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Original Research Article

A Study of Correlation of Perceived Stress with Serum Prolactin Levels in Newly Diagnosed Cases of Hypothyroidism in Females in an Urban Population of a Developing Country

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ABSTRACT

Background: Prolactin affects the brain and mood but, on the other hand, personality traits and environmental factors may stimulate the secretion of prolactin and may play a role in the genesis of the disease.
Aims: To study correlation of Prolactin levels and Perceived stress scores in newly diagnosed hypothyroid females and subjects with clinical and subclinical hypothyroidism Materials and methods:
Materials and methods: This pilot study was conducted in Burdwan Medical College on two hundred and sixty newly diagnosed female hypothyroid subjects in a period of 12 months after taking institutional ethical clearance and informed consent of the subjects. Anthropometric Measurements - Height, Weigh, Body mass index (BMI), Presumptive stressful Life Event Stress Scale (PSLES) scores, Perceived stress scale(PSS) scores, TSH, fT4, serum prolactin levels. Subjects were divided into two groups: Hypothyroid individuals and subjects with subclinical Hypothyroidism. The computer software "Statistical Package for the Social Sciences (SPSS) version 16 (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.)" was used to analyze the data.
Results: 260 newly diagnosed hypothyroid females were enrolled in our study. Among these 130 were clinical hypothyroid and 130 were subclinical hypothyroid patients. There was no difference in socioeconomic status, dietary habits, educational status, age between the two groups. 126 subjects in the subclinical hypothyroid group were married and 128 in the hypothyroid group. Significant difference was found between clinical and subclinical hypothyroid females for mean BMI($p=0.002$), mean TSH($p<0.0001$), mean fT4($p<0.0001$), mean PRL($p=0.002$), mean PSLES($p<0.0001$), mean PSS($p<0.0001$) (Table1).TSH values, PRL were positively correlated with PSS values while ft4 values were negatively correlated with PSS values in both clinical and subclinical hypothyroid subjects.
Conclusions: Hypothyroid female individuals may have hyperprolactinemia and perceived stress may be a significant contributor to this problem.
Keywords: Prolactin, stress, hypothyroidism.

INTRODUCTION

Stress is body's way of responding to the demand which is caused by both good and bad events/experiences. The body reacts by releasing chemicals in the blood to combat this demand by a complex repertoire

of behavioral and physiologic adaptive responses. Prolactin (PRL) is a pleiotropic pituitary hormone and acts as a neuropeptide to promote physiological responses related to reproduction, stress adaptation, neurogenesis, and neuroprotection. PRL regulates neurogenesis both in the subventricular zone (SVZ) and hippocampus. So, alterations in the PRL system due to stress may contribute to maladaptive responses and pathological behavioral outcomes. [1]

Stress is a critical factor that may lead to depressive disorders. Stress exposure activates the HPA axis, triggering the release of corticotrophin releasing hormone (CRH) in the paraventricular nucleus (PVN), and promotes the secretion of adrenocorticotrophin (ACTH) from the pituitary. ACTH triggers the release of glucocorticoids from the adrenal glands. PRL is also secreted from the pituitary in response to a number of stressors. Chronic stress exposure and depressive states are known to affect neurogenesis. Prolactin is a regulator of neurogenesis. PRL receptors are expressed in the SVZ and the hippocampus. Prolactin alters neural circuits to help the individual to cope with stress. Reduced activation of neural inputs, activation of ionic channels, and modulation of several signaling pathways are some of the putative mechanisms of action underlying the effects of PRL on brain circuits. It is still unknown how PRL regulates the HPA axis function during the stress response. PRL may affect hypothalamic and/or hippocampal activity to regulate emotionality. [1]

The effects of acute stress during a parachute jump on hormonal responses were studied by Schedlowski M et al in 12 experienced and 11 inexperienced military parachutists. Each subject performed two jumps. Prior to and immediately after each jump blood samples were drawn and analysed for plasma levels of cortisol, prolactin, thyrotropin (TSH), somatotropin (STH), and luteinizing hormone (LH). There was a significant increase in cortisol, prolactin and TSH levels after both jumps. [2]

Significant correlation was found between day-to-day changes in anxiety and stress hormones, cortisol and prolactin by Jeffcoate WJ et al in their studies. [3] The relations between prolactin, emotions and feelings are circular, i.e., prolactin affects the brain and mood and, on the other hand, personality traits and environmental factors may stimulate the secretion of prolactin and play a role in the genesis of disease. There are several intersections between prolactin, psychological stress and the environment in humans reported in different studies. [4]

Thyroid diseases are common worldwide. It has been estimated that about 42 million people in India suffer from thyroid diseases. [5] Thyro-stress is defined as an emotional state, characterized by extreme apprehension, discomfort or dejection, caused by the challenges and demand of living with thyroid disorders such as hypothyroidism. There is significant evidence to show that hypothyroidism is usually associated with an impact on psychological health. The physical complaints, may impair emotional health and quality of life. However, not much attention has been paid till date to the psychosocial aspects of hypothyroidism. [6]

A potential explanation for the positive TSH-cortisol relationship is that hypothyroidism - subclinical or clinical - is associated with subtle metabolic stress. Metabolic stress may impose an effect on the adrenocorticotropin hormone-adrenal axis leading to an increase in stress hormone (i.e., cortisol) release and production and this hypothesis has been confirmed through the measurement of other stress hormones including the catecholamines, norepinephrine/epinephrine, and/or prolactin. [7] Thus it may evident from the above discussion that there exit a complex correlation of stress, cortisol, thyroid hormones and prolactin interactions.

The postmodern over-industrialized and highly competitive metropolitan culture has added up to stresses in human lives at

many levels. Women have major roles to play in the collective social wellbeing. Stress may lead to early aging and death or sometimes in reduced levels of performances in females. Eighty-seven percent of Indian women feel stressed most of the times, with eighty-two percent having insufficient time to relax. [8] In a survey conducted in 2011 [8] it was found the highest stress is perceived by women between 25-55 years who have to manage multiple roles in various fields. Women have a life-expectancy advantage over men, but a marked disadvantage with regards to morbidity. Individual differences in physical and mental health are further notably explained by the degree of stress individuals endure, with women being more affected by stressors than men. [8]

Hypothyroidism is a common health problem in India and worldwide. It has a wide clinical spectrum ranging from myxedema, end organ damage, multisystem failure to a subclinical condition. In patients with primary hypothyroidism there is increased levels of Thyroid realising hormone (TRH) which can cause rise of prolactin levels and these patients may have galactorrhea. [9-14] The increasing numbers of hypothyroid subjects with hyperprolactinemia found in different studies deserve special attention.

In a study by Bahar A et al in 2011 [15] in Sari, Iran, prolactin levels of 481 subclinical hypothyroid patients were assessed. Prolactin measurement was performed using chemiluminescent immunoassay. Prevalence of hyperprolactinemia in subclinical hypothyroidism was 20.4%. (11% in men and 22% in women, p=0.05). This study showed that prevalence of hyperprolactinemia in subclinical hypothyroidism is notable and this disorder is more common in female subclinical hypothyroidism than the men.

In the above context the present study was conducted to assess correlation of Prolactin levels and Perceived stress scores in newly diagnosed hypothyroid females and subjects with clinical and subclinical hypothyroidism, so that early lifestyle modification with stress management programmes may improve the quality of life in these individuals. Females of reproductive age group were selected for this study as from previous discussion it is evident that they are more exposed to multiple stressors as they have to play multiple roles in the present day society. A study in these subjects on these parameters in a rural population of West Bengal is lacking and the study may have some positive impact in this population.

MATERIALS AND METHODS

This pilot study was conducted in Burdwan Medical College on two hundred and sixty newly diagnosed female hypothyroid subjects in a period of 12 months after taking institutional ethical clearance and informed consent of the subjects. The formula used to calculate the size of the required sample was $n= (z)^2p (1-p)/d^2$, where, n= sample size, z= z statistic for a level of confidence(95% level of confidence used, therefore z value is 1.96), p= expected prevalence of proportion, d= desired precision, (taken as 6%), and previous studies were taken into consideration. [16-17]

Inclusion criteria:

Newly diagnosed hypothyroid females in the reproductive age group attending in the Department of Biochemistry, Burdwan Medical College.

Exclusion criteria:

1.Pregnancy
2.Lactation
3.Liver diseases
4.Renal diseases
5.Any history suggestive of visual field defect/headache/seizure
6. Postmenopausal females, subjects on treatment from psychiatry problem or with past history of treatment were excluded.
7. Adolescent girls, perimenopausal women, sportswomen, yogis, subjects on regular meditation and exercise regime were not included.

Parameters studied:

Anthropometric Measurements -Height, Weigh, Body mass index (BMI)

PSLES scores, PSS scores.

TSH, fT4, serum prolactin levels.

Methods:

1. Approval from the institutional ethics committee of Burdwan Medical College was taken before conduction of the study
2. Participants were recruited by random sampling.
3. Informed consent was taken from participating subjects
4. PSLES scores were assessed.
5. Detailed history was taken regarding any past or current illness, hospitalization, medication, smoking and alcoholism and daily habits.
6. Participants were further screened based on the inclusion and exclusion criteria for final selection.
7. Pre-test instructions were given to avoid consumption of any drugs that may alter the Cardio respiratory parameters 48 hours prior to the test. The subjects were advised for a good restful sleep and to fast at least for 12 hours after a light dinner at the night before the test day. On the day of the test, no cigarette, nicotine, coffee, or drugs were permitted.
8. PSLES scores were assessed.

Perceived stress of the subjects was measured by using Perceived stress scale of Sheldon Cohen.

9. Anthropometric measurements were recorded.
10. Blood samples were drawn from subjects by sterile needle and syringes and sent to biochemical laboratory in sterile vials for analysis.

Increased sympathetic activity has been observed during the premenstrual phase and this was positively correlated with the stress levels in previous studies. To avoid stress effects of the premenstrual phase, we examined our subjects during the postmenstrual phase.

All subjects were on non-vegetarian diet and their dietary habits were nearly similar.

Presumptive stressful Life Event Stress Scale [18]

Using an open-ended question along with Holme's and Rahe's Social Readjustment Rating Schedule, Singh et al., on a sample of 200 adult subjects, constructed a suitable scale of stressful life events experienced by the Indian population, the Presumptive Stressful Life Events Scale (PSLES). Stress level of the subjects was assessed according to presumptive life event stress scale. Subjects were asked to tally a list of 43 life events based on a relative score experienced in past one year. The standardized and statistically tested PSLES Scale was designed by Indian scientist Gurmeet Singh. In this scale, 43 different variables (life events) were found to be experienced by the normal Indian population in the past one year. For each life event, a mean stress score was given. All the subjects were asked to tick the life events which were applicable to them (Yes/No). The total score was obtained for each subject by adding all the applicable life events scores.

The Perceived Stress Scale (PSS) of Sheldon Cohen, the most widely used psychological instrument for measuring the perception of stress, was used. It is a measure of the degree to which situations in one's life are appraised to be stressful. The questions in the PSS ask about feelings and thoughts during the last month. It comprises of 10 items, four of which are reverse-scored, measured on a 5-point scale from 0 to 4. PSS scores are obtained by reversing responses (e.g., 0 = 4, 1 = 3, 2 = 2, 3 = 1 and 4 = 0) to the four positively stated items (items 4, 5, 7 and 8) and then summing across all scale items. Total score ranges from 0 to 40. [19]

Subjects were divided into 2 groups: G1 Hypothyroid subjects and G2 subjects with subclinical hypothyroidism.

Hypothyroidism was defined as Clinical as an elevated TSH (>6.16μIU/ml) with a decreased serum fT4 level(<0.8ng/dl) and Subclinical as an elevated TSH (>6.16 μ

IU/ml) together with normal fT4 levels(0.8-2.0 ng/dl)[10-11].

Estimation of Serum TSH level was done by Quantitative determination of TSH concentration by Microplate immuno-enzymometric assay using Monobind Inc., USA manufactured TSH AccuBind ELISA Kit.

Estimation of Serum fT4 level was done by Quantitative determination of fT4 concentration by Microplate Enzyme Immuno assay using Monobind Inc.,USA manufactured Free T4 AccuBind ELISA Kit.

Estimation of Serum Prolactin level was done by Quantitative determination of Prolactin concentration by Microplate immunoenzymometric assay using Monobind Inc.,USA manufactured Prolactin AccuBind ELISA Kit.

Statistical analysis:

The computer software "Statistical Package for the Social Sciences (SPSS) version 16 (SPSS Inc. Released 2007. SPSS for Windows, Version 16.0. Chicago, SPSS Inc.)" was used to analyze the data, $P < 0.05^{*}$ was considered as significant and $P < 0.01^{**}$ was considered as highly significant.

RESULTS

260 newly diagnosed hypothyroid females were enrolled in our study. Among these 130 were clinical hypothyroid and 130 were subclinical hypothyroid patients. There was no difference in socioeconomic status, dietary habits, educational status, age between the two groups. 126 subjects in the subclinical hypothyroid group were married and 128 in the hypothyroid group.

Significant difference was found between clinical and subclinical hypothyroid females for mean BMI (p=0.002), mean TSH (p<0.0001), mean fT4 (p<0.0001), mean PRL (p=0.002), mean PSLES (p<0.0001), mean PSS (p<0.0001) (Table1, Figure1). TSH values, PRL were positively correlated with PSS values while ft4 values were negatively correlated with PSS values in both clinical and subclinical hypothyroidism (Table2).

Table 1: Shows Age, BMI, TSH values, fT4 values, PRL and PSS values of clinical, subclinical hypothyroid subjects

Parameter	Clinical hypothyroidism (mean ±SD)	Subclinical hypothyroidism (mean ± SD)	P value
Age(yrs)	30.89±7.69	31.05±7.6	0.52
BMI(kg/m2)	25.14±2.83	22.85±1.44	<0.001**
TSH (µIU/ml)	23.85±8.15	9.45±2.17	<0.0001**
fT4(ng/dl)	0.61±0.56	1.338±0.28	<0.0001**
PRL(ng/ml)	20.52±18.14	15.3±6.22	0.002**
PSS	28.40±3.09	25.17±3.08	< 0.0001**
PSLES	319.39±28.01	211.23±37.01	< 0.0001**

P value < 0.05* significant; <0.01** highly significant

Table 1 shows that the difference of BMI, TSH values, ft4 values, PRL, PSLES and PSS values between clinical and subclinical hypothyroidism were highly significant.

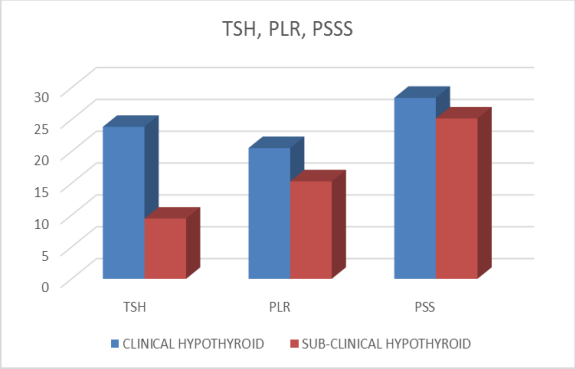

Figure 1 shows that the difference of TSH values and PSS values between clinical and subclinical hypothyroid subjects.

Table 2: shows that the correlation of TSH values, ft4 values, PRL with PSS values in clinical and subclinical hypothyroidism.

Parameter	Clinical hypothyroidism r value	Subclinical hypothyroidism r value	P value
TSH (µIU/ml)	0.04	0.03	<0.0001**
fT4(ng/dl)	-0.172	-0.116	<0.0001**
PRL(ng/ml)	0.052	0.016	0.002**

P value < 0.05* significant; <0.01** highly significant

DISCUSSION

As has already been stated in the introductory chapter, stress can have serious implications in mental and physical well-being of any individual.

Major determinants of mind – body reciprocity are: [20-21]

1. Nervous system
2. Endocrine system
3. Immune system

Optimally interaction of these three systems maintains Homeostasis and wellness [20]

The human stress response involves a complex signaling pathway among neurons and somatic cells. Exposure to hostile conditions initiates the secretion of several hormones, including Corticosterone/Cortisol, Catecholamines, Prolactin, Oxytocin, and Renin, as part of the survival mechanism. The hormones released in response to stressors often are referred to as "stress hormones" and their secretion is regulated by neural circuits impinging on hypothalamic neurons. [1-3]

Vital components of the stress system are the hypothalamic-pituitary-adrenal (HPA) axis which are, in turn, mediated by the hippocampus and the autonomic nervous system (ANS), which interact with other vital centers in the central nervous system (CNS) and tissues/organs in the periphery as well as the locus Coeruleus (LC) and other catecholaminergic, norepinephrine (NE)synthesizing cell groups of the medulla and pons (central sympathetic nervous system) to create a successful stress response against stressor(s). Stress stimulates the release of corticotropin-releasing hormone (CRH) from the hypothalamic Paraventricular nucleus (PVN), into the hypophysial-portal circulation, where it induces the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary and glucocorticoids from the adrenal glands. [1-4, 20-21]

The present study was conducted to study the effects of perceived stress on prolactin levels in newly diagnosed hypothyroid and sub clinical hypothyroid individuals. Significant positive correlation was observed in both groups in respect of PSS and PRL levels.

A link between the prolactin response to a serotonergic challenge and affective symptoms, such as depression and guilt, has been reported in one study. [22] In a study examining 439 patients, 118 of whom were diagnosed as having psychosis, Pompili et al. [23] found slightly reduced prolactin levels in those patients who had attempted suicide.

Riecher-Rössler et al. [24] suggested that stress leads to an increased level of prolactin, which triggers dopamine release by a feedback mechanism; this increase in dopamine transmission may mediate the link between stress and psychosis.

The aim a study by Montalvo I et al was to explore whether high plasma prolactin levels are associated with poorer cognitive functioning in subjects with early psychoses. They studied 107 participants: 29 healthy subjects and 78 subjects with an early psychosis (55 psychotic disorders with <3 years of illness, 23 high-risk subjects). Cognitive assessment was performed with the MATRICS Cognitive Consensus Cognitive Battery, and prolactin levels were determined as well as total cortisol levels in plasma. Psychopathological status was assessed and the use of psychopharmacological treatments (antipsychotics, antidepressants, benzodiazepines) recorded. Prolactin levels were negatively associated with cognitive performance in processing speed, in patients with a psychotic disorder and high-risk subjects. In the latter group, increased prolactin levels were also associated with impaired reasoning and problem solving and poorer general cognition. The results suggested that increased prolactin levels are associated with impaired processing speed in early psychosis. The researchers suggested that strategies targeting reduction of prolactin levels may improve cognition in this population. [25]

Relations between prolactin, emotions and feelings are circular, i.e., prolactin affects the brain and mood but, on the other hand, personality traits and environmental factors may stimulate the secretion of prolactin and may play a role in the genesis of the disease. [4]

Observations in adults under conditions of real life stress suggest that higher prolactin levels are associated with passive coping strategies. A major difficulty with the interpretation of the studies of

prolactin responses to psychological stress and of its adaptive role, or perversions thereof, is the absence of a theoretical frame of reference capable of serving as an attractor for testable hypotheses and observations from different quarters. Although cortisol is the most commonly observed hormonal response to "psychological stress" prolactin is also, sometimes, stimulated. Besides, cortisol and prolactin secretion have been described to behave as alternative, not complementary, responses to psychological stress in humans both in acute conditions and in some chronic states. [4] So in the present study prolactin levels of subjects were measured and its correlation with perceived was studied.

Pelúcio L et al in 2016 [26] reviewed scientific articles that investigated psychiatric comorbidities and quality of life in patients with hypothyroidism. A search was conducted in three databases; ISI Web of Science, Pubmed and PsycInfo and 1333 references were found. 27 articles were chosen for this review. Thyroid disease, be it an excess or a deficiency of thyroid hormones, may produce psychiatric symptoms. The results of 14 studies selected for this review positively proved the association of thyroid disease with psychiatric disorders affecting the quality of life. Anxiety and depression disorders were highly prevalent in the population with hypothyroidism. This is the reason we had selected hypothyroid subjects in our study.

In a study conducted by Goel P et al in 2015 [27] in Meerut, India, consecutive patients presenting for various thyroid related problems were segregated into two groups subclinical and overt hypothyroidism. Newly diagnosed 75 patients in each group were enrolled. Similar number of age and sex matched controls were selected. All subjects filled a predesigned questionnaire for the evaluation of hypothyroid symptoms. Thyroid profile for T3, T4 (total and free), TSH and prolactin were determined in all the subjects and analyzed. Prolactin elevation was found in 16 patients (21.33 %) with overt hypothyroidism, and in six patients (8%) with subclinical hypothyroidism. The control group and subclinical hypothyroid patients exhibited no significant difference in terms of total and free T3, total and free T4. For TSH and prolactin on the other hand, a statistically significant elevation was found in patients with overt hypothyroidism when compared with subclinical hypothyroidism; and in patients with subclinical hypothyroidism when compared to the controls. Similar results have also been observed in the present study.

Health programmes directed towards women have typically had a narrow focus on reproductive health and fertility control, especially in developing countries. The preoccupations of health planners, aid agencies and researchers are not necessarily shared by the women towards whom these programmes are directed. [28] There is considerable evidence of stress due to dual role of women at home and work place which is inherent in health care system and negatively impacts their quality of life. So the mental aspect of health problems needs to be addressed in health programmes for women.

The present study demonstrated a significant positive correlation between hypothyroidism, hyperprolactinemia and perceived stress in females of reproductive age group. Though this was a cross sectional study it may contribute a valuable management strategy in hypothyroid individuals having hyperprolactinemia with inclusion of stress management programmes as a part and parcel of treatment.

CONCLUSIONS

Hypothyroid female individuals may have hyperprolactinemia and perceived stress may be a significant contributor to this problem.

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