

## Study of oxidative stress and immune variation in the patients of hypothyroidism on the basis of hormonal changes

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### Abstract

Hypothyroidism is most common autoimmune disorders generally caused by an attack on the thyroid gland it results in inflammation and damage of the thyroid cells, linked to hormonal, human immune system under pathophysiology and psychological stress. The study focuses on the level of thyroid and stress hormone and its impact on the immune system, and oxidative stress. Two hundred human subjects were randomly selected. Hormonal analysis was conducted of both thyroid and stress hormones and level of cytokines, both pro and anti-inflammatory and oxidative stress was performed using serum samples. Statistical analysis was performed of all the parameters. The study depicts significant increase in TSH and decrease in T3 and T4 hormones. Also rise in stress hormones was observed. The rise in the hormones resulted in significant increase in the level of pro and decrease in anti-inflammatory cytokines. Increase in oxidative stress have lead to increase in normal process of the body, the production of free radicals, the reactive oxygen species (ROS), GSH, GPx, LPx which naturally created stress in the body. The level of superoxide dismutase (SOD), repaired cells and reduced the damage done by superoxide. It also acts as anti-inflammatory, neutralizing the free radicals maintaining the levels of hormones which proved to be same in hypothyroidism when compared with the normal groups. Apart from the physical and emotional point of view, our study focused on the immunological changes in the body in correlation with other metabolic processes in patients of hypothyroidism caused due to stress.

**Keywords:** autoimmunity, cytokines, Hashimoto's thyroiditis, oxidative stress, psychological-physiological stress

### Introduction

All living organisms maintain a complex dynamic equilibrium, or homeostasis, which is constantly challenged by internal or external adverse effects. Stress is defined as a state in which homeostasis is actually threatened or perceived to be so <sup>[1]</sup>. Homeostasis is reestablished by a complex repertoire of behavioral and physiological adaptive responses of the organism <sup>[2]</sup>.

Stress may cause immunodepression but may also exert an immunoenhancing effect on cell numbers same stressor may have a positive effect rather than a negative one, depending on its duration or intensity <sup>[3]</sup>. According to investigations, stress has been classified as acute laboratory stress and natural stress. Stressor induced neurosensory signals are processed in the paraventricular nucleus (PVN) of the hypothalamus. In response to stressors, hypothalamus secretes corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP). CRH-containing neurons have different pathways and projections to noradrenergic centers in the brain stem and spinal cord <sup>[4]</sup>. CRH activates hypothalamic pituitary axis (HPA), leading to release of peptides from the pituitary and adrenocorticotrophic hormone, enkephalins, and endorphins. Adrenocorticotrophic hormone induces release of glucocorticoids from the adrenal cortex and CRH and central nervous system (CNS) together stimulate noradrenergic neurons resulting in secretion of norepinephrine (NE) by peripheral sympathetic nervous system (SNS) and release of epinephrine (EPI) from the adrenal medulla. The activation of these two neurochemical

pathways and release of hormones and transmitters have profound effects of immune function <sup>[5]</sup>.

Stress hormones influence numerous physiologic processes; they regulate inflammatory diseases, their effects maintaining balance between cell-mediate and humoral immunity and on neurogenic inflammation in peripheral tissues. The hypothalamic-pituitary-adrenal (HPA) axis and SNS represent the peripheral stress system, its activation occurs in CNS in response to distinct blood-borne, neurosensory signals <sup>[6, 7]</sup>. Homeostasis within the immune system is largely dependent on cytokines, the chemical messengers between immune cells, which play crucial roles in mediating inflammatory and immune responses, for instance, immune challenges such as blood-borne stressor of infections with bacteria release bacterial lipopolysaccharides (LPS), which induce the nuclear factor (NF) mediated secretion of IL-1 and IL-6 and activates HPA axis and stimulates the hypothalamic stress response <sup>[8]</sup>. The HPA axis regulates a wide variety of immune functions affecting cell trafficking, migration, maturation and differentiation; this regulation is the result of several neuroendocrine pathways including hormones.

Thyroid dysfunction is an important cause of depression <sup>[9]</sup>. Hypothyroidism is considered a potentially reversible cause of depression, and both disorders have symptoms that may complicate studies attempting to clarify the relationship between them <sup>[10, 11]</sup>. The changes occur due to hormone involving both stress and thyroid hormones, among these cortisol, prolactin, thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4) are the major <sup>[12]</sup>.

The normal process of the body is the production of free radicals, the reactive oxygen species (ROS) and reactive nitrogen species (RNS) which naturally creates stress in the body, different enzymes act as antioxidant which helps in maintaining the oxidative stress [13]. The level of superoxide dismutase (SOD), one of the antioxidant, repairs cells and reduces the damage done by superoxide. It also acts as anti-inflammatory, neutralizing the free radicals maintaining the levels of hormones which proved to be same in hypothyroidism when compared with the normal groups.

Cytokines encompasses all the immunomodulating agents that trigger inflammation and respond to infections. It includes two classes pro-inflammatory and anti-inflammatory. Stress hormones changes stress system activity through modulating pro or anti-inflammatory cytokines, TNF- $\alpha$ , IFN $\gamma$ , IL-2, IL-6, IL-4 and IL-10, balance by stimulating or suppressing the progression of this autoimmune disease [14].

Present study emphasizes on the effect caused due to stress, oxidative stress on an autoimmune hypothyroidism and changes in the immune system by the stressors, also study will be a stepping stone in understanding the thyroid–depression interaction, people suffering from hypothyroidism and the one with psychological disorder.

## Materials and methods

### Study Participants

200 samples, 100 as patients and 100 as normal healthy control, age ranging from 20-60 years from different outdoor patients (OPD) of hospitals of Bhopal. Clinical examination included height and body weight measurements, and body mass index (BMI). Blood pressure, medical histories, bleeding and smoking habits, were recorded, heart disease, diabetes, stroke or other neurological disorders or depression; significant medication use beta-blockers, inhaled beta agonists, hormonal contraceptives, corticosteroid use within prior three months, psychotropic medication use within prior eight weeks; psychiatric hospitalization within past year; was confirmed at the beginning of the study.

### Blood collection and sample preparation

After the Institutional Ethical committee (IEC) clearance, 10ml blood was withdrawn in serum separation vials from selected subjects after overnight fasting with dry disposable syringe and needle by venous puncture under aseptic conditions. Serum was separated after 30 minutes by centrifuging at 3000 rpm for 10 minutes; this sample was then used for all the assays.

### Hormonal Analysis

All the tests were performed using commercially available enzyme immunoassay kits (from Krishgen Biosystems, Mumbai, India). The level of the hormones in serum sample of the subjects was determined by ELISA.

### Thyroid hormone analysis

The specific thyroid hormone (TSH, T3, and T4) enzyme linked immunosorbent assay (ELISA) applies quantitative sandwich immunoassay. The microtiter plate was pre-coated with a monoclonal antibody specific for the hormone. Standards, samples and control (25 $\mu$ L) were added to the microtiter plate wells and the hormone if present binds to the

antibody pre-coated wells. In order to quantitatively determine the amount of hormone present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for the hormone was added (100  $\mu$ L) to each well to sandwich the hormone immobilized on the plate. The microtiter plate was incubated (60 minutes), and then the wells were thoroughly washed by working washing solution 5 times (300  $\mu$ L) to remove all unbound components. TMB (3, 3', 5, 5' tetramethylbenzidine) substrate solution was then added (100  $\mu$ L) to each well. The enzyme (HRP) and substrate were allowed to react for a short incubation period in dark (20 minutes). Only those wells that contain the specific hormone and enzyme-conjugated antibody for it exhibit a change in color. The enzyme substrate reaction is terminated by the addition of the stopping reagent (150  $\mu$ L) (1 N sulphuric acid solution) and the color change was measured by the ELISA reader at a wavelength of 450 nm [15, 16].

### Stress hormone analysis

The stress hormones (cortisol, prolactin) immunoassay was performed using competitive microplate enzyme immunoassay. Plate coated with anti-cortisol antibodies was used. Serum reference, patient specimens and control (25  $\mu$ L) was first added to the microplate well. Enzyme- conjugate (100 $\mu$ L) was added. The conjugate binds with antibody coated microplate to form an antigen-antibody complex. Unbound conjugate was removed by working washing solution 5 times (300  $\mu$ l each time). The enzyme activity in the antibody-bound fraction is inversely proportional to the native stress hormone concentration. The enzyme activity was revealed by a color change in TMB-Substrate solution (100 $\mu$ L). The plate was incubated for 20-30 minutes at room temperature in the dark. Stop Reagent (150 $\mu$ l) was added into each well at the same timed intervals and absorbance was taken by the ELISA reader at 450nm [17, 18].

### Cytokine Analysis

#### Proinflammatory Cytokines

The procedure is an enzyme-linked immunosorbent assay for quantitative detection of human proinflammatory cytokine (TNF- $\alpha$ , IFN- $\gamma$ ) in cell culture supernatants, human plasma (EDTA, heparin and citrate), serum, cerebrospinal fluid, urine, synovial fluid or other body fluids. Two-fold serial dilution of standards (2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml 125pg/ml, 62.5pg/ml, and 31.3pg/ml) and samples (100  $\mu$ l) was pipette into the wells. The plate was incubated for 2 hours at room temperature then washed with working washing solution 4 times (300 $\mu$ l each time) to remove unbound labeled antibodies. Detection antibody (100  $\mu$ l) was pipette to the wells. The plate was incubated for 2 hours at room temperature and were again washed using same working washing solution following same procedure. Streptavidin-HRP (100  $\mu$ l) was pipette to the wells. The plate was incubated for 30 minutes at room temperature and were again washed using same working washing solution. TMB substrate solution was added (100  $\mu$ l) to the wells, resulting in color development proportional to the amount of specific cytokine bound. The plate was incubated for 15 minutes at room temperature in dark. The stop reagent changes the color from blue to yellow, and the intensity of the color was measured at 450 nm.

### Anti-inflammatory cytokines

Human anti-inflammatory cytokines (IL-4, IL-6, IL-10) ELISA assay employs an antibody specific for human anti-inflammatory cytokine coated on microtiter plate. Two-fold serial dilution of standards (2000 pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml and 31.5pg/ml), samples (50 µl) and biotinylated anti-human specific cytokine (50µl) was pipette into the wells. The plate was incubated for 1 hour 30 minutes at room temperature. Cytokine present in a sample is captured by the antibody immobilized to the wells and by the biotinylated specific detection antibody wells were washed with working washing solution 5 times (300µl each time) to remove unbound labeled antibodies. HRP-conjugated streptavidin (100 µl) was pipetted to the wells. The plate was again incubated for 30 minutes at room temperature and were again washed using same working washing solution following same procedure. Following the second wash step, TMB substrate solution (50 µl) was added to the wells, resulting in color development proportional to the amount of cytokine bound. The plate was incubated for 20 minutes at room temperature in dark. The stop solution (25µl) changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

### Oxidative stress Analysis

#### Reduced glutathione (GSH)

The method is based on the fact that DTNB (5,5- dithio bis (2- nitro benzoic acid) is reduced by sulfhydryl group (SH), present in reduced glutathione to form one mole of 2-nitro-5-mercapto benzoic acid per mole of SH [19]. 0.2 ml of homogenate was taken. 1.8 ml of chilled Distilled Water and 3.0 ml Sulphosalicylic acid was added and vortexed at 2500 rpm for 15 minutes. 0.5 ml of supernatant was taken into a new tube, 1.0 ml of Phosphate buffer and 0.5 ml of DTNB was add and incubated at room temperature for five minutes. Absorbance was taken at 412 nm.

#### Superoxide dismutase (SOD)

Superoxide dismutase catalyses the breakdown of the superoxide free radical (O<sub>2</sub>). This method is based on the formation of NADH-phenazine methosulphate-nitroblue tetrazolium formazan complex. Acetic acid in the concentration used was found to arrest the formazan formation. Colour intensity of the chromogen was measured at 560 nm. To a test tube add 1.2ml of sodium pyrophosphate, 0.3 ml of phenazine methosulphate, 0.3 ml of nitrobluetetrazolium, 0.2 ml of homogenate, 0.8 ml of distilled water and 0.2 ml of NADH solution was added. Control was also kept in which water was added in place of homogenate. Violet coloured was observed and it was

incubated at 37°C for 90 seconds. Reaction was stopped by adding 1 ml of acetic acid. After 10 minutes absorbance was read at 560 nm.

#### Glutathione peroxidase (GPx)

Selenium-containing glutathione peroxidase present in the cytoplasm of cells, catalyzes the oxidation of reduced glutathione (GSH) to oxidized glutathione (GSSG) at the expense of hydrogen peroxide that is converted to water. 0.2 ml of phosphate buffer, 0.2 ml GSH, 0.1 ml distilled water, 0.1 ml of hydrogen peroxide was added in a tube containing 0.3 ml of homogenate. This was incubated at 37°C for 15 minutes. 0.5 ml of TCA was added to terminate the reaction. Above mixture was centrifuged at 2000 rpm for 15 minutes. After centrifugation, 0.5 ml of supernatant was taken into a new tube. 0.7 ml of Na<sub>2</sub>HPO<sub>4</sub> and 0.2 ml of DTNB. Absorbance was taken at 420nm.

#### Lipid peroxide (LPx)

Malonaldehyde (MDA), a decomposition product of lipid hydro peroxides, used as an indicator of oxidative damage to cells and tissues. Thiobarbituric acid test is one of the most frequently test for measuring the production of peroxidation of fatty acids, membranes and food products. 0.4 ml of tissue homogenate was taken and mixed with 0.1 ml of Sodium Dodecyl Sulphate and vortexed on cyclomixer. This was incubated at room temperature for 10 minutes. 0.75 ml of acetic acid and 0.75 ml of thiobarbituric acid was added and kept in a boiling water bath for 60 minutes. After cooling 2.5 ml of butanol and pyridine was added and centrifuged at 2500 rpm for 10minutes at room temperature. Carefully absorbance of pink colour layered was taken at 532 nm against blank.

#### Statistical Analysis

Statistical analysis were carried out by using the statistical packages for GraphPad Prism 6.0 for Windows (GraphPad Software Inc. California, CA, USA). Mean and standard deviation (SD) were calculated for continuous variables. The group size was small t-test was used to assess the differences of the variables. One tailed p values were considered statistically significant at p<0.0001.

### Results

#### Thyroid Hormones

The changes in thyroid hormones of patients are compared with the control. Data is represented in Mean ± SEM (n = 100). Values among thyroid patients are significantly higher in case of TSH (P< 0.0001) and significantly not different in case of T3 (P< 0.1116) and T4 (P< 0.0773) from control (Table 1). The values are illustrated in Fig. 1.

**Table 1:** Comparative values of thyroid hormones of patients with normal healthy controls.

S. No.	Groups	TSH (µIU/ml) Mean ± SEM	T3 (ng/ml) Mean ± SEM	T4 (nmol/l) Mean ± SEM
1.	Control	2.11 ± 0.09	0.83 ± 0.02	83. ± 09
2.	Patients	9.30 ± 0.83	0.97 ± 0.11	91.81 ± 5.85

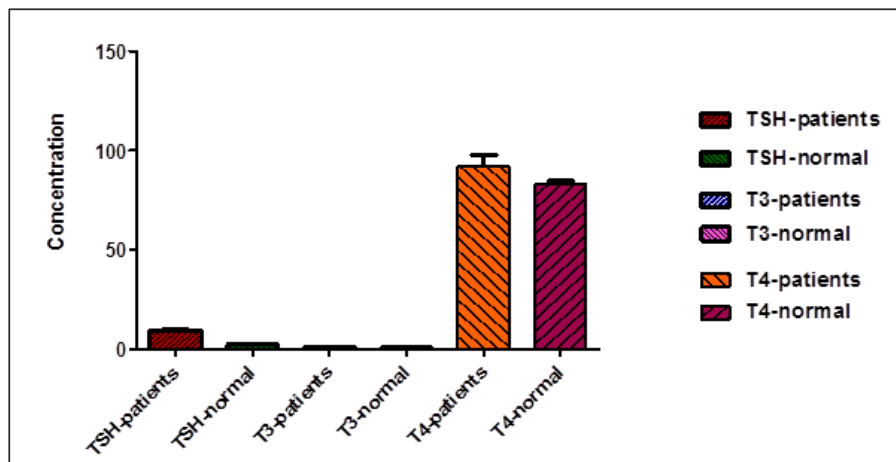


Fig 1: Mean concentration of thyroid hormones

**Stress Hormones**

The changes in stress hormones cortisol among patients as compared with normal healthy control in Table 2. Data is

represented in Mean ± SEM (n = 100) are highly significantly higher (P< 0.0001) from normal healthy control. (Fig. 2. represents the mean ± sem of the hormone).

Table 2: Data representing values of stress hormones

S. No.	Groups	Cortisol (nmol/l) Mean ± SEM	Prolactin(mIU/l) Mean ± SEM
1.	Control	289.30 ± 17.12	115.50 ± 19.69
2.	Patients	471.4 ± 29.07	168.90 ± 10.51

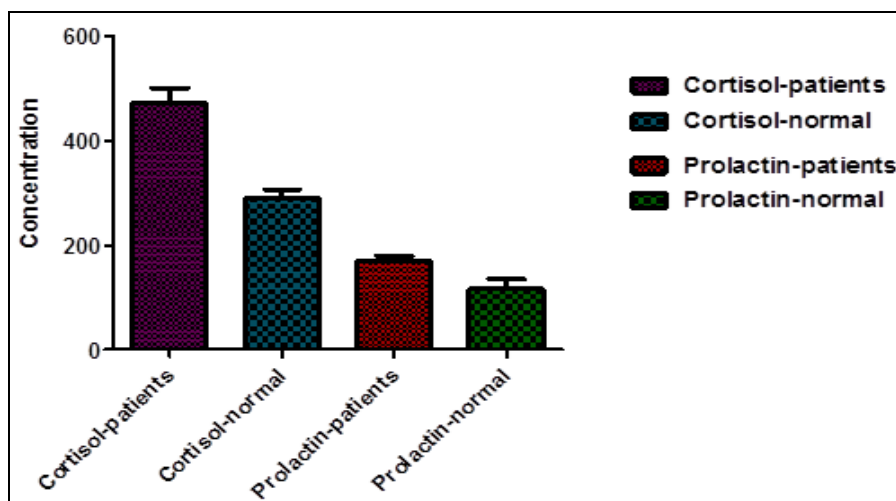


Fig 2: Mean ± sem values of stress hormones.

**Cytokines**

**Anti-Inflammatory Cytokines and Pro-inflammatory Cytokines**

The level of changes in cytokines of thyroid patients as compared with that of the normal healthy control. (Data represented in Mean ± SEM (n = 100)) are highly

significantly different (P< 0.0001) from normal healthy control Table. 3.

It has been also observed that the level of pro-inflammatory cytokines value (TNF-α, IFN-γ) was significantly raised as comparison to anti-inflammatory cytokines (IL-4, IL-6 and IL-10) Fig. 3.

Table 3: Values of anti-inflammatory and pro-inflammatory cytokines

S. No.	Groups	†IL – 4 (pg/ml) Mean ± SEM	†IL – 6 (pg/ml) Mean ± SEM	†IL – 10 (pg/ml) Mean ± SEM	*TNF – α (pg/ml) Mean ± SEM	*IFN – γ (pg/ml) Mean ± SEM
1.	Control	545.6 ± 21.64	147.70 ± 8.11	82.03 ± 3.59	553.9 ± 21.75	265.20 ± 10.4
2.	Patients	1006 ± 36.64	73.78 ± 6.56	51.63 ± 5.62	1193.0 ± 32.51	1081.00 ± 33.8

† Anti-inflammatory cytokines, \* Pro-inflammatory cytokines

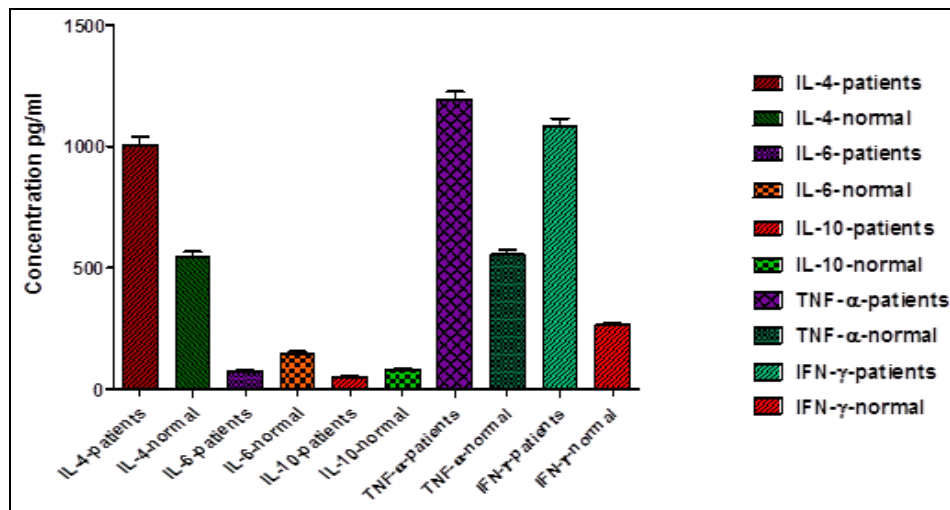


Fig 3: Concentration of anti and pro-inflammatory cytokines

**Oxidative Stress**

Depicts changes in oxidative stress in comparison to control. Data represented in Mean ± SEM (n = 100). Values of

patients are significantly different in all (P< 0.0001) except GPx (P< 0.0040) from control. Table 4. Fig. 4

Table 4: Values of oxidative stress compared between control and patients

S. No.	Groups	GSH (μmol/l) Mean ± SEM	SOD (Units/ml) Mean ± SEM	GPx (mmol/l) Mean ± SEM	LPx (nmol/l) Mean ± SEM
1.	Control	7.89 ± 0.28	174.70 ± 4.35	3.14 ± 0.10	0.89 ± 0.05
2.	Patients	11.23 ± 0.44	258.30 ± 4.04	2.74 ± 0.10	2.78 ± 0.10

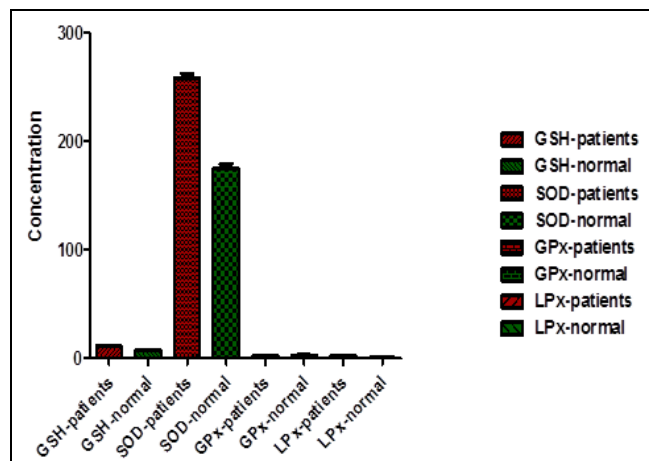


Fig 4: Overall representation of the concentration of oxidative stress

**Discussion**

The elevated basal levels of stress hormones like cortisol and prolactin have shown association with chronic stress suppress immunity by directly affecting cytokine profiles. Cytokines are communicatory molecules produced primarily by immune cells [20]. Proinflammatory cytokines mediate acute inflammatory reactions. Th1 cytokines mediate cellular immunity by stimulating natural killer cells and cytotoxic T cells, immune cells that target intracellular pathogens (e.g., viruses). Finally, Th2 cytokines mediate humoral immunity by stimulating B cells to produce antibody, which “tags” extracellular pathogens (e.g., bacteria) for removal. In a meta-analysis of over 30 years of research, intermediate that stressors in life style could promote a Th2 shift (i.e., an increase in Th2 cytokines relative to Th1 cytokines) [20]. A

Th2 shift has the effect of suppressing cellular immunity in favor of humoral immunity. In response to more chronic stressors (e.g., long-term caregiving for a dementia patient), proinflammatory, Th1, and Th2 cytokines become dysregulated and lead both to suppressed humoral and cellular immunity [21]. Intermediate and chronic stressors are associated with slower wound healing and recovery from surgery, poorer antibody responses to vaccination, and antiviral deficits that are believed to contribute to increased vulnerability to viral infections (e.g., reductions in natural killer cell cytotoxicity [22]).

Immune challenges such as infections with bacteria release bacterial lipopolysaccharides (LPS), which induce the nuclear factor (NF) kB mediated secretion of IL-1 and IL-6, which stimulate the hypothalamic stress response [6, 7, 23]. Immune responses are regulated by antigen presenting cells (APC), such as monocytes/macrophages, dendritic cells, and other phagocytic cells that are components of innate immunity, and by the helper T-lymphocytes subclasses Th1, Th2, and Treg that are components of adaptive immunity. Homeostatis within the immune system is largely dependent on cytokines, the chemical messengers between immune cells, which play crucial roles in mediating inflammatory and immune responses. It has also been found that increase in cytokine like (IFN)-gamma, TNF-alpha lead to cell-mediated immunity; whereas increase in IL-4, TL-10 leads to the cell stimulation enhance humoral immunity [24, 25]. Naïve T cells (Th0) are precursors of Th1 and Th2 cells, and IL-12 (produced by APCs) is the major inducer of Th1 differentiation and hence, cellular immunity. Thus, IFN-gamma inhibit Th2, whereas IL-4 and IL-10 inhibit Th1 cell activities. IL-4 and IL-10 promote humoral immunity by stimulating the growth and activation of mast cells and eosinophils, the differentiation of

B cells into antibody secreting B cells, and immunoglobulin switching to IgE. Importantly, these cytokines also inhibit macrophage activation, T-cell proliferation, and the production of proinflammatory cytokines [26, 27].

We have found the significant increase in level of TSH and decrease status in T3 and T4 hormone. As thyroid hormones makes and stores hormones that help regulate the heart rate, blood pressure, body temperature, and the rate at which food is converted into energy [28]. It achieves this by manufacturing the hormones, thyroxine (T4) and triiodothyronine (T3) and secreting them into the blood stream. Thyroid stimulating hormone (TSH) secreted by the pituitary gland stimulates the release of other thyroid hormones T4 and T3 which further enhances all the cells of the body to metabolize at a faster rate [29]. On a broad extent two types of hypothyroidism has been studied, primary hypothyroidism caused by decreased production of T4 and T3 due to thyroid dysfunction increase production of TSH; by pituitary (TSH) or hypothalamic (TRH) disease [30, 1].

Hypothyroidism is generally caused by an attack on the thyroid gland it results in inflammation and damage of the thyroid cells [31]. We have seen changes due to hormone involving both stress and thyroid hormones, among these prolactin, cortisol, thyroid stimulating hormone (TSH), triiodothyronine (T3), and thyroxine (T4) are the major [32].

Hypothyroidism is routinely considered in the differential diagnosis of depressive and anergic states, and is screened for with determinations of serum thyroxine (T4), triiodothyronine (T3), and basal thyroid-stimulating hormone (TSH) [33]. Thyroid failure with its predilection for behavioral presentation is much more likely to manifest as depression or lack of energy to a psychiatrist.

Cytokines are involved in common endocrine diseases, such as diabetes mellitus and autoimmune thyroid disease (ATD). The hormones mediate the differentiation of Th0 (naïve T Helper cells) towards the Th2 humoral immune response to the detriment of the Th1 cell-mediated response. APC's secrete cytokines that mediate Th1 differentiation, however the presence of bacterial products such as LPS that bind to Toll-like Receptors induce the production of IL-1 and IL-6, which cross the blood-brain barrier and trigger the hypothalamic CRH-stress response [35]. In this manner, a blood borne stressor of infectious nature can activate the HPA axis. Th1 effects are mediated by the cytokines IL-12, IL-18, IL-2 and  $\gamma$ Interferon and T cells and Macrophages. Th2 responses are mediated by IL-4, IL-6, IL-13 and B Cells, Eosinophils and Mast Cells. CRH: Corticotropin releasing Hormone; NE: Norepinephrin; Th0: Naïve Helper cells; APC: Antigen Presenting Cell; LPS: Lipopolysaccharide; HPA: Hypothalamic-Pituitary-Adrenal Axis [36].

Cytokines include two classes of it pro and anti-inflammatory. We have found stress hormone changes stress system activity through modulating pro or anti-inflammatory cytokines. Increase in significant level of pro-inflammatory cytokines of TNF- $\alpha$  and IFN- $\gamma$  and significant decrease in IL-4 and IL-10. Stress hormones changes stress system activity through modulating pro or anti-inflammatory cytokines, TNF- $\alpha$ , IL-6, IFN- $\gamma$ , IL-4 and IL-10, balance by stimulating or suppressing the progression of autoimmune diseases.

Increase in oxidative stress have lead to increase in normal process of the body is the production of free radicals, the

reactive oxygen species (ROS) and reactive nitrogen species (RNS) which naturally creates stress in the body. Different enzymes act as antioxidant which helps in maintaining the oxidative stress [13]. The level of superoxide dismutase (SOD), one of the antioxidant, repairs cells and reduces the damage done by superoxide. It also acts as anti-inflammatory, neutralizing the free radicals maintaining the levels of hormones which proved to be same in hypothyroidism when compared with the normal groups

Studies from humans and animal models have revealed significant new insights into the complex role of cytokines in the pathogenesis of AITD. Modulating cytokine responses have yielded highly encouraging results and they hold considerable promise in the treatment of autoimmune diseases. Pro-inflammatory cytokines such as GM-CSF and IL1b can contribute to Foxp3 + Treg expansion, whereas a regulatory suppressor cytokine such as TGF- $\beta$  can initiate a pathogenic Th17 T cell response. These observations highlight the paradoxical effects of cytokines and their critical roles in maintaining a delicate balance between health and disease. Therefore, additional studies to understand the complex interplay between different cytokines and their effects on the different components of the immune system in the context of a particular disease are essential.

## Conclusion

Present era is full of stress that to of environmental, psychological, physiological and physical stress and all of it compound to produce severe impact. Any of above if dominates leads to affect our body physiology directly. Surprisingly out of the above psychological stress, life style pattern and physiological interaction leads to progressive disturbance in producing disease syndrome.

Study interprets a significant rise in stress hormones like cortisol and prolactin, leading to immune regulation of body homeostasis. We have found that stress hormone after recognizing the immune cells which promote the mother T and B cells and Th cells type Th1 and Th2 are the most affected. The balance of Th1 and Th2 has been disturbed leading to autoimmune process. Increase in cytokines alpha-TNF and gamma interferon attacked on thyroid cells destruction and predominates for Th2 mediated immune response promoting antigen specific B cells. These B cells produce anti-TSH receptor antibodies leading to hypothyroidism.

The study concludes that to regulate the hypothyroidism like autoimmune disorder one has to take proper care of compound stress increasing day by day particularly in immune suppression autoimmune conditions. Supportive and suggestive combination therapy in addition to regular treatment will benefit the patient of hypothyroidism because stress, immunity and infection play a major role not only hypothyroidism but may lead to other autoimmune diseases.

## Conflict of Interest

There are nonfinancial competing interests (political, personal, religious, ideological, academic, intellectual, commercial, or any other) to declare in relation to this manuscript.

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