hypothalamic legacy may be an important factor (adaptoptosis and ontenogenic models). Hypothalamus being key in endocrine regulations, it must be rejuvenated to restore normalcy. Pineal gland regulates the biorhythms. Hormones are cyclically secreted on a pulsatile fashion depending on the time of the day. Hence, pineal gland must have relationships with hormone regulations. As

hormone regulation is intricately hypothalamic, pineal gland must maintain a relationship with hypothalamus and that is probably through melatonin. The study shows some of the effects of melatonin in hormone balance in a diabetic.

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