

**INTERRELATIONSHIP OF VITAMIN B12 AND CORTISOL LEVEL****Dr. Rubeeya Lodhi\* and Shaik Abdul Nadim**

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Pharmacology, Deccan  
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Hyderabad, India.**ABSTRACT**

Stress, both physical and psychological, is attracting increasing attention among neuro researchers. In the last 20 decades, there has been a surge of interest in the research of stress induced manifestations and this approach has resulted in the development of more appropriate animal models for stress associated pathologies and its therapeutic management. These stress models are an easy and convenient method for inducing both psychological and physical stress. To understand the behavioral changes underlying major depression, molecular and cellular studies are required. Dysregulation of the stress system may lead to disturbances in growth and development, and may this may further lead to the development of various other disorders. This article reviews the interrelation of Vitamin B<sub>12</sub>, androgens and cortisol in chronic stress model and their neurobiology, including the different neurotransmitters and heart function affected. There are various

complications associated with stress and their management through various pharmacological and Non-Pharmacological techniques. The use of Vitamin B<sub>12</sub> in the treatment of stress related problems is in practice in both Indian and Western societies, Examination of the hyper-responsiveness of the Hypothalamic-Pituitary-Adrenal axis, consequent elevated serum cortisol, Androgens plus the effects of this upon brain structure and function, provides a model for understanding how chronic stress may be a causal vector in the development of major organ dysfunction like CVS dysfunction.

**KEYWORDS:** Chronic Stress, Vitamin B<sub>12</sub>, Cortisol.**INTRODUCTION**

The body's principal adaptive responses to stress stimuli are mediated by an intricate stress system, which includes the hypothalamic-pituitary-adrenocortical (HPA) axis and the

sympathoadrenal system (SAS). Dysregulation of the system, caused by the cumulative burden of repetitive or chronic environmental stress challenges (allostatic load) contributes to the development of a variety of illnesses including hypertension, atherosclerosis, and the insulin-resistance– dyslipidemia syndrome, as well as certain disorders of immune function. The brain's limbic system, particularly the hippocampus and amygdala, is also intimately involved in the stress response.<sup>[1],[2]</sup>

Chronically elevated corticosteroid levels induced by persisting stress may adversely affect hippocampal structure and function, producing deficits of both memory and cognition. The ability of stress to cause illness in humans is most clearly exemplified by post-traumatic stress disorder (PTSD), which consists of a predictable constellation of distressing behavioral symptoms and physiological features<sup>[3],[4]</sup>

An appreciable proportion of the observed variance in vulnerability to PTSD is attributable to genetic factors.<sup>[5]</sup>

Vitamin B12 has key role in the normal functioning of the brain and nervous system, and for the formation of RBC and blood. Chronic stress leads to decrease the level of vitamin b12 in the body by destructing the parietal cells (which secretes intrinsic factor for vitamin b12 absorption) in the stomach.<sup>[6],[7]</sup>

So, Mal-absorption of vitamin b12 takes place in the absence of intrinsic factor, which leads to vitamin b12 deficiency. This lost in functionality of vitamin B12 can be measured clinically as an increased in the homocysteine level in vitro. In the metabolism of Homocysteine during remethylation process, Hcy is reconverted into Methionine.<sup>[8]</sup>

This reaction is catalyzed by methionine synthase. Methionine synthase uses Vitamin B12 as a co-factor. So. in absence of vitamin b12, the process of Methionine synthesis disturbs and resulted in over accumulation of Homocysteine. Increase the level of homocysteine called as Homocysteinaemia is, which is associated with mostly cardiovascular disease (CVD).<sup>[9]</sup>

HCy increased thrombogenicity, increased oxidative stress, increased inflammatory activation, impaired endothelial function, and finally atherogenesis.<sup>[10]</sup>

Reproductive activity is one of the main functions that becomes altered and inactivates during the adaptive response to stress. chronic exposure to stressors increases HPA (Hypothalamus-

Pituitary Adrenal) axis activity and concomitantly reduces HPG (Hypothalamus-Pituitary-Gonadal) axis activity.<sup>[11]</sup>

This antagonistic relationship between both these axes has been proposed to underlie the inhibition of reproductive function due to stress. The hyperactivity of the hypothalamic-adrenal axis is involved in mediating the effect of stress on the testes and increased glucocorticoid levels are associated with reduction of testosterone biosynthesis by leydig cells which are the primary site of glucocorticoid binding in the testis.<sup>[12],[13]</sup>

### Experimental Method and Material

**Animals:** Adult male Sprague–Dawley rats, weighing 250-350 g were used in the study.

#### Animals were divided into 5 Groups

- 1) Normal control,
- 2) Normal control and vitamin b12,
- 3) Chronic stress
- 4) Chronic stress and vitamin b12,

For all groups of rats were housed per cage in a room with temperature regulated at  $22 \pm 2$  °C, with a 12/12 h light/dark cycle (lights on 07:00 h, lights off 19:00 h). Standard chow pellets and water were given ad libitum, except during the experimental period when food or water deprivation was applied.

**Drugs:** Vitamin B12 (500µg/kg)

**Stress Procedure:** All the rats were acclimatized under laboratory conditions and handled daily for a week prior to the commencement of experiment. Two different stress models were used, acute and chronic unpredictable stress. Acute stress was produced by restraining 12 h fasted rats for 150 min inside a cylindrical steel tube (7 cm diameter, 17.5 cm long, with holes for ventilation). Chronic unpredictable stress procedure includes fasting, tail pinching, restraint, overnight wet cage bedding, isolation, forced swimming, day–night reversal, cold-restraint, water deprivation. Individual stressors and time of exposure during chronic unpredictable stress on every day have been summarized in Table 1.<sup>[17],[18],[19]</sup>

**Schedule:** Animals were randomly divided into various groups as describe above, each group containing 6 rats. The respective groups consist of non-stress, acute stress, chronic

unpredictable stress along with Vitamin b12.

Treatment of Vitamin B12 was given daily by oral route and testosterone enanthate was given twice in a week by intra muscular route for 28 days.

Measurement of parameters After 28 days of stressor schedule various parameters like body weight and physical behaviour were measured. Physical behaviour was measured by: Elevated plus maze model, Open field model, Despair swim test, Sucrose consumption test. Blood was collected by retro-orbital route to check blood parameters like Vitamin B12 level, homocysteine level and cortisol level. Animals were sacrificed then; thoracic aorta was isolated to record the Ach induced relaxation of thoracic aorta precontracted with Phenylneprine.

### Statistical Analysis

The result was expressed as mean  $\pm$  S.E.M. where n represents the number of rats. Statistical differences between two groups were checked by unpaired t-test and among the groups were checked by one way ANOVA followed by Tukey's multiple comparison tests. The statistical comparisons were carried out using the Sigma Plot software, version 11. A value of  $P < 0.05$  level was accepted as significant throughout. Result: After 28th days of stressor schedule following behavior test were perform: 1) Behavior tests A) Elevated Plus maze model.

### DISCUSSION

Chronic stress influences the Sympathoadrenal system and the hypothalamic pituitary adrenocortical (HPA) axis, which are, in turn, mediated by the hippocampus. Stress stimulates the release of corticotropin-releasing factor (CRF), from the hypothalamic paraventricular nucleus (PVN), into the hypophyseal-portal circulation, where it induces the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary and glucocorticoids (cortisol in humans; corticosterone in rodents) from the adrenal glands.

Activation of this axis, results in glucocorticoid release into systemic circulation. Stress and glucocorticoids have specific effects on cognitive functions in humans and in animal models. These hormones trigger physiological "fight-or-flight" mechanisms, which include increases in heart rate, respiration rate, fat and carbohydrate breakdown, and blood pressure. Chronic stress leads to decrease the level of vitamin B12 in the body by destructing the parietal cells (which secretes intrinsic factor for vitamin b12 absorption) in the stomach. So, malabsorption

of vitamin B12 takes place in the absence of intrinsic factor, which leads to vitamin B12 deficiency.

Stress disrupts the circadian rhythmic secretion of cortisol. An effective method to phase-shift circadian rhythm is a combination of bright-light exposure and methylcobalamin. Methylcobalamin is thought to assist bright light in resetting the circadian rhythm by enhancing the light sensitivity of the circadian clock. Methylcobalamin also appears to generate the right quality of sleep activity by both reducing sleep time and improving sleep quality, resulting in feeling refreshed upon waking.

Plasma Homocysteine concentrations accumulate with B12 deficiency thus providing a functional biomarker of vitamin B12 status. Elevated total plasma Homocysteine (tHcy) is an independent risk factor for peripheral vascular, cerebrovascular, and coronary artery disease (CAD).

In general, there is a great increase in serum adrenal steroids for a short period after acute stress. With the prolonged exposure to extreme stress, serum adrenal steroids concentrations become subnormal and appear to affect directly or indirectly the depression of serum reproductive steroids like cortisol and testosterone.

The increased body weight of the rats is especially responsive to the administration of androgens Testosterone enanthate, at a dose of 0.5mg/kg, produced a significant increase in body weight. The analyses of the organs of rat treated with testosterone demonstrated that the hormones definitely stimulated the synthesis of protein independently of each other. These studies are solely based on chronic stress induced depressive behaviour. Results obtained from EPM test clearly indicates that an increase tendency of rodents to enter in the open-arm and their time spent in that arm is an index of anti-anxiety behaviour of rodents. Similarly, the observations of OFT demonstrates the tendency of rodents to avoid brightly illuminated areas, and this avoidance is confused as a symptom of anxiety.

Normal rats generally show increased ambulation and rearing in a novel open field. Whereas, stressed rats display decreased ambulation and rearing in a novel open field. Considering immense validity and reliability of depressive-like states in Learned Helplessness (LH) nature of depression, stressed animals have loss of weight, agitation, decreased locomotors activity, sleep disturbances, decreased libido, reduced learning in some tests but not in spatial learning.

tests, and alterations in the HPA axis, with elevated corticosterone. Anhedonia has been postulated as a central symptom of depression, which can be monitored in animals using sucrose preference tests.

Vitamin B12 and treated group's shows significant response in elevated plus maze model, open field model, despair swim test and sucrose consumption tests. In elevated plus maze model no. of entries increases in open arm by normal control groups and treatment control groups as compare to disease control group. Same expected responses were seen in open field model, sucrose consumption test and despair swim test, also significant decreases were measured in vitamin B12 level and increases the level of homocysteine and cortisol in blood parameters. By doing correlation co-efficient analysis there was good correlation seen between all stress parameters (behaviour parameters) and blood parameters, which justify the title of thesis that there is significant interrelation occur between Vitamin B12, androgens and cortisol in chronic stress and associated vascular dysfunction. By dose response curve it had been noted that vascular relaxation property was reduced in chronic stress condition as compare to normal control and treatment treated groups. Thus, from the above result it can be concluded that there is significant interrelationship present in vitamin B12, androgens and cortisol in chronic stress conditions and associated vascular dysfunction. Elevation in either of these three-blood parameter may lead to potential biomarker parameter for chronic stress condition and related vascular dysfunction.

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