List of Abbreviations

Abbreviations	Terms
ACTH	Adrenocorticotropic hormone
ADH	Antidiuretic hormone
ANOVA	Analysis of variance
AVP	Arginine vasopressin
cAMP	Cyclic adenosine monophosphate
COX	Cyclooxygenase
CRH	Corticotropic releasing hormone
DLC	Differential leukocyte count
EDTA	Ethylene Diamino Tetra Acetic acid
FSH	Follicle stimulating hormone
GH	Growth hormone
GHIH	Growth hormone-inhibitory hormone
GHRH	Growth hormone-releasing hormone
GnRH	Gonadotropine-releasing hormone
Hb.	Hemoglobin
HPA	Hypothalamic-pituitary-adrenal
HPG	Hypothalamic-pituitary-gonadotropic
HPT	Hypothalamic-pituitary-thyroid
HS	Highly stressed
IL	Interleukin
LH	Luteinizing hormone
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume

Abbreviations	Terms
MS	Moderately stressed
MSH	Melanocyte-stimulating hormone
NE	Norepinephrine
NK	Natural killer cell
NS	Non-sressed
PCV	Packed cell volume
PFC	Prefrontal cortex
PGs	Prostoglandins
PIH	Prolactin-inhibitory hormone
PTSD	Post-traumatic stress disorder
PVN	Paraventricular nucleus
RBC	Red blood cell
SEM	Standard error of the mean
T_3	Triiodothyronine
T_4	Thyroxine
TRH	Thyrotropine-releasing hormone
TSH	Thyroid-stimulating hormone
WBC	White blood cell
WHO	World health organization

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Ali

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Chapter One

Introduction and Literature Review

1.1 Introduction

Stress is simply a fact of nature...forces from the outside world affecting the individual. Stress has existed as long as humans have walked on the earth and is the body's way of ensuring survival in every day situations that present new challenges (Panzarino *et al.*, 2007). Scientifically, stress is defined as the response of the body to any threatening demand (Selye, 1974). Stress may cause disruption of homeostasis and stimuli that challenge homeostasis are designated as stressors (Rivier and Rivest, 1991; Van de Kar and Blair, 1999; Dong *et al.,* 2004).

Stressors can be divided into three general categories (Pacak *et al*., 1998; Tillbrook *et al*., 2000; Dong *et al.*, 2004; Majzoub, 2006):

1) Physical (for example, restraint, foot shook, and exercise);

2) Psychological, including anxiety, fear or mental frustration; and

3) Metabolic, including heat exposure, hypoglycemia, and hemorrhage. Stress has been further subdivided based on duration (Dong *et al.,* 2004; Miller and Smith, 2006; Malhotra, 2007): acute (single, intermittent, and time-limited exposures) and chronic (intermittent- and-prolonged or continuous exposures). Prolonged spells of stress may result in post traumatic stress disorder (PTSD) (Chaundhury *et al.,* 2006; and Ramasubramanian *et al*., 2007). Accumulated evidence has demonstrated that stress results in longstanding changes in biological stress response systems that underlie many of the symptoms of PTSD (McEwen, 2000; Vermetten *et al.*, 2002).

During stress an adaptive response originating in the Hypothalamic-pituitary-adrenal (HPA) axis is activated to sustain homeostasis (Moberg, 1987; and Xiao *et al.*, 1999). Stress from a variety of stimuli exerts a profound suppression of the reproductive axis (Chrousos and Gold, 1992; and Tillbrook, 2000).

Stress alters immune system responses and suppresses the digestive system, the reproductive system and growth processes (Hardy *et al.*, 2002; Breen and Karsch, 2004; and Maeda and Tsukamura, 2006).

Iraq has witnessed after 2003 war a very troubled era characterized by large scale killing, bombing and wide spread public fear during daily life. The impacts of these troubles are needed to be studied by various teams or investigators e.g.: sociologist, psychologist and other interested bodies. In the present study, an attempt was made to investigate the effects of psychological stress or PTSD on male young Iraqi students that live in Nahrain University hostel in Al-Jaderriya, who came from different areas of Iraq. Parameters that are important and more or less available now in Iraq were adopted in this study: hematological and hormonal were used. The outcome of this study may open the way for further studies in the same direction.

1.2 Literature Review

1.2.1 Stress concept

1.2.1.1 Historical review and definition of stress

A key to the understanding of the negative aspects of stress is the concept of *milieu intérieur* (the internal environment of the body), which was first advanced in 1878 by the French physiologist, Claude Bernard. In this concept, he described the principles of dynamic equilibrium. In dynamic equilibrium, constancy, a steady state (situation) in the internal bodily environment, is essential to survival. Cannon in 1929 was the first to introduce the term "homeostasis" to describe the "coordinated physiological processes which maintain most of the steady states in the organism." He turned his attention to the sympathetic nervous system as an essential homeostatic system that serves to restore stress-induced disturbed homeostasis and to promote survival of the organism. Cannon also mentioned the issue of specificity of stress responses since he showed, for example, that the specific stabilizing or homeostatic reaction to lack of oxygen is quite different from that with which the body responds to exposure to cold; this, in turn, is virtually the reverse of that required to resist heat . However, Cannon, (1929) never used the term "stress".

Selye in 1936 introduced stress as a medical and scientific idea (Pacak and Palkovits, 2001). The starting point for the elaboration of his stress theory was his report, published as a letter to *Nature* in 1936 (Selye, 1936), describing a pathological triad (**1.**adrenal enlargement, **2.** gastrointestinal ulceration, and **3.** thymicolymphatic involution) elicited by any of a variety of stressors. He defined stress as the nonspecific response (revealed after subtraction of the specific components from the total response) of the body to any demand, emphasizing that the same pathological triad "stress syndrome"

would result from exposure to any stressor. Selye mainly focused on the HPA axis as the key effecter of the stress response. He considered the adrenal cortex "the organ of integration which participates in the normal and pathological physiology of virtually all tissues in the body," by virtue of its endocrine function (Selye, 1950). Indeed, administration of ACTH can elicit all three components of the pathological triad (Selye, 1950). However, Selye did not assert that HPA activation attending stress reflected the organ pathology in the pathological triad. Selye also asserted the converse. Selye also introduced the term "general adaptation syndrome" with its three successive phases: the alarm, resistance, and exhaustion stages. He wrote that during the stages of the "general adaptation syndrome" the intensity of the stress response might vary; however, the neural and endocrine patterns characterizing the stage of "alarm" would be essentially the same as those characterizing the other stages. He and others proposed a large list of diseases of adaptation including hyperfunctional and dysfunctional conditions such as Cushing's disease, hypertension, adrenal tumors, and others. Hypofunctional states included Addison's disease and cancer (Selye, 1974; Chrousos and Gold, 1992; Goldstein, 1995; Chrousos, 1998; Matsuwaki, 2004). Later, in 1974 Selye proposed that most of the stressful stimuli induce two types of responses:

1) A general stress response, which is common to all stressors and involves the release of ACTH and adrenal corticosterone, and 2) individual stress responses mediated by "conditioning factors," such as genetically determined predispositions.

In contrast to Selye, Cannon recognized the importance of psychological as opposed to physical responses during stress (Pacak and Palkovits, 2001). From an evolutionary perspective he questioned whether a stereotyped response pattern could be adaptive, recognizing that a nonspecific

stress response would not have provided an advantage in natural selection and thus, would not have evolved. Others, like Mason, in 1971 properly noted that in response to different stressors, activity of the HPA axis could increase, decrease, or remain unchanged, implying that the presence of a pathological triad may not indicate the occurrence of stress (Munch *et al.*, 1984; and Goldstein, 1995). (Mason, 1971) proposed that elicitation of an emotion such as anxiety or fear constituted the basis for similar neuroendocrine responses to different stressors.

In 1992, Chrousos and Gold defined stress as a state of disharmony or of threatened homeostasis evoking both specific and nonspecific responses.

Goldstein, (1995) Defined stress as a condition where expectations, whether genetically programmed, established by prior learning, or deduced from circumstances, do not match the current or anticipated perceptions of the internal or external environment. This discrepancy between what is observed or sensed and what is expected or programmed elicits patterned, compensatory responses.

In 1998 McEwen incorporated the term allostasis as the process of adaptation of the body upon the exposure to various stressors.

Many current views concerning what stress means and how to define and approach its existence, but none has been widely accepted (Pacak and Palkovits, 2001; Panzarino *et al.*, 2007).

1.2.1.2 Classification of stressful stimuli

Stressors can be divided into four main categories: **1)** physical stressors that have either a negative or, in some situations, a positive psychological component; **2)** psychological stressors that reflect a learned response to previously experienced adverse conditions; **3)** social stressors reflecting disturbed interactions among individuals; and **4)** stressors that challenge cardiovascular and metabolic homeostasis (McCartey, 1989; Pacak *et al.*,1998; Van de Kar and Blair,1999; Pacak and Palkovits, 2001; Majzoub, 2006). Physical stressors include cold, heat, intense radiation, noise, vibration, and many others. Other stressors that may be classified as physical include all poisons. Pain stress may be elicited by many different chemical and physical agents. Psychological stressors profoundly affect emotional processes and may result in behavioral changes such as anxiety, fear, or frustration (Dong *et al*., 2004).

In terms of duration, stressors may be divided into two main categories: **acute** (single, intermittent, and time-limited exposure**, chronic** (intermittent and prolonged exposure *vs.* continuous exposure) stressors (Weninger *et al*., 1999; Pacak and Palkovits, 2001; Miller and Smith, 2006). It should be noted that many stressors differ in their intensity (Vermetten and Bremner, 2001; Panzarino *et al.*, 2007).

1.2.1.3 Stress effects

Animal and human research has taught us much about our internal stress systems. When laboratory animals are exposed to a prolonged stress (usually food deprivation, mild electrical stimulation of the foot, or handling), they develop a stress syndrome (Chrousos, 1995). This syndrome consists of high blood pressure (hypertension), loss of appetite, weight loss, muscle

wasting, gastrointestinal ulcers, loss of reproductive function, suppression of the immune system, and depression (Rivier and Rivest, 1991; Vermetten and Bremner, 2002). Females exposed to chronic stress may present with delayed puberty, anovulatory cycles, and spontaneous abortions, and their infants have increased mortality (Pacak and Palkovits, 2001). In males, chronic stress induces inhibition of testosterone secretion associated with abnormal spermatogenesis and decreased libido (Matsuwaki *et al.*, 2003; Eskiocak *et al.*, 2004; Eskiocak *et al.*, 2006). Psychosocial dwarfism is another example of the effects of chronic emotional stress (Meczekalski and Szymankiewicz, 1999; Majzoub, 2006).

There is now evidence that points to abnormal stress responses as being involved in causing various diseases or conditions. These include anxiety disorders, depression, hypertension, and cardiovascular disease, certain gastrointestinal diseases, some cancers, and even the process of aging itself. Stress also seems to increase the frequency and severity of migraine headaches, episodes of asthma, and fluctuations of blood sugar in diabetics. There is also scientific evidence showing that people experiencing psychological stress are more prone to develop colds and other infections than their less-stressed peers. Overwhelming psychological stress (such as the events of 9-11) can cause both temporary (transient) and long-lasting (chronic) symptoms of a serious psychiatric illness called post traumatic stress disorder (PTSD) (Schweiger *et al.*, 1999; Hardy *et al.*, 2002; Panzarino and Schoenfield, 2007; Panzarino *et al.*, 2007).

1.2.1.4 Post traumatic stress disorder (PTSD)

Post-traumatic stress disorder (PTSD) is an anxiety disorder that some people develop after seeing or living through an event that caused or threatened serious harm or death. Symptoms include flashbacks or bad dreams, emotional numbness, intense guilt or worry, angry outbursts, feeling "on edge," or avoiding thoughts and situations that remind them of the trauma. In PTSD, these symptoms last at least one month (Anonymous, 2007; Ramasubramanian *et al.*, 2007).

Post-traumatic stress disorder (PTSD), as such, has been a part of organized psychiatry for only the past twenty years. The concept of PTSD, however, has been well known for over a hundred years under a variety of different names (Rosch, 2007).

During World War I, PTSD was called shell shock, and during WW II, it was referred to as combat fatigue. After the Vietnam War, it was often mistakenly called the Post-Vietnam Syndrome. Indeed, about 15 % of Vietnam veterans suffered from this disorder. There are medical and surgical events such as cancer diagnosis or open heart surgery have been documented to meet the criteria of PTSD. Such cases of PTSD following several types of traumatic events like experiencing Tsunami, witnessing violence, and perceiving threat (Panzarino and Schoenfield, 2007; George *et al.*, 2007; Patra and Kumar, 2007).

Post-traumatic stress disorder is defined in terms of the trauma itself and the person's response to the trauma. Trauma occurs when a person has experienced, witnessed, or been confronted with a terrible event that is an actual occurrence. Alternatively, the person may have been threatened with a terrible event, perhaps injury (physical or psychological) or death to

themselves or others. Then, the person's response to the event or to the threat involves intense fear, helplessness, and/or horror (Malhotra, 2007; George *et al.*, 2007).

It is important to note, however, that having strong reactions to trauma is normal. What's more, there is a range (spectrum) of expected reactions depending on a person's prior exposure to trauma and even on hereditary (genetic) factors (Gurvits *et al.*, 2000; Vermetten and Bremner, 2002; Panzarino and Schoenfield, 2007).

Studying parts of the brain involved in dealing with fear and stress also helps researchers to better understand possible causes of PTSD. One such brain structure is the amygdala, known for its role in emotion, learning, and memory. The amygdala appears to be active in fear acquisition, or learning to fear an event (such as touching a hot stove), as well as in the early stages of fear extinction, or learning not to fear (Milad and Quirk, 2002).

Storing extinction memories and dampening the original fear response appears to involve the prefrontal cortex (PFC) area of the brain (**figure 1-1**), (Milad and Quirk, 2002) involved in tasks such as decision-making, problem-solving, and judgment. Certain areas of the PFC play slightly different roles. For example, when it deems a source of stress controllable, the medial PFC suppresses the amygdala an alarm center deep in the brain stem and controls the stress response (Amat *et al.*, 2005). The ventromedial PFC helps sustain long-term extinction of fearful memories, and the size of this brain area may affect its ability to do so (Milad *et al.*, 2005).

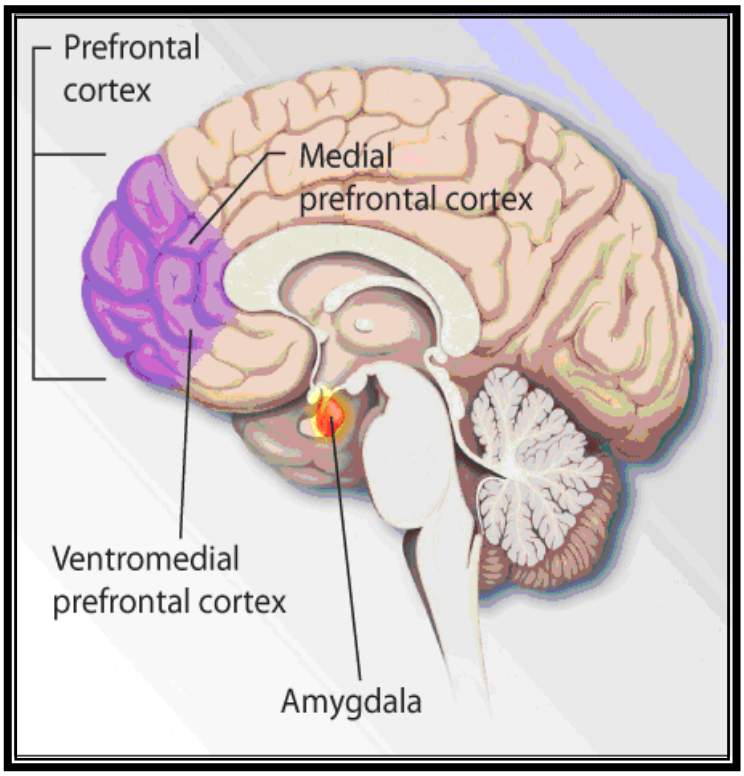

***Figure (1-1) Brain structures involved in dealing with fear and stress*.**

Adapted from (Anonymous, 2007)

1.2.1.5 Coping with stress

In terms of health consequences during exposure to various stressors, the mechanisms of coping with stress and relevant feedback mechanisms are essential for an organism to develop less severe stress-related health consequences and to survive. Coping responses during stress may be defined as cognitive and behavioral responses to manage stress (Weiss, 1971; Delongis and Preece, 2000). There are a number of factors that determine whether an individual will cope effectively with a particular stressor. One of these factors, called the "relevant feedback," is the appropriate feedback from coping responses (Delongis and Preece, 2000). For example, if the relevant feedback to a stressor (unsignaled shock) is low, stress related symptomatology, *e.g.*, gastric ulceration, increases, while if the relevant

feedback is high (signaled shock) less symptomatology is present. Other factors involve appropriate neuroendocrine responses (Dong *et al.*, 2004).

The role of neuroendocrine responses in coping with stress is well recognized, since without these responses an organism would be less likely to survive many stressful situations (Majzoub, 2006). One important feature of successful coping with stress is that physiological systems are not only turned on efficiently by a particular stressor but are also turned off again after a stressor has ceased (McEwen, 1997). Thus, when these systems (*e.g.*, neuroendocrine systems) are not rapidly mobilized and then appropriately reduced, elevated hormone levels become dangerous for an organism, resulting in various stress related diseases (*e.g*., hypertension, stroke, diabetes, obesity, autoimmune and inflammatory disorders, etc.) (Delongis and Preece, 2000). The extent to which an individual can cope with stressful situations varies, and these differences are products of genetics, developmental influences, experience, training, social support, and current mental and physical health (Vermetten and Bremner, 2002).

1.2.1.6 Stress response

Stress response, often referred to as the "fight-or-flight" reaction, is the body's rapid and automatic switch into "high gear." It is easy to imagine how this reaction helps body to deal with a physical threat. You need the energy, speed, concentration and ability either to protect itself or to run as fast as possible (Guyton and Hall, 2000a).

When a subject encounters such a threat, the hypothalamus, at (the base of the brain), sets off an alarm system in the body. Through a combination of nerve and hormonal signals, this system prompts adrenal glands, situated on

the top of the kidneys, to release a surge of hormones, the most abundant being adrenaline and cortisol (Vermetten and Bremner, 2002; and Matsuwaki *et al*., 2004).

Adrenaline increases heart rate, elevates blood pressure and boosts energy supplies. Cortisol, the primary stress hormone, increases sugars (glucose) in the bloodstream, enhances the brain's use of glucose and increases the availability of substances that repair tissues (Anonymous, 2006).

Cortisol also curbs functions that would be nonessential or detrimental in a fight-or-flight situation. It alters immune system responses and suppresses the digestive system, the reproductive system and growth processes (Hardy *et al.,* 2002; Breen and Karsch, 2004; and Maeda Tsukamura, 2006).

The complex alarm system also communicates with regions of the brain that control mood, motivation and fear. Also, many of our modern stressful circumstances, unlike most physical threats, tend to be prolonged. Consequently, you may be running on the fight-or-flight reaction longer than it's intended to operate. What's good for the body in a short-term crisis can be very harmful over long periods (Anonymous, 2006).

The long-term activation of the stress-response system and the subsequent overexposure to cortisol and other stress hormones can disrupt almost all body's processes, increasing the risk of obesity, insomnia, digestive problems, heart disease, depression, memory impairment, immune suppression, physical illnesses and other complications (Guyton and Hall, 2000a).

1.2.2 The Hypothalamus

The hypothalamus, despite its very small size of only a few cubic centimeters, has two way communicating pathways with all levels of the limbic system (Guyton, 1993). In turn, it and its closely allied structures send output signals in three directions (Rivier and Rivest, 1991; Guyton and Hall, 2000a): (**1**) downwards to the brain stem, mainly into the reticular areas of the mesencephalon, pons, and medulla and from these areas into the peripheral nerves of the autonomic nervous system; (**2**) upwards toward many higher areas of the diencephalons and cerebrum, especially to the anterior thalamus and limbic cortex; (**3**) into the hypothalamic infundibulum to control or partially control most of the secretory functions of both the posterior and the anterior pituitary glands.

The hypothalamus, which represents less than 1 percent of the brain mass, is one of the most important of the control pathways of the limbic system. It controls most of the vegetative and endocrine function of the body as well as, many aspects of emotional behavior (Aron, 2004).

1.2.2.1 The Hypothalamus controls Pituitary secretions

Almost all secretion by the pituitary is controlled by either hormonal or nervous signals from the hypothalamus (Chrousos, 1995). Secretion from the posterior pituitary is controlled by nerve signals that originate in the hypothalamus and terminate in the posterior pituitary (Ganong, 1999b). In contrast, secretion by the anterior pituitary is controlled by hormones called *hypothalamic releasing* and *hypothalamic inhibitory hormones* (or *factors*) secreted within the hypothalamus itself and then conducted to the anterior pituitary through minute blood vessels called *hypothalamic-hypophysial portal vessels* (Guyton and Hall, 2000a). In the anterior pituitary, these

releasing and inhibitory hormones act on the glandular cells to control their secretion (Aron, 2004).

The hypothalamus in turn receives signals from many sources in the nervous system (Meczekalski and Szymankiewicz, 1999). Thus, when a person is exposed to pain, a portion of the pain signal is transmitted into the hypothalamus. Likewise, when a person experiences some powerful depressing or exiting thought, a portion of the signal is transmitted into the hypothalamus. Olfactory stimuli denoting pleasant or unpleasant smells transmit strong signal components directly and through the amygdaloid nuclei into the hypothalamus (Tsigos and Chrousos, 2002). Even the concentrations of nutrients, electrolytes, water, and various hormones in the blood excite or inhibit various portions of the hypothalamus. Thus, the hypothalamus is a collecting center for information concerning the internal well-being of the body, and in turn, much of this information is used to control secretions of the many globally important pituitary hormones (Guyton and Hall, 2000a; and Maeda Tsukamura, 2006).

1.2.2.2 Hypothalamic-Hypophysial portal system

The anterior pituitary is a highly vascular gland with extensive capillary sinuses among the glandular cells (Guyton, 1993). Almost all the blood that enters these sinuses passes first through another capillary bed in the lower hypothalamus. The blood then flows through small *hypothalamic-hypophysial portal vessels* into the anterior pituitary sinuses (Guyton and Hall, 2000a). Figure (**1-2**) shows the lowermost portion of the hypothalamus called the *median eminence* that connects inferiorly with the pituitary stalk. Small arteries penetrate into the substance of the median eminence and then additional small vessels return to its surface, coalescing to form hypothalamic-hypophysial portal vessels. These in turn pass downward along

the pituitary stalk to supply blood to the anterior pituitary sinuses (Ganong, 1999b; and Aron, 2004).

Special neurons in the hypothalamus synthesize and secrete the *hypothalamic releasing* and *inhibitory hormones* that control secretion of the anterior pituitary hormones. These neurons originate in various parts of the hypothalamus and send their nerve fibers to the median eminence and *tuber cinereum*, an extension of hypothalamic tissue into the pituitary stalk (Seeley *et al.*, 1996). The endings of these fibers are different from most endings in the central nervous system in that their function is not to transmit signals from one neuron to another but rather to secrete the hypothalamic releasing and inhibitory hormones into the tissue fluids. These hormones are immediately absorbed into the hypothalamic-hypophysial portal system and carried directly to the sinuses of the anterior pituitary gland (Aron, 2004; Maeda and Tsukamura, 2006).

The major hypothalamic releasing and inhibitory hormones are the following (Vander *et al.*, 1998):

1. *Thyrotropin-releasing hormone* (TRH), which causes release of thyroid-stimulating hormone.
2. *Corticotropin-releasing hormone* (CRH), which causes release of adrenocorticotropin (ACTH).
3. *Growth hormone-releasing hormone* (GHRH) which causes release of growth hormone, and *growth hormone inhibitory hormone* (GHIH), also called *Somatostatin*, which inhibits release of growth hormone.
4. *Gonadotropin-releasing hormone* (GnRH), which causes release of the two gonadotropic hormones, Luteinizing hormone and follicle-stimulating hormone.

5. *Prolactin inhibitory hormone* (PIH), which causes inhibition of prolactin secretion.

There are some additional hypothalamic hormones including one that stimulates prolactin secretion and perhaps others that inhibit release of the anterior pituitary hormones (Rivier and Rivest, 1991).

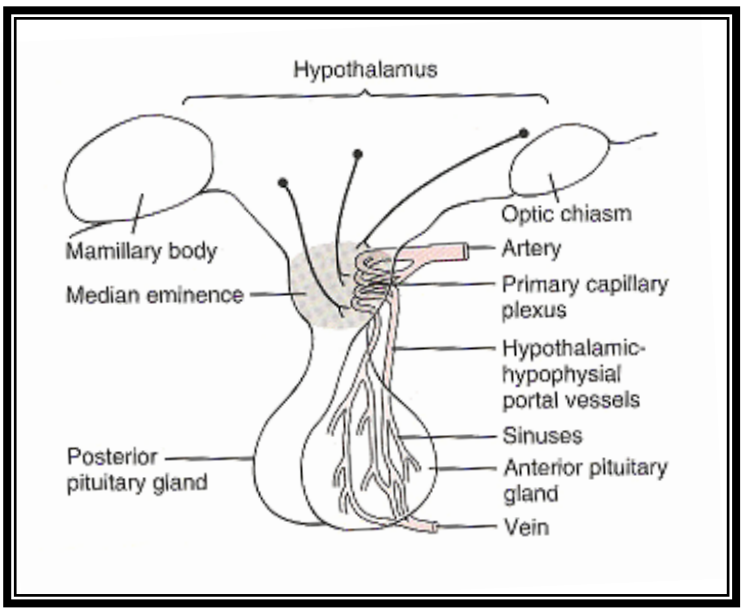

Figure (1-2): Hypothalamic-hypophysial portal system (Guyton and Hall, 2000a)

1.2.3 Pituitary Gland

The pituitary gland also called the *hypophysis* is a small gland that lies in the *sella turcica*, a bony cavity at the base of the brain, and is connected to the hypothalamus by the *pituitary* (or *hypophysial*) stalk, while the other part is called posterior pituitary or *neurohypophysis* (Guyton and Hall, 2000a). Physiologically, the pituitary gland is divisible into two distinct portions: the anterior pituitary, also known as the *adenohypophysis* and the posterior pituitary also known *neurohypophysis* (Seeley *et al*., 1996). The

hypothalamus is an important autonomic nervous system and endocrine control center of the brain (Rivier and Rivest, 1991; Rich and Romero, 2005).

1.2.3.1 Hormones of the Anterior Pituitary

The anterior pituitary is made up of epithelial cells derived from the embryonic oral cavity, six important peptide hormones are secreted by the anterior pituitary include (Guyton and Hall, 2000a; Aron, 2004):-

Growth hormone, Thyroid-stimulating hormone (TSH), Melanocyte-stimulating hormone (MSH), Luteinizing hormone (LH), Follicle- stimulating hormone (FSH) and Prolactin (Seeley *et al.*, 1996)**,** Secretion of the anterior pituitary hormones is largely regulated by hormones produced by the hypothalamus as indicated above.

1.2.3.1.1 Luteinizing Hormone (LH)

LH is a small glycoprotein having molecular weight of about 30,000 (Granner, 1988), LH exerts intracellular actions by stimulating formation of the second messenger cyclic adenosine monophosphate (cAMP) inside the cell membrane. The cAMP in turn causes subsequent intracellular effects of the hormone (Vander *et al.,* 1998). The target tissues of LH hormone are testis in males and ovary in females. It promotes testosterone synthesis and support for sperm cell production in testis, ovulation and progesterone production in the ovary (Seeley *et al.*, 1996). In both males and females, LH is essential for reproduction, in females, at the time of menstruation, FSH initiates follicular growth; also, LH receptors are expressed on the maturing follicle that produces an increasing amount of estradiol, eventually at the time of the maturation of the follicle (Ganong, 1999b). In both sexes, LH stimulates secretion of sex steroids from the gonads. In the testes, LH binds to receptors on Leydig cells, stimulating synthesis and secretion of testosterone (Rivier

and Rivest, 1991)**.** LH stimulates the Leydig cells to secrete testosterone. Some times, it is given the name interstitial stimulating hormone because it controls the production of testosterone by the interstitial cells that are scattered in the spaces between semniferous tubules (Matsuwaki *et al.,* 2003; Dong *et al.,* 2004; Majzoub, 2006).

1.2.3.1.2 Follicle-stimulating Hormone (FSH)

Follicle-stimulating hormone is a small glycoprotein having molecular weight of about 30,000 (Granner, 1988); FSH uses the adenylyl cyclase-cAMP second messenger system (Guyton, 1993). The target tissues of FSH hormone are semniferous tubules in males and follicles in ovary in females. It promotes sperm cell production in testis, follicle maturation and estrogen secretion in ovary (Seeley *et al.*, 1996). Pituitary gonadotropins FSH and LH hormones stimulate the gonads by regulating germ cell proliferation and differentiation. FSH receptors localized to testicular sertoli cells and ovarian granulosa cells and are coupled to activation of the adenylyl cyclase and other signaling pathways **(**Dierich *et al.*, 1998). FSH is also critical for sperm production; it supports the function of Sertoli cells, which in turn support many aspects of sperm cell maturation (Bowen, 2004; and Aron, 2004). FSH stimulates the sertoli cells in the semniferous tubules to facilitate sperm development and to secrete inhibin and activin, which regulates the FSH secretion (Vander *et al.,* 1998).

1.2.3.2 Hormones of the posterior pituitary

Posterior pituitary is an extension of the brain and is made up of nerve cells (Aron, 2004), and its hormones include-

Antidiuretic hormone (ADH) which increase water reabsorption and oxytocin which increase uterine contractions (Seeley *et al*., 1996). Vasopressin and oxytocin are also produced in other areas of the brain and serve in those sites as neurotransmitters or neuromodulators (Guyton and Hall, 2000a).

1.2.4 Thyroid gland

The thyroid gland is made up of two lobes connected by a narrow band called the isthmus**,** the thyroid gland is one of the largest endocrine glands, it is highly vascular, appears more red than surrounding tissues (Seeley *et al*., 1996; Mathur and Shiel, 2007).

1.2.4.1 Thyroid hormones

The main function of the thyroid gland is to secrete thyroid hormones, which regulate the rate of metabolism in the body. Without a normal rate of thyroid hormone secretion, growth and development cannot proceed normally (Vander *et al.*, 1998), Hormones include:

Thyroxin (T4), and Triiodothyronine (T3) (are amine hormones that increase gene transcription in the cell nucleus), and calcitonin (peptide hormone) are the major thyroid hormones (Seeley *et al*., 1996; Ganong, 1999b).

1.2.5 Adrenal gland and hormones

The adrenal glands are two small glands that are located superior to each kidney. Each adrenal gland has adrenal cortex and adrenal medulla. Hormones include Aldosterone, cortisol, and adrenal androgens (Guyton, 1993; Meczecalski and Szymankiewicz, 1999). The adrenal medulla, the central 20 percent of the gland, is functionally related to the sympathetic

nervous system; it secretes the hormones *epinephrine* and *norepinephrine* in response to sympathetic stimulation. In turn, these hormones cause almost the same effects as direct stimulation of the sympathetic nerves in all parts of the body (Rivier and Rivest, 1991; Aron, 2004).

The adrenal cortex secretes an entirely different group of hormones, called *corticosteroids*. These hormones are all synthesized from the steroid cholesterol, and they all have similar chemical formulas (Granner, 1988). However, slight differences in their molecular structures give them several different but very important functions (Guyton and Hall, 2000a).

1.2.6 Testis

The testes secrete several sex hormones, which are collectively called *androgens,* including testosterone, dihydrotestosterone, and androstenidione. In addition to production of sperm cells (Guyton and Hall, 2000a). The main hormone produced is testosterone. It is responsible for the growth and development of the male reproductive structures, muscle enlargement, growth of body hair and increase male sexual drive (Seeley *et al*., 1996). The contractile "myoid cells" surrounding the semniferous tubules serve as a barrier to the penetration of substances into the germinal epithelium (Silverberg and Turner, 2001). The efferent ductules lead from the testes to a tightly coiled series of threadlike tubules that form a comma-shaped structure on the posterior side of the testes called the epididymis, within the epididymis, sperm cells develop the capacity to swim and the ability to bind to the secondary oocyte (Guyton, 1993; Ganong, 1999b).

1.2.6.1 Testosterone

Testosterone is a steroid hormone that can be synthesized either from cholesterol or directly from acetyl coenzyme A, which is the major male hormone secreted by the testes (Granner, 1988), during puberty, testosterone causes the enlargement and differentiation of male genitals and reproductive duct system, is necessary for spermatogenesis, and encourages the development of male secondary sexual characteristics, in addition to its essential paracrine action within the testes on spermatogenesis(Seeley *et al.*, 1996).

Testosterone is necessary for proper sperm production, development and maintenance of male reproductive organs and stimulates development of male secondary sexual characteristics; testosterone has a negative feed-back effect of the hypothalamus and pituitary gland to reduce LH and FSH secretion (Guyton and Hall, 2000a).

Testosterone is essential in males for development of sex drive at puberty, and in the adult male (Ganong, 1999b).

1.2.7 Stress Mechanism

1.2.7.1 HPA - axis

The Para ventricular nucleus (PVN) is the site of the majority of CRH-containing neurons in the hypothalamus and is an important site in effecting cardiovascular and neuroendocrine responses to stress (Rivier and Rivest, 1991; Guyton and Hall, 2000a; Imak *et al.*, 2001; Mayers and Altermatt, 2007). Adrenocorticotropin hormone (ACTH) secretion is primarily controlled by hypothalamic secretion of CRF into pituitary portal blood. However, arginine vasopressin (AVP), which is co-localized in the PVN, can modulate the actions of CRH (Weninger *et al.*, 1999; Vermetten and Bremner,

2002; Maeda and Tsukamura, 2006). Both neuropeptides are capable of potentiating each others' action and create a system in which hypothalamic control of the pituitary-adrenocortical system has a remarkable degree of redundancy (Makara, 1992; Meczecalski and Szymnakiewicz, 1999). Even though a dual ACTH-secreting pathway in anterior pituitary cells has been described, which may be controlled separately by AVP and CRH, corticotrope cells require CRH to maintain their capacity to secrete ACTH (Antoni, 1993). When CRH is released from the hypothalamus, this causes release of ACTH from the anterior pituitary, which stimulates release of cortisol (the major stress hormone) from the adrenals (Schweiger *et al.*, 1999; Tsigos and Chrousos, 2002; Breen and Karsch, 2004). This axis is turned "on" by stress and is regulated negatively by glucocorticoids in a negative feedback loop (as well as regulatory feedback with the noradrenergic system) (Chrousos, 1995; Majzoub, 2006), see **figure (1-3).**

1.2.7.2 HPG - axis

In terms of the hypothalamic-gonadal axis **(figure 1-4)**, exposure of animals and humans to acute stress is associated with a small and often short-lived increase in plasma LH and androgens (Rivier and Rivest, 1991). Acute stress-induced increases in androgen appear to be caused by an alteration in plasma volume and decrease in androgen metabolic clearance, rather than as a result of a true alteration of androgen release. The mechanisms by which LH is increased under various stress conditions are not clear, but one possibility is that high levels of ACTH may stimulate GnRH neurons (Jeong *et al.*, 1999; Pacak and Palkovits, 2001), while others (Breen *et al*, 2004b; Matsuwaki *et al.*, 2006) stated that, stress-suppression of GnRH release is mediated by prostaglandins (PGs) in the brain **(figure 1-5).**

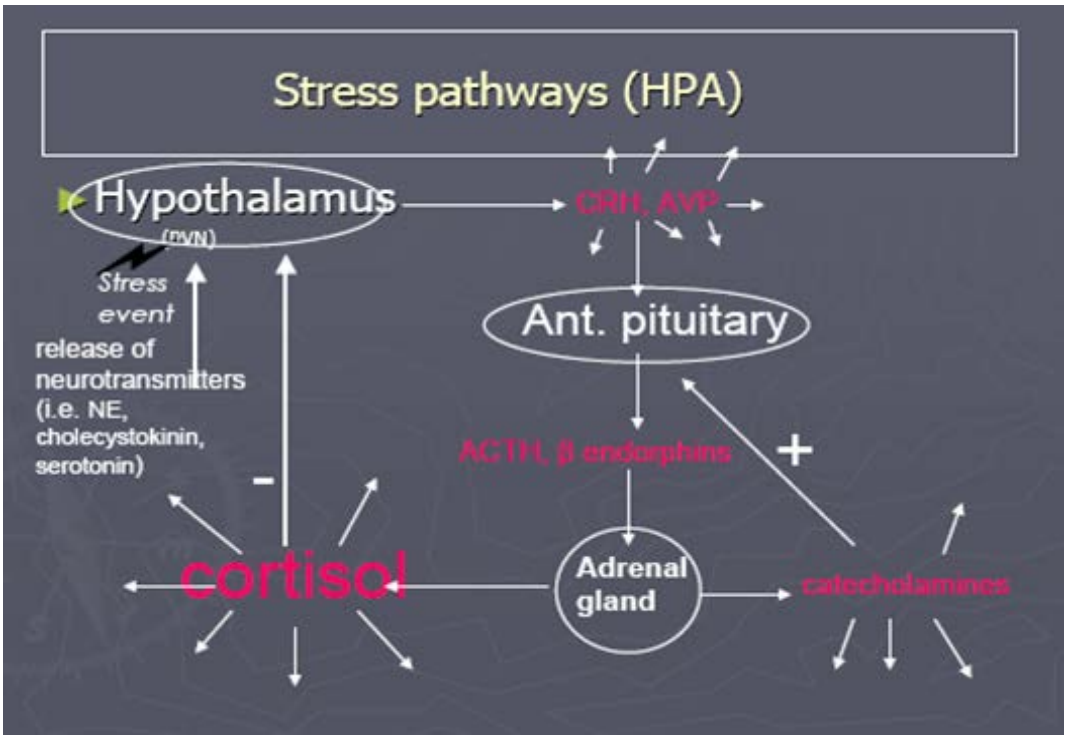

Figure (1-3): Hypothalamic-pituitary-adrenal axis.

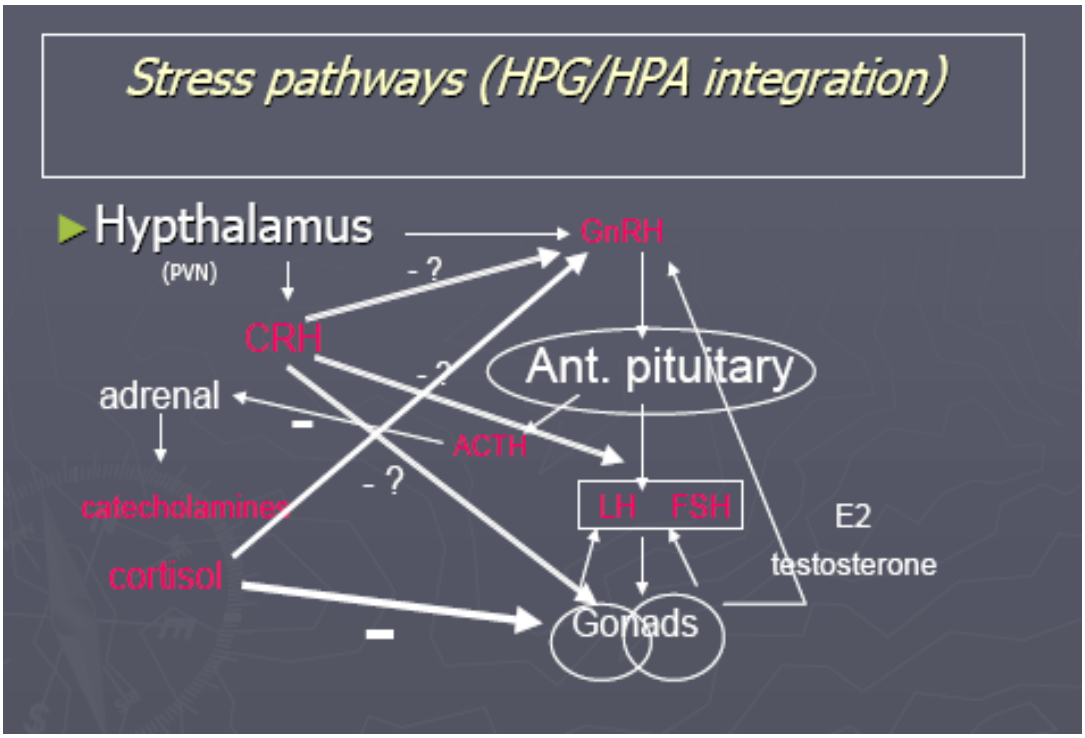

Figure (1-4): Hypothalamic-pituitary-gonadal axis

*Figures (1-3) and (1-4) were taken from (**Mayer and Altermatt, 2007**).

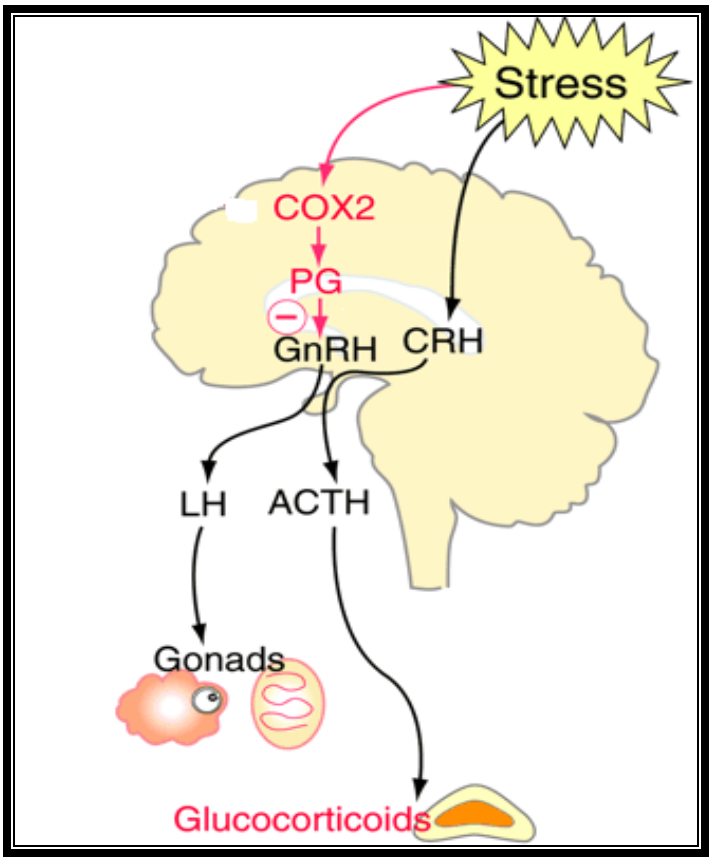

***Figure (1-5): Stress activation of the HPA axis and suppression of the HPG axis* (Maeda and Tsukamura, 2006)**

1.2.7.3 HPT axis

In terms of the hypothalamic-pituitary-thyroid axis, acute (most often) stressors with high intensity induce inhibition of the thyroid axis. This is reflected by the decrease in release/ production of hypothalamic TRH, decreased production/ secretion of thyroid-stimulating hormone, and by inhibition of conversion of T4 to more biological active T3 in peripheral tissues. CRH, somatostatin, and cytokines (*e.g.*, IL-1 and IL-6) most likely contribute to acute stress-induced inhibition of the thyroid axis (Chrousos, 1998; Guyton and Hall, 2000a; Chrousos, 2000; Tsigos and Chrousos, 2002; Mayers and Altermatt, 2007).

1.2.8 Blood components

Normally, 7-8% of human body weight is blood. This essential fluid carries out the critical functions of transporting oxygen and nutrients to cells and getting rid of carbon dioxide and other waste products (Anonymous, 1999a). In addition, it plays a vital role in the immune system and in maintaining a relatively constant body temperature. Blood is a highly specialized tissue composed of many different kinds of components. Four of the most important ones are red cells, white cells, platelets, and plasma. All humans produce these blood components (Sood, 1989).

1.2.8.1 Red cells

Red cells, or erythrocytes, are relatively large microscopic cells without nuclei in the human. In this latter trait, they are similar to the primitive prokaryotic cells of bacteria. Red cells normally make up 40-50% of the total blood volume. They transport oxygen from the lungs to all of the living tissues of the body and carry away carbon dioxide (Haen, 1995). The red cells are produced continuously in bone marrow from stem cells (Pluripotent stem cells) (Theml *et al.*, 2004). Hemoglobin is the gas transporting protein. It is a molecule that makes up 95% of a red cell. Each red cell has about 270,000,000 iron-rich hemoglobin molecules. People who are anemic generally have a deficiency in red cells. The red color of blood is primarily due to oxygenated red cells (Guyton, 1993).

1.2.8.1.1 Morphology and physiology of erythrocytes

Erythrocytes are the most abundant cells in the human body (~5.4 million cells/mm3 blood in a healthy male and ~4.8 million cells/mm^3 blood in a healthy female) (Theml *et al.*, 2004). Erythrocytes are biconcave discs with an average diameter of 7.8 µm, a thickness of 2.5 µm in periphery, 1µm

in the center, and a volume of 85–91 μm3 (Sood, 1989). The flexible, biconcave shape enables erythrocytes to squeeze through narrow capillaries, which may be only 3 μm wide. Mature erythrocytes are quite simple in structure. They lack a nucleus and other organelles. Their plasma membrane encloses hemoglobin, a heme-containing protein that is responsible for O2–CO2 binding inside the erythrocytes (Telen, 1993). Thus, erythrocytes are a highly specialized O2 carrier system in the body (Guyton and Hall, 2000b). Because a nucleus is absent, all the intracellular space is available for O2 transport. Also, because mitochondria are absent and because energy is generated anaerobically in erythrocytes, these cells do not consume any of the oxygen they are carrying (Gothoskar, 2004).

Erythrocytes live only about 120 days because of wear and tear on their plasma membranes as they squeeze through the narrow blood capillaries. Worn-out erythrocytes are removed from circulation and destroyed in the spleen and liver, and the breakdown products are recycled (Ganong, 1999a). The process of erythrocyte formation within the body is known as *erythropoiesis*. In a mature human being, erythrocytes are produced in red bone marrow under the regulation of a hemopoietic hormone called *erythropoietin* (Torotra and Grabowski, 1993).

1.2.8.1.2 Red blood cell indices

a) Definition

Red blood cell indices are measurements that describe the size and oxygen-carrying protein (hemoglobin) content of red blood cells. The indices are used to help in the differential diagnosis of anemia. They are also called red cell absolute values or erythrocyte indices (Nordenson, 2006).

b) Mean corpuscular volume (MCV)

MCV is the index most often used. It measures the average volume of a red blood cell by dividing the hematocrit by the number of RBC. The MCV categorizes red blood cells by size (Haen, 1995). Cells of normal size are called normocytic, smaller cells are microcytic, and larger cells are macrocytic. These size categories are used to classify anemias. Normocytic anemias have normal-sized cells and a normal MCV; microcytic anemias have small cells and a decreased MCV; and macrocytic anemias have large cells and an increased MCV. Under a microscope, stained red blood cells with a high MCV appear larger than cells with a normal or low MCV (Guyton and Hall, 2000b; Nordenson, 2006).

c) Mean corpuscular hemoglobin concentration (MCHC)

The MCHC measures the average concentration of hemoglobin in a red blood cell. This index is calculated by dividing the hemoglobin by the hematocrit. The MCHC categorizes red blood cells according to their concentration of hemoglobin. Cells with a normal concentration of hemoglobin are called normochromic; cells with a lower than normal concentration are called hypochromic. Because there is a physical limit to the amount of hemoglobin that can fit in a cell, there is no hyperchromic category (Sood, 1989; Nordenson, 2006).

Just as MCV relates to the size of the cells, MCHC relates to the color of the cells. Hemoglobin contains iron, which gives blood its characteristic red color. When examined under a microscope, normal red blood cells that contain a normal amount of hemoglobin stain pinkish red with a paler area in the center. These normochromic cells have a normal MCHC. Cells with too

little hemoglobin are lighter in color with a larger pale area in the center. These hypochromic cells have a low MCHC. Anemias are categorized as hypochromic or normochromic according to the MCHC index (Hauser, 2001; Theml *et al.*, 2004; Nordenson, 2006).

d) Mean corpuscular hemoglobin (MCH)

The average weight of hemoglobin in a red blood cell is measured by the MCH. The formula for this index is the sum of the hemoglobin multiplied by 10 and divided by the number of RBC. MCH values usually rise or fall as the MCV is increased or decreased (Ganong, 1999a).

1.2.8.2 White cells

White cells (leukocytes), exist in variable numbers and types but make up a very small part of blood's volume, normally only about 1%. Most are produced in the bone marrow from the same kind of stem cells that produce red cells (Guyton, 1993). Signals include interleukin 1 (IL-1), a molecule secreted by macrophages that contributes to the fever of infections, and histamine, which is released by circulating basophiles and tissue mast cells, and contributes to allergic reactions (Haen, 1995). In response to these signals, the WBCs leave the blood vessel by squeezing through pores in the blood vessel wall. They migrate to the source of the signal and help begin the healing process (Guyton and Hall, 2000b).

1.2.8.2.1 White Cells functions

1. Granulocytes

a) Neutrophils: They are cells with segmented nuclei serve mostly to defend against bacteria. Predominantly outside the vascular system, in "inflamed" tissue, they phagocytose and lyse bacteria. The blood merely transports the granulocytes to their site of action (Ganong, 1999a).

b) Eosinophiles: Its function is to defend against parasites; they have a direct cytotoxic action on parasites and their eggs and larvae. They also play a role in the down-regulation of anaphylactic shock reactions and autoimmune responses, thus controlling the influence of basophilic cells (Sood, 1989).

c) Basophiles: They are equivalents to (tissue mast cells). Its function is to regulate circulation through the release of substances such as histamine, serotonin, and heparin. These tissue hormones increase vascular permeability at the site of various local antigen activity and thus regulate the influx of the other inflammatory cells (Haen, 1995).

2. Agranulocytes

a) Monocytes: These cells help in the defense against bacteria, fungi, viruses, and foreign bodies. Defensive activities take place mostly outside the vessels by phagocytosis. Monocytes also break down endogenous cells (e.g., erythrocytes) at the end of their life cycles, and they are assumed to perform a similar function in defense against tumors. Outside the bloodstream, Monocytes develop into histiocytes; macrophages in the endothelium of the body cavities; epithelioid cells; foreign body macrophages (including Langhans' giant cells); and many other cells (Hauser, 2001; Theml *et al.*, 2004).

b) Lymphocytes: These are divided into two major basic groups according to function. Thymus-dependent T-lymphocytes, which make up about 70% of lymphocytes, provide local defense against antigens from organic and inorganic foreign bodies in the form of delayed-type hypersensitivity, as classically exemplified by the tuberculin reaction. T-lymphocytes are divided into helper cells and suppressor cells. The small group of natural killer cells (NK), which have a direct cytotoxic function, is closely related to the T-cell group (Guyton, 1993; Ganong, 1999a).

1.2.8.3 Platelets

Platelets, or thrombocytes, are structures that help in clotting blood at the site of wounds, by adhering to the walls of blood vessels, thereby plugging the rupture of vascular wall. They also can release coagulating chemicals that cause clots to form in the blood that can plug up narrowed blood vessels (Theml *et al.*, 2004). There are more than a dozen types of blood clotting factors and platelets that need to interact in the blood clotting process (Sood, 1989). Recent research has shown that platelets help fight infections by releasing proteins that kill invading bacteria and some other microorganisms (Gothoscar, 2004). In addition platelets stimulate the immune system. Individual platelets are about 1/3 the size of red cells. They have a lifespan of 9 to 10 days. Like the red and white blood cells, platelets are produced in bone marrow from megakaryocytes stem cells (Ganong, 1999a).

1.2.8.4 Plasma

Plasma is the relatively clear liquid protein and salt solution that carries the red cells, white cells, and platelets. Normally, 55% of our blood's volume is made up of plasma. About 95% of it consists of water. As the heart pumps

blood to cells throughout the body, plasma brings nourishment to them and removes the waste products of metabolism (Guyton, 1993). Plasma also contains blood clotting factors, sugars, lipids, vitamins, minerals, hormones, enzymes, antibodies, and other proteins. It is likely that plasma contains some of every protein produced by the body approximately 500 have been identified in human plasma so far (Haen, 1995).

Chapter Two

Materials and Methods

2.1 Subjects:

Ninety-eight healthy students from Al-Jaderriya compass of Al-Nahrain University belonging to the following colleges: Engineering, Information technology, Science, and Political sciences with an age ranging from 18 to 26 years and body weight ranged between (51-108) kg were included in this study. The participants were asked to complete a questionnaire including Sociodemographic, Medical details (figure2-1). This step was taken to exclude any systemic diseases that may affect the results of the parameters to be studied, particularly; Diabetes, Chronic diseases, and Drug intake, otherwise the subjects were excluded from this study. The study design including a prospective study by direct questionnaire through an interview, and a retrospective study through taking additional informations (Complete Blood Count, Hormonal Assay, and Seminal Fluid Analysis).The study spanned over a period of four months from March to June 2007.

2.2 Study design

Depending on the outcome of the post-traumatic stress disorder (PTSD) evaluation questionnaire form (questionnaire form no.2); the subjects were classified into three groups (Al-Salihi, 2007):

- Group I, included 44 subjects who were considered to be unstressed (NS group).
- Group II, included 27 subjects who were considered to be moderately stressed (MS group).

- Group III, included 27 subjects who were considered to be highly stressed (HS group).

2.3 Chemicals and Reagents:

- Turke's Fluid for total WBCs count (Fluka, Switzerland).
- Sodium citrate solution for total RBCs count (Riedel-Dehaeny, Germany).
- Leishman's stain for differential WBCs count (Crescent Diagnostics, Saudi Arabia).
- Aqueous solution of Eosin for sperm count.

This solution was prepared from the followings (Bearden and Fuquay, 1992):

Material	Volume
Eosin	2 ml
Formalin	5ml
D.Water	93ml

Questionnaire form no. (1) filled by all subjects in the study.

Subject no. ( ) Date:…….. Time:……

General informations

Name:	***Confidential***	Age:	
College and department:		Grade:	
Marital status	Single:	Married:	Divorced:
Residence address:	City:	Village:	Hostel:
Family income:	Poor	Middle	Rich
Physical data:	Weight(kg):	Height(m):	*BMI(kg/m^2):

Life style, Environmental, and Occupational hazards:

Factor	**Y = yes, N = No**
Cigarettes smoking	
Alcohol consumption	
Drug abuse	
Sport	
Exposure to chemicals or radiation	

Past medical and surgical history:

Traumatized testis	Recent febrile illness:		Diabetes Mellitus
Urinary tract infection	Mumps orchitis		Thyroid diseases
Blood pressure:	Inguinal hernia	Varricocele	Others:

Questionnaire form no. (2) for assessment of post-traumatic stress disorder (PTSD) prepared by Adil Al-Salihi (2007).

Are you involved in an event (witnessing or experiencing) that threatens your life, which causes you to be severely scared, failure, and panic?
☐ yes ☐no

Do you manifest with re-experiencing the traumatic event in at least one of the followings?
- Flash back of sad events/or dreams?

☐ yes ☐no
- The behavior or feeling, as if you were re-experiencing the traumatic event?

☐ yes ☐no
- Physical tension / or emotional numbing when you manifest activities reminiscing you with traumatic event?

☐ yes ☐no

Do you avoid what reminds you with traumatic event and feeling with numbing in comparison with the way you feel before in three or more of the followings:
- Avoid ideas, or feelings, or talk about event?

☐ yes ☐no
- Avoidance of activities, or places, or people who reminiscing you?

☐ yes ☐no
- Inability to remember influential part of event?

☐ yes ☐no
- Loss of interest in daily life activities?

☐ yes ☐no
- The mental detachment from other people?

☐ yes ☐ no
- Numbing of emotions?

☐ yes ☐no
- Feeling of restricted progression in your future (e.g. you are not expected to have a job, marriage, children, normal life)?

☐ yes ☐no

Are you upset in two or more of the followings?
- Sleeping problem?

☐ yes ☐no
- Anger outburst or irritability?

☐ yes ☐no
- Loss of concentration?

☐ yes ☐no
- Hyper arousal state?

☐ yes ☐no
- Hyper reflexia state?

☐ yes ☐no

Do these symptoms interfere with your daily life?
☐ yes ☐no

Did these symptoms you were suffering with spanned for at least one month?
☐ yes ☐no

If you suffer from other problems which need treatment, please answer the following questions:
- Do you have a change in sleep pattern or dietary habit?

☐ yes ☐no

Do you feel in most days with:
- Grief or depression?

☐ yes ☐no
- Loss of Interest in life?

☐ yes ☐ no
- Feeling of guilt or usefulness?

☐ yes ☐no

In the last year, Alcohol or drug abuse:
- Lead to your failure in performing responsibilities of work, school, and family?

☐ yes ☐no
- Put you in critical situation, e.g. driving under substance effect.

☐ yes ☐no
- To be arrested??

☐ yes ☐no
- To continue in spite of problems to you and / or your relatives?

☐ yes ☐no

2.4 Apparatuses:

Table (2-1): Manufactures and Origin of Apparatus

Apparatus	Company	Origin
Air Incubator	Memmert	Germany
Hemocytometer	Neubauer improved	Germany
Light Microscope	Olympus	Japan
Microcentrifuge	Hematocrit	United Kingdom
Micropipette	Slamed	Germany
Portable Centrifuge	Hermlxe Labortech Nik	Germany
Refrigerator	Ishtar	Iraq
Sensitive Balance	Mettler	Switzerland
Spectrophotometer	Aurora Instrument Ltd.	United Kingdom

2.5 Evaluation of the Level of stress:

For determining the stress level of the participants, Al-Salihi (2007) questionnaire from for assessment of post traumatic stress disorder (PTSD) (questionnaire from no. 2) was used. The participants were asked to fill the questionnaire form which includes 26 questions. The answers were either yes or no, yes were indicate one score, while no indicate zero score. Total scores ranged from 0 to 26. Zero scores indicate no stress (NS), 1-10 scores indicating moderate stress, and 11- 26 scores indicate high stress (HS).

2.6 Body mass index (Styne, 2004)

The body mass index is calculated from the following formula:

BMI = weight (kg)/ (height (m)) ^{2}.

2.7 Laboratory tests

2.7.1 Blood tests

Venous blood (10ml) was drawn from arm vein using disposable needle and plastic syringe from each subject. Blood samples for endocrine investigation were obtained before masturbation (Hellhammer *et al*., 1985). The samples were divided immediately into two parts; 4 ml of blood were transferred into plastic tubes containing anticoagulating substance (EDTA) for hematological tests. The rest (6ml) were transferred into clean plastic disposable tubes and left at room temperature for 10 minutes for clotting, centrifuged (3000) rpm for 10 minutes, then serum was transferred into clean plastic cuvettes and stored at (-20°C) for hormonal assay (Naito,1987).

2.7.1.1 Total red cell count (Theml *et al*., 2004)

1. Dilution of blood was made by adding 20µl of the sample to 4ml of red cell solution (sodium citrate) in a glass tube.
2. The mixture was rotated for 2 minutes.
3. The counting chamber was filled with a Pasteur pipette or capillary glass tube.
4. The red cell count was made using the following formula (Haen, 1995);

$$\text{RBC/ µl} = \frac{\text{cells counted in 0.2 mm}^2 \times \text{dilution factor}}{\text{Volume}}$$

Where, 0.2 mm^2 is the area of 5 squares

Volume is the depth (0.1 mm) × area counted (0.2 mm^2) = 0.02 mm^3.

$$\text{RBCs/ µl} = \frac{N \times 200}{0.02} = N \times 10000$$

RBC/L = RBC/ µl × 10^6.

2.7.1.2 Total leukocyte count (Theml *et al.*, 2004)

1. Dilution of blood was made by adding 20µl of the sample to 380 µl of leukocyte solution (Turke's fluid) in a glass tube.
2. The mixture was rotated for 2 minutes.
3. The counting chamber was filled with a Pasteur pipette or capillary glass tube.
4. The leukocyte count was made using the following formula :

$$\text{WBC/µl} = \frac{\text{cells counted in 4 squares} \times \text{dilution factor}}{\text{Volume (µl)}}$$

Where, dilution factor = 20; volume = area counted in all 4 corner squares ($4mm^2$) × depth (0.1mm) = $0.4mm^3$.

$$\text{WBC/µl} = \frac{N \times 20}{0.4} = N \times 50.$$

$$\text{WBC/L} = \text{WBC/µl} \times 10^6.$$

2.7.1.3 Differential leukocyte count (DLC) (Haen, 1995)

1. Blood (1-2 drops) were placed in the center of a clean and dried glass slide.
2. The blood was spread on the slide using another slide to obtain a monolayer of blood.
3. The film was stained with Leishman's stain for 5 - 7 minutes.
4. The film was washed with buffered water for 10 minutes, dried, and examined.
5. The DLC consists of identifying and counting the first 100 WBCs encountered.

This gives the percentages of the cells present.

2.7.1.4 Packed cell volume (Sood, 1989)

Packed cell volume (PCV) was obtained from microhematocrit method that includes:

1. The anticoagulated blood is drawn into the capillary action.
2. The dry ends of each capillary tube are sealed with specially manufactured sealing clay.
3. Centrifugation with speed of 10000 rpm for 5 minutes.
4. The microhematocrit result is read with a graphic reading device that allows the hematocrit to be read directly as a percentage of the total.

2.7.1.5 Hemoglobin (Haen, 1995)

1. Add 0.02 ml (=20µl) of blood to 5 ml of Drabkin's solution, mix thoroughly and left to 5 minutes for reaction.
2. Read the absorbancy of spectrophotometer at wavelength of 540-nanometer for the standard and the sample.
3. the hemoglobin(Hb.) was calculated using the following formula:

$$\text{Hb.} = \frac{\text{A sample}}{\text{A standard}} \times \text{n} = \text{g/dl}$$

Where, A: absorbance at 540 n.m.

n: concentration of standard = 14.3g/dl

2.7.1.6 Red blood cells indices (Haen, 1995; Theml *et al*., 2004)

The quality of erythrocytes is characterized by their Mean cell volume (MCV); their mean cell hemoglobin content (MCH), and the mean cellular hemoglobin concentration (MCHC).

MCV in femtoliter is calculated as follows:

$$\text{MCV (fl)} = \frac{\text{PCV (\%)} \times 10}{\text{RBC count } (\times 10^{12})}$$

MCH (in picograms per erythrocyte) is calculated using the following formula:

$$\text{MCH (pg)} = \frac{\text{Hemoglobin (g/dL)} \times 10}{\text{RBC count } (\times 10^{12})}$$

MCHC is determined using the following formula:

$$\text{MCHC (g/dl)} = \frac{\text{Hemoglobin (g/dL)}}{\text{PCV (1/1)}}$$

2.7.1.7 Hormonal assay

Serum concentrations of LH, FSH, Total testosterone, T3, T4 and TSH were determined in the central laboratory of AL-Kadhimiya Teaching hospital using Addendum -mini VIDAS apparatus (VIDAS 12 model, 1992, Biomerieux company, France), through an enzyme linked fluorescent assay (ELFA) technique.

2.7.2 Seminal fluid analysis

2.7.2.1 Sample collection

Every subject was asked to provide semen by masturbation after an abstinence period ranging between 3-5 days and not exceeding 7 days. The ejaculate was collected into a clean, wide mouthed plastic and disposable Petri dish labeled with subject name. Collected samples were immediately placed in an incubator at 37 ◌̊C, waiting for liquefaction (Silverberg and Turner, 2001).

2.7.2.2 Macroscopic evaluation

The following parameters were estimated:

a) Appearance (Rrumbullaka, 2003)

The semen sample was first evaluated by simple inspection. Normal sample has a gray-opalescent and homogenous appearance. The sample may appear clear if the sperm concentration is too low. It may also appear brown when RBCs are present (hematospermia).

b) Odor

The odor is strong and pungent.

c) Liquefaction (Silverberg and Turner, 2001)

Semen coagulum liquefaction is completed within 60 minutes at room temperature and can be accelerated at 37 ◌̊ C which is the procedure employed in the present study.

d) Consistency (Comhaire and Vermeulen, 1995; Anonymous, 2002)

The consistency (viscosity) of the liquefied sample was assessed by estimation how fast the sample runs out of a pipette by gravity and observing the length of the thread formed.

e) Volume (Anonymous, 1999b; WHO, 1999)

The volume was measured by aspirating the whole semen sample into a 15 ml graduated conical tube. A normal semen volume is considered to be ≥ 2ml.

f) pH (Silverberg and Turner, 2001)

The pH of the liquefied sample was assessed using litmus paper, ranging from 6.1 to 10.0; the paper was dipped inside the semen sample, and after 30 seconds, read the pH.

2.7.2.3 Microscopic evaluation

2.7.2.3.1 Wet preparation (WHO, 1999; Anonymous, 2002)

Immediately after liquefaction has completed, a fixed volume of well mixed semen (a drop of 10µl) was put on a clean microscope slide and covered with a 22 × 22 mm cover slip. This gave the preparation a depth of about 20 µm .Examination began as soon as ' the flow ' in the preparation has ceased. If drifting doesn't cease within 60 seconds, the preparation is discarded and a new one is prepared. The preparation was then examined under a magnification of 400×. Several randomly chosen fields were examined and the mean number was taken for the following semen parameters: motility, percentage of normal sperm morphology, agglutination, and round cells.

2.7.2.3.2 Examination of a wet preparation

a) Sperm motility (Anonymous, 2002)

Assessment of sperm motility should begin immediately to avoid artifacts caused by either a temperature decrease or dehydration of the preparation. Sperm motility is determined by counting all motile and immotile sperms in several randomly chosen different fields using light microscope.

Sperm motility was categorized into four groups of motility at 37 ° C (WHO, 1999):

WHO category	**"code"**	**corresponding velocity**
Rapid progressive	A	≥ 25µm/second (≥5 sperm head lengths)
Slow progressive	B	5-24 µm/second
Non progressive	C	<5 µm/second
Immotile	D	_

The four categories were expressed as percentages. The mean number of spermatozoa in each motility group was divided by the mean total number of sperms to obtain percentages for the four groups (A-D)

$$\text{Sperm motility\% for each group} = \frac{\text{The mean number of spermatozoa in each motility group}}{\text{The mean total number of spermatozoa}} \times 100$$

Sperm motility percent for progressive spermatozoa was then calculated by taking the sum of percentages for grades (A+B). The mean numbers of total motile progressive sperm count per ejaculate was calculated using the following formula:

TMS = semen volume (ml) × sperm concentration (million/ml) × percent Progressive motility (A+B)

b) Sperm morphology (WHO, 1999; Silverberg and Turner, 2001)

Using the morphology methods recommended by the 1999 world health organization manual, only sperms with a regular outline (4.0-5.0µm long and 2.5-3.5µm wide of the head) with a pale anterior part (acrosomal body); 40-70 % of the head area) .The length/width ratio of the head should

be 1.5-2. Only one tail should be attached to the base of the sperm head (about 45 µm long), not coiled, nicked or bent over itself, behind the head, the first part of the tail, the midpiece, should be somewhat thicker (maximum width = 1 µm) and about 7-8 µm long.

In this study, the morphology was evaluated using a wet slide preparation method .At least 100 sperms were counted under a magnification of 400 ×.

$$\text{Normal sperm morphology \%} = \frac{\text{The mean number of normal sperms}}{\text{The mean total number of sperms}} \times 100$$

c) Sperm agglutination (Silverberg and Turner, 2001)

Sperm agglutination was determined in several randomly chosen fields, away from the cover slip edges. The percentage of agglutinated sperm was then calculated from the following equation:

$$\text{Sperm agglutination\%} = \frac{\text{The mean number of agglutinated sperms}}{\text{The mean total number of sperms}} \times 100$$

d) Other cells and debris

These findings were assessed in several fields and then the average number was taken. They include:

1. **Round cells (Johanisson *et al.*, 2000; Anonymous, 2002)**

   These cells basically round in shape. They may be white blood cells (WBCs), immature gametes or perhaps large cell bodies (usually without nucleus). Also, cells of prostate origin appear round in the ejaculate. By conventional light microscopy or sperm staining

techniques, it is not possible to reliably differentiate WBCs from immature germ cells in the semen. The round cells were assessed in several fields and the result was reported as an average number multiplied by a factor of 10^6 (one million) to obtain number of round cells/ml.

2. **Epithelial cells (Anonymous, 2002)**

   These cells (squamous, cubic, and transitional) are usual in semen in low numbers. Increased percent is not related to any specific functional impairment or presence of infection.

3. **Red blood cells (Anonymous, 2002)**

   These cells should not be found in semen, although a few can be present without indicating pathology.

4. **Debris (Johanisson *et al.*, 2000)**

   The presence of no debris is a very unusual situation. It includes any thing observed in the semen that can't be identified as sperm, round cells, or other common cellular components. Larger amounts of debris are, however, abnormal.

2.7.2.3.3 Sperm concentration (Bearden and Fuquay, 1992)

Sperm cell concentrations are expressed as number of cells per ml. The Hemocytometer used to count the sperm concentration, with a known depth and area (0.1mm × $1mm^2$ respectively) the number of sperms can be determined for a specific volume. A dilution rate of 1:100 is normal. The diluent used must kill the sperms so that counting can be accomplished. The authors have found that a 2% aqueous solution of eosin accomplishes this and as the additional advantage of staining the sperm head so that they are easier to count the number of sperms within the desired number of squares. When 25

squares are counted, the volume represented is $0.1mm^3$. The number of sperms/ml can be found by using the following formula:

No. sperms/ml = no. sperms in $0.1mm^3 \times 10 \times$ dilution $\times 1000$

The Hemocytometer is a time consuming and tedious method for determining sperm concentration, but provides accurate information (Parthalingam *et al.,* 2006).

2.8 Statistical analysis

Computerized statistical analysis was performed using Minitab program, 2001; Excel program, 2003. The values for each variable were expressed as mean ± standard error of the mean (SE). Analyses of variance (ANOVA) test was used to assess the significance of statistical differences in the mean within each group and between different groups. Probabilities of less than 0.05 were regarded as statistically significant ($p<0.05$), while p values more than 0.05 were regarded as statistically not significant ($p>0.05$).

Chapter Three

Results and Discussion

3.1 Age of subjects

Distribution of the participants in this study according to age is shown in figure (3-1). It was found that 46.4% of subjects belonging to NS group were of age range between 18 to 20 years, 44.4% their age range were between 24 to 26 years, and 44% were of age range between 21 to 23 years. Concerning the MS group, 28.6% of subjects were of age range between 18 to 20 years, 25% were of age range between 21 to 23 years, and 16.6% were of age range between 24 to 26 years. As for HS group, 39% of subjects were of age range between 24 to 26 years, followed by age range between 21 to 23 years, and 25% were of age range between 20 to 24 years.

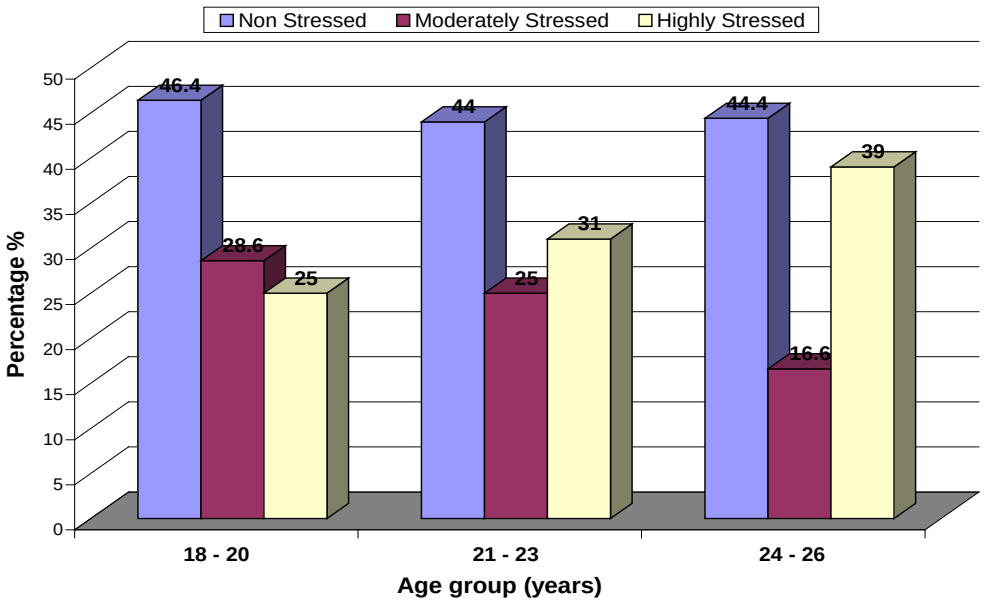

Figure (3-1): Percentages of subjects according to age

3.2 Educational level of subjects

As shown in table (3-1), 29 subjects in NS group were undergraduate students (48.3%), 15 (39.5%) were postgraduates. Concerning the MS group, 12 (31.6%) were postgraduates, 14(23.3%) were undergraduates. With respect to HS group, 11 subjects were postgraduates (28.9%) and 17(28.4) were undergraduates.

Table (3-1): Number (n.) and percentage (%) of subjects according to educational level

Educational level	Group					
	NS		MS		HS	
	n.	%	n.	%	n.	%
Undergraduates	29	48.3	14	23.3	17	28.4
Postgraduates	15	39.5	12	31.6	11	28.9

3.3 Life style of subjects

In this study, it was found that none of the subjects in each group was alcohol drinker or drug abuser, besides; none of them was exposed to chemicals or radiation. Distribution of participants according to life style is shown in figures (3-2) and (3-3).

a) Smoking habit

Results indicated that, total number of smokers and non-smokers in all sample of subjects studied was distributed as follows: non-stressed subjects (NS) had 11.2 % from the total sample were smokers, while MS group had

12.2 % and HS had 17.4 % smokers (these subjects were classified as negative life style).

Concerning non-smokers, it was found that about 33.7 % of total sample were non-smokers and non-stressed, 14.3 % were moderately stressed, and 11.2 % from total subjects were highly stressed (HS).

b) Sport

Results indicated that, total number of sport practitioners and non-practitioners in all sample of subjects studied was distributed as follows: non-stressed group (NS) had 22.4 % of subjects were sport practitioners, while MS group had 10.2 % and HS group had 8.3 % of subjects who were sport practitioners (these subjects were classified as positive life style).

Concerning non-sport practitioners, it was found that 22.4 % of total subjects were non-stressed (NS), 16.3 % were moderately stressed, and 20.4 % of all subjects which were non-sport practitioners were highly stressed.

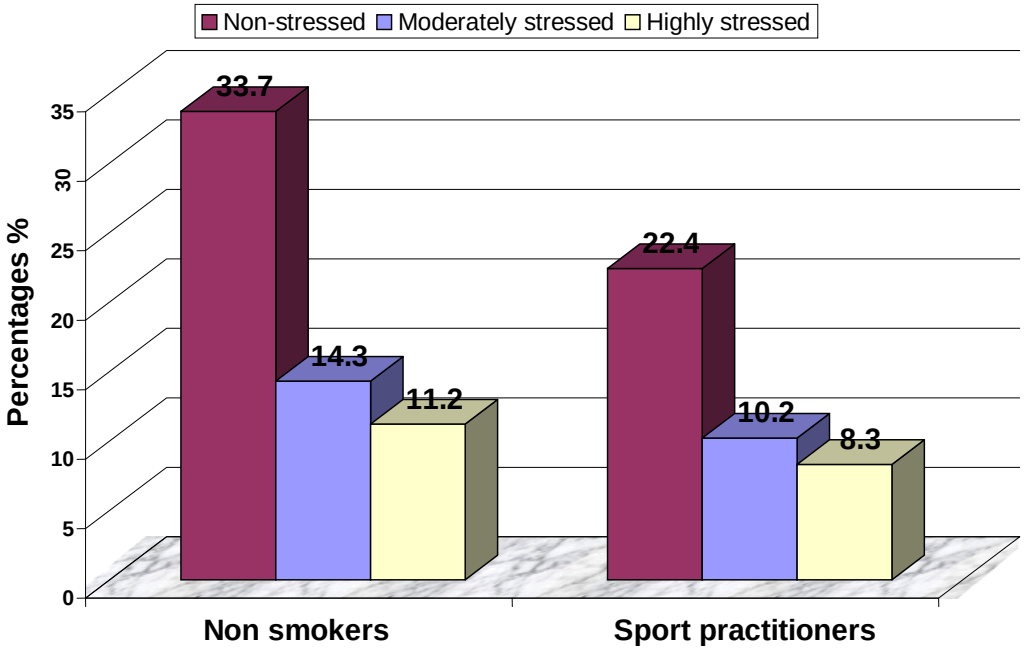

Figure (3-2): Positive life style

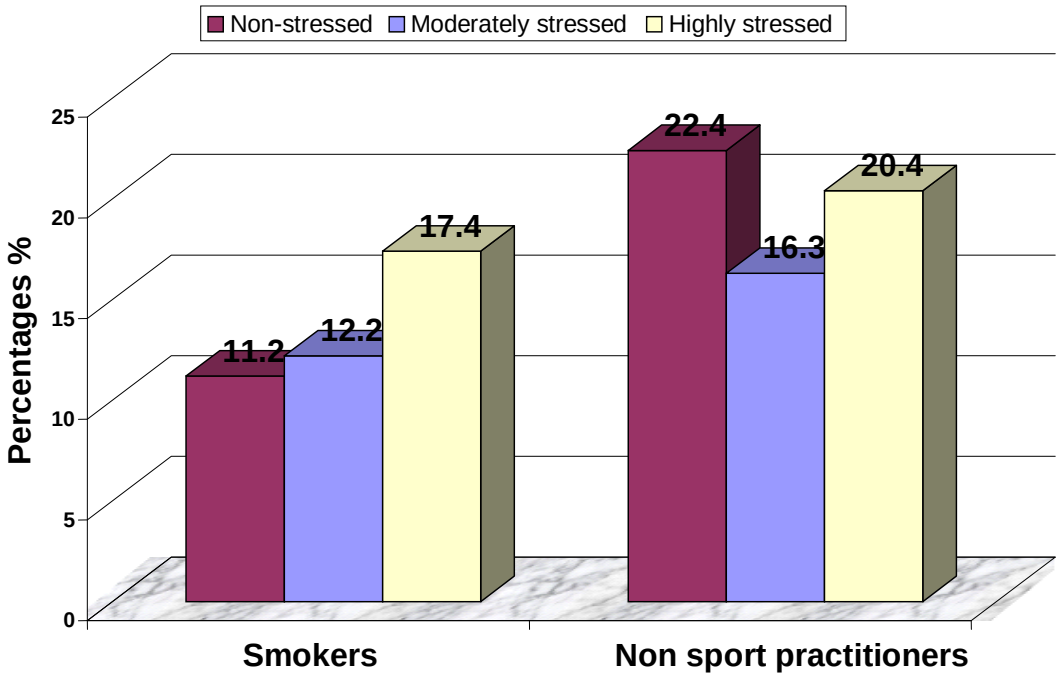

Figure (3-3): Negative life style

3.4 Effect of residency area on subjects

As shown in figure (3-4), 60.3% of subjects were living in relatively safe areas, 26.6% in troubled areas, were non-stressed (NS group). Concerning MS group, it was found that it comprises 37.7% of subjects of troubled areas, and 17% of subjects of relatively safe areas. Subjects living in troubled areas had showed a percentage of 35.7% of highly stressed (HS group) compared to 22.7% of subjects living in relatively safe areas.

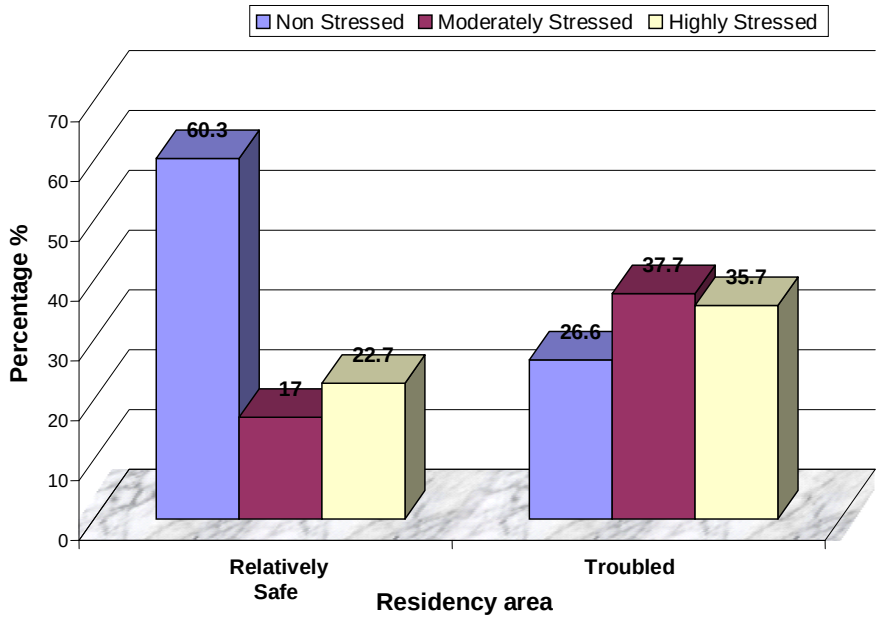

Figure (3-4): Percentages of stress groups according to residency area

3.5 Basic physical data of subjects

As shown in table (3-2), all groups of subjects were more or less closer in: age, body mass index. While other parameters showed a different trend, since there is some non-significant decrease ($p>0.05$) in subject's body weight in MS and HS groups as compared to other groups (figure 3-5). However there was a significant decrease ($p<0.05$) in subjects body height in HS group as compared to NS group (figure 3-6).

It is well known that, environmental stress activates HPA mechanism (Chrousos and Gold, 1992; Guyton and Hall, 2000a). Hypothalamic CRH increases upon stress (Breen *et al.*, 2004a) which plays an important role in inhibiting GH via Somatostatin (Wingfield *et al.*, 1997; and Sapolsky *et al.*, 2000), CRH also inhibits TRH and eventually TSH, thus suppressing growth

and functions of the thyroid gland (Chrousos, 2000; Guyton and Hall, 2000; Tsigos and Chrousos, 2002). CRH was found to inhibit appetite, thus leading to weight loss (Tsigos and Chrousos, 2002; Bale and Vale, 2004).

Table (3-2): Changes in basic physical data of subjects of different groups

Characteristic	NS group(n=44)	MS group(n=27)	HS group(n=27)
Age (yr)	21.68 ± 0.29	21.88 ± 0.39	22.18 ± 0.47
Height (m)	1.76 ± 0.009	1.74 ± 0.013	*****1.73 ± 0.014
Weight(kg)	74.36 ± 1.7	74.15 ± 2.27	68.96 ± 2.12
BMI (kg/m^2)	23.89 ± 0.5	24.41 ± 0.67	23.37 ± 0.56

Values are mean ± SE

n = Number of subjects

***** Significant with the NS group.

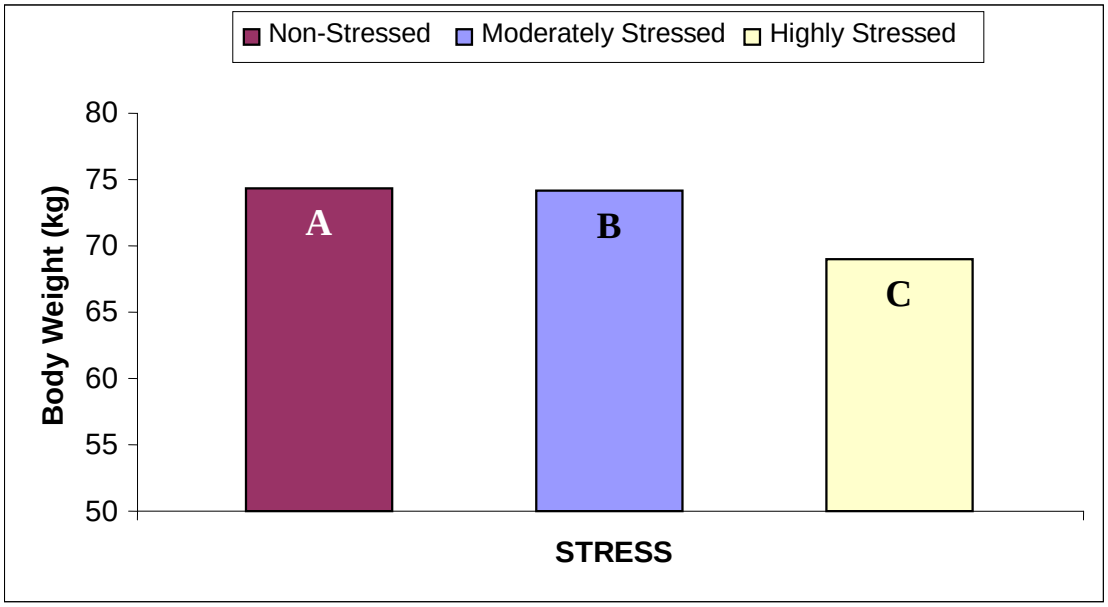

Figure (3-5): Body weight in non-stressed, moderately stressed, and highly stressed subjects

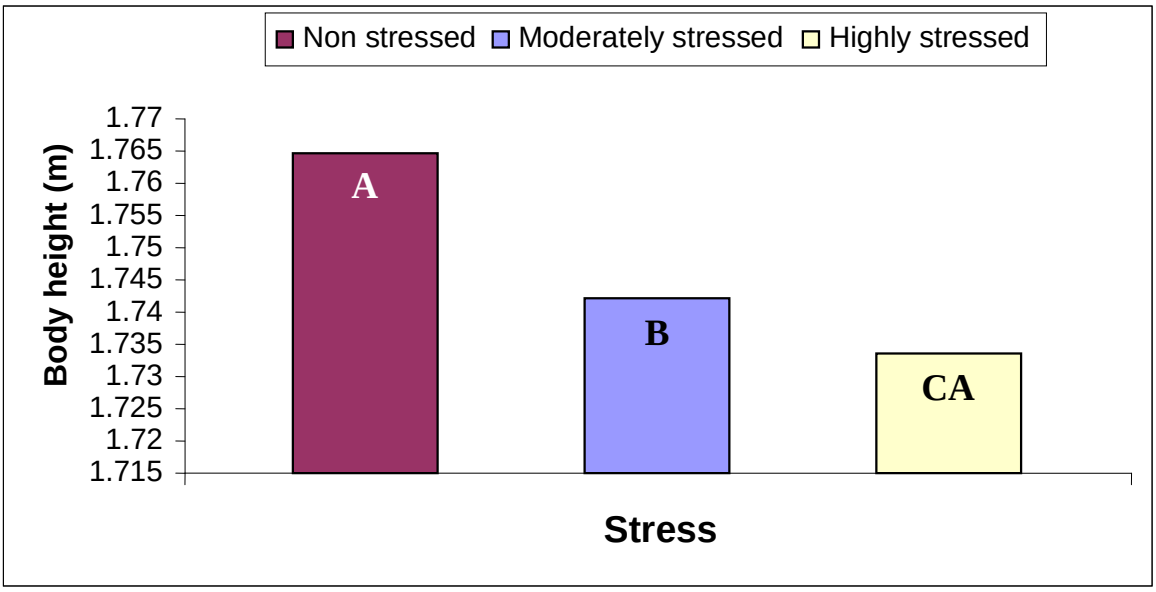

Figure (3-6): Body height in non-stressed, moderately stressed, and highly stressed subjects

* Single letter = non-significant difference ($p>0.05$).

* Two different letters = significant difference ($p<0.05$).

3.6 Changes in different hematological parameters

3.6.1 Changes in RBCs

There were no significant changes in both PCV and hemoglobin although both were nearly lower than the normal known values (figure 3-7). Concerning total RBC count, there was a significant increase in HS group ($p < 0.02$) as compared with NS group (figure 3-8). As for red blood indices, the mean size of RBC (MCV) decreased non-significantly in MS group ($p = 0.09$) and significantly in HS group ($p<0.05$) in comparison to NS group (figure 3-9). No changes in the mean hemoglobin content in a red blood cell (MCH) and mean hemoglobin concentration per unit volume of packed blood cells (MCHC) were obtained (figure 3-9).

It has been shown that chronic stress is associated with hypothalamic releasing of CRH (Steckler and Holster, 1999) and CRH stimulate release of ACTH from the anterior pituitary (Adamec *et al*., 1998; Madrigal *et al*., 2003) and the latter stimulates release of cortisol "the major stress hormone" from the cortex of the adrenal gland (Dallman, 1993; Marti *et al.*, 1994; Dallman and Bhatnagar, 2001). Cortisol is known to increase production of red blood cells and the cause of which is unknown (Guyton and Hall, 2000a). No reports concerning changes in red blood cell indices due to stress has been found in available literatures to compare these results with. The significant decrease in MCV may be indicating of decrease in the cellular size of RBCs, since the total RBC count increased significantly, but PCV did not.

3.6.2 Changes in leukocytes (total and differential)

Total WBC count decreased significantly ($p<0.05$) in both groups (MS and HS) in comparison with the NS group (figure 3-8). However differential WBC count showed: Neutrophiles percentage increased significantly ($p<0.05$)

in HS group when compared to NS and MS groups, lymphocytes, both MS and HS group showed a non-significant ($p = 0.07$) and significant ($p<0.05$) decrease respectively in comparison to NS group, the decrease in lymphocyte percentage may be due to increase in Neutrophiles production (figure 3-10). As for Monocytes, on the other hand showed no significant changes in all groups (figure 3-11). Concerning Eosinophiles (figure 3-11), there was a significant decrease ($p<0.05$) in HS group when compared to NS group. Basophiles showed no significant changes among all groups (figure 3-12).

As pointed out earlier, almost any type of physical or mental stress can lead to strong activation of hypothalamic-pituitary adrenal axis and hence secretion of ACTH and consequently cortisol from adrenal cortex (Mann *et al*., 1985; Demyttenaere *et al*., 1994; Bremner *et al*., 1999; Bremner and Vermetten, 2001; Matsuwaki *et al*., 2004). Cortisol is known to prevent the development of inflammation through different mechanisms, one of which by suppression of immune system through inducing decrease lymphocytes production (Irwin *et al*., 1990; Kiecolt and Glaser, 1991; Reichlin, 1993). Cortisol also decreases the number of Eosinophiles (Eosinopenia) and Lymphocytes (Lymphocytopenia) and raises the Neutrophiles number (Neutrophilia) in the blood (Harbuz and Lightman, 1992; Dallman, 1993; Chrousos, 1995; Mayers and Altermatt, 2007). Guyton and Hall (2000a) stated that Lymphocytopenia or eosinopenia are an important diagnostic criterion for overproduction of cortisol by adrenal gland.

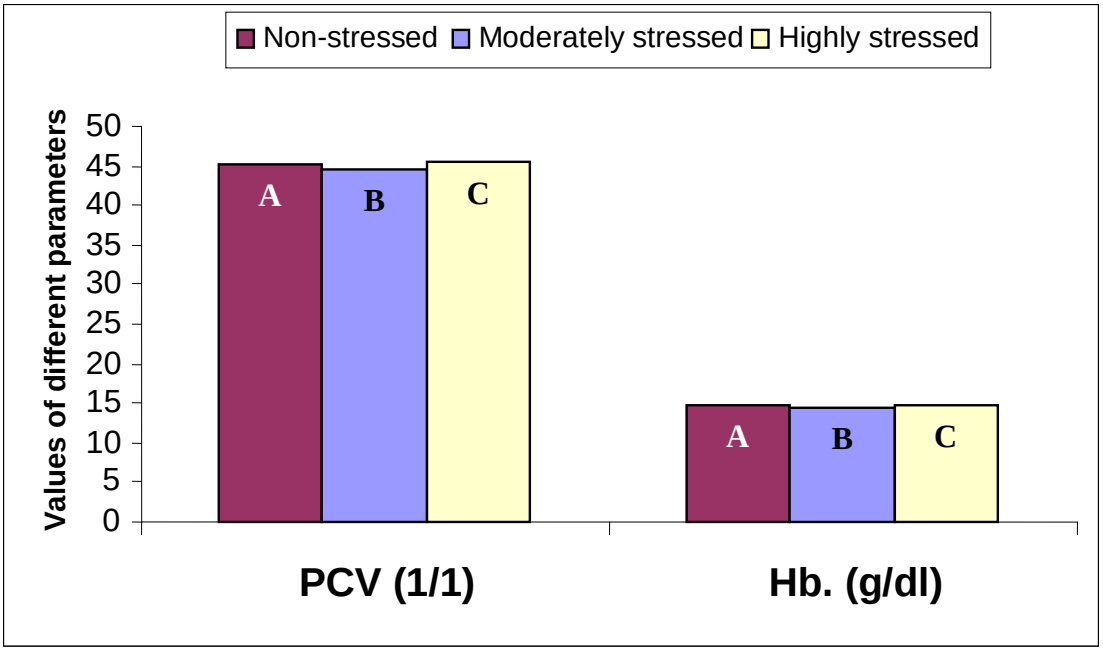

Figure (3-7): PCV and Hb. in non-stressed, moderately stressed, and highly stressed subjects

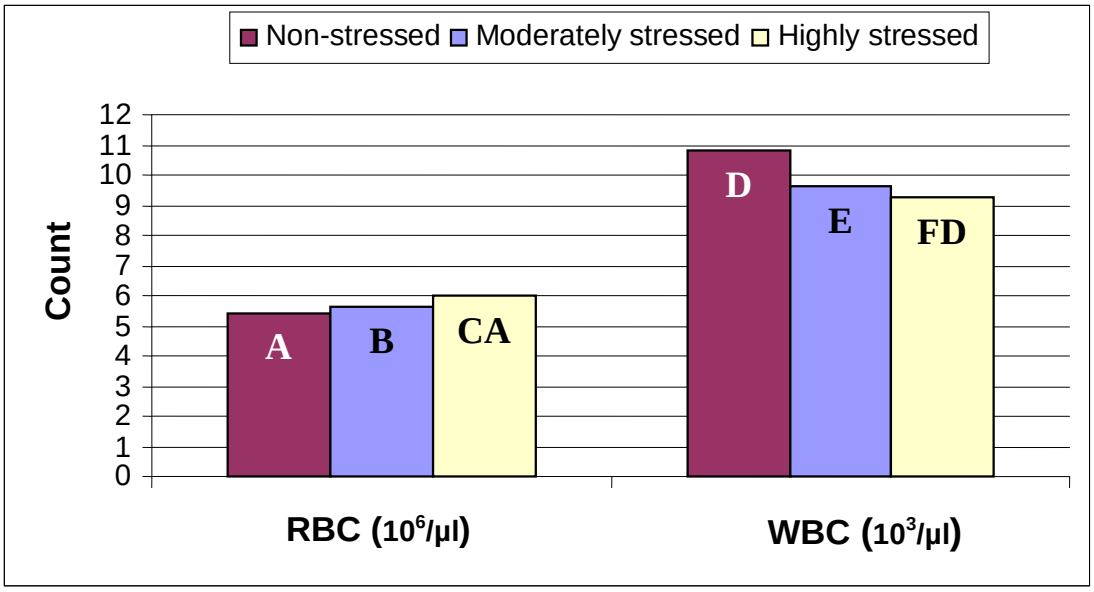

Figure (3-8): Total RBC and WBC count in non-stressed, moderately stressed, and highly stressed subjects

* Single letter = non-significant difference ($p>0.05$).

* Two different letters = significant difference ($p<0.05$).

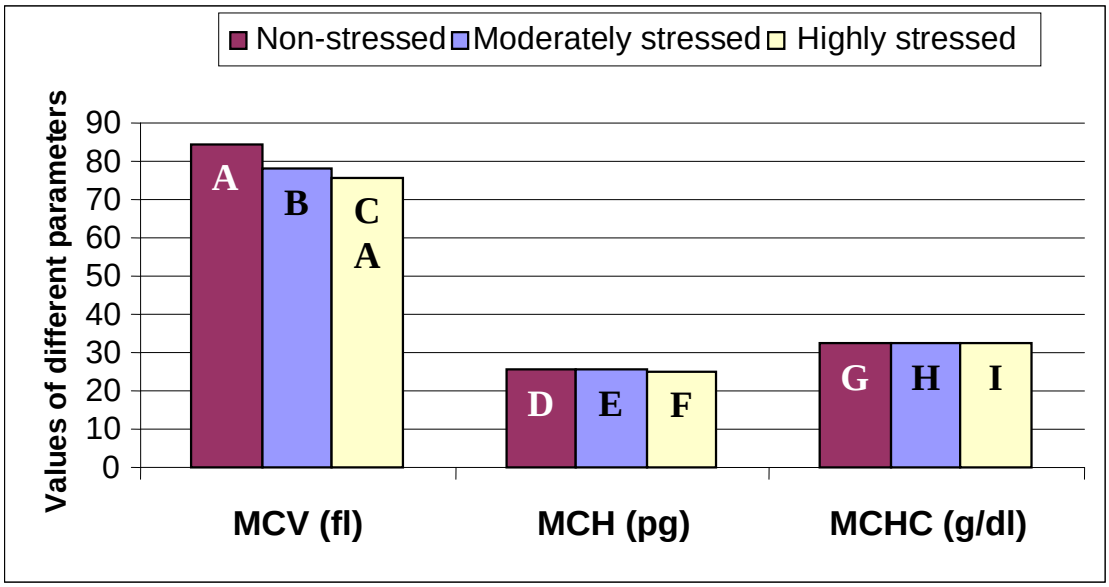

Figure (3-9): Red blood cells indices in non-stressed, moderately stressed, and highly stressed subjects

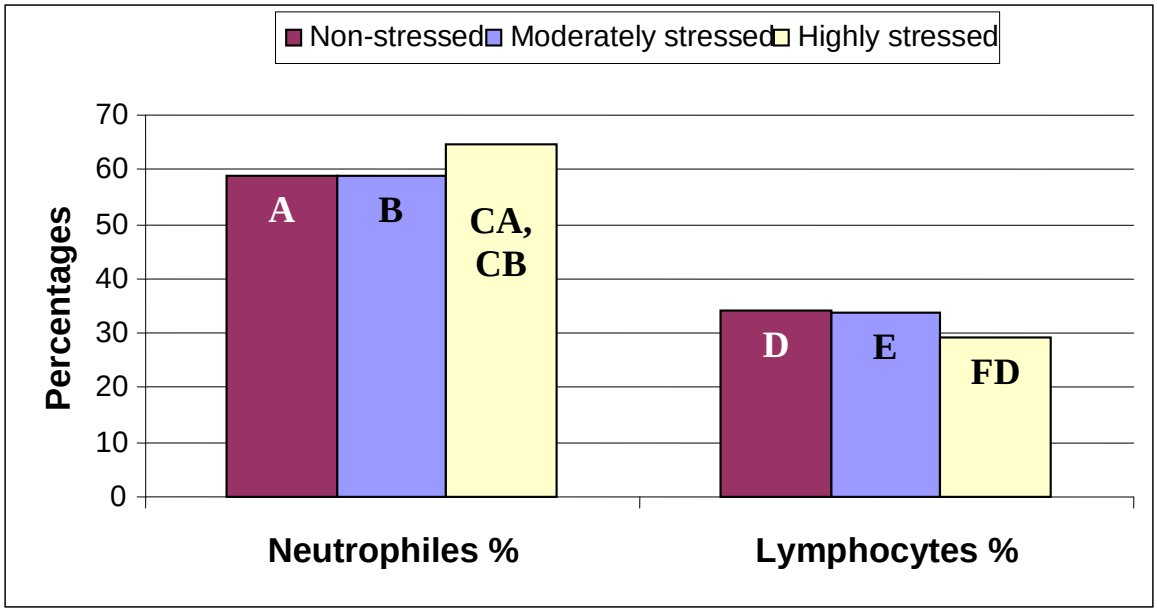

Figure (3-10): Neutrophiles and Lymphocytes percentages in non-stressed, moderately stressed, and highly stressed subjects

* Single letter = non-significant difference ($p>0.05$).

* Two different letters = significant difference ($p<0.05$).

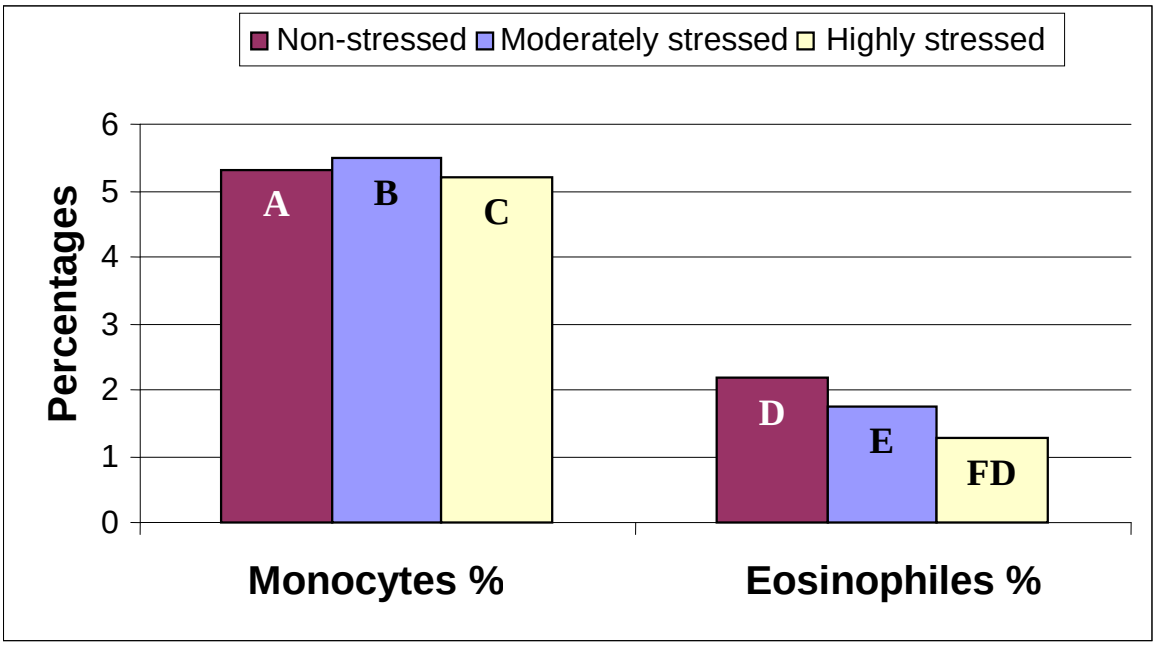

Figure (3-11): Monocytes and Eosinophiles percentages in non-stressed, moderately stressed, and highly stressed subjects

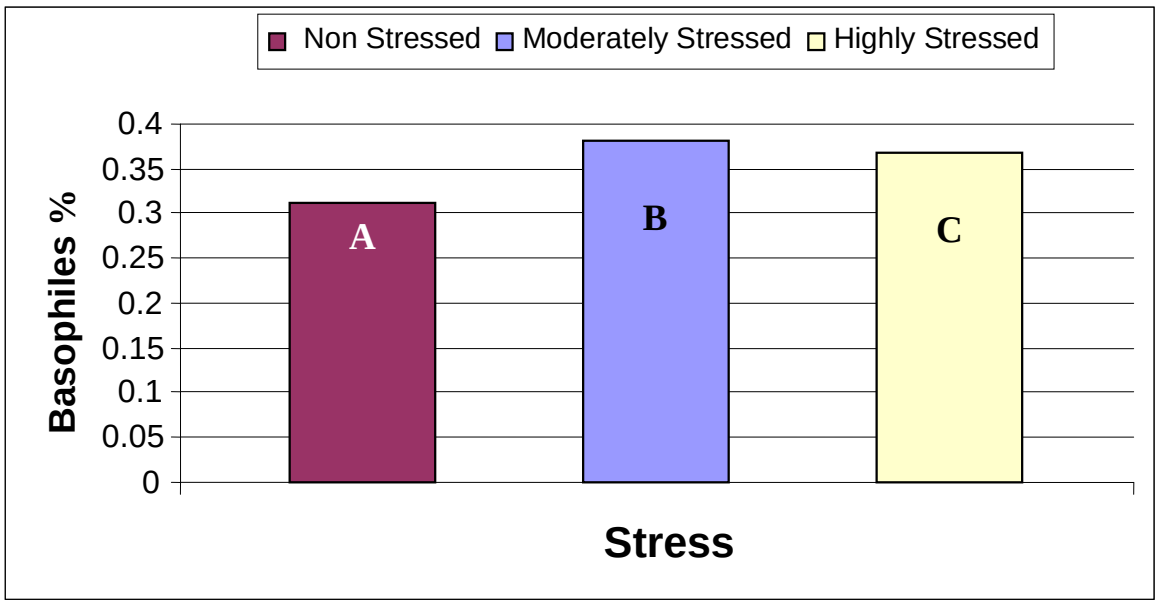

Figure (3-12): Basophiles percentages in non-stressed, moderately stressed, and highly stressed subjects

* Single letter = non-significant difference ($p>0.05$).

* Two different letters = significant difference ($p<0.05$).

3.7 Changes in seminal fluid parameters

3.7.1 Physical

The majority of semen samples had normal whitish - opaque color. All semen samples had normal viscosity and alkaline pH. The mean values of semen volume of all groups were within normal range, no significant changes were found (figure 3-13).There were also no significant changes in the liquefaction time in all groups (figure 3-14).

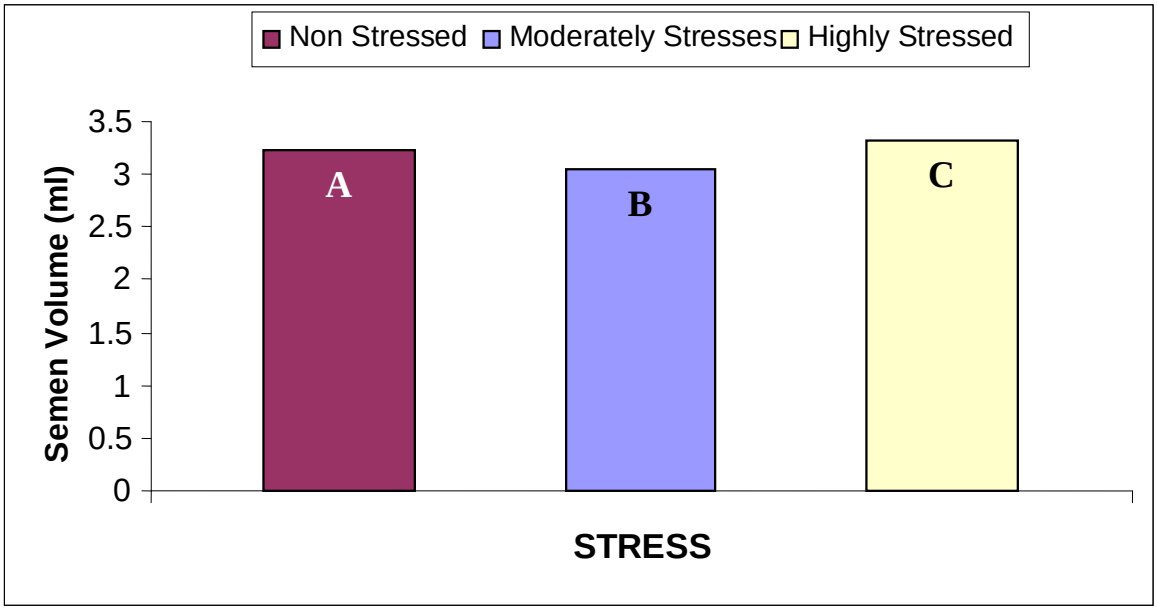

Figure (3-13): Semen volume in studied subjects of all groups

*Single letter = non significant difference (p>0.05).

3.7.2 Microscopic evaluation

The results showed that, there was a non-significant decrease ($p>0.05$) in sperm concentration in both MS and HS groups as compared to NS group (figure 3-14). The grades of motility however, were significantly changed (figure 3-15). In HS group (rapid linear progressive motility) "grade A" indicated a significant decrease ($p<0.05$) in comparison to NS group, while the other grades of motility, HS group of (slow progressive motility) "grade B" manifested a significant increase ($p<0.05$) in comparison with NS group. Concerning (non-progressive motility) "grade C", MS and HS groups showed a significant decrease for both groups ($p<0.01$ and $p<0.05$) respectively when compared to NS group. As for grade D (immotile sperms), it was found that there is a non-significant increase in HS group with respect to NS group.

There was a non-significant decrease in normal sperm morphology percent ($p>0.05$) in HS group (figure 3-14). Concerning total progressive motile sperm per ejaculate (figure 3-14), there was a non-significant decrease in HS group. A significant increase ($p<0.05$) in sperm agglutination percentage was found in HS group as compared to MS group. While a significant increase ($p<0.05$) in round cells concentration was noticed in HS group as compared to MS group in one hand, on the other hand, a significant decrease ($p<0.05$) in MS group as compared to NS group (figure 3-16).

It has been established that, stress exerts a broad influence on the neuroendocrine system, resulting in the activation of the HPA axis and the suppression of the HPG or reproductive axis (Rivier and Rivest, 1991; VanBockstaele *et al*., 1998; Ferin, 1999; Matsuwaki *et al.,* 2004). Such an inhibition occurs at hypothalamic (inhibition of GnRH secretion), and consequently the anterior pituitary (inhibition of LH release), leading to gonadal sex hormones secretion. (Herman and Cullinan, 1997; Jeong *et al*., 1999; Breen *et al*., 2004). On the other hand, activation of the HPA axis by

stress may be the main factor contributing to HPG inhibition, since CRH and glucocorticoids (cortisol) are known to inhibit the GnRH, and glucocorticoids themselves exert inhibitory effects at the level of anterior pituitary and gonads (Orth, 1992; Anisman *etal*., 1998; Bale *et al*., 2000; Frias *et al*., 2002; Karimzadeh *et al*., 2006). Such suppressive effects of stress on pituitary and gonads leads to decreased LH, Testosterone, and eventually a negative effect on sperm parameters related with quality of the semen such as concentration, grades of motility, and morphology of the sperm cells (Fenster *et al*., 1997; Sheiner *et al*., 2003; Eskiocak *et al*., 2004; Mehta and Kumar, 2005).

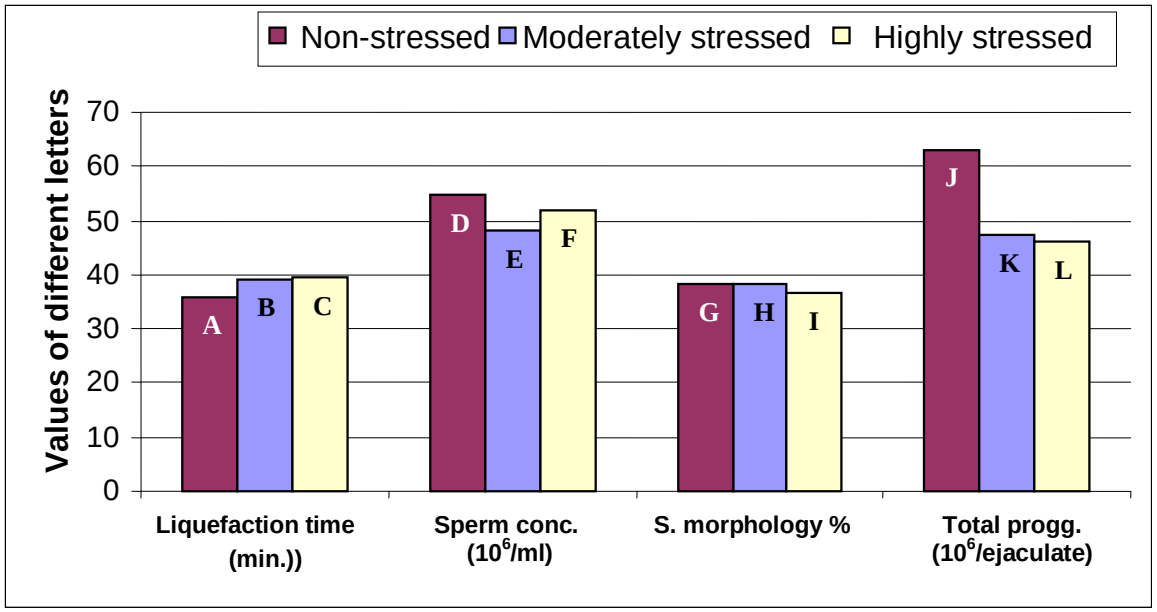

Figure (3-14): Different Semen parameters in studied subjects of the study

*Single letter = non-significant difference (p>0.05).

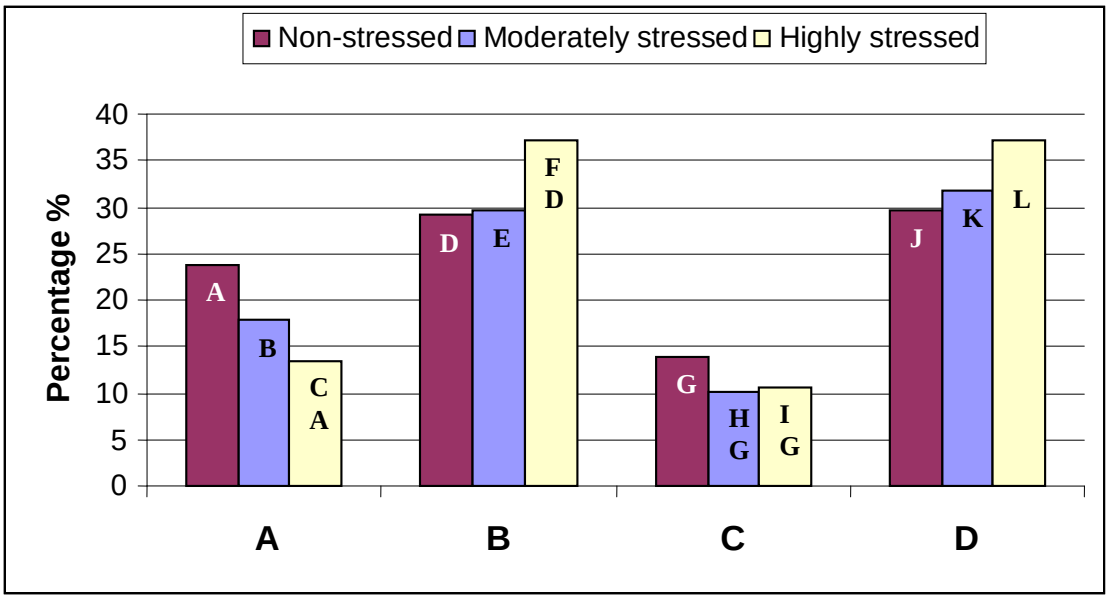

Figure (3-15): Grades of sperm motility (A, B, C and D) in non-stressed, moderately stressed, and highly stressed subjects

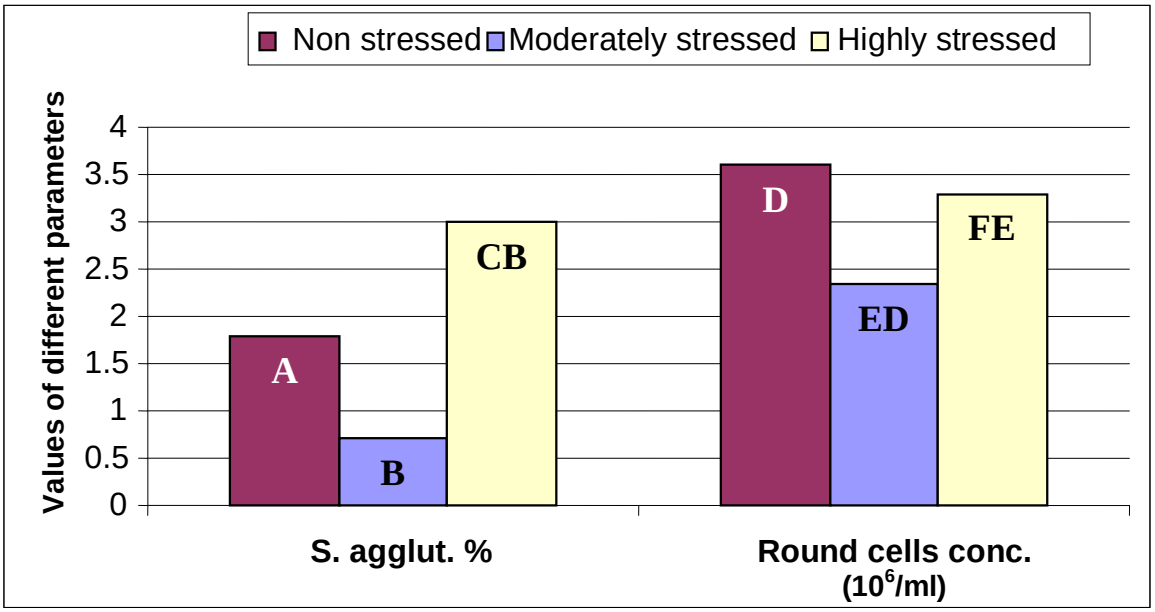

Figure (3-16): Sperm agglutination percentage and round cells conc. in studied subjects

*Single letter = non-significant difference ($p>0.05$).

*Two different letters = significant difference ($p<0.05$).

3.8 Serum hormonal changes

A) Changes in Reproductive hormones (gonadotrophins and testosterone):

Hormonal Profile changes include:

1) LH and testosterone: a significant decrease ($p<0.05$) in HS as compared to NS group (figure 3-17).

2) FSH: showed no significant changes in all groups (figure 3-17).

A variety of stimuli exerts a profound suppression of the reproductive axis (Brann and Mahesh, 1991; Chen *et al.*, 1996; Yoo *et al.*, 1997; Tillbrook *et al*., 2000; Sapolsky *et al*., 2000; Dong *et al*., 2004). Hormones that comprise components of the HPA axis, such as CRH, ACTH, and Cortisol have all been shown to inhibit GnRH / gonadotropin secretion at the hypothalamic and / or pituitary levels. Stress induced suppression of GnRH release is mediated by prostaglandins in the brain (Meczekalski and Szymnakiewicz, 1999; Dong *et al.*, 2004; Maeda and Tsukamura, 2006). Direct roles in inhibiting GnRH release by CRH are not well defined, Cortisol may inhibits pulsatile secretion of LH (Mayers Altermatt, 2007). This inhibitory effect resulted in lowering serum LH release (the main tropic stimulus of Testosterone production in Leydig cells) decreases Testosterone Production (Maric *et al*., 1996; McEwen, 2000; Pitteloud *et al*., 2004; Hardy *et al*., 2005). In 1999, Ferin stated that stress was lower FSH, while Pacak and Palkovitz (2001) demonstrated that FSH did not affect upon stress.

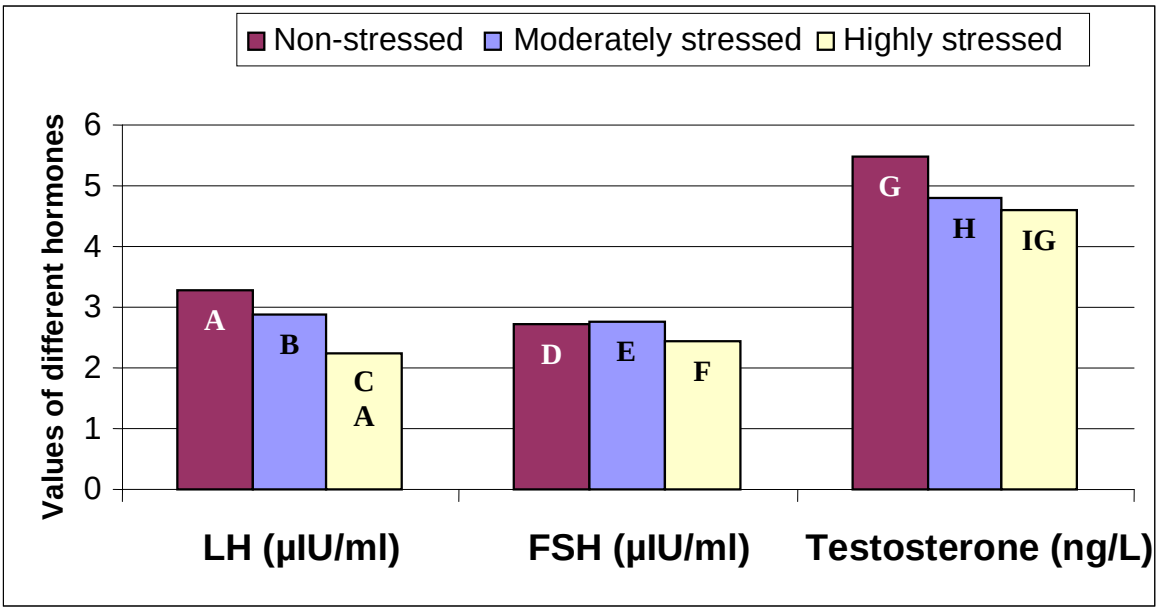

Figure (3-17): Serum LH, FSH, and Testosterone in non-stressed, moderately stressed, and highly stressed subjects

*Single letter = non-significant difference ($p>0.05$).

*Two different letters = significant difference ($p<0.05$).

B) Changes in Thyroid hormones

There were no significant changes in both TSH and T4 among different groups, while T3 in HS group showed a significant decrease ($p<0.05$) in comparison with NS group (figures 3-18 and 3-19).

In terms of hypothalamic - pituitary - thyroid axis, stress induces inhibition of thyroid axis, via CRH, that suppresses thyroid through Somatostatin, that inhibits or decreases the hypothalamic TRH, decreased production / secretion of TSH, and by inhibition of conversion of T4 to more biological active T3 in peripheral tissues (Guyton and Hall, 2000a; Pacak and Palkovitz, 2001; Tsigos and Chrousos, 2002).

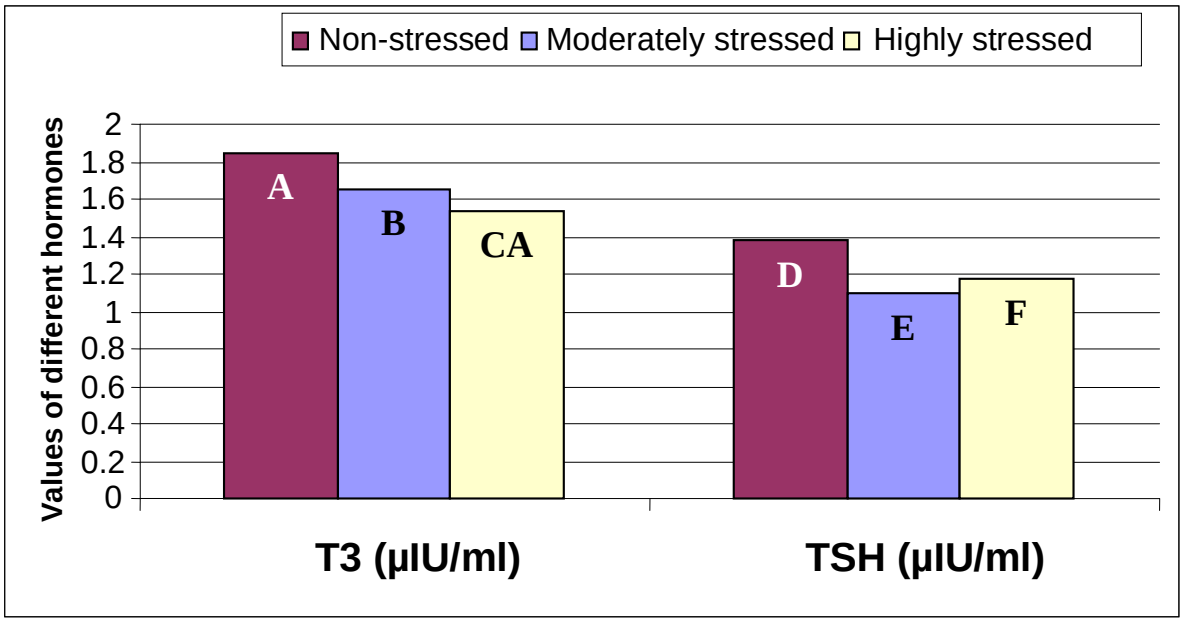

Figure (3-18): Serum T3, and TSH in non-stressed, moderately stressed, and highly stressed subjects

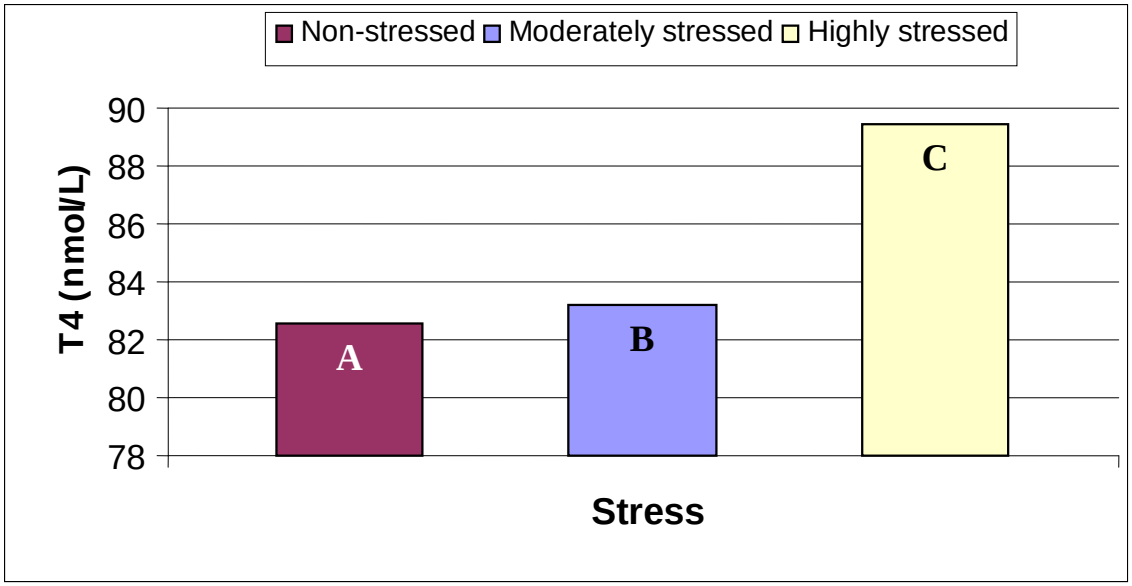

Figure (3-19): Serum T4 in non stressed, moderately stressed, and highly stressed subjects

*Single letter = non-significant difference ($p>0.05$).

*Two different letters = significant difference ($p<0.05$).

Committee Certification

We, the examining committee, certify that we have read this thesis and examined the student in its contents and that according to our opinion is accepted as a thesis for the degree of Master of Science in Biotechnology.

Signature:
Name:
Scientific Degree:
Date:
(Chairman)

Signature:
Name:
Scientific Degree:
Date:
(Member)

Signature:
Name:
Scientific Degree:
Date:
(Member)

Signature:
Name:
Scientific Degree:
Date:
(Supervisor and Member)

I hereby certify upon the decision of the examining committee

Signature:

Name: Dr. Laith Abdul-Aziz Al-Ani

Scientific Degree: Assist. Prof.

Title: Dean of College of Science

Date:

Conclusions

From the results of the present study, the following conclusions have been arrived at:

1. Highly stressed young people for a long period showed less body height.
2. Psychological stress induces some hematological changes, *vs.* increase in total RBC, and decreasing in the MCV and total WBC count. Associated with marked neutrophilia and lymphocytopenia as well as eosinopenia.
3. Semen quality was adversely affected: decrease in grades A and C sperm motility in HS group, associated with decrease in MS group in grade C, while grade B increased significantly ($p<0.05$) as compared to NS group. An increase in sperm agglutination percentage and decrease in round cells concentration was also recorded.
4. Hormones showed decrease in LH, testosterone and T3.
5. As far as, we know this study is first of its kind concerning stress of present situations in Iraq is done.

Recommendations

1. Further studies are needed in this important area of stress using some other techniques e.g. immunological effect, tumor stimulating.
2. Studies using other ages of male and female humans.
3. Samples from military constructions may show stronger results.

CONTENTS

Subject **page**

CHAPTER TWO: MATERIALS AND METHODS

CHAPTER THREE: RESULTS AND DISCUSSION

CONCLUSIONS AND RECOMMENDATIONS

Republic of Iraq
Ministry of Higher Education
and Scientific Research
Al-Nahrain University
College of Science
Department of Biotechnology

Impact of Psychological Stress on Some Hematological, Reproductive, and Hormonal Parameters of Al-Jaderriya Campus Students

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By

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Summary

In this study, the influence of psychological stress on male reproductive health and certain hematological and hormonal parameters was investigated. Ninety-eight healthy male students from Al-Jaderriya compass of Nahrain University were included in the study. The full medical history of subjects was studied. Depending on questionnaire form, 44 of subjects were classified as non-stressed (NS group) (age = 21.68 ± 0.29 years), while 27 subjects were moderately stressed (MS group) (age = 21.88 ± 0.39 years). Concerning highly stressed subjects were also 27 (age = 22.18 ± 0.47 years). Forty three (43) of subjects from all groups accepted to provide us with semen samples. Blood was collected from ninety eight subjects for determination of hematological and hormonal changes. Changes between different groups were compared statistically. Results indicated that;

Body height change, a significant decrease ($p<0.05$) of body height in HS group as compared to NS group.

Hematological changes, there was a significant increase in total RBC count ($p<0.05$), while there was a significant decrease in the mean size of a red blood cell (MCV) of HS group. Concerning **leukocytes changes** (total WBC count), there was a significant decrease in HS group ($p<0.05$), both groups of MS and HS showed a significant decrease ($p<0.05$) when compared to NS group. Concerning differential count, there were significant changes in HS group, either increase in Neutrophiles percentage (Neutrophilia) and/or decrease in Lymphocytes percentage (Lymphocytopenia) as well as for Eosinophiles percentage (Eosinopenia).

Changes in seminal fluid parameters have showed; a significant decrease ($p<0.05$) of 2 grades of sperm motility A and C in HS group, also there was a significant decrease ($p<0.01$) in MS group of grade C, while

grade B of HS group has increased significantly ($p<0.05$) as compared to NS group. A significant increase ($p<0.01$) of sperm agglutination percentage in HS group as compared to MS group, while round cells concentration in MS group was decreased significantly as compared to NS group and HS group.

Changes of hormonal profile: A significant decrease ($p<0.05$) in LH, testosterone and T3 was reported in the HS group in comparison to NS group.

Supervisor Certification

We certify that this thesis was prepared under my supervision at the Department of Biotechnology, College of Science, Al-Nahrain University as partial requirements for the Degree of Science in Biotechnology.

Signature:
Supervisor:
Prof. Dr. Adnan Salih Al-Janabi

In review of available recommendations, I forward this thesis for debate by Examining Committee.

Signature:
Prof. Dr. Kadhim Mohammad Ibrahim
Head of Biotechnology Department
College of Science
Al-Nahrain University

Dedication

To my Father,

For the Uncompromising Principles That are Guiding Him.

To my Mother,

For Making Everything Worthwhile.

To my Sister and Brothers,

For Their Abundant Support, for Their Patience and Understanding, and For Their Love.

To my Teachers,

For Showing Me the Excitement and Joy of Science.

بِسْمِ اللهِ الرَحْمن الرَحِيم

اَلَمْ نَشْرَحْ لَكَ صَدْرَكَ ﴿1﴾ وَ وَضَعْنَا عَنْكَ وِزْرَكَ ﴿2﴾
اَلَّذِي اَنْقَضَ ظَهْرَكَ ﴿3﴾ وَ رَفَعْنَا لَكَ ذِكْرَكَ ﴿4﴾ فَاِنَّ مَعَ
الْعُسْرِ يُسْرًا ﴿5﴾ اِنَّ مَعَ الْعُسْرِ يُسْرًا ﴿6﴾ فَاِذَا فَرَغْتَ
فَانْصَبْ ﴿7﴾ وَ اِلَى رَبِّكَ فَارْغَبْ ﴿8﴾

صَدَقَ اللهُ العَلّي العَظِيم

سورة الإنشراح

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الخلاصة

أجريت هذه الدراسة لبحث تأثير الإجهاد النفسي علي صحة الرجل الإنجابية و بعض المعايير الدموية و الهرمونية. اذ شملت هذه الدراسة ثمانية و تسعون طالباً من جامعة النهرين في مجمع الجادرية الذين تطوعوا للمشاركة في هذه الدراسة، و قد اخذ التاريخ الطبي لكل شخص. اعتمادا على قائمة التقييم النفسي، قسم الأشخاص إلى ثلاثة مجاميع: مجموعة غير المجهدين وعددهم أربعة و أربعون طالباً (العمر=٢١,٦٨±٠,٢٩)، عدد المجهدين بصورة متوسطة بلغ سبعة و عشرون (العمر=٢٢,١٨±٠,٤٧)، فيما يتعلق بمجموعة المجهدين بصورة شديدة بلغ عددهم سبعة و عشرون و معدل أعمارهم (٢١,٨٨±٠,٣٩). ثلاثة و أربعون متطوعا زودونا بعينات سائل منوي، ثمانية و تسعون متطوعا سحبت عينات دم منهم لقياس التغيرات في بعض المعايير الدموية و الهرمونية. الفروقات بين المجاميع المختلفة قورنت إحصائياً، و قد بينت النتائج التالي:

بينت مجموعة المجهدين بصورة شديدة انخفاضاً معنوياً في طول الجسم ($p<0.05$) مقارنةً مع مجموعة غير المجهدين.

التغيرات في المعايير الدموية اظهرت ازديادا معنويا في عدد كريات الدم الحمر ($p<0.05$)، و انخفاضا معنويا في معدل حجم خلية الدم الحمراء (MCV) في مجموعة المجهدين بصورة شديدة. فيما يخص التغيرات في كريات الدم البيض، وجد أن هناك انخفاضاً معنويا في العدد الإجمالي لكريات الدم البيضاء في مجموعة المجهدين بصورة شديدة ($p<0.05$) مقارنةً مع كل من مجموعتي غير المجهدين و المجهدين بصورة متوسطة. أظهرت الدراسة أن مجموعة المجهدين بصورة شديدة أبدت ارتفاعا معنوياً ($p<0.05$) في النسبة المئوية لخلايا العدلة في حين أظهرت كلاً من الخلايا اللمفاوية و خلايا الحمضة انخفاضاً معنوياً.

فيما يتعلق بمعايير السائل المنوي فقد بينت الدراسة انخفاضاً معنوياً ($p<0.05$) في درجات حركة النطف (أ) و هي نمط الحركة التقدمية الخطية و كذلك الدرجة (ج) أو "الحركة اللاتقدمية أو الموقعية" في مجموعة المجهدين بصورة شديدة، أيضاً هناك انخفاضا معنوي ($p<0.01$) في مجموعة المجهدين بصورة متوسطة مقارنةً مع مجموعة غير المجهدين. في حين أظهرت الدرجة (ب) أو الحركة التقدمية البطيئة لمجموعة المجهدين بصورة شديدة ازدياداً معنوياً ($p<0.05$) بالمقارنة مع مجموعة غير المجهدين. لوحظ أن نسبة تلاصق "تلازن" النطف قد ازداد بصورة معنوية ($p<0.01$) في مجموعة المجهدين بصورة شديدة عندما قورنت مع مجموعة المجهدين بصورة متوسطة. و قد لوحظ أيضاً أن عدد الخلايا الكروية قد انخفض بصورة معنوية مقارنةً مع مجموعة غير المجهدين او المجهدين بصورة شديدة كلاً على حدة.

فيما يخص الهرمونات التي قيس مستوياتها، فقد وجد أن هناك انخفاضاً معنويا في مستويات كل من الهرمون اللوتيني (LH) وهرمون الشحمون الخصوي أو التيستوستيرون وهرمون الغدة الدرقية (T_3) لمجموعة المجهدين بصورة شديدة مقارنةً بمجموعة غير المجهدين.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم ألتقانة ألإحيائية

تأثير الأجهاد النفسي في بعض المؤشرات الدموية والتناسلية والهرمونية للطلبة في مجمع الجادرية

نزك ب

ف خ ل ب ك و ق ي ب ك ع ك ل ج ل ع ب ك ه ي م

ه ى ج؟ ل م ل ك ة ه ك خ ن ج ب ل ج ز ق ذ ع ك ل غ ى ل ق ئ ب ل ح ق ي ب

مِنْ قِبَلْ

علي حيدر محمد علي الحمر

بكالوريوس تقانة إحيائية-جامعة النهرين- ٢٠٠٥

باشراف

الاستاذ الدكتور عدنان صالح الجنابي

كانون الثاني ٢٠٠٨

ذي الحجة ١٤٢٨