EFFECT OF CHRONIC STRESS ON LACTOGENESIS IN HUMANS

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Certificate

This is to certify that this thesis entitled ***"EFFECT OF CHRONIC STRESS ON LACTOGENESIS IN HUMANS"-*** is a bonafied work of Mrs. Vandali Jyothi and was carried out under our supervision and guidance.

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DECLARATION

I declare that the thesis entitled ***"EFFECT OF CHRONIC STRESS ON LACTOGENESIS IN HUMANS"*** has been prepared by me under the guidance of Dr. Manjunath Aithala, Professor & Head, Department of Physiology, BLDE University's Shri B.M. Patil Medical College, Hospital and Research Centre, Vijayapura, Karnataka, India and co-guidance of Dr. Neerja Shastri, Associate Professor of Department of Physiology, Prathima Institute of Medical Sciences, Nagnuru, Karimnagar, Telangana State. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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DEDICATION

I dedicate this thesis to

my beloved parents

and

In-laws

ABSTRACT

INTRODUCTION: Chronic stress is a state of ongoing physiological arousal. The concept of chronic stress is based on how frequently the stressors appear over a period of time. Cumulative effects of changing life events with failure of coping reflex leads to chronic stress, which repeatedly activates hypothalamic-pituitary-adrenal (HPA) axis without relaxation response, to release excess Cortisol, the principal stress hormone. Chronic stress causes undesirable consequences like preeclampsia, preterm labor, spontaneous abortions and may also affect the lactogenesis. Studies in animals indicate that various types of stressful stimuli can depress lactation, but there is less information in humans.

AIM: To assess the effect of chronic stress on lactogenesis in humans.

METHODS: Pregnant women in the reproductive age (21-45 yrs) attending the antenatal clinic were selected for the study. 96 women of similar demographic background and health condition were assessed for the level of stress with the help of Holmes and Rahe stress scale. We assessed the relation between stress and socio-demographic parameters like occupation, family type, religion, economic and educational status of study subjects. Serum Cortisol (µg/dl) was estimated in all 3 trimesters and postpartum. Serum Prolactin (µg/L) was estimated by electrochemiluminescence immunoassay before delivery and up to 5 days postpartum. Measurement of milk volume (ml) was done by baby test weighing method up to 7 days postpartum.

RESULTS: 37.5% pregnant women were mildly stressed, 35.41% were moderately and 27.08% were severely stressed. Serum Prolactin levels were insignificantly low ($p>0.05$)in moderate and severely stressed women. Serum Cortisol levels were significantly high ($p<0.05$) in moderately and severely stressed women with significant reduction in milk volume when compared to mildly stressed women.

CONCLUSION: Moderate and severe stressful events reduce milk volume output in humans.

KEYWORDS: Chronic stress, serum Cortisol, serum Prolactin, milk volume.

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
BM	Basement membrane
bpm	Beats per minute
BP	Blood Pressure
Cm	Centimeter
CPI	Consumer Price Index
CRH	Corticotropin Releasing Hormone
dl	Deci liter
DHEA	5-Dehydroepiandrosterone
D	Desmosome

EDD	End Date Delivery
FDA	Fat depleted adipocyte
GJ	Gap junction
GAS	General Adaptation Syndrome
g	Gram
HPA axis	Hypothalamic – pituitary – adrenal axis
IgA	Immunoglobulin A
IGF-I	Insulin like Growth Factor – I
L- I	Lactogenesis stage – I
L - II	Lactogenesis stage – II
LMP	Last menstrual period
LCU	Life Change Unit

L	Liter
µg	Microgram
ml	Milliliter
ME cell	Myoepithelial cell
N	Nucleus
No	Number
PC	Plasma Cell
PP	Postpartum
PSA	Pregnancy Specific Anxiety
PRL	Prolactin
RER	Rough Endoplasmic Reticulum
SIgA	Secretory IgA

SV	Secretory Vescicle
TJ	Tight Junction
Tri	Trimester
WHO	World Health Organization
Yrs	Years
>	More than
<	Less than
=	Equals to
±	Plus or minus
/	Per
%	Percentage

CHAPTER - I

INTRODUCTION

1.1.BACKGROUND :

Stress has become an integral part of our daily life. Generally, stress phenomenon is a physiologic response to psychological and physical demands. The threats are known as stressors. Stress develops due to demand – capability imbalance in the organism's homeostatic mechanism[1]. Lazarus defined stress as an inward process that happens when a person confronted with a demand that is seen to surpass the assets accessible to successfully react to it and where inability to effectively respond to it, has undesirable consequences[2].

Stress is associated with some changes in the structure as well as chemical composition of the body. Stress leads to many manifestations and changes which also include body's defensive adaptive processes[2]. Hypertension, varicose veins, insomnia, fatigue, feeling jittery, poor concentration, general illness etc. are sensations of stress[3].

1.2. ACUTE AND CHRONIC STRESS:

Stress can be acute or chronic. Acute stress is a short-lived stress. The physiological response to this type of stress causes effective resolution to the stressors such as demand or threat. Chronic stress is long-lived stress, generally lasting weeks to months to years. The physiological response does not have effective resolution to the stressors[4]. The basis for chronic stress depends upon frequent occurrence of stressors over a period of time[5]. So, chronic stress is a state of continuous arousal of physiological response. This is because when a body experiences too many threats and demands or only one stressor continuously

for a long period of time, but it does not have the capacity to activate the relaxation response. This type of stress develops due to everyday stressors which are neglected or poorly managed or in response to traumatic events[6]

Acute stress is not dangerous to health. It can actually be beneficial in certain circumstances of life, as it can increase alertness and performance. Stress for a prolonged time i.e. chronic stress has been linked to ill-health consequences. So, stress hormones are to blame[7]. Pregnancy is one of stressful events in woman's life, as pregnancy is a time of physiological change which requires huge psychological adjustment[8]. Studies indicate that high stress or anxiety levels during pregnancy are at increased risk for preeclampsia, spontaneous abortion, preterm labor and delayed fetal growth[9-11].

1.3. PHYSIOLOGY OF STRESS RESPONSE:

Autonomic Nervous System (ANS) and Hypothalamic – pituitary – adrenal axis(HPA) axis get activated in response to stress[12]. With chronic stress, both the systems are repetitively activated. This results in persistent physiologic effects[13]. Prolonged stress alters HPA axis, malfunctions negative feedback loop resulting in excess production of Corticotrophin Releasing Hormone (CRH) from hypothalamus. This stimulates the anterior pituitary for the systemic release of ACTH. This subsequently signals the adrenal glands to release glucocorticoids predominantly Cortisol[14,15] .

Chronic stress response leads to pregnenolone steal, which causes production of huge amount of Cortisol (the principal stress hormone). So, serum cortisol level can be considered as a more objective measure of chronic stress. Excessive Cortisol levels during

pregnancy has been associated with adverse pregnancy outcomes[16,17]. Keeping in view of this, present study was conducted to assess effects of chronic stress on lactogenesis.

1.4. INTERACTION BETWEEN STRESS AND LACTATION:

The primary lactogenic hormones associated with milk synthesis and ejection are Prolactin, Oxytocin, Glucocorticoids and also other hormones such as Insulin, Growth Hormone, Leptin and Opiates. The data available from both animal and human studies indicate that the physiological trigger for lactogenesis is a fall in progesterone, maintained Prolactin and Cortisol after child birth [18]

There are no clinical studies demonstrating a direct correlation between measures of maternal stress and decreased lactation. Animal studies indicate that stress depresses lactation directly by acting on hypothalamus which inhibits secretion of Prolactin and Oxytocin from anterior and posterior pituitary respectively or indirectly by activating sympatho-adrenomedullary system, which secrete norepinephrine, epinephrine and cause the peripheral inhibition of milk ejection. This is due to vasoconstriction at the level of mammary glands, stimulation of mammary myoepithelial cells and potential increase in mammary ductal tone[19 - 21].

Animal experiments have demonstrated suppression of lactation when animals were exposed to certain types of stressful stimuli[22]. In humans, lactation insufficiency may be due to the stress imposed by preterm delivery, infant medical condition, maternal life style or life events [23, 24]. Duration of stress and its sites of action lead to decrease in milk synthesis or ejection which causes the suppression of lactation[25, 26]. As per the human experiment done by

Newton and Newton, various types of acute stressful stimuli decreased the milk ejection reflex[27]. Udea T et al have shown that mental stress and noise stress have reduced milk volume[24]. These studies were demonstrated during established lactation and not during lactogenesis. This research indicate the effect of acute stress on lactation i.e. Milk volume was measured before the induction of stress, during the time of stress and after the removal of stress. Milk volume was normal before induction of stress, volume was reduced during the time of stress as the milk ejection was affected and normal milk volume was regained after the recovery from stress. The effects of chronic stress on lactogenesis are not yet known. So, this study was conducted to assess the effect of chronic stress on lactogenesis.

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CHAPTER –II

REVIEW OF LITERATURE

2.1. SHORT HISTORICAL BACKGROUND OF STRESS:

Since the late 17th century, the concept of stress has been developing and has been systematically conceptualized. It has been a subject of research in the early 19th century.

Walter Cannon coined the term fight-or-flight [1]during the research conducted from1913- 1920s. He first described the short-term stress response as a theory where an animal reacts to a sudden accident or danger "with a general discharge of the sympathetic nervous system".

Hans Selye borrowed the term stress from the field of engineering in 1936. According to Selye, determinants of stress response were nonspecific. Hans Selye proposed a model of human stress response ie, General Adaptation Syndrome (GAS) . GAS explains about alarm stage, resistance stage and exhaustion stage[2].

In 1953, the concept of 'a dynamic state' involving adaptation to demand was discovered by Selye and Wolff [3].

In 1966, Lazarus suggested that stress can be an organizing concept for understanding a wide range of phenomena of great importance in human adaptation.

In 1977, Lazarus and Cohen explained about the types of stressors as i) major changes, often cataclysmic and affecting large number of persons, ii) major changes affecting one / few persons and may be iii) daily hassles[4].

In 1974, Holmes & Masuda have indicated that life-events and 'daily hassles' are the causes for chronic stress[5].

Lazarus and Folkman in 1984 have suggested that the daily hassles which occur in day to day life may be as much or more stressful than that of the major life events. Cognitive appraisal and coping are the two central processes in Lazarus theory that determine the extent of stress experiences in a given situation.

Cognitive appraisal- The determinants of why and to what extent a situation is perceived as stressful by an individual is the Cognitive appraisal. So, it is an evaluative process.

Coping - The extent to which a particular situation is experienced as stressful, also the individual's capacity of dealing with the situation, depends on his / her coping capacity. According to Lazarus, stress is an internal physiological process that occurs when a person is faced with a demand or a threat. If it is perceived as highly stressful that exceed the resources available to effectively respond to it, and leads to undesirable consequences [6].

2.2. STRESS AND WOMEN:

Nielsen's study was conducted from February to April of 2011, polled almost 6,500 women throughout 21 developed and developing countries including those in Asia, Europe, and America. The results of the polls showed that an astounding 87% of Indian women claim feeling stressed most of the time. The top 10 countries in which women are highly stressed are -India (87%), Mexico (74%), Russia (69%), Brazil (67%), Spain (66%), France (65%), South Africa (64%), Italy (64%), Nigeria (58%) and Turkey (56%).This type of chronic stress in Indian

women may be due to modern lifestyle. Heavy traffic and high – pressured jobs are everyday stressors, which keep the body in a state of perceived threat and chronic stress [7].

Pregnancy is recognized as one of the stressful events in woman's life.It is a time of physiological change that needs huge psychologicaladjustment[8] .Women's health care providers have investigated and are increasingly aware that the chronic stressors i.e. ongoing perceived stress/threat and anxiety are due to poverty, intimate partner violence and experiences of racism. They are associated with an increased incidence of preterm birth and low birth weight of the baby in United States[9] .

Stress during pregnancy is more among the teenagers, low educational status, discriminated group of population or with low socioeconomic status[10] . Presence of major events in the life affects the daily activities of a person. Some of the studies have shown significant association of stressful life events during pregnancy on reducing age of gestation. Presence of two or more major life events during pregnancy has been linked to birth of baby with prematurity and low birth weight[11, 12].

Rondo et al in 2003, have reported the prevalence of stress and distress was varying from 22.1 % to 52.9 % in a Brazilian cohort with 865 pregnant women[13].

Eriksson et al in 2006, observed around 20% of low-risk pregnancies in western countries had intense childbirth fear and also from 6 % to 10 % women were seriously incapacitated by childbirth fear [14] .

Prospective study by Grant et al in 2008 have investigated about the course of maternal anxiety from pregnancy till seven months among 100 Australian pregnant women. They have reported about 21% anxiety disorders in female individuals[15] .

Esperat et al in 2007 have indicated that poverty is one of the important stressors. Socio-Economic Status (SES) of an individual can be assessed by taking in to account of educational status, income, occupation and housing conditions. They may be used as proxy measures for assessing poverty. If there is lack of money during pregnancy, individual may be incapable of utilizing health care facilities. This may lead to increased percentage of poor maternal outcome, which in turn may result in increase in morbidity and mortality of both mother and child [16] .

Hodnett in 2002 and Rondo et al.in 2003 have found high prevalence of anxiety disorder among pregnant women which was found to be undiagnosed and untreated [13,17] . Anxiety and depression in pregnancy leads to prematurity and low birth weight of the baby. Few studies have reported that pregnancy related stress was one of the predictors of preterm delivery [13, 18, 19] . Anxiety during pregnancy is known as Pregnancy Specific Anxiety (PSA), which is due to worries and fears about pregnancy and childbirth, also concerns about the health of infant and future parenting[18]. Lee et al in 2007 and Teixeira et al in 2009 reported that about 54% of low risk pregnant women had pregnancy anxiety. The anxiety levels varied at different trimesters of pregnancy and showed U pattern of high levels of anxiety during first and third trimesters [20, 21] .

Thus, stress is a state of threatened homeostasis or disharmony caused by intrinsic or extrinsic adverse forces. It is counteracted by physiologic and behavioral responses that in turn try to reestablish the challenged body equilibrium.

2.3. A MODEL OF HUMAN STRESS RESPONSE :

In 1956, Hans Seyle investigated and proposed a model which reveals the physiological stress response, known as "General Adaptation Syndrome" (GAS). It is a triphasic phenomenon – stage of alarm reaction, stage of resistance and stage of exhaustion[22].

The initial phase, Selye refers to as "alarm" stage, which represents the body's defense mechanism. It is also known as fight or flight response. Increased heart rate and hormonal activity are the physiological responses to the alarm stage. They are adaptive physiological changes in the body.

The second phase of general adaptation syndrome is called "stage of resistance". In this stage, the body tries to reestablish and maintain homeostasis. The physiological changes are increased with the release of stress hormones. Sympathetic activity reduces than during alarm stage, but it will be higher than normal range. Energy sources are exhausted in this stage.

Throughout one's life, stages 1 and 2 may be repeated. If stress persists for a long period or any other form of stress is encountered, then the body enters in to 3rd stage i.e., stage of exhaustion. This causes destruction of body's defense system. There will be depletion of energy resources and muscles become fatigued. If stress exposure is continued, may lead to body disorders or diseases [22]. Overstress causes many deleterious effects like ulcers, heart diseases, visceral obesity, metabolic complications etc [23].

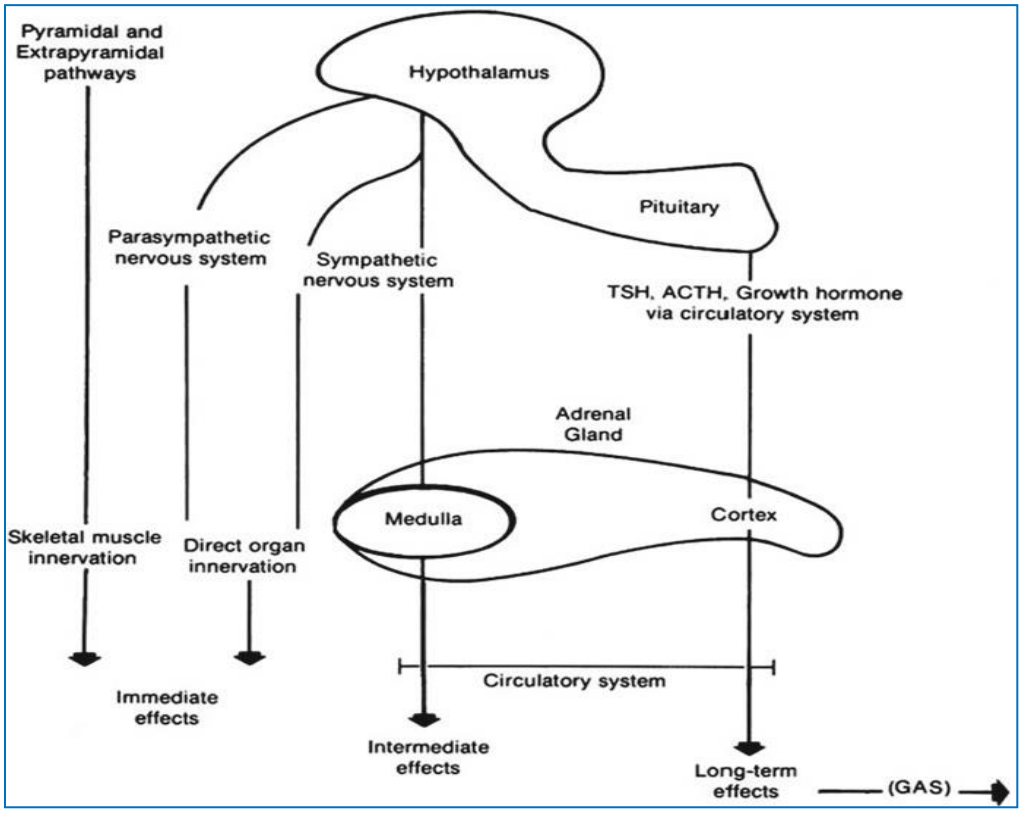


Figure-1 : The sequential activation of the stress-response axes[22] :

The direct neural innervations of end organs cause fast and immediate physiological response. The neuroendocrine fight-or-flight axis causes intermediate physiological response.

The next and final physiological response to the stressful situation is endocrine axis. Higher intensity stimuli cause activation of this axis for a long time and is known as chronic stress response [22].

2.4. THEORIES OF STRESS :

Cognitive theory of stress explained by Lazarus consists of the three factors – i. stressor, ii. stress appraisal, iii. stress response.

i. Stressor - It can be a situation, due to external or internal stimulus, which causes a physical or psychological challenge.

ii. stress appraisal - The way an individual senses the stress situation is called stress appraisal.

iii. stress response - The physiological reactions and psychological changes occur in the body due to the stress appraisal are known as stress responses[24].

A stressor can be perceived in many ways, may be as harm / loss or threat / challenge. A person perceives a stressor as stressful situation mainly is dependent on the cognitive appraisal, when an individual doesn't have enough resources to face the situation. Thus, stress perception is subjective in most of the situations. Any event/condition can be stressful for one person, which may not be a stressful situation for another person[24] .

In 1994, "biopsychosocial model" for stress was proposed by Bernard and Krupat. This model explains about three factors – i. external component, ii. internal component, iii. interaction between the external and internal components.

i. External component – It may be environmental event which elicits a physiological stress response.

ii. Internal component – It explains a set of physiological and neurological reactions to stress. Selye dealt with an internal component in General Adaptation Syndrome (GAS).

iii. The interaction between external and internal components - It involves the cognitive processes of a person. It is explained in Lazarus's cognitive theory of stress[25].

Reformulation of cognitive theory was explained by Dienstbier. He explained about emotional consequences of appraising an event as a stressor or as a challenge, which leads to different physiological consequences than when it is appraised as a harm / loss / threat. He used term Stress to refer to the conditions that led only to negative emotions. He defined the term Challenge to describe a condition that could lead both to positive as well as negative emotions. Marianne Frankenhaeuser and colleagues studied on the same concept. They supported Dienstbier's concept i.e. stressor evaluated as a challenge should be considered more positively than a loss / threat / harmful situation [26] .

Frankenhaeuser found three factors for physiological responses to stress –i. Effort with distress, ii. Effort without distress, iii. Distress without effort.

i. Effort with distress - It is due to daily hassles, which leads to increased secretion of catecholamines and Cortisol. Such stressors are felt as negative emotions. This component of the theory explains about the negative emotions present in an event appraised as a loss / as a threat.

ii. Effort without distress – It leads to an increase in catecholamine secretion and decrease in Cortisol. Such stressors/ situations are experienced as positive emotions. Thus, it represents the positive emotions in the conditions appraised as challenging.

iii. Distress without effort – It causes an increased secretion of the hormone Cortisol but not necessarily catecholamine release. In depressed subjects, these physiological responses are found[27] .

2.5. TYPES OF STRESS :

i. Short term or acute stress : generally lasts from days to weeks.

ii. Long term or chronic stress : generally lasts from weeks to months to years.

i. Acute stress : It may be due to an accident or immediate perceived threat. The physiological responses are activation of autonomic nervous system with high levels of Cortisol and Adrenaline, increased heart rate and respiratory rate etc. This is fight-or-flight response. In 1920s, Walter Cannon coined the term 'fight-or-flight' response. When the physiological response to acute stress is over, the body tries to return back to normal or homeostasis is re-established. This can be achieved by activating the relaxation response. This is the reverse of the fight-or-flight stress response. So, there will be decrease in heart rate, production of stress hormones, breathing, vasodilation etc.

ii. Chronic stress : It may be a state of on-going physiological arousal as the body of an individual experiences multiple stressors at a time/ within less time or a single stressor continuously for long period, so that the body does not have the ability or opportunity to activate the relaxation response [24] . Chronic stress may develop due to everyday stressors or stressful

situations, which are not managed or in response to the traumatic events in life. It has many negative consequences including suppression of the immune system, increased risk of heart attacks and stroke, diabetes, speeding up of process of ageing, infertility etc [22].

2.6. MEASUREMENT OF STRESS :

Self report of stress i.e. Perceived stress scale, stressor exposure measurements i.e. 'major life events' stress scale or Holmes and Rahe stress scale are used to measure stress.

Holmes and Rahe stress scale– A list of major events which occur in life is given. The subjects are asked to indicate which events, also how many times they have experienced. Each event is given a weighted score called 'life change unit'(LCU). The overall score is estimated to each subject by adding the weighted scores of all events indicated. Stress related illnesses are observed in the subjects with high score for a prolonged period[25, 26] .

2.7. FACTORS AFFECTING STRESS RESPONSE :

Studies indicate that the events which are "unpredictable and uncontrollable" are more stressful than the events which are "predictable and controllable". If there is a belief that some coping actions can be taken regarding a given challenge, then it is perceived as less stressful[27].Therefore, first and very important component influencing appraisal of a given stressor is lack of control. The other component is suddenness. It is usually easier to face an event that one can foresee. The third component is ambiguity. It also influences the stress appraisal. So, the well defined situation can be faced easily as the nature of the stressor is known to the person whereas, ambiguity needs lot of energy[28].

2.8. PHYSIOLOGICAL STRESS RESPONSE (HPA AXIS) :

Stress stimulates the neuroendocrine system and causes certain changes in the body metabolism. When the neuroendocrine neurons of hypothalamus (HPA AXIS) are stimulated, hypothalamus secretes more Corticotropin Releasing Hormone. This then stimulates the anterior pituitary gland to secrete higher amount of Adreno-Cortico-Tropic-Hormone [29] . The ACTH then helps the adrenal glands to secrete aldosterone which effects the kidneys [30], Cortisol that effects the immune system also brain [31], 5-De- Hydro- Epi-Androsterone (DHEA) effects the body's metabolism and Adrenaline which effects the cardiovascular system and respiration. Activation of Sympathetic nervous system results in secretion of noradrenaline [32]. Chronic stress causes the stress response to occur for a long period without relaxation response. This stimulates continuously the adrenal glands to secrete more amounts of cortisol the "principal stress hormone."

Generally, adrenal glands secrete the hormones like DHEA, Aldosterone, Cortisol, Testosterone, Estrogens and Progesterone. All these hormones have the only and common precursor, master hormone "pregnenolone". During chronic stress, there will be hyperstimulation to adrenal glands and the pregnenolone is diverted or stolen from other pathways to produce the stress hormone, cortisol in excess. Pregnenolone steal or cortisol escape mechanism is the body's hormonal response to prolonged periods of stress. So, during chronic stress, body utilizes most of pregnenolone for the synthesis of the principal stress hormone, Cortisol as shown in figure-2 [33, 34]. Thus, chronic stress causes the HPA axis dysfunction. So, there will be hypercortisolism and also diurnal dysrhythmia [35, 36] .The hypercortisolism phase

may last for several years. Chronic stressors which also threaten physical integrity are uncontrollable or involve trauma will tend to result in malfunctioning of HPA axis, which in turn may result in destruction of diurnal profile of Cortisol release [37].This leads to imbalance and deficiency of all other hormones in the body leading to deleterious effects[33, 34].

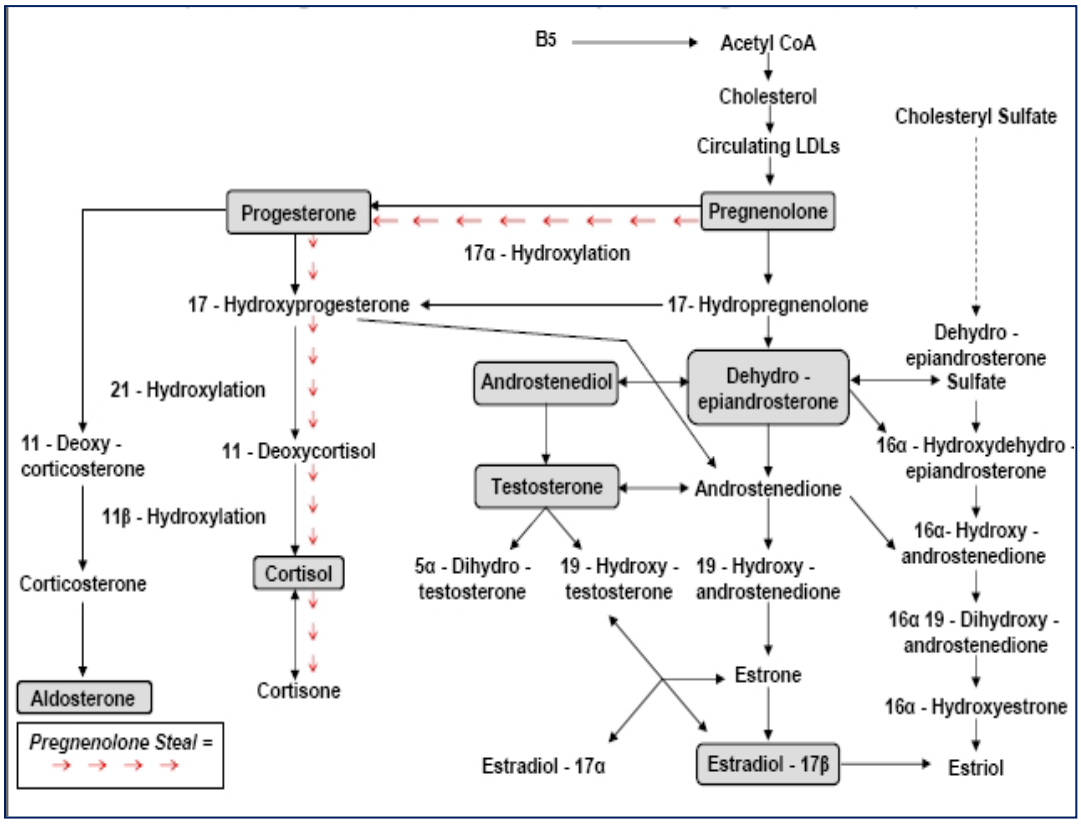


Figure-2 :Chronic stress response[33] :

It is evident that chronic stress is mostly due to the negative emotional state/situation [38, 39] . Cortisol released during the stress response crosses the blood brain barrier, in turn binds to receptors in the hippocampus. So, long term and excess production of stress hormones cause shrinking also atrophy of the hippocampus and induce memory impairments[40, 41]

There are many stress related illnesses[22, 23].The present study is conducted to assess the interaction between chronic stress and lactation. Human milk is a carefully engineered substance, has unique composition ideal for the growth and development of infants. Availability of mother's milk is conceivable only if lactation is sustained. To safeguard lactation, it is necessary to know the physiology of lactation, how to initiate and maintain maternal interest in breastfeeding particularly at the time of stress. The initiation of lactation or milk production is based on research conducted over the past 20 to 25 years[42, 43].

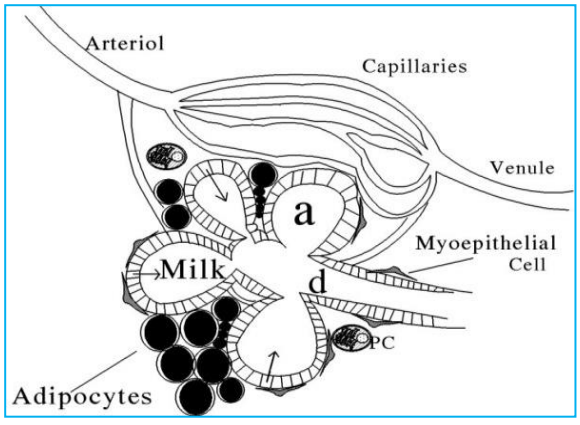
2.9. HUMAN MILK:

Mammalia - This class mammals have the ability to produce milk, designed specifically to nourish the young ones. Milk composition is species specific. Human milk is a complex biological fluid[43]. Many components such as growth factors, long chain polyunsaturated fatty acids, the protein lactoferrin, bile salt stimulated lipase and anti-infectious oligosaccharides and glycol-conjugates are present only in the human milk[43]. According to WHO, in humans, breast milk is the most appropriate source of nutrition up to the age of 6 months[44].

2.10. FUNCTIONAL ANATOMY OF HUMAN BREAST DURING PREGNANCY :

The alveolar complexes of mammary glands increase in number during pregnancy. The cells lining the alveoli and small ducts mature, acquire the capability to secrete milk. During pregnancy, secretion of milk is kept in check by high concentration of circulating sex steroids, primarily Progesterone. A series of programmed changes occur during parturition, which transform the cells into the fully secretory state. This transformation leads to lactogenesis. The receptors present in the areola around the nipple carries sensory information about suckling to the higher centers like spinal cord and brain. This helps to regulate the release of oxytocin from the posterior pituitary and release of prolactin from the anterior pituitary gland. Beneath the areola, the mammary ducts expand slightly to form sinuses. Once lactogenesis occurs, milk is secreted and stored in the alveolar lumens until there is let-down reflex. Suckling reflex brings about contraction of the myoepithelial cells, forcing milk through the ducts to the sinuses beneath the areola where it becomes available to the suckling infant[45].

Figure 3 :THE MAMMARY ALVEOLUS :



(a)Alveolus (d) Duct. PC - Plasma cells[45]

In alveolar units, milk is produced and stored. Frequent contraction of myoepithelial cells causes removal of milk from alveoli into small sinuses as they near the areola where they open directly on the nipple[45].This is known as let-down reflex of milk and milk ejection reflex.

2.11. SYNTHESIS OF NUTRIENT COMPONENTS OF HUMAN MILK :

Mammary epithelial cells convert most of the precursors into milk constituents and transport them to the mammary lumen, so are responsible for milk ejection from the breasts. Neville MC, Allen JC et al explained that the mammary glands contain adipocytes, fibroblasts, plasma cells and blood vessels. During lactation, the blood flow is greatly expanded to make available the large amounts of substrate required for milk synthesis[44]. The stromal adipocytes and fibroblasts are known to be the source for growth factors such as hepatic growth factor and IGF-1,which in turn help for the synthesis of the enzyme, lipoprotein lipase. This is necessary for the milk lipid synthesis.

B - lymphocytes migrate to the mammary gland during lactation. They become plasma cells and stored in the interstitial spaces. They will produce the immunoglobulins that ultimately find their way into milk. Thus the mammary epithelium acts as an integrator of activities for the synthesis of milk[44].

2.12. CELLULAR MECHANISMS FOR MILK SYNTHESIS AND SECRETION:

Neville in his study explained about the secretory processes which are well organized in the mammary epithelial cells of the mammary gland during lactation [46] as shown in figure- 3. Linzel and Peaker in their study have mentioned regarding the mechanism of secretion of milk [47].

I. Exocytosis

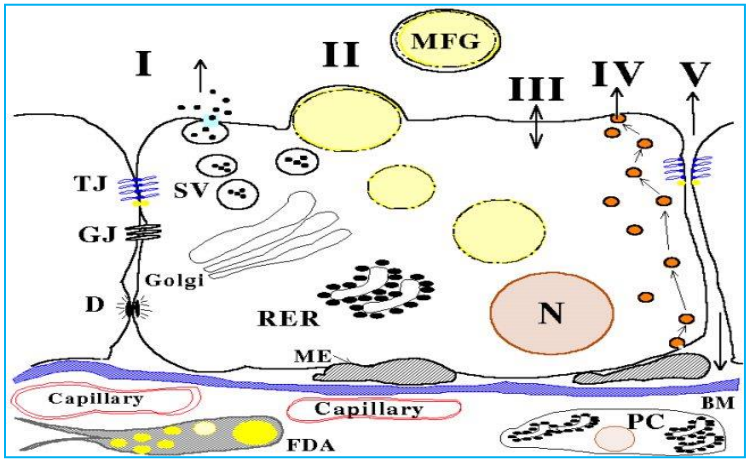
II. Synthesis of lipids.

III. Transport of ions and water across the apical membrane.

IV. Transcytosis of interstitial molecules.

V. Paracellular transport.

Figure 4 :Alveolar Cell of lactating mammary gland[46] :



N- nucleus, TJ- tight junction, GJ- gap junction, D- desmosome, SV- secretory vesicle, FDA- fat-depleted adipocyte, PC- Plasma Cell, BM- basement membrane, ME- cross section through process of myoepithelial cell, RER- rough endoplasmic reticulum. I, II, III, IV, V :Secretory pathways.

I. Exocytosis:

The proteins are synthesized on the ribosomes. They are transferred to rough endoplasmic reticulum. There their signal sequences are cleaved and the protein molecules are folded. Then they are transferred by the vesicles to the Golgi apparatus. Golgi vesicles also synthesize lactose from UDP-galactose and glucose. These precursors are from cytoplasm. Trans - Golgi has

swollen appearance. In the cis-Golgi with condensation of casein molecules, there is formation of casein micelle. With the addition of calcium, there will be maturation of the casein micelles.

The source for most of the constituents of the aqueous phase of milk including Calcium, Phosphate, Citrate, nucleotides, probably monovalent ions and glucose are the secretory vesicles. These vesicles fuse with plasma membrane release their contents into the milk space by exocytosis. After parturition, exocytosis begins and continues with the lactation period [48]. Thus, many components of milk are secreted by exocytosis.

II. Synthesis of lipids :

The smooth endoplasmic reticulum synthesize triglycerides from precursors, fatty acids and glycerol. They combine to form large droplets. Then, they separate from the cell as milk fat globule [46].

III. Transport across the apical membrane of alveolar cells:

Linzell and Peakerin 1971 demonstrated that Sodium, Potassium, Chloride, monosaccharides and water directly permeate the epithlelial membrane[47] but Calcium, Phosphate and Citrate did not permeate [49]. Linzel and Peaker in 1975 observed the presence of chloride-bicarbonate exchange at the apical membrane[50].Neville et al in 1990,have proved the presence of a glucose pathway across the apical membrane. Stable isotope investigations were carried out to study human mammary glands [44]. Fleishaker & McNamarain (1988) in their study indicated that across both basolateral and apical membranes in mammary alveolar cell, drugs enter by direct transfer [51].

IV. Transcytosis of Interstitial Molecules:

By transcytosis or through paracellular pathway, intact proteins cross the mammary epithelium from the interstitial fluid. During lactation, only transcytotic pathway works. Immunoglobulins enter milk via transcytosis. In 1983, Hayward investigated that IgA is synthesized by plasma cells of the mammary gland in most non-ruminants [52]. IgA binds to receptor at apical membrane. At the apical membrane, extracellular portion of the receptor is cleaved. The cleaved receptor portion is called secretory component. So, the secreted product is called secretory IgA or sIgA. By this pathway, hormones, proteins and growth factors are also secreted.

V. The Paracellular Pathway:

Lin et al. in 1995[53] explained that tight junctions between epithelial cells will not allow the substances to pass during lactation. They become leaky and allow many components from the interstitial space to pass into the milk during pregnancy with mastitis and after involution as studied by Morton in 1994[54].

2.13. LACTOGENESIS:

Lactogenesis is the onset of milk secretion. Hartmann (1973) and Fleet et al (1975) divided lactogenesis into two stages.

Lactogenesis Stage - 1 (L-I) : Mammary gland becomes sufficiently differentiated for the secretion of specific milk called colostrums. It is secreted in very small quantities.

Lactogenesis Stage – 2 (L-II) : It is defined as the onset of copious milk secretion[43, 55].

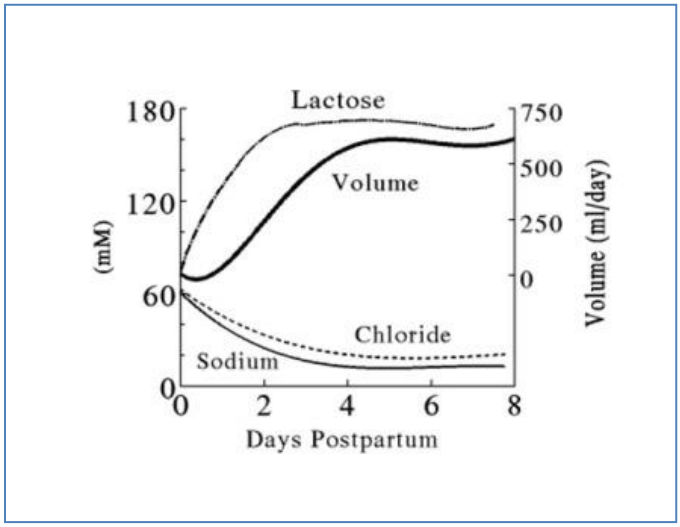
Lactogenesis occurs due to sudden fall in the progesterone levels during parturition and also in the presence of maintained Prolactin levels. The secretion product of the mammary gland in early post-partum period is known as colostrum. It contains high levels of immunoglobulins and lactoferrin, the protective protein. In species such as ruminants there is no transplacental transport of the immunoglobulins. In humans, there is transplacental transport of immunoglobulins. It provides the humoral immunity in the early post-partum period. The secretory IgA, lactoferrin and high concentrations of oligosaccharides protect the mucosal surfaces from infection[56].

2.14. CHANGES IN MILK COMPOSITION AND VOLUME IN EARLY POSTPARTUM PERIOD :

Kulski JK and Hartmann PE in 1981 suggested that lactogenesis represents a profound and rapid series of changes in the activity of differentiated mammary epithelial cells from a quiescent state to a fully active secretory state. During the first week postpartum, a series of events occur - there is closure of the tight junctions between the myoepithelial cells followed

by a transient increase in the secretion of the protective proteins sIgA and lactoferrin in the mammary glands. The synthesis of all other components of milk occurs after about 36 hrs. The synthesis of mature milk begins, that completes around day 5 postpartum in humans as shown in figure-5 [57].

Figure 5 : Composition and volume changes of human milk [57] :



In the above graph, the concentration scale for lactose Naand Cl, whose concentrations change immediately afterdelivery, is on the left. Volume increases from 50 ml/day on the day 1 to 600 ml/day on day 5 with the greatest increase taking place between second to fourth day [57].

Neville et al. in 1991explained that the first change that occurs is a fall in Sodium, Chloride levels and increase in lactose levels in human milk. This occurs immediately after child birth and mostly completes by 72 hours Postpartum[44]. After 36 hours postpartum, there is onset of copious milk production ie, there is the increase in milk volume due to the closure of the tight

junctions in the mammary epithelium. The paracellular pathway also blocks and so lactose cannot pass into the plasma. The Sodium and Chloride ions also cannot pass from the interstitial space into the lumina of the mammary alveoli. So, this phase is characterized by rapid decline in sodium, chloride ions of milk but a rapid increase in the lactose levels in human milk.

There is an increase in sIgA and lactoferrin, which comprise 10% by weight during the first 48 hrs postpartum. Linzell JL and Peaker M in 1974 suggested that Colostrum contains high levels of lymphocytes, macrophages, neutrophils and sloughed secretory epithelium [58].

Kulski JK and Hartmann PE in 1981 suggested that on first day after postpartum the milk volume is about 50 ml/day, between 36-48hrs volume reaches around 250-300ml/day, on fifth day it reaches to 500-750ml/day ie, it is about 10 fold increase when compared with the first day volume [57].

Studies by Vonderhaar BK and Bremel RD in 1997 indicated that the factors most essential for the initiation of series of events and changes that occur in the mammary epithelium that constitute lactogenesis stage-II. It is also necessary that there should be Progesterone withdrawal, maintained plasma Prolactin and removal of milk from the breast at regular intervals after postpartum. But the molecular mechanisms by which prolactin regulates milk protein synthesis are still unclear [59].The specific mechanisms by which Progesterone and milk removal interact with the mammary epithelial cell at parturition have not been studied, perhaps because no in vitro model system exists that mimics lactogenesis stage-II or because of complexity of changes that must be coordinated during the process of milk synthesis and secretion [59].

2.15. STRESS AND LACTATION :

Human milk has unique composition ideal for the growth and development of human infants[44] .With the benefits of human milk well recognized, it is essential to focus on how to optimize lactation for the benefit of newborns particularly at the times of stress. Stress interferes with lactation performance of humans. Lactation insufficiency for many times is blamed on stresses such as those imposed by preterm delivery, infant medical conditions, stressful life events, maternal lifestyle etc[60, 61].

The research on the effect of stress on lactation was started with animal experiments. Animal studies have demonstrated suppression of lactation when exposed to certain types of stressful stimuli[62] .

Major factors associated with the interaction of stress and lactation based on animal studies are - stress may hamper lactation directly by inhibiting Prolactin, Oxytocin or indirectly by acting on specific regions in the CNS such as through activation of sympathetic CNS[63, 64, 65, 66, 67]. Sympatho-adreno-medullary (SAM) system also has been demonstrated for the peripheral inhibition of milk ejection. This occurs by vasoconstriction at the level of the mammary glands, stimulation of mammary myoepithelial cells or potential increase in mammary ductal tone [63, 68,69]. The effect of stress on lactation is a complex process. Depending on the duration of stress and the site of action, there will be suppression of lactation and may result from reduction in milk synthesis or ejection [70].Most of the studies were performed during

established lactation, not during lactogenesis. So, this study is conducted to assess the effect of chronic stress on lactogenesis.

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CHAPTER - III

AIMS, OBJECTIVES AND HYPOTHESIS

3.1 OBJECTIVES OF THE STUDY:

Objectives of the present study are -

1. To determine the relationship between chronic stress, level of maternal Cortisol in 1st, 2nd and 3rd trimesters of pregnancy.

2. To evaluate the association of socio-demographic parameters with maternal stress.

3. To observe the relation between maternal Prolactin and milk volume in the first week of postpartum.

4. To assess the relation between chronic stress and milk output.

5. To evaluate the effect of chronic stress on lactogenesis in different age groups.

3.2 HYPOTHESIS:

NULL HYPOTHESIS (H_0):

Chronic stress may not cause significant increase in maternal serum cortisol level. There may not be significant difference in milk volume output in subjects at different levels of stress. Chronic stress may not have significant effect on lactation in humans.

ALTERNATE HYPOTHESIS (H_1):

Chronic stress causes significant increase in maternal serum cortisol levels. There is significant difference in milk volume output in subjects at different levels of stress. Chronic stress has significant effect on lactation in humans.

CHAPTER – IV

MATERIALS AND METHODS

CHAPTER – IV :MATERIALS AND METHODS :

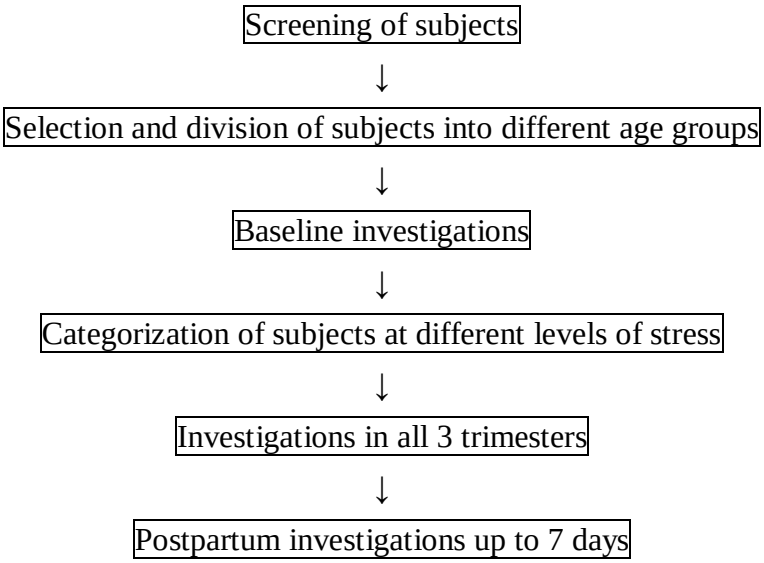
4.1. STUDY DESIGN:

A cross sectional prospective study was conducted in pregnant women in the reproductive age between 21 and 45 years. Volunteers were screened at visits 1-10. Out of 10 visits, 3 were held before delivery and 7 were held during postpartum period. It is a follow-up study. The pregnant women were screened in 1st, 2nd and 3rd trimesters as well as during postpartum up to 7 days.

4.2. JUSTIFICATION FOR STUDY DESIGN:

As per the guidelines of WHO, Geneva, baseline for the study was designed [1] . The study was conducted up to 7 days postpartum as per the research conducted by Dewey KG et al in 2001 [2] , Neville M and Morton J [3] , Newton M and Newton N R in 1948 [4] and Lau C in 2001[5].

CHART-1. Flow chart showing participants under study :



4.3. STUDY PARTICIPANTS:

The study participants were in the reproductive age between 21-45 years. Pregnant women attending the antenatal clinic at Prathima Institute of Medical Sciences, Nagunur, Karimnagar were recruited in the study. Women were from and around Karimnagar.

4.4. SAMPLE SIZE:

A total of 128 pregnant women were included in the study. With 95% confidence level and margin of error of ±10%, a sample size of 96 subjects were allowed to participate in the study to determine the effect of different chronic stress levels on lactogenesis.

The formula[6] used was:

$$n = \frac{z^2 p(1-p)}{d^2}$$

Where, n= sample size

z = z statistic at 5% level of significance (1.96)

d = margin of error

p = anticipated prevalence rate (50%)

$$n = \frac{1.96 \times 1.96 \times 0.5 (1-0.5)}{0.1 \times 0.1}$$

$$n = \frac{1.96 \times 1.96 \times 0.5 \times 0.5}{0.01}$$

$$n = \frac{0.9604}{0.01}$$

n = 96.04

Out of 128 pregnant women who were selected for the study, 32 women did not co-operate for follow up study. So, 96 women remained in the study group.

Total sample size = 96 + 33% of 96 (non- response rate)

= 96 +32

= 128

4.5. INCLUSION AND EXCLUSION CRITERIA:

a. INCLUSION CRITERIA:

Criteria for including subjects in the study group were:

i. Participants were pregnant women.

ii. Women were in the reproductive age between 21 – 45 yrs.

iii. Women who fulfilled the selection criteria as per Holmes and Rahe stress scale.

iv. Women who agreed to be in the study group till 7 days postpartum.

b. EXCLUSION CRITERIA:

Criteria for excluding subjects in the study group were:

i. Pregnant women with history of non lactogenesis.

ii. Women with no intention to breastfeed.

iii. Alcoholics, smokers, known diabetics, with h/o major endocrinal abnormality.

iv. Women who were HbSAg or HIV positive.

4.6. CRITERIA FOR DISCONTINUATION :

i. Participant refusal during follow-up study.

ii. Participant refusal for baby test weighing method.

4.7. ETHICS:

a. INFORMED CONSENT :

Participants were informed about the purpose of the study and the procedures involved. Subjects were explained about their role in the study. Participants were assured that despite entering in the study they could withdraw at any time they wish and their information would be kept confidential. Informed written consent was obtained from each of volunteered participants (Appendix – I).

b. INSTITUTIONAL APPROVAL :

The study was approved by institutional ethical committee and ethical clearance certificate was obtained in this regard.

c. DECLARATION FROM HELSINKI :

Declaration of Helsinki were followed during the entire study.

4.8. STUDY PERIOD :

A cross sectional prospective study was conducted during the period 2012 – 2015 in Karimnagar, Telangana state.

4.9. STUDY PROTOCOL :

The participants (n= 128) were screened as per our inclusion and exclusion criteria from the antenatal clinic of Prathima Institute of Medical Sciences, Nagunur, Karimnagar and were enrolled for the study accordingly.

i. Informed consent was obtained from volunteered subjects (Appendix – I).

ii. At the onset of the study, a proforma was filled with information of individual subject in regard of general health status, history of past illness, obstetric history along with personal and family history (Appendix – II).

Women were divided into 5 age groups.

1. 21-25 years
2. 26-30 years
3. 31-35 years
4. 36-40 years
5. 41-45 years

4.10. BASELINE CHARACTERISTICS OF PARTICIPANTS:

The baseline characteristics of the participants for study were as follows:

- All participants were pregnant and were in the reproductive age.
- Participants were from rural area.
- All the participants accepted for the follow-up study.
- All participants were at different stress levels as per Holmes and Rahe stress scale.
- Physiological and anthropometric parameters of participants were in normal range.
- All the participants accepted for the baby test weighing method for milk volume measurements for 7 days after delivery.

4.11. DETAILS OF EXAMINATIONS AT EACH VISIT :

EXAMINATIONS BEFORE DELIVERY :

VISIT – I

i. Participant was in reproductive age between 21 – 45 years.

ii. Participant was in 1st trimester.

iii. Medical history, demographics and personal history were obtained.

iv. Pregnancy test was reported to be positive.

v. LMP and EDD were noted.

vi. Subjects were screened with Holmes and Rahe stress scale. Life Change Unit (LCU) score was obtained. Accordingly study group was categorized into different sub groups.

vii. Anthropometric parameters were recorded.

viii. Physiological parameters were recorded.

ix. Blood samples were collected with all aseptic precautions following standard protocol.Hematological and biochemical parameters were recorded.

x. With blood samples hormonal assays were done.

All the recordings were entered in concerned proforma (Appendix – II).

VISIT – II

i. Participant was in 2nd trimester.

ii. Screened with Holmes and Rahe stress scale, Life Change Unit (LCU) score was obtained.

iii. Anthropometric parameters were recorded.

iv. Physiological parameters were recorded.

v. Blood samples were collected with all aseptic precautions following standard protocol. Hematological and biochemical parameters were recorded.

vi. With blood samples hormonal assays were done.

All the recordings were entered in concerned proforma (Appendix – II).

VISIT – III

i. Participant was in 3rd trimester.

ii. Screened with Holmes and Rahe stress scale, Life Change Unit (LCU) score was obtained.

iii. Anthropometric parameters were recorded.

iv. Physiological parameters were recorded.

v. Blood samples were collected with all aseptic precautions following standard protocol. Hematological and biochemical parameters were recorded.

vi. With blood samples hormonal assays were done.

All the recordings were entered in concerned proforma (Appendix – II).

POSTPARTUM EXAMINATIONS:

VISIT – IV

i. Visit is on 1st day postpartum.

ii. Explained about importance of 1st day milk (colostrum) feeding and requested to feed the baby for 10 times / day.

iii. Milk volume was measured by baby test weighing method.

iv. Blood sample was collected after 1st breast feeding in the morning for hormonal investigations such as determining levels of Prolactin and Cortisol.

All the recordings were entered in concerned proforma (Appendix – II).

VISIT – V

i. Visit is on 2nd day postpartum.

ii. Mother was requested to feed the baby for 10 times / day.

iii. Milk volume was measured by baby test weighing method.

iv. Blood sample was collected after 1st breast feeding in the morning for determination of level of prolactin.

All the recordings were entered in concerned proforma (Appendix – II).

VISIT – VI

i. Visit is on 3rd day postpartum.

ii. Mother was requested to feed the baby for 10 times / day.

iii. Milk volume was measured by baby test weighing method.

iv. Blood sample was collected after 1st breast feeding in the morning for determination of level of prolactin.

All the recordings were entered in concerned proforma (Appendix – II).

VISIT – VII

i. Visit is on 4th day postpartum.

ii. Mother was requested to feed the baby for 10 times / day.

iii. Milk volume was measured by baby test weighing method.

iv. Blood sample was collected after 1st breast feeding in the morning for determination of level of prolactin.

All the recordings were entered in concerned proforma (Appendix – II).

VISIT – VIII

i. Visit is on 5th day postpartum.

ii. Mother was requested to feed the baby for 10 times / day.

iii. Milk volume was measured by baby test weighing method.

iv. Blood sample was collected after 1st breast feeding in the morning for determination of level of prolactin.

All the recordings were entered in concerned proforma (Appendix – II).

VISIT – IX

i. Visit is on 6th day postpartum.

ii. Mother was requested to feed the baby for 10 times / day.

iii. Milk volume was measured by baby test weighing method.

All the recordings were entered in concerned proforma (Appendix – II).

VISIT – X

i. Visit is on 7th day postpartum.

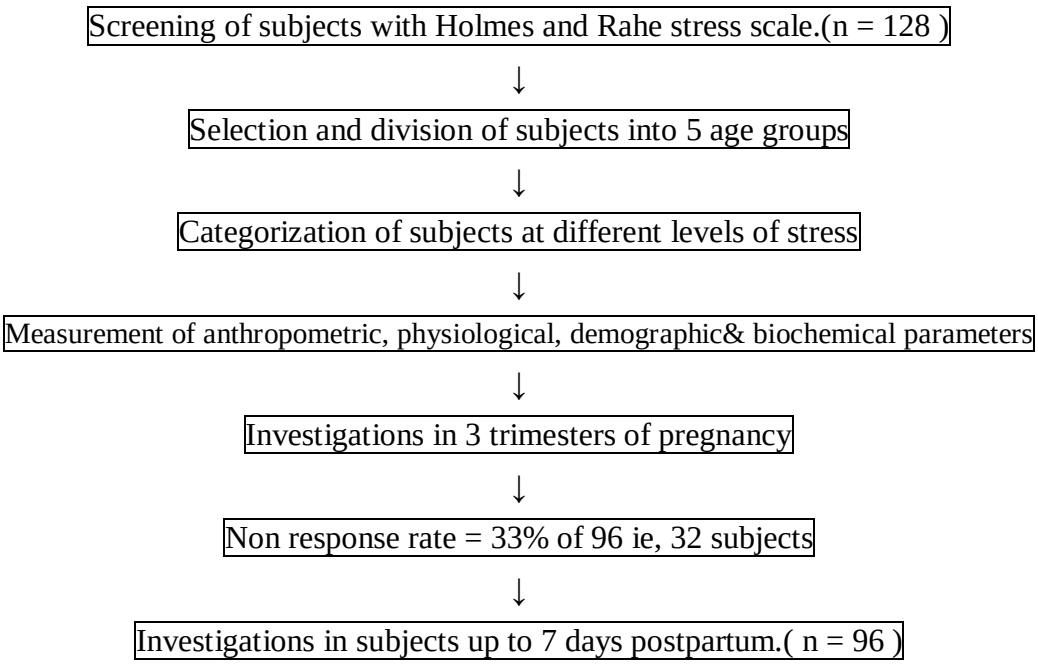
ii. Mother was requested to feed the baby for 10 times / day.

iii. Milk volume was measured by baby test weighing method.

All the recordings were entered in concerned proforma (Appendix – II).

Participants who were selected from screening at visit – 1 were allocated an identification number i.e. subject number. This allocated subject number was used to identify the volunteer during the entire study. They were then grouped under mild stress / control group, moderate and severe stress groups.

CHART -2. Flow chart showing participants after follow-up :



Pregnant women visiting the antenatal clinic were screened with Holmes and Rahe stress scale and selected for our study were = 128. Few participants rejected the follow-up study during pregnancy. Few participants refused for baby test weighing method. So, the number of subjects remained up to the end of our study were 96.

4.12. DETAILS OF PARAMETERS INCLUDED IN THE STUDY :

A. RECORDING OF ANTHROPOMETRIC PARAMETERS :

i. HEIGHT :

Height was measured by using stadiometer with subject standing in erect posture without foot wear to the nearest 0.5cm with shoulders in relaxed position and arms hanging freely and arms hanging freely and expressed in centimeters (cms).

ii. BODY WEIGHT :

Digital weighing machine was used to measure body weight with an accuracy of ±100g. Subjects were weighed without foot wears in light clothing and standing in errect posture.

iii. BODY MASS INDEX (BMI) :

Body Mass Index of each participant was calculated as body weight in kilograms divided by square of body height in meters.BMI = kg/m^2

B. RECORDING OF PHYSIOLOGICAL PARAMETERS :

i. HEART RATE (HR) :

Heart rate of individual participant was recorded in sitting posture. The radial pulse is examined by compressing the radial artery against the head of radius. For elucidation of the pulse, the forearm of the subject should be semipronated and the wrist was slightly flexed. Heart rate was recorded as beats/min.

ii. BLOOD PRESSURE (BP) :

Systolic and diastolic blood pressures were recorded (in mmHg) in each subject by using a sphygmomanometer in sitting posture. Three recordings were taken for each subject. Average of 3 measurements was taken for calculation.

C. MEASUREMENT OF STRESS BY HOLMES AND RAHE STRESS SCALE :

The Holmes and Rahe stress scale contains a list of 43 stressful life events which contribute to illness in adults. In 1967, Thomas Holmes and Richard Rahe (psychiatrists) tested about 5,000 medical patients to evaluate whether the stressful events might cause illness and got a positive correlation of 0.118 [7], so this scale is also known as Social Readjustment Rating Scale (SRRS) [8] . It is more commonly known as Holmes and Rahe stress scale [9] . Validity of the scale was done in 1970 by Rahe [10] . The scale was also assessed against different

population within United States with African, Mexican and white American[11] , also with Japanese [12] and Malaysian [13] groups.

We have used this scale for our study. Holmes and Rahe stress scale was used to measure stress. The number of "Life Change Units" (LCU) that apply to the events happened in the past year of an individual's life were added. The final score of LCU gave an estimate of how stress affects health. Accordingly stress was categorized into 3 levels of stress.

LCU <150 : Mild stress

LCU 150-299 : Moderate stress

LCU >300 : Severe stress

We have included questionnaire in regard of life events which occurred in past one year like death of spouse, divorce, marital separation, imprisonment, pregnancy, marriage, marital reconciliation, sexual difficulties, dismissal from work, change in financial state, change in sleeping or eating habits etc. to estimate the LCU score. Accordingly participants were categorized into mildly, moderately and severely stressed groups.

D. SOCIO-DEMOGRAPHIC PARAMETERS :

Participants were asked about –

i. Social class[14] - Economic status of the participant was assessed. Basing on per capita income, subjects were categorized into class-I to class-V.

The B. G. Prasad's scale first appeared in the Journal of Indian Medical Association in the year 1961.It was based on the consumer price index of 1960[15] . In India, B. G. Prasad's classification is one of the most commonly used scales for determining the socioeconomic class of an individual by researchers. Socio Economic Status is regarded as one of the important determinants of health, nutritional status and morbidity and mortality of an individual[16]. This is a classification based on per capita income of an individual. Hence it has to be constantly updated to take into account of inflation and depreciation of rupee. It can be applied to both urban as well as rural participants. The Consumer Price Index (CPI) for Industrial Workers (IW) is used to calculate the updated income categories. The Consumer Price Index should be updated every month. This is available at the Labour Bureau of India website on the last day of every month. There are state-specific CPI values, which are also available on the Department of Labour website. It should be used to determine more accurate income categories for the required study area and population[14]

ii. Educational status – divided into $<10^{th}$standard, $< 12^{th}$ standard, graduation and above.

iii. Occupation – divided into house wife, working women.

iv. Religion – categorized into Hindu, Muslim and Christian.

v. Family type – number of persons in the family was noted and categorized into joint/nuclear family.

COLLECTION OF BLOOD SAMPLES FOR HORMONAL ASSAY :

Collection of blood samples was done using standard aseptic precautions and in minimal time interval. Venous blood samples were collected from participants after an overnight fasting between 8.30am – 10.30am. Samples were collected in Ethylene Diamine Tetra Acetic acid (EDTA) bulbs. The samples were thoroughly but gently mixed with anticoagulant immediately. The blood samples were placed in evacuated tubes and then taken to laboratory in ice box. Serum samples for hormone assays were frozen at -20ºC. Serum levels of Cortisol and Prolactin were determined and analyzed.

E. ESTIMATION OF SERUM CORTISOL LEVEL:

Objective measurement was done by measuring the serum cortisol levels in all the three trimesters of pregnancy and postpartum. Blood samples(5-7ml) were collected by venepuncture between 8.30am – 10.30am with standard aseptic precautions. Serum samples were frozen at -20ºC. Elecsys cortisol reagent kit, Cat. No. 11875116 was used for quantitative determination of Serum cortisol by electrochemiluminescence immunoassay.

Principle :

Elecsys Cortisol makes use of a competition test principle using a polyclonal antibody which is cortisol specific. Endogeneous cortisol in the sample which has been liberated from the binding protein with Danazol competes with exogeneous cortisol derivative in the test which has been labeled with ruthenium complex i.e. Tris(2,2^{1}- bipyridyl) ruthenium(II) - complex

Ru(bpy)$^{2+3}$, for binding sites on the biotinylated antibody. The chemiluminescent emission was measured by photomultiplier.

Reagents :

Elecsys Cortisol reagent kit, Cat. No. 11875116 – 100 tests

M - Streptavidin coated microparticles, 1 bottle, 6.5 ml :

Streptavidin coated microparticles, 0.72 mg/ml; binding capacity; 470 ng biotin/mg microparticles; preservative.

R1- Anti-cortisol-Ab-biotin, 1 bottle, 9ml:

Biotinylated polyclonal anti-cortisol antibody (ovine) 90 ng/ml; MES buffer 100 mmol/L, pH 6.0; preservative.

R2 - Cortisol-peptide-Ru(bpy)$^{2+3}$, 1 bottle, 9 ml:

Cortisol derivative (synthetic), labeled with a ruthenium-complex 25 ng/ml; danazol 20 µg/ml; MES buffer 100 mmol/L, pH 6.0; preservative.

Procedure :

Total duration of assay is 18 min.

- 1stincubation: 20 µl of serum sample is incubated with a cortisol - specific biotinylated antibody and a ruthenium complex labeled cortisol derivative. Depending on the concentration of the analyte in the sample and the formation of the respective immune complex, the labeled antibody binding site is occupied in part with ruthenylatedhapten.

- 2nd incubation: After the addition of streptavidin - coated microparticles, the complex becomes bound to the solid phase via the interaction of biotin and streptavidin.
- The reaction mixture is aspired into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Procell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

F. ESTIMATION OF SERUM PROLACTIN LEVEL:

Antenatal and postpartum blood samples (5ml) were collected from 1st to 5th day with standard aseptic precautions. Serum samples for hormone assays were frozen at -20ºC. Elecsys Prolactin reagent kit, Cat. No. 03203093 – 190 was used for Serum Prolactin (µg/L) quantitative determination by electrochemiluminescence immunoassay.

Principle :

The Elecsys prolactin makes use of sandwich principle.Elecsys prolactin uses two monoclonal antibodies specifically directed against human prolactin. Both antibodies show a low reactivity with most forms of macroprolactin.

Reagents :

Elecsysprolactinreagent kit, Cat. No. 03203093 – 190 for 100 tests

M - Streptavidin coated microparticles, 1 bottle, 6.5 ml :

Streptavidin coated microparticles, 0.72 mg/ml; preservative.

R1- Anti- prolactin -Ab-biotin, 1 bottle, 10 ml :

Biotinylated monoclonal anti- prolactin antibody (mouse) 0.7 mg/ L ; phosphate buffer 50 mmol/L, pH 7.0; preservative.

R2 - Anti-prolactin-Ab-Ru(bpy)$^{2+3}$, 1 bottle, 10 ml:

Monoclonal anti- prolactin antibody labeled with a ruthenium-complex 0.35 mg/ L; phosphate buffer 50 mmol/L, pH 7.0; preservative.

Procedure :

Total duration of assay is 18 min.

- 1st incubation : 10 µl of serum sample and a biotinylated monoclonal prolactin-specific antibody form a first complex.
- 2nd incubation : After addition of a monoclonal prolactin-specific antibody labeled with ruthenium complex ie, Tris(2,2^{1}- bipyridyl)ruthenium(II)-complex Ru(bpy) $^{2+3}$ and streptavidin - coated microparticles, a sandwich complex is formed and becomes bound to the solid phase via the interaction of biotin and streptavidin.
- The reaction mixture is aspired into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Procell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

G. MEASUREMENT OF MILK VOLUMES :

Women were encouraged to feed the baby for 10 times per day (24 hrs) from day 1 to day 7 postpartum. Milk volume was measured by test weighing method on electronic digital weighing scale to the nearest of 0.01g [17].

Test weighing method : Goldtech digital baby weighing scale was used to measure the weight of the baby.

Procedure:

The infant was undressed and weighed by an appointed nurse on the digital baby weighing scale. The weight of the baby was noted. The baby was wrapped in a cloth and given immediately to the mother for breastfeeding. The time was noted. After 15 minutes, baby was taken from the mother, weighed undressed and noted the weight in grams. The difference in the weight of the baby before and after breast feeding was calculated. The difference in the weight gives the amount of milk fed to the baby [17]. Savenije and Brand[18] in their study concluded that the weight of the breast milk in grams is nearly equivalent to its volume in milliliters and is one of the precise method of determining the amount of breast milk produced. [19, 20, 21, 22, 23]

This was the procedure for baby test weighing method for one session.

A log book was maintained. The date of the test, time of each session and duration of each breastfeeding session were noted. The milk volumes of 12 sessions were added to get total milk volume per day.

This method was repeated for 12 times per day for 7 days after the delivery.

4.13. STATISTICAL ANALYSIS:

Results were recorded. Data was analyzed. All characteristics were summarized descriptively.

For all continuous variables, the summary statistics of N, mean and standard deviation (SD) were used.

For all categorical data, numbers and percentages (%) were used in the data summaries. Chi-square (χ^2)/Freeman-Halton Fisher exact test was employed to determine the significance of differences between groups for categorical data.

The difference of the means of analysis variables between two independent groups was tested by unpaired t test. The difference of the means of analysis variables between two time points in same group was tested by paired t test. The difference of the means of analysis variables between more than two independent groups was tested by ANOVA and F test of testing the equality of Variance. Bivariate correlation analysis using Pearson's correlation coefficient (r) was used to test the strength and direction of relationships between the interval levels of variables.

If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant.

Data were analyzed using SPSS software v.23.0. and Microsoft office.

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CHAPTER – V

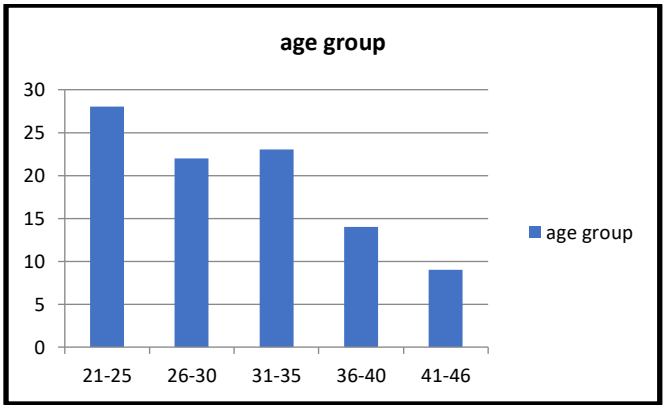
RESULTS

Study participants were divided into 5 age groups. These groups are made to observe the relation between, lactogenesis and age variation.

TABLE-1 : Age-wise distribution of pregnant women included in the study :

Sl no	Age groups (yrs)	No. of subjects
1	21-25	28
2	26-30	22
3	31-35	23
4	36-40	14
5	41-45	9
	Total	96

GRAPH-1 : No. of pregnant women and age wise distribution :

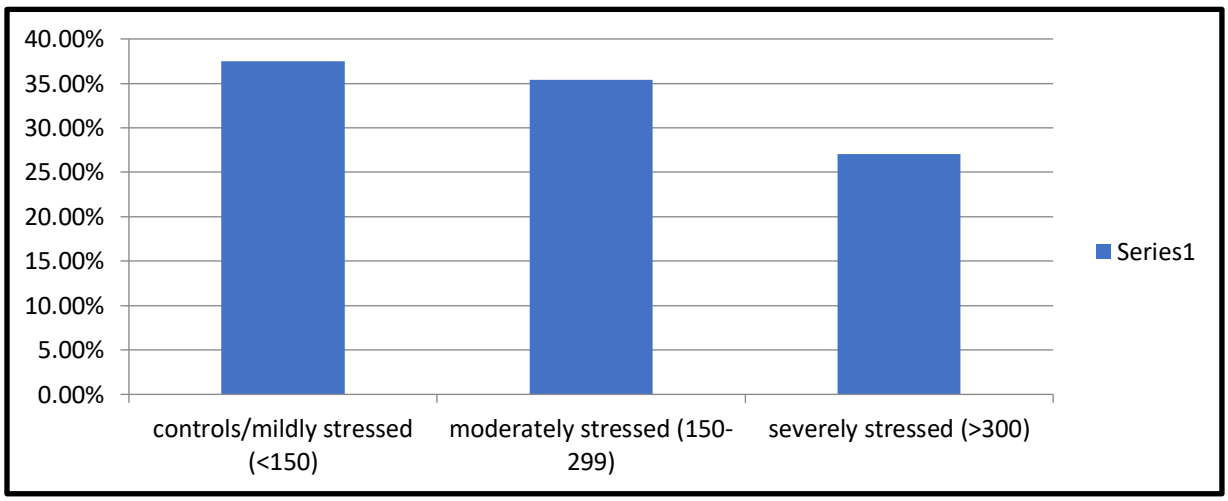


In the age group 21-25 years, the number of subjects were 28. In the age group 26-30 years, the number of subjects were 22. In the age group 31-35 years, the number of subjects were 23. In the age group 36-40 years, the number of subjects were 14. In the age group 41-45 years, the number of subjects were 9. Thus, the total number of participants in our study was 96.

TABLE-2 : Stress levels in different age groups :

Age groups (yrs)	Control or mildly stressed (<150)	Moderately stressed(150-299)	Severely stressed (>300)	Total
20 – 25	14	7	7	28
26 – 30	8	9	5	22
31 – 35	8	9	6	23
36 – 40	4	7	3	14
41 – 45	2	3	4	9
	36(37.5%)	35 (36.5%)	25 (26%)	96

GRAPH-2 : Number of subjects (%) at different stress levels :



Subjects were categorized into groups as per Holmes and Rahe stress scale. As pregnancy itself can cause stress, mildly stressed women are considered as control (n = 36). Among total number of study subjects 37.5% were mildly stressed, 36.5% were moderately stressed and 26 % were severely stressed.

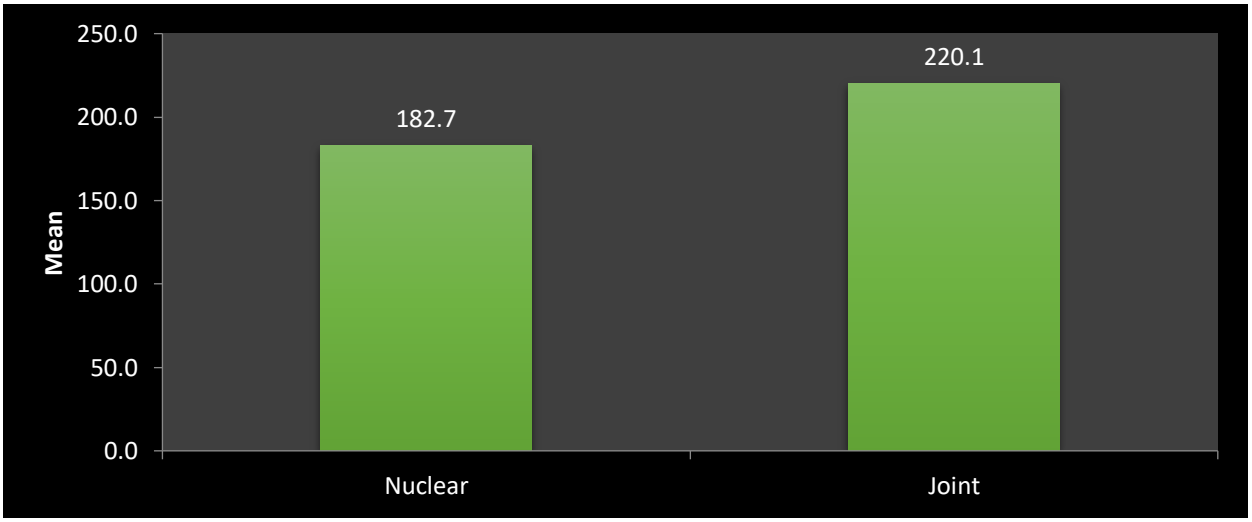
RELATION BETWEEN SOCIO-DEMOGRAPHIC PARAMETERS AND STRESS :

FAMILY TYPE (NUCLEAR/ JOINT) :

Table - 3: Relation between family type (nuclear/joint) and stress:

Type of Family	No. of subjects	Min	Max	Mean	SD	t value	p value
Nuclear	18 (19%)	100	313	182.7	68.4	-1.6	0.108
Joint	78 (81%)	100	408	220.1	92.0		

Graph-3 : Mean Stress levels between family types :



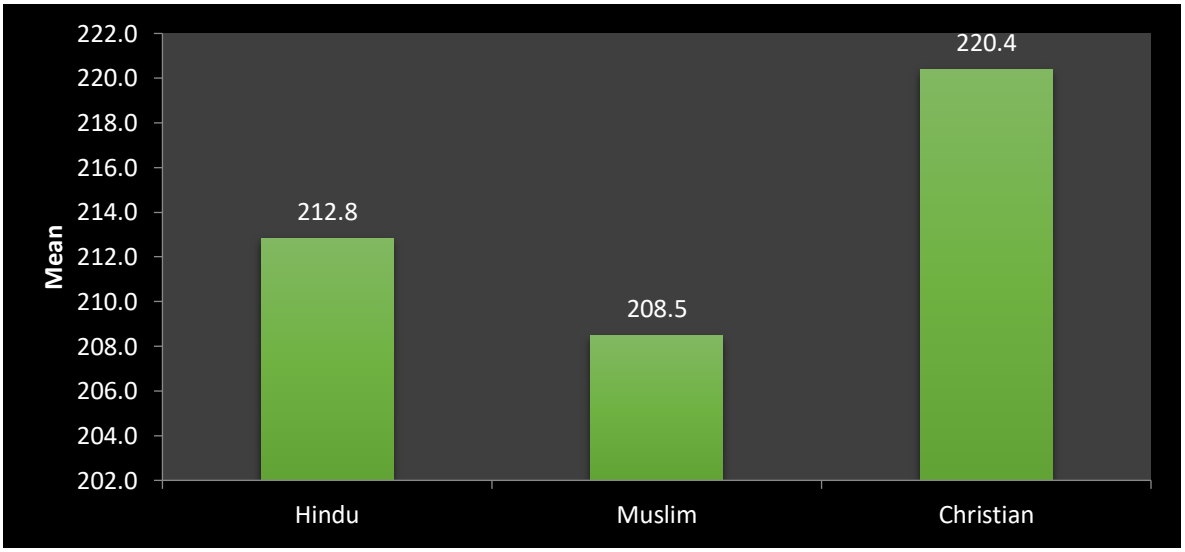
Basing on the number of members in the family and their relationship, women were categorized into two groups ie, nuclear/joint. Mean and standard deviation of the level of stress in both types were estimated. Nuclear family women had the mean stress level of 182.7±68.4, joint family women had the stress level of 220.1±92.0. Stress level was comparatively higher in women of joint family when compared to women of nuclear family. But no significant difference was observed ie, no significant effect of stress was observed between the two family types.

RELIGION :

Table-4: Relation between religion and stress:

Religion	No.of subjects	Min	Max	Mean	SD	F value	p value
Hindu	48 (50%)	100	408	212.8	96.2	0.1	0.902
Muslim	28 (29%)	100	340	208.5	86.6		
Christian	20 (21%)	120	372	220.4	77.2		

Graph-4 : Mean Stress level between Religions :



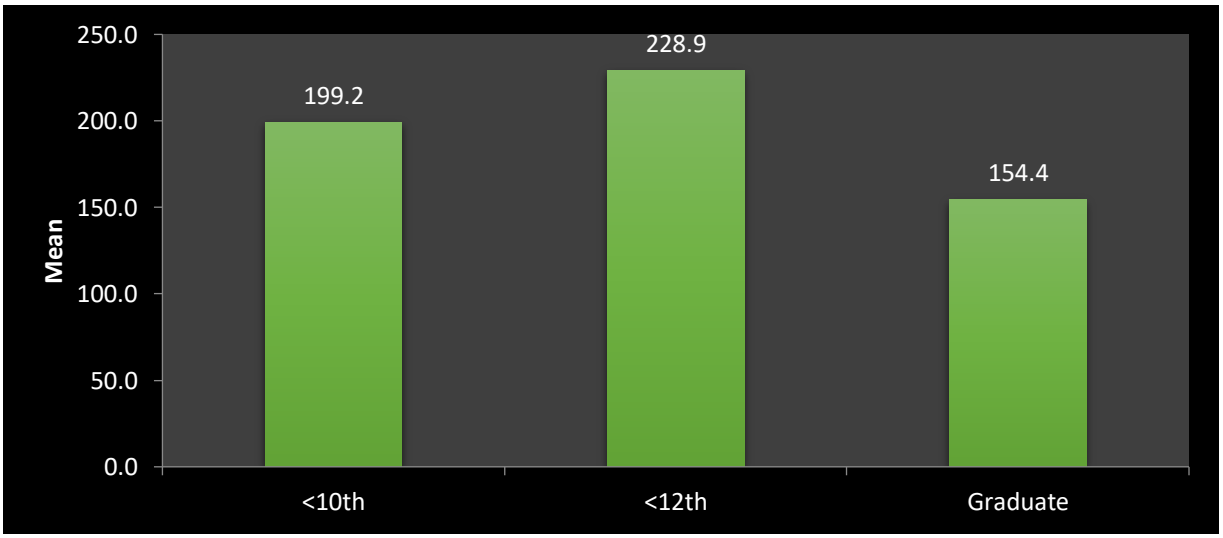
Participants were inquired about their religion and categorized into Hindus, Muslims and Christians. The mean stress level in Hindu females was 212.8±96.2, Muslim was 208.5±86.6 and Christian was 220.4±77.2. Very small variation indicates no much difference in the effect of stress on different religions.

EDUCATION :

Table-5: Relation between educational status and stress:

Education	No. of subjects	Min	Max	Mean	SD	F value	ANOVA p value
<10th	31 (32%)	104	408	199.2	84.5	3.16	0.047*
<12th	57 (60%)	100	402	228.9	90.3		
Graduate& above	8 (8%)	100	310	154.4	68.8		

Graph-5 : Mean Stress level at different education levels :



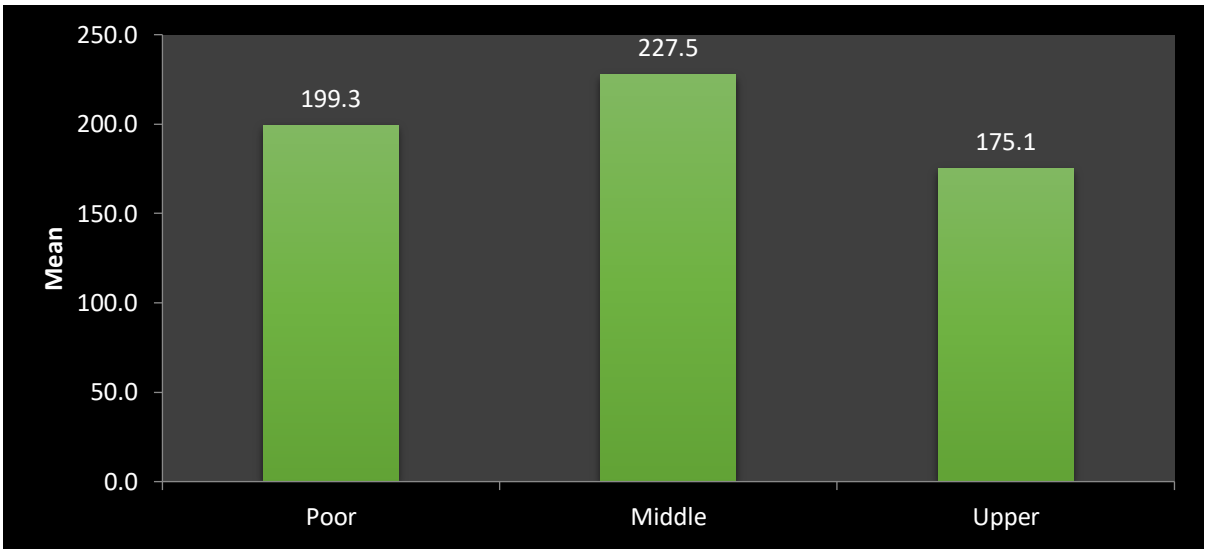
Based on education, women were categorized into <10th class, <12th standard and graduates& above. The mean stress levels were 199.2±84.5, 228.9±90.3 and 154.4±68.8 respectively. Stress level was significantly lower in graduates when compared with other categories. It indicates the awareness of well-educated females regarding stress and its adverse effects.

ECONOMIC STATUS :

Table-6: Relation between different social class and stress:

Economic status	No. of subjects	Min	Max	Mean	SD	F value	p value
Class I	23 (24%)	100	408	199.3	89.0	2.40	0.097
Class II	59 (61%)	100	402	227.5	90.0		
Class III-V	14 (15%)	104	324	175.1	73.8		

Graph-6 : Mean stress level and economic status of subjects :



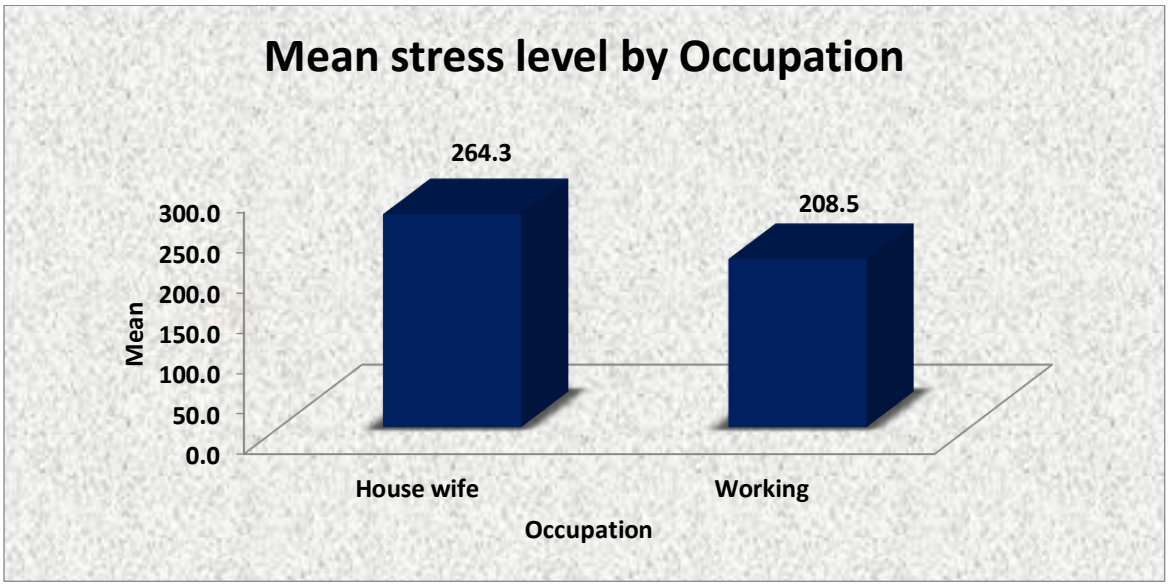
The mean stress level in class I was 199.3±89.0, in class-II was 227.5±90.0 and class III-V was 175.1±73.8. Mean stress in class III-V females was less but was statistically insignificant.

OCCUPATION :

Table-7: Relation between occupation of subjects and stress:

Occupation	No. of subjects	Min	Max	Mean	SD	t value	p value
Working	8(8%)	112	394	264.3	98.9	-1.7	0.09
House wife	88 (92%)	100	408	208.5	87.2		

Graph – 7 :Mean stress level vs occupation :



Participants were categorized into working and non-working group. The mean stress levels were 264.3± 98.9 and 208.5± 87.2 respectively indicating no significant difference. So occupation status may not affect the stress.

INFLUENCE OF CORTISOL DURING PREGNANCY AND POSTPARTUM:

Table-8: Comparison of the Cortisol level (µg/dl) with Stress level for different age groups (Years):

Age group	Trimester & Post partum	Cortisol level (Mean±SD)			F value	ANOVA p value	Tukey test multiple comparison		
		In mildly stressed	In moderately stressed	In severely stressed			p value b/w Mild & Moderate	p value b/w Moderate & Severe	p value b/w Mild & Severe
21-25	1st trim	13.3±1.7	20.5±1.6	21.7±1.7	71.7	<0.001*	<0.001*	0.427	<0.001*
	2nd trim	21.7±1.8	26.3±1.2	27.1±1.1	38.6	<0.001*	<0.001*	0.583	<0.001*
	3rd trim	26.2±1.5	29.5±3.2	32.4±2.3	19.4	<0.001*	0.009*	0.054	<0.001*
	Post Partum	26.2±1.4	31.7±3.3	34.6±3	31.9	<0.001*	<0.001*	0.084	<0.001*
26-30	1st trim	13.5±1.4	20.5±1.3	22.2±0.9	98.5	<0.001*	<0.001*	0.068	<0.001*
	2nd trim	21.6±1.3	25.1±1.8	26.3±2.6	12.8	<0.001*	0.002*	0.505	0.001*
	3rd trim	26.5±1.7	30±2.8	32.4±2.4	9.9	0.001*	0.022*	0.178	0.001*
	Post Partum	26.5±1.9	31±3.6	33.7±3.1	9.9	0.001*	0.016*	0.244	0.001*
31-35	1st trim	15.3±1.6	21±2.6	24.1±3.2	23.4	<0.001*	<0.001*	0.065	<0.001*
	2nd trim	23.4±1.6	26.2±3	28.9±5	5.0	0.017*	0.19	0.291	0.014*
	3rd trim	27.2±2.4	29.7±2.7	33.8±3	10.5	0.001*	0.145	0.024*	0.001*
	Post Partum	27±2.5	30.4±2.8	34.5±3.2	12.1	<0.001*	0.061*	0.029*	<0.001*
36-40	1st trim	14.8±2.7	20.8±1.2	25.8±1.9	30.1	<0.001*	0.001*	0.007*	<0.001*
	2nd trim	21.1±3.3	26.7±1.6	27.8±0.3	11.7	0.002*	0.003*	0.752	0.004*
	3rd trim	25.9±2	29.9±3.2	35±4.8	6.7	0.013*	0.166	0.106	0.01*
	Post Partum	25.8±2.2	30.8±3.8	35.2±5	5.6	0.021*	0.127	0.235	0.017*
41-45	1st trim	16.5±0.2	19.7±1.1	22.4±2.5	6.6	0.03*	0.231	0.23	0.026*
	2nd trim	25±0.6	26.6±2.8	26.5±3.3	0.3	0.785	0.806	1	0.802
	3rd trim	26.6±3.6	29.3±4.6	33.2±4.1	1.8	0.243	0.768	0.49	0.244
	Post Partum	27.5±3.4	29.7±5	33.3±4	1.4	0.321	0.835	0.553	0.326

Mean Cortisol levels in 1st trimester of mild, moderate and severely stressed women were between 13-16 µg/dl, 19-21µg/dl and 21-25 µg/dl respectively. Mean Cortisol levels in 2nd trimester of mild, moderate and severely stressed women were between 21-25 µg/dl, 25-26µg/dl and 26-28 µg/dl respectively. Mean Cortisol levels in 3rd trimester of mild, moderate and severely stressed women were between 25-27 µg/dl, 29-30µg/dl and 32-35 µg/dl respectively. Mean Cortisol levels were rising from 1st to 3rd trimester of pregnancy. Cortisol levels were

significantly increased in moderately and severely stressed women in comparison with mildly stressed women.

Table-9 : Mean and SD of cortisol levels of study subjects in 3 trimesters& postpartum at different stress levels :

Stress levels	Cortisol in 1st tri	Cortisol in 2nd tri	Cortisol in 3rd tri	Postpartum cortisol
<150	14.15±1.91	22.16±1.99	26.48±1.85	26.50±1.91
150-299	20.44±1.33	25.91±1.79	29.72±2.95	30.79±3.39
>300	23.10±2.53	27.56±3.09	33.06±2.97	34.15±3.19

Graph-8 : Mean and SD of cortisol levels of all study subjects in 3 trimesters, postpartum at different stress levels :

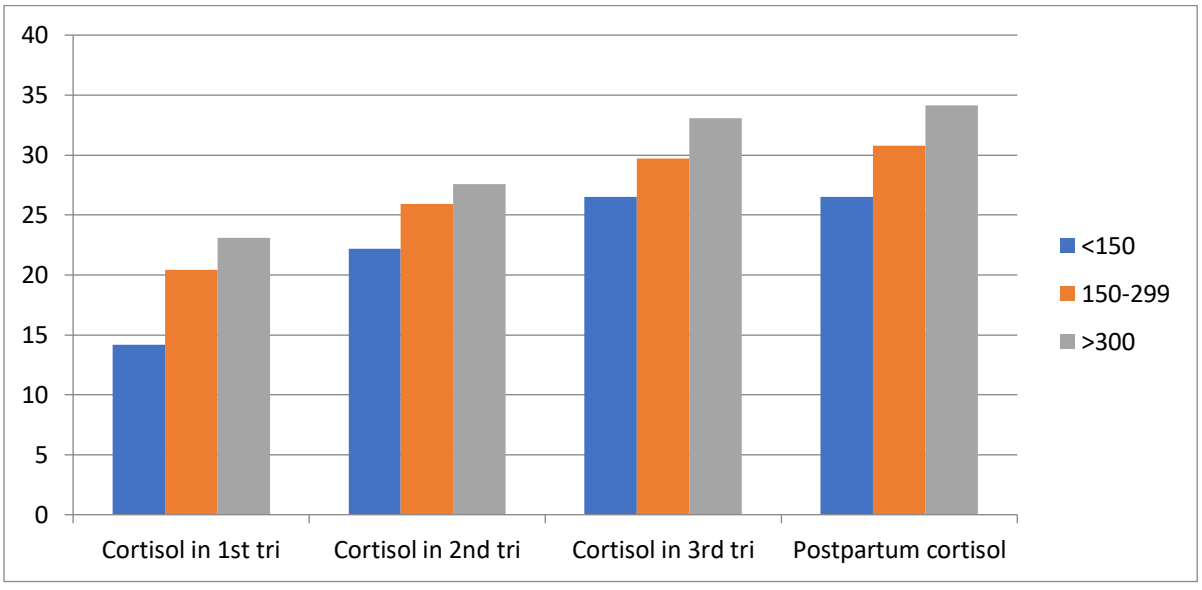


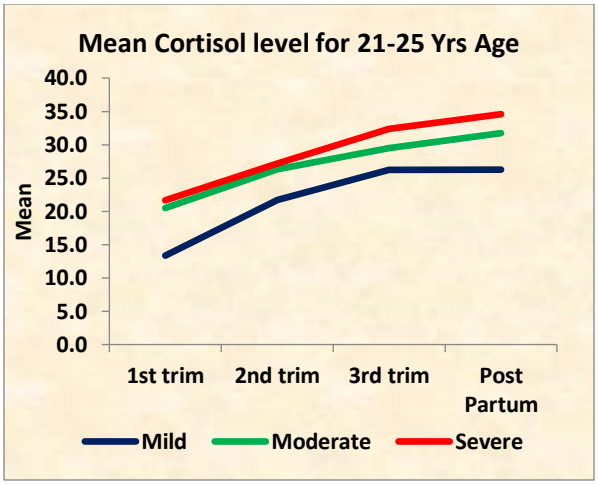
Table-10: Comparison of age, stress level and mean Cortisol level (µg/dl) for 3rd trimester & Postpartum:

Age group	Stress level	Cortisol level (Mean±SD)		t value	p value
		3rd trimester	Post Partum		
21-25	Mild	26.2±1.5	26.2±1.4	-0.5	0.62
	Moderate	29.5±3.2	31.7±3.3	-5.2	0.002*
	Severe	32.4±2.3	34.6±3	-2.9	0.027*
26-30	Mild	26.5±1.7	26.5±1.9	0.0	0.979
	Moderate	30±2.8	31±3.6	-3.4	0.009*
	Severe	32.4±2.4	33.7±3.1	-1.8	0.152
31-35	Mild	27.2±2.4	27±2.5	2.1	0.077
	Moderate	29.7±2.7	30.4±2.8	-3.0	0.016*
	Severe	33.8±3	34.5±3.2	-2.7	0.043*
36-40	Mild	25.9±2	25.8±2.2	0.8	0.507
	Moderate	29.9±3.2	30.8±3.8	-2.3	0.062
	Severe	35±4.8	35.2±5	-1.2	0.362
41-45	Mild	26.6±3.6	27.5±3.4	-7.2	0.088
	Moderate	29.3±4.6	29.7±5	-1.8	0.214
	Severe	33.2±4.1	33.3±4	-1.5	0.241

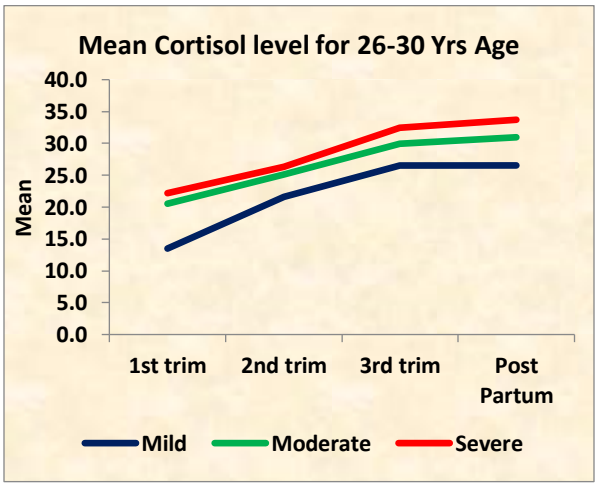
Note: *significant at 5% level of significance

Mean cortisol level in 3rd trimester of mildly stressed and postpartum were between 25-27.2µg/dl and 25-27.5µg/dl respectively. Mean cortisol levels in 3rd trimester of moderately stressed and postpartum were between 29-30 µg/dl and29-31 µg/dl respectively. Mean cortisol levels in 3rd trimester of severely stressed and postpartum were between 32-35 µg/dl and33-35.2 µg/dl respectively. No significant difference between 3rd trimester and postpartal cortisol levels.

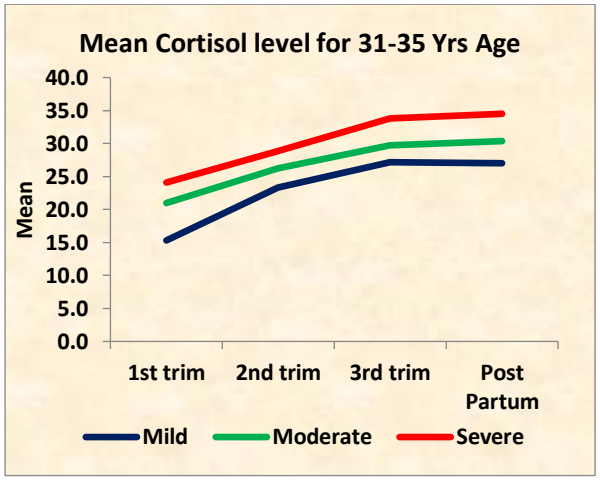
Graph-9 :Mean Cortisol level vs Stress level in 21-25 Yrs age group at 3 trimesters & Postpartum



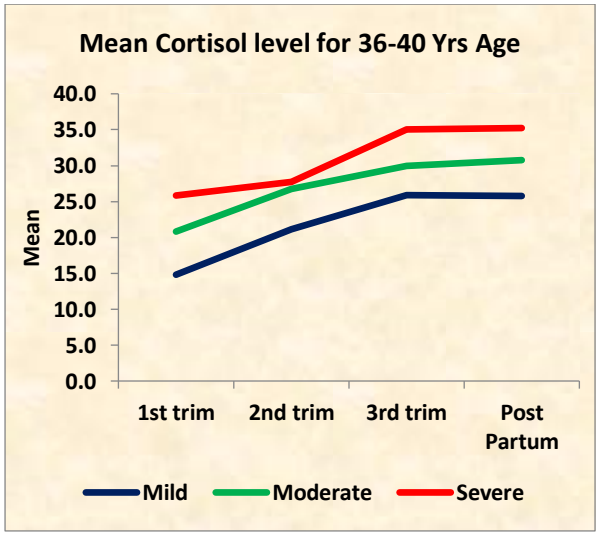
Graph-10 :Mean Cortisol level vs Stress level in 26-30 Yrs age group at 3 trimesters & Postpartum



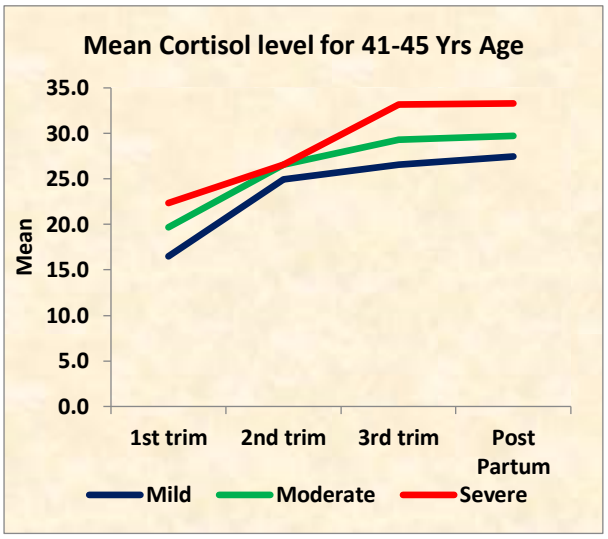
Graph-11 :Mean Cortisol level vs stress level in 31-35 Yrs age group at 3 trimesters & Postpartum :



Graph-12 :Mean Cortisol level vs stress level in 36-40 Yrs age group at 3 trimesters & Postpartum :



Graph-13 :Mean Cortisol level vs stress level in 41-45 Yrs age group at 3 trimesters & Postpartum:



INFLUENCE OF PROLATIN DURING PREGNANCY AND 5 DAYS POSTPARTUM:

Table 11: Comparison of mean Prolactin level (µg/L) and Stress levels:

Age group (yrs)	Days	Prolactin levels (µg/L)						ANOVA		Tukey test multiple comparison		
		In mildly stressed (control group)		In moderately stressed		In severely stressed				p value b/w Mild & Moderate	p value b/w Moderate & Severe	p value b/w Mild & Severe
		N	Mean±SD	N	Mean±SD	N	Mean±SD	F value	p value			
21-25	Ante natal Prolactin level	14	190.8±26.6	7	167.9±29.7	7	158.7±17.2	5.3	0.012*	0.052	0.372	0.004*
	Post partum day 1	14	194.8±28.3	7	162.1±38.8	7	149.6±18.9	9.5	0.001*	0.054	0.241	<0.001*
	Post partum day 2	14	207.6±33.1	7	164.3±39.7	7	148.6±19.5	12.7	<0.001*	0.214	0.162	<0.001*
	Post partum day 3	14	221±28.6	7	176.3±12.3	7	136.9±17.6	32.2	<0.001*	<0.001*	0.001*	<0.001*
	Post partum day 4	14	241.4±37.9	7	188.7±16	7	161.4±13.8	19.6	<0.001*	<0.001*	0.006*	<0.001*
	Post partum day 5	14	259.9±38.5	7	202.1±13.8	7	170.7±17.4	23.2	<0.001*	<0.001*	0.003*	<0.001*
26-30	Ante natal Prolactin level	8	180.8±26.3	9	168.9±22.6	5	166.6±22.8	0.7	0.497	0.338	0.861	0.330
	Post partum day 1	8	174±24.9	9	171.8±18.7	5	170±23.2	0.1	0.949	0.842	0.887	0.775
	Post partum day 2	8	182.5±23.6	9	173.8±21.1	5	165.6±24.9	0.9	0.437	0.439	0.553	0.259
	Post partum day 3	8	194±29.8	9	159±19	5	148.2±21.4	7.0	0.005*	0.016*	0.378	0.009*
	Post partum day 4	8	211.8±43	9	174.3±20.4	5	172.6±15	4.1	0.033*	0.051	0.862	0.043*
	Post partum day 5	8	243.8±58.8	9	204.9±20	5	179.4±9.8	4.7	0.022*	0.113	0.009*	0.019*
31-35	Ante natal Prolactin level	8	157±25.2	9	145.1±19.8	6	139±27.2	1.1	0.362	0.303	0.649	0.235
	Post partum day 1	8	148.5±25.7	9	136±23.2	6	124.8±30.4	1.4	0.260	0.313	0.466	0.158
	Post partum day 2	8	160.6±19.6	9	149.3±22.5	6	137.5±32.9	1.5	0.244	0.287	0.465	0.170
	Post partum day 3	8	176.5±18.6	9	161.7±15.5	6	155.5±26.6	2.2	0.142	0.100	0.622	0.137
	Post partum day 4	8	191.6±16.2	9	170.4±12.7	6	168±25.1	4.1	0.032*	0.011*	0.836	0.079
	Post partum day 5	8	226.4±21.3	9	172.2±16.3	6	170.2±10.4	27.0	<0.001*	<0.001*	0.777	<0.001*
36-40	Ante natal Prolactin level	4	151.3±21.3	7	147.1±28.2	3	128.3±16.8	0.8	0.459	0.788	0.240	0.186
	Post partum day 1	4	144.5±23.4	7	139.4±27.9	3	117±17.1	1.2	0.350	0.756	0.172	0.147
	Post partum day 2	4	157.3±20.4	7	144.7±23.6	3	136.7±10.1	0.9	0.438	0.383	0.477	0.154
	Post partum day 3	4	171.5±16.2	7	162.1±15.5	3	154±16.7	1.1	0.379	0.383	0.525	0.237
	Post partum day 4	4	187±9.8	7	176.4±7.3	3	171.7±17.4	2.1	0.172	0.133	0.696	0.305
	Post partum day 5	4	200±13.3	7	172.7±7.5	3	170.3±6.8	12.9	0.001*	0.019*	0.646	0.018*
41-45	Ante natal Prolactin level	2	171.5±40.3	3	159±31.2	4	149.3±27.5	0.3	0.723	0.774	0.691	0.611
	Post partum day 1	2	155±32.5	3	149.7±29	4	146±27.2	0.1	0.937	0.869	0.872	0.793
	Post partum day 2	2	167.5±23.3	3	154.7±22.1	4	152.3±16.3	0.4	0.677	0.602	0.884	0.560
	Post partum day 3	2	182±12.7	3	166.3±21.2	4	163.3±20.9	0.6	0.571	0.410	0.861	0.268
	Post partum day 4	2	197.5±0.7	3	173.7±4.5	4	171.3±13.2	5.3	0.047*	0.012*	0.757	0.029*
	Post partum day 5	2	228±17	3	172.3±14	4	170.5±6.5	18.6	0.003*	0.162	0.855	0.136

In mildly stressed women, the mean prolactin levels of antenatal and day-1 postpartum of age group 21-25 years were between 190-194 µg/L, age group 26-30 years were between 174-180 µg/L and age group 31-35 years were between 148-157 µg/L, age group 36-40 years were between 144-151 µg/L and age group 41-45 years were between 155-171 µg/L.

In moderately stressed women, the mean prolactin levels of antenatal and day-1 postpartum of age group 21-25 years were between 162-167 µg/L, age group 26-30 years were between 168-171 µg/L and age group 31-35 years were between 136-145 µg/L, age group 36-40 years were between 139-147 µg/L and age group 41-45 years were between 149-159 µg/L.

In severely stressed women, the mean prolactin levels of antenatal and day-1 postpartum of age group 21-25 years were between 149-158 µg/L, age group 26-30 years were between 166-170 µg/L and age group 31-35 years were between 124-139 µg/L, age group 36-40 years were between 117-128 µg/L and age group 41-45 years were between 146-149 µg/L.

TABLE-12 :Mean and SD of Prolactin levels of all study subjects before delivery, for first 5 days postpartum at different levels of stress :

Stress levels	Prl Before delivery	PP Prl on D-1	PP Prl on D-2	PP Prl on D-3	PP Prl on D-4	PP Prl on D-5
<150	175.58±29.30	172.08±32.68	183.75±33.05	197.44±31.50	215.27±38.44	240.44±41.84
150-299	148.85±22.19	141.44±21.41	151.85±20.50	162.97±15.52	179.52±14.94	204.5±15.55
>300	133.73±21.98	125.11±23.66	137.11±22.41	152.30±20.02	170.46±17.20	191.92±12.79

GRAPH-14 : Mean and SD of Prolactin levels of all study subjects before delivery, for first 5 days postpartum at different levels of stress :

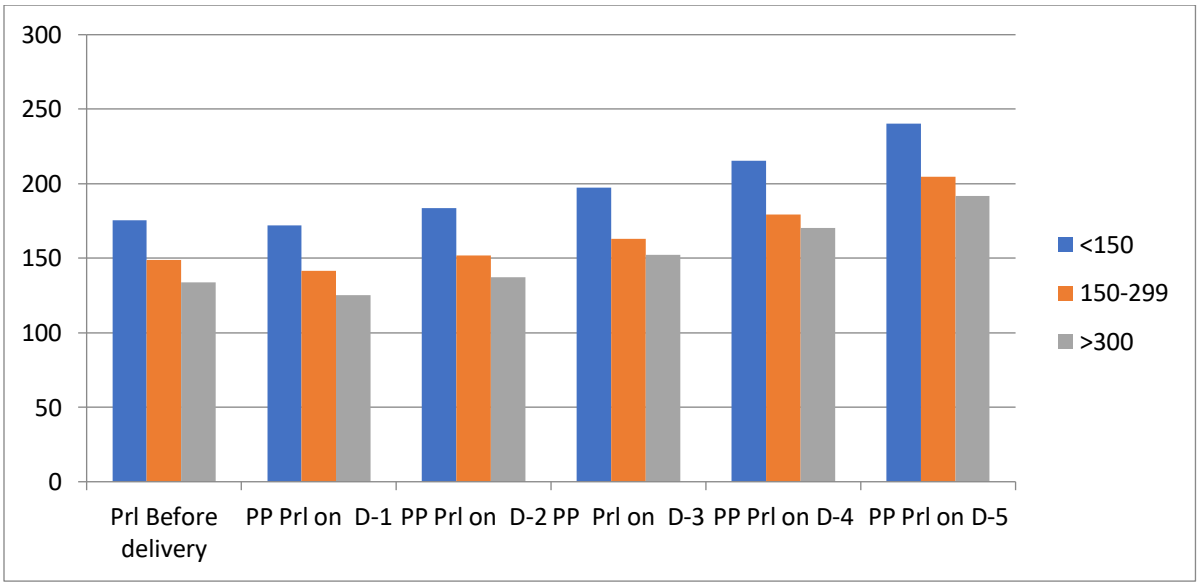


Table-13 : Comparison of age group, stress levels & mean Prolactin levels during antenatal and on day 5 after delivery

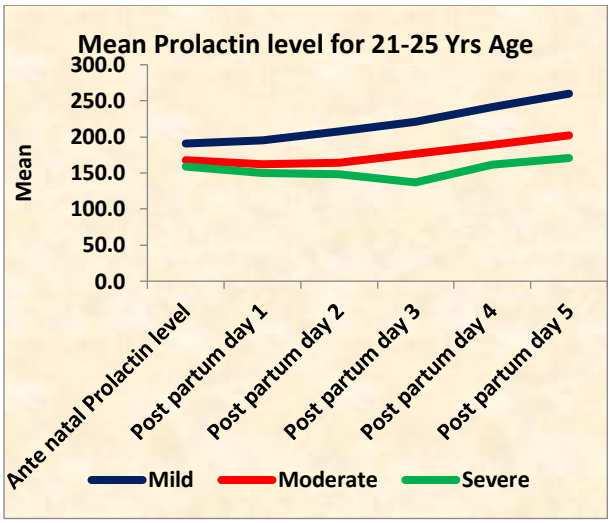
Age group	Stress level	N	Prolactin level (Mean±SD)		t value	p value
			Ante natal Prolactin level	Post partum day 5		
21-25	Mild	14	190.8±26.6	259.9±38.5	-5.5	<0.001*
	Moderate	7	167.9±19.7	202.1±13.8	-3.8	0.004*
	Severe	7	158.7±17.2	170.7±17.4	-1.3	0.221
26-30	Mild	8	180.8±26.3	243.8±58.8	-2.8	0.022*
	Moderate	9	168.9±22.6	204.9±20	-3.6	0.003*
	Severe	5	166.6±22.8	179.4±9.8	-1.2	0.301
31-35	Mild	8	157±25.2	226.4±21.3	-5.9	<0.001*
	Moderate	9.0	145.1±19.8	172.2±16.3	-3.2	0.006*
	Severe	6	139±27.2	170.2±10.4	-2.6	0.039*
36-40	Mild	4	151.3±21.3	200±13.3	-3.9	0.012*
	Moderate	7.0	147.1±28.2	172.7±7.5	-2.3	0.059
	Severe	3	128.3±16.8	170.3±6.8	-4.0	0.057
41-45	Mild	2	171.5±40.3	228±17	-1.8	0.319
	Moderate	3.0	159±31.2	172.3±14	-0.7	0.570
	Severe	4	149.3±27.5	170.5±6.5	-1.5	0.230

Note: *significant at 5% level of significance

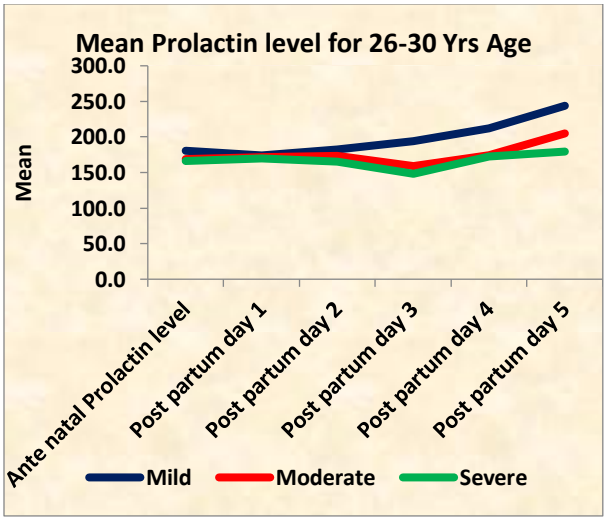
In all the age groups of antenatal and day-1 postpartum, the difference in mean prolactin levels were very slight and up to day 5 postpartum there was no significant increase. The serum

prolactin levels were not significantly altered in moderate and severely stressed groups when compared with mildly stressed group.

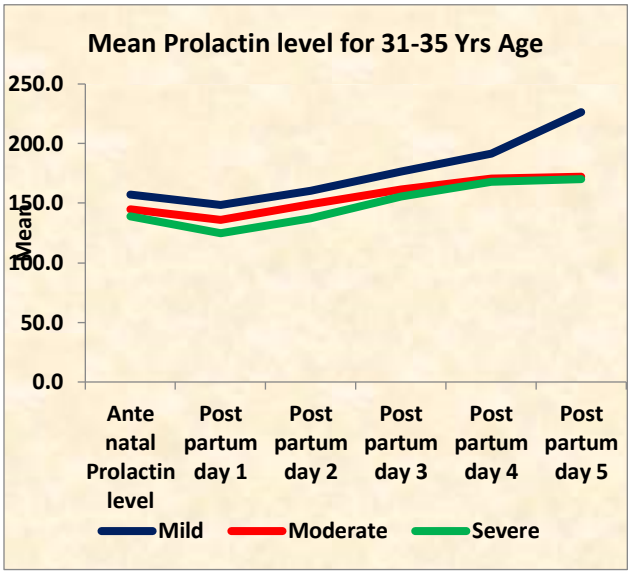
Graph-15 : Mean Prolactin level vs stress level in 21-25 yrs age group on antenatal & upto 5 days postpartum:



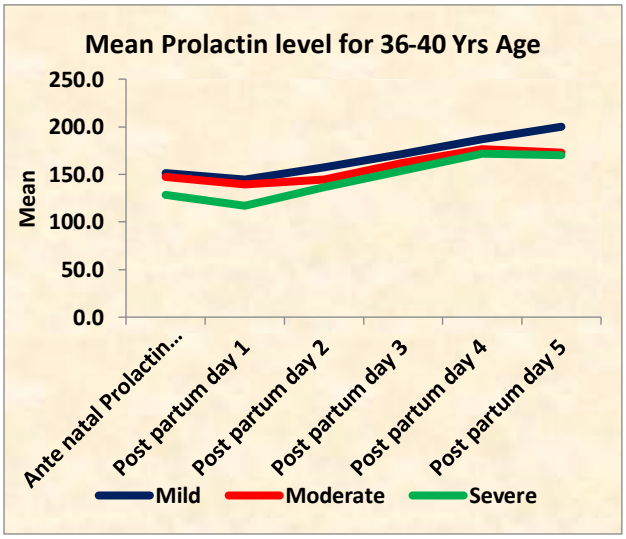
Graph-16 :Mean Prolactin level vs stress level in 26-30 yrs age group on antenatal & upto 5 days postpartum:



Graph-17 :Mean Prolactin level vs stress level in 31-35 yrs age group on antenatal & upto 5 days postpartum:



Graph-18 :Mean Prolactin level vs stress level in 36-40 yrs age group on antenatal & upto 5 days postpartum:



Graph-19 :Mean Prolactin level vs stress level in 41-45 yrs age group on antenatal & upto 5 days postpartum:

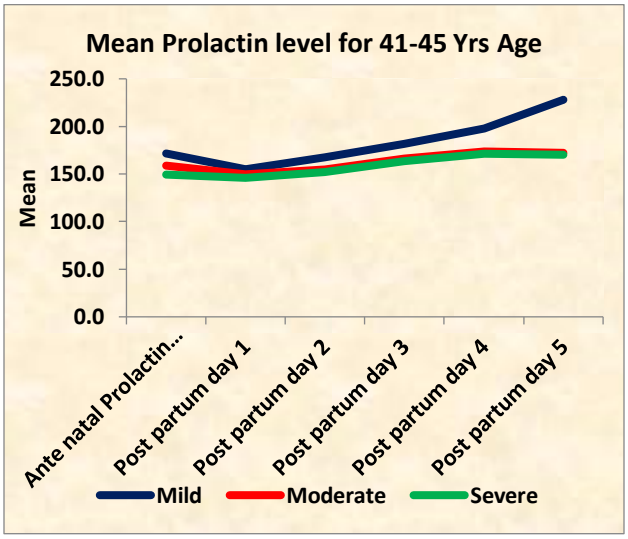


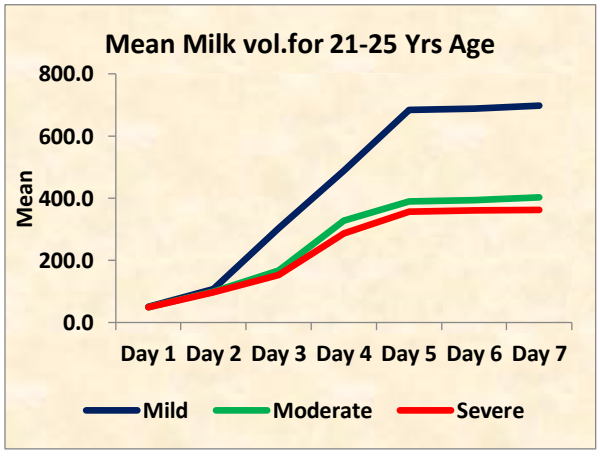
Table -14 Comparison of the Mean Milk Volume (ml) with Stress levels in different age groups:

	Days	Milk Volume (ml)						ANOVA		Tukey test multiple comparison		
		In mildly stressed (control group)		In moderately stressed		In severely stressed				p value b/w Mild & Moderate	p value b/w Moderate & Severe	p value b/w Mild & Severe
		N	Mean±SD	N	Mean±SD	N	Mean±SD	F value	p value			
21-25	Day 1	14	50.5±3.8	7	49.2±1.8	7	49±1.7	0.8	0.473	0.302	0.835	0.228
	Day 2	14	107.6±8.4	7	100±12.8	7	96.6±4.6	7.4	0.003*	0.057	0.129	0.001*
	Day 3	14	302.3±55	7	166.7±14.1	7	152.4±9.8	43.6	<0.001*	<0.001*	0.052	<0.001*
	Day 4	14	489.1±59.5	7	326.8±11.9	7	287.4±11.9	62.2	<0.001*	<0.001*	<0.001*	<0.001*
	Day 5	14	683.5±22.7	7	389.4±15.7	7	356.1±13.5	916.6	<0.001*	<0.001*	0.001*	<0.001*
	Day 6	14	687.1±21.7	7	393.1±14.1	7	360.9±16.8	939.1	<0.001*	<0.001*	0.003*	<0.001*
	Day 7	14	697.7±21.9	7	402.6±8.5	7	362.2±19.3	985.0	<0.001*	<0.001*	0.001*	<0.001*
26-30	Day 1	8	49±3.5	9	49.2±1.7	5	48±2	0.4	0.694	0.886	0.294	0.527
	Day 2	8	114.6±28.3	9	102±2.1	5	100±2	1.5	0.241	0.249	0.116	0.189
	Day 3	8	342.6±48	9	195.9±14.1	5	126.9±11.1	85.9	<0.001*	<0.001*	<0.001*	<0.001*
	Day 4	8	510.1±87.7	9	278.7±14.5	5	232.2±12.4	54.2	<0.001*	<0.001*	<0.001*	<0.001*
	Day 5	8	684.2±40.4	9	464.5±15.8	5	387.8±12.3	223.3	<0.001*	<0.001*	<0.001*	<0.001*
	Day 6	8	686.9±38.6	9	470.1±16.9	5	392.5±9.9	234.6	<0.001*	<0.001*	<0.001*	<0.001*
	Day 7	8	692.1±39.9	9	478.9±15.6	5	399.7±6.8	226.9	<0.001*	<0.001*	<0.001*	<0.001*
31-35	Day 1	8	49.4±2.9	9	41.1±2	6	39.1±1.2	45.5	<0.001*	<0.001*	0.032*	<0.001*
	Day 2	8	105.4±6.2	9	87.1±2.5	6	80.3±2.4	71.1	<0.001*	<0.001*	<0.001*	<0.001*
	Day 3	8	348.6±45.5	9	160.5±15.3	6	117.5±8.5	135.5	<0.001*	<0.001*	<0.001*	<0.001*
	Day 4	8	516.3±76	9	278.9±41.9	6	172.5±10.3	81.7	<0.001*	<0.001*	<0.001*	<0.001*
	Day 5	8	690.5±39.9	9	421.6±16.4	6	371.1±11.8	318.2	<0.001*	<0.001*	<0.001*	<0.001*
	Day 6	8	692.9±39	9	422±14.4	6	377.1±10.9	343.1	<0.001*	<0.001*	<0.001*	<0.001*
	Day 7	8	698.3±38	9	430.4±16.6	6	383.6±8	345.5	<0.001*	<0.001*	<0.001*	<0.001*
36-40	Day 1	4	46.7±6.2	7	42.5±1.4	3	32.9±1.7	13.9	0.001*	0.274	0.003*	0.024*
	Day 2	4	105.2±10.8	7	79.9±2.6	3	68.6±1.1	36.7	<0.001*	0.019*	<0.001*	0.007*
	Day 3	4	347.9±32.9	7	166.4±11.4	3	136.2±7.6	140.3	<0.001*	0.002*	0.004*	0.001*
	Day 4	4	508.1±48.4	7	290.5±8.7	3	189.1±10.3	141.1	<0.001*	0.003*	0.001*	0.001*
	Day 5	4	701.2±24.4	7	369±9.6	3	285.2±6.1	865.0	<0.001*	<0.001*	<0.001*	<0.001*
	Day 6	4	707.9±20.6	7	370.7±8.4	3	293.1±7.3	1171.1	<0.001*	<0.001*	<0.001*	<0.001*
	Day 7	4	712.7±14.3	7	378.2±7.2	3	301.6±1.3	2237.3	<0.001*	<0.001*	<0.001*	<0.001*
41-45	Day 1	2	49.2±0.9	3	38.6±1.2	4	31.2±1.3	149.3	<0.001*	0.008*	0.001*	<0.001*
	Day 2	2	113±7.4	3	76.8±1.3	4	66.9±1.1	140.5	<0.001*	0.092	0.002*	0.072
	Day 3	2	305.7±9.8	3	156.2±7.6	4	137.3±14.7	141.6	<0.001*	0.035*	0.092	<0.001*
	Day 4	2	546.3±8.1	3	281.1±8.8	4	178.9±8.6	1226.1	<0.001*	0.001*	<0.001*	<0.001*
	Day 5	2	724.8±8.3	3	347±7.2	4	295.9±9.3	1820.3	<0.001*	<0.001*	0.001*	<0.001*
	Day 6	2	725±15.5	3	354.4±13.1	4	294.4±8.2	997.9	<0.001*	0.023*	0.006*	0.017*
	Day 7	2	727.4±14.7	3	376.4±18.8	4	317.4±21.8	301.7	<0.001*	0.002*	0.019*	<0.001*

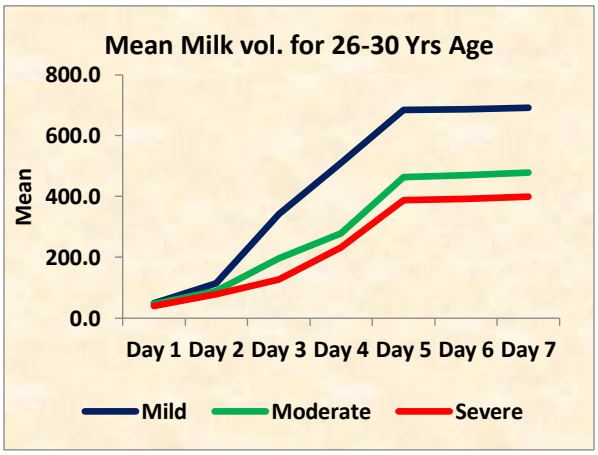
In the age group of 21-30 years, the effect of chronic stress in moderate and severely stressed women on lactogenesis was very slight for first two days when compared with mildly stressed women. The difference was statistically insignificant. From third day to seventh day, moderately stressed women showed increase in milk volume. But in severely stressed women, the decrease in milk volume was continued even up to seven days indicating chronic stress has profound negative effect in severely stressed women of these age groups.

In the age group of 31-45 years, the effect of chronic stress in moderate and severely stressed women on lactogenesis was observed when compared with mildly stressed women up to seven days postpartum. The difference was statistically significant. In this age group, there was no much difference in the milk output in moderate and severely stressed women indicating that they had similar negative effect on lactogenesis.

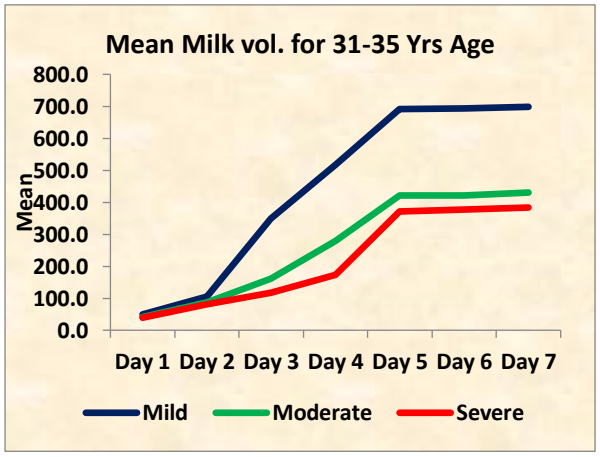
GRAPH – 20 : Mean and SD of milk volumes of 21-25 yrs age group for first 7 days postpartum at different stress levels :



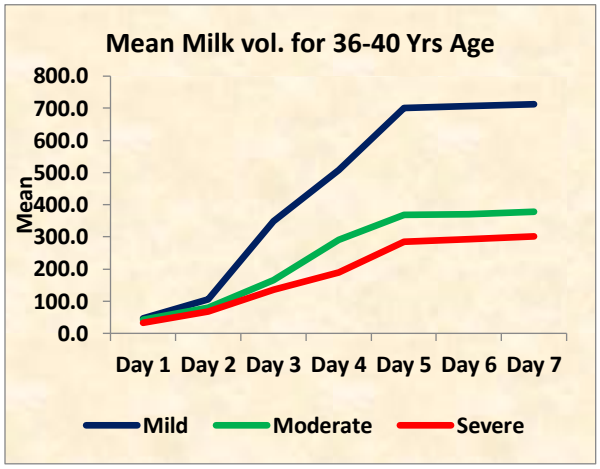
GRAPH – 21 : Mean and SD of milk volumes of 26-30 yrs age group for first 7 days postpartum at different stress levels :



GRAPH – 22 : Mean and SD of milk volumes of 31-35 yrs age group for first 7 days postpartum at different stress levels :



GRAPH – 23 : Mean and SD of milk volumes of 36-40 yrs age group for first 7 days postpartum at different stress levels :



GRAPH – 24 : Mean and SD of milk volumes of 41-45 yrs age group for first 7 days postpartum at different stress levels :

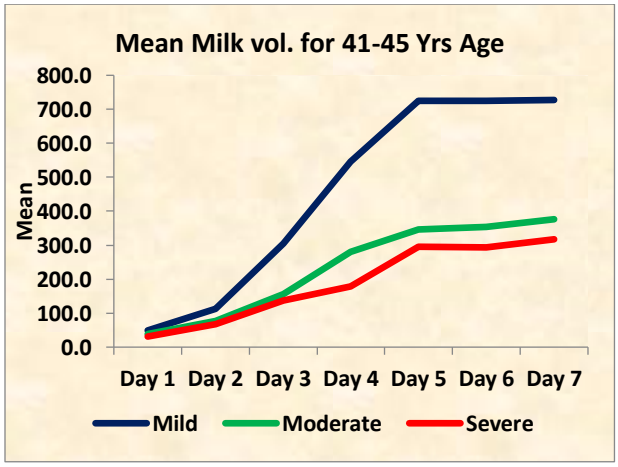


Table 15 : Comparison of the Mean Milk Volume by 5th, 6th and 7th day for different stress level & age groups :

Age group	Stress level	Milk Vol (Mean±SD)			p value b/w Day 5 & Day 6	p value b/w Day 6 & Day 7
		At Day 5	At Day 6	At Day 7		
21-25	Mild	683.5±22.7	687.1±21.7	697.7±21.9	0.119	0.015*
	Moderate	389.4±15.7	393.1±14.1	402.6±8.5	0.161	0.019*
	Severe	356.1±13.5	360.9±16.8	362.2±19.3	0.081	0.468
26-30	Mild	684.2±40.4	686.9±38.6	692.1±39.9	0.085	0.006*
	Moderate	464.5±15.8	470.1±16.9	478.9±15.6	0.008*	0.001*
	Severe	387.8±12.3	392.5±9.9	399.7±6.8	0.274	0.023*
31-35	Mild	690.5±39.9	692.9±39	698.3±38	0.088	0.072
	Moderate	421.6±16.4	422±14.4	430.4±16.6	0.832	<0.001*
	Severe	371.1±11.8	377.1±10.9	383.6±8	0.001*	0.019*
36-40	Mild	701.2±24.4	707.9±20.6	712.7±14.3	0.127	0.347
	Moderate	369±9.6	370.7±8.4	378.2±7.2	0.559	0.005*
	Severe	285.2±6.1	293.1±7.3	301.6±1.3	0.106	0.141
41-45	Mild	724.8±8.3	725±15.5	727.4±14.7	0.975	0.141
	Moderate	347±7.2	354.4±13.1	376.4±18.8	0.238	0.197
	Severe	295.9±9.3	294.4±8.2	317.4±21.8	0.774	0.064

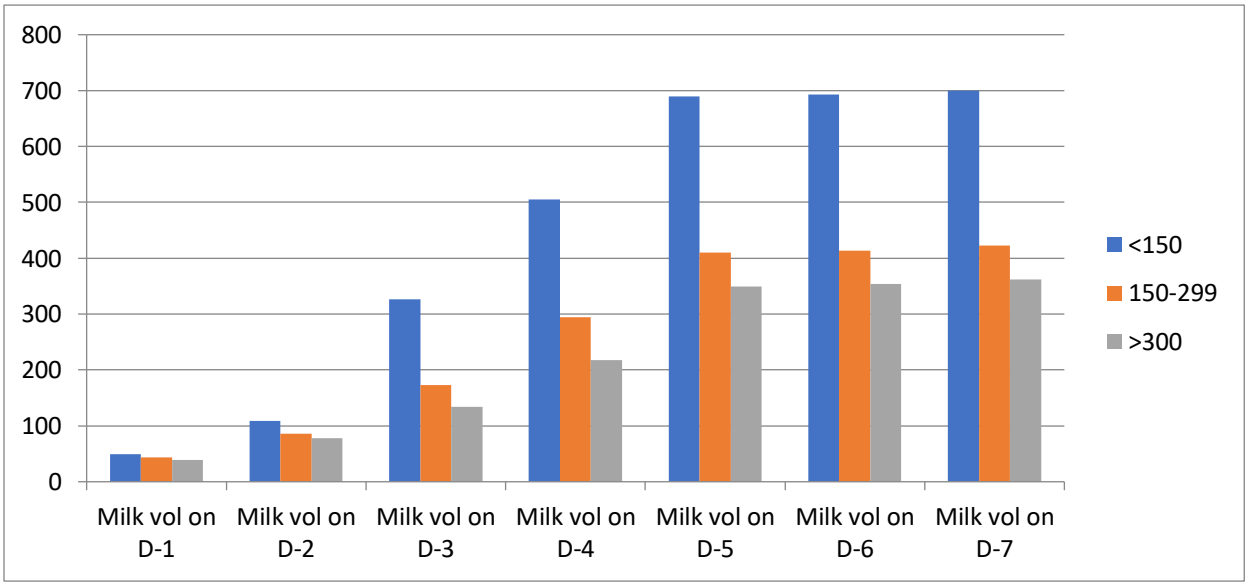
Note: *significant at 5% level of significance

There is no much difference in the milk volume on 6th and 7th day after delivery in all the age groups. This is because volume increase and lactation establishment is usually up to 5th day after postpartum. From 6th day onwards there will be maintenance of lactation.

TABLE-16 :Mean and SD of milk volumes of study subjects for first 7 days postpartum at different stress levels :

Stress levels	Milk vol on D-1	Milk vol on D-2	Milk vol on D-3	Milk vol on D-4	Milk vol on D-5	Milk vol on D-6	Milk vol on D-7
<150	49.43±3.77	108.68±14.80	326.77±50.76	505.10±66.65	689.45±31.56	692.76±30.41	699.89±29.77
150-299	43.60±3.28	85.89±5.15	172.81±18.59	294.54±20.53	409.83±42.80	413.20±43.02	423.09±41.47
>300	38.82±4.89	78.26±7.51	134.44±16.13	217.70±49.22	349.53±39.38	353.76±40.30	361.65±36.85

GRAPH –25 :Mean and SD of milk volumes of all study subjects for first 7 days postpartum at different stress levels :

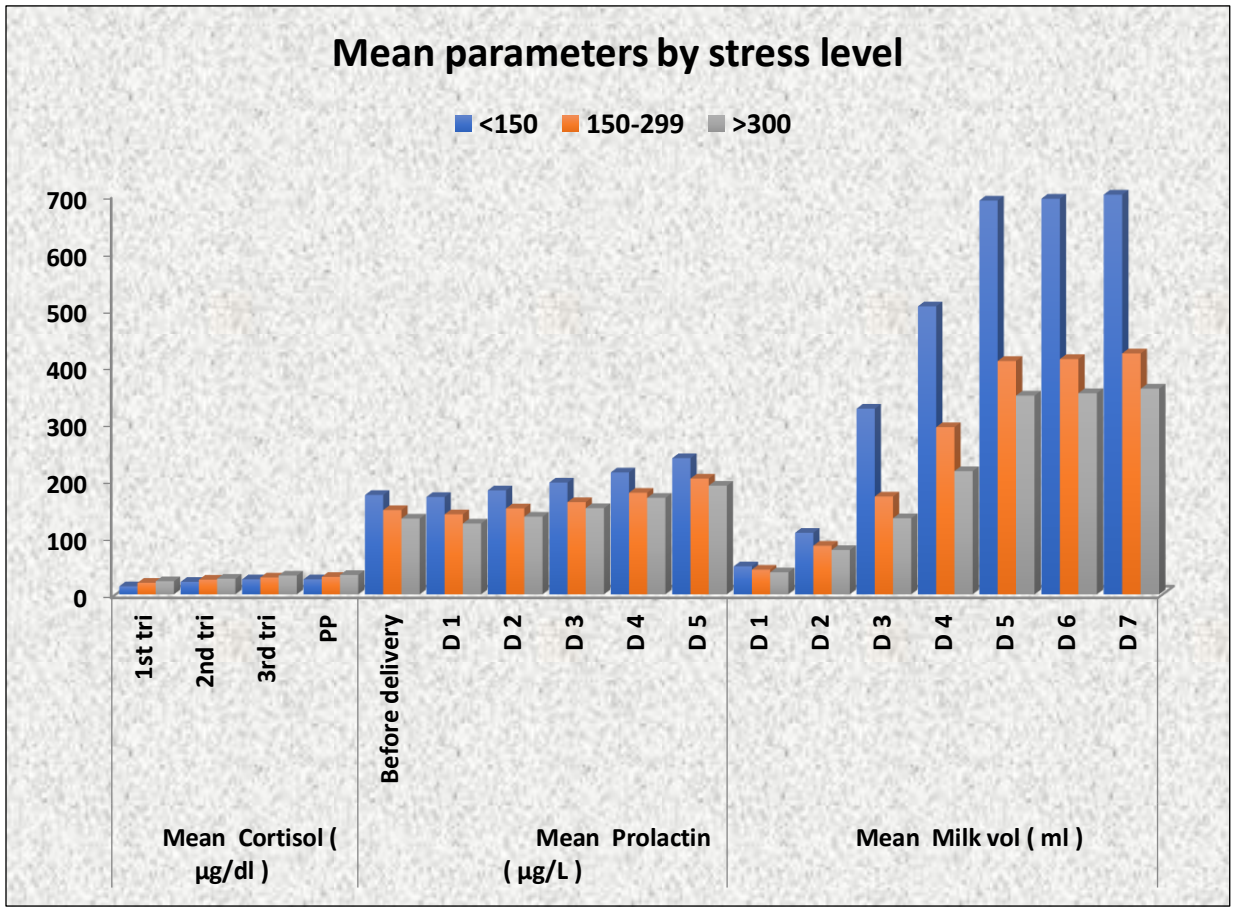


Total milk volumes of all subjects were measured at different stress levels. It was observed that mildly stressed women produced ≈ 700ml of milk, moderately stressed women produced ≈ 425ml and severely stressed women produced ≈ 360 ml of milk. Milk volumes were reduced by ≈ 40% in moderately stressed women and ≈ 50% in severely stressed women when compared with milk volumes of mildly stressed women (table -16).

Table-17 : Master table showing all recorded parameters in study subjects :

Stress Level	Mean Cortisol (µg/dl)				Mean Prolactin (µg/L)						Mean Milk vol (ml)						
	1st tri	2nd tri	3rd tri	PP	Before delivery	D 1	D 2	D 3	D 4	D 5	D 1	D 2	D 3	D 4	D 5	D 6	D 7
<150	14.15	22.16	26.48	26.5	175.58	172.08	183.75	197.44	215.27	240.44	49.43	108.68	326.77	505.1	689.45	692.76	699.89
150-299	20.44	25.91	29.72	30.79	148.85	141.44	151.85	162.97	179.52	204.5	43.6	85.89	172.81	294.54	409.83	413.2	423.09
>300	23.1	27.56	33.06	34.15	133.73	125.11	137.11	152.3	170.46	191.92	38.82	78.26	134.44	217.7	349.53	353.76	361.65

Graph-26. Master graph showing all recorded parameters in study subjects :



All recorded parameters of study subjects were represented in a master table (table –17). The parameters included are –

i. Mean Cortisol levels (µg/dl) in 1st, 2nd and 3rd trimesters during pregnancy and after delivery.

ii. Mean Prolactin levels before delivery and from 1st day to 5th day after delivery.

iii. Mean milk volumes from 1st day to 7th day after delivery.

CHAPTER – VI

DISCUSSION

CHAPTER-VI. DISCUSSION:

Life events like change in financial state, dismissal from work, change in eating or sleeping habits, divorce, death of spouse, marital separation, pregnancy, marriage, marital reconciliation, imprisonment etc. are the causes of chronic stress[1-6]. This leads to deleterious effects on health – may be cardiovascular diseases such as heart attacks and strokes, complications of obesity and diabetes, speeding up of the process of ageing, suppression of immune functions, infertility etc [7]. Stress during pregnancy leads to hypertension, diabetes mellitus, preterm delivery and fetal loss [8 – 23] .This work was conducted to observe the effect of chronic stress on lactogenesis in women of reproductive age 21-45 years (table-1).

Neilsen's study indicates the prevalence of stress in women in different countries such as - in India (87%), Mexico (74%), Russia (69%), Brazil (67%), Spain (66%), France (65%), Italy (64%), Turkey (56%). India is the fastest developing country and Indian women are becoming the most stressed women on earth. The reason may be the societal and family structures have yet to adapt to the evolving work patterns in India. Women are trying to fulfill the responsibility of both modern career and traditional home life. In developing countries, the prevalence of stress during pregnancy has been found to range between 6-52.9% [24 - 28]. In our study, 37.5% pregnant women were mildly stressed, 35.41% were moderately stressed and 27.08% were severely stressed on the basis of Holmes and Rahe stress scale (table-2).

Studies indicate that about 20% of pregnant women experience severe stress due to marital difficulties, lack of partner, socio-demographic parameters such as low socioeconomic status, occupational status, low literacy rate etc[29, 30]. Our observations revealed that joint and nuclear family patterns do not bear any significant difference in the stress levels (table-3). Women belonging to different religious faithssuch as Hindu, Muslim and Christian bear the same stress level due to life events, as they have almost same way of living (table-4). In our study we found that formally educated (graduation and above) women are able to deal appropriately with stressful conditions. As only 10% of graduates are either moderately or severely stressed, while stress of this severity is prevalent in more than 60% of the undergraduates (table-5). Education might be helping them to develop coping response/ability to decelerate the stress generated by life events. Though the stressful situations in the life of economically sound and economically poor women are different, the overall stress experienced by women of both economical strata is same. The women of middle economical group experience slightly higher stress, but statistically the difference is insignificant (table-6). Mean stress in working women is quite higher as compared to house wives. This might be because working women have to carry out multitasking and meet the professional stress (table-7).

Serum Cortisol levels of moderate and severely stressed women were significantly raised in comparison with mildly stressed women (table-8). Similar results were observed by Dallman etal[31], Harvaline etal[32] and Obel etal [33] and also by few other studies [34 - 42]. The difference in the 3rd trimester and postpartal Cortisol level was insignificant indicating that

chronically stressed women remain in the same stressed state even in the postpartal period (tables – 9& 10).

In our study, serum Prolactin levels were not significantly altered in moderate and severely stressed groups when compared with mildly stressed group (table-11). Ueda et al in 1994 conducted a similar study in breastfeeding women assigned with noise stress and mental stress. They also found that Prolactin concentration did not vary significantly with levels of stress [43].

In the age group 21-30 years, for the first two postpartal days (initiation phase), the lactogenesis in moderate and severely stressed women was reduced insignificantly when compared with mildly stressed women. It indicates milk ejection is affected due to decreased secretion of Oxytocin. In the next five days, lactogenesis improved in moderately stressed women while it remained suppressed in severely stressed women.It indicates milk synthesis and ejection improved in moderately stressed women in this phase of lactation. In severely stressed women both milk synthesis and ejection get affected. This shows severe stress produces significant negative effect on lactogenesis (table-14).

In moderately and severely stressed women of age group 31-45 years, lactogenesis was reduced significantly when compared to mildly stressed women in all phases (up to 7 days) of lactation. It indicates that both milk synthesis and ejection got affected. Milk ejection is a neuroendocrinal reflex. When milk synthesis is decreased, there is negative effect

over the reflex action leading to decreased milk output. So, moderate and severe chronic stress generated due to various life events get compounded and has suppressive effect on all phases of lactation in the middle and elderly aged mothers (table-14).

On 6^{th} and 7^{th} day after delivery, there was no much difference in milk volumes in all the age groups. This was because volume increase and lactation establishment is usually up to 5^{th} day after postpartum. From 6^{th} day onwards, there will be maintenance of lactation (table-15).

Total milk volumes of all subjects were measured at different stress levels. It was observed that mildly stressed women produced ≈ 700ml of milk, moderately stressed women produced ≈ 425ml and severely stressed women produced ≈ 360 ml of milk. Milk volumes were reduced by ≈ 40% in moderately stressed women and ≈ 50% in severely stressed women when compared with milk volumes of mildly stressed women (table -16).

Decrease in the milk volume in stressed women correlates to the first study conducted by Newton and Newton in 1948[44 - 48] . They have shown that the maternal acute stress suppresses the lactation. A study by Feher, Berger etal in 1989 estimated the milk volume output from the mothers who gave birth prematurely[49]. They also observed that the mothers were in stress and the milk volume was reduced when compared with the milk volume of control.

All recorded parameters of study subjects were represented in a master table (table –17). The parameters included are – i. Mean Cortisol levels (µg/dl) in 1^{st} , 2^{nd} and 3^{rd}

trimesters during pregnancy and after delivery. ii. Mean Prolactin levels before delivery and from 1st day to 5th day after delivery. iii. Mean milk volumes from 1st day to 7th day after delivery.

Our findings indicate that cumulative effects of changing life events and daily hassles with failure of coping reflex leads to chronic stress which causes decreased lactation.

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CHAPTER – VII

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION:

- 62.49 % of Indian women are living with stress, of which 35.41% are moderately and 27.08% are severely stressed.s
- Stress is equally prevalent in women of different religious faith.
- Formally educated women are well equipped to deal effectively with stress than uneducated women in rural India.
- Mean stress in working women is quite higher as compared to house wives.
- Chronically stressed women remain in the same stressed state even in the postpartal period.
- Chronic stress of different levels did not show much effect on serum prolactin levels during pregnancy and postpartum.
- Moderate stress of up to 299 on Holmes and Rahe stress scale in younger age group (21-30 years) suppresses the initial phase (first two days) of lactation, but lactation improves from third day. Severe stress of >300 in the same age group has deleterious effect on all phases of lactation (even up to seventh day).
- In the women of middle and elderly age group (above 30 years), moderate and severe stress has compounding negative effect leading to difficulty in initiation and establishment of lactation.
- Moderate and severe stressful life events reduce milk volume output in humans. It is essential to bring awareness in population about deleterious effects of stress on lactation. Chronic stress due to life events cannot be avoided but can be

reduced. So, the stress assessment in the 1st trimester and proper measures to reduce it may partially nullify the effects of chronic stress on lactogenesis and normal lactation can be established.

- To conclude, apart from the routine investigations of infective diseases and hematological tests, the stress assessment parameters may be included in the assessment of antenatal health of pregnant woman.

LIMITATIONS OF THE STUDY:

- Milk volumes were measured up to 7 days postpartum.
- Study confined to subjects from rural area.

SCOPE FOR FUTURE STUDY:

- Milk volumes can also be measured even after 7 days postpartum.
- Further studies are required to observe chronic stress status during pregnancy in larger population.
- Study can also be extended in subjects from urban area.

APPENDIX

APPENDIX- I

BLDEU' S SHRI B.M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE, BIJAPUR

INFORMED CONSENT FORM

Title of the study: "**EFFECT OF CHRONIC STRESS ON LACTOGENESIS IN HUMANS**"

Name of the principal investigator: Vandali Jyothi

Name of the PhD Guide: DR. MANJUNATH AITHALA
PROF & HOD
DEPARTMENT OF PHYSIOLOGY

PURPOSE OF RESEARCH: I have been informed that this study will assess the effect of chronic stress on lactogenesis in rural women.

This study will be useful academically and clinically to find the association between prolonged stress and lactation.

PROCEDURE:

I understand that, the study procedure involves recording of various physiological, hematological, hormonal tests. As it is a follow-up study, I have to involve in the study

group from 1st trimester up to 7 days postpartum. I also understand that the study procedure involves baby test weighing method for the measurement of milk volume.

RISKS AND DISCOMFORTS:

I understand that, there is no drug trial in this research study. So, this study will not cause discomfort to me and do not involve any risk to my health and my baby.

INJURY STATEMENT:

I understand that in unlikely event of injury to me resulting directly from my participation in this study, if such injury were reported promptly, the medical treatment will be available to me, but no further compensation would be provided.

FREEDOM TO ASK MORE INFORMATION:

I understand that I can ask more questions about the research study at any time. A copy of this consent form will be given to me to keep for careful re-reading.

FREEDOM TO WITHDRAW FROM THE STUDY:

I understand that my participation is voluntary. I may refuse, withdraw my consent and

discontinue participation in the study at any time. I also understand that researcher may request to terminate my participation in the study at any time after explaining the reasons for doing so.

BENEFITS:

I understand that my participation in this study may not have a direct benefit to me but this may have a potential benefit for larger population in the community.

CONFIDENTIALITY:

I understand that the personal and medical information produced during this study will be kept confidential. If the data are used for publication and for teaching purposes, no names will be used and other identities such as photographs, audio and video tapes will be used only with my special written permission. I understand I may see the photographs and the video tapes and have the audio tapes before giving this permission.

I understand that by my agreement to participate in this study I am not waiving any of my legal rights.

Signature of participant

Signature of investigator

APPENDIX- II

PROFORMA

Date:……
Place: ……..
Participant No:……..

Name	:	
Age (Years)	:	
Sex	:	Female
Marital Status	:	Married
Address	:	
Occupation	:	House wife / Working
Monthly Income (SES)	:	Rs……
Members in the family	:	Adults……. Children…….
Education	:	< 10^{th}std/ < 12^{th} std / graduate or above
Religion	:	Hindu / Muslim / Christian
Family type	:	Nuclear / Joint

Family history of Diabetes Mellitus: Maternal …… Paternal relative ……

History of endocrinal abnormality:

LMP	:
Weight in Kg	:
Height in Cms	:
Blood Pressure (mmHg)	:
Blood glucose	:
Hb g%	:
History of non-lactogenesis	:
Any bad obstetric history	:

Life Change Units (LCU) by Holmes & Rahe stress scale:

Blood collected in 1st trimester …….

Serum Cortisol level in 1st trimester ……

Blood collected in 2nd trimester …….

Serum Cortisol level in 2nd trimester ……

Blood collected in 3rd trimester …….

Serum Cortisol level in 3rd trimester ……

Serum Prolactin level before delivery …….

Date of delivery .....

Time of delivery.....

Mode of delivery .....

Baby wt .....

Maturity of baby .....

Sex of baby .....

Blood collected on 1st day postpartum …….

Serum Cortisol and Prolactin levels on 1st day postpartum …….

Blood collected on 2nd day postpartum …….

Serum Prolactin level on 2nd day postpartum …….

Blood collected on 3rd day postpartum …….

Serum Prolactin level on 3rd day postpartum …….

Blood collected on 4th day postpartum …….

Serum Prolactin level on 4th day postpartum …….

Blood collected on 5th day postpartum …….

Serum Prolactin level on 5th day postpartum …….

Volume of milk on 1st day postpartum .....

Volume of milk on 2nd day postpartum.....

Volume of milk on 3rd day postpartum .....

Volume of milk on 4th day postpartum .....

Volume of milk on 5th day postpartum .....

Volume of milk on 6th day postpartum .....

Volume of milk on 7th day postpartum .....

Signature of investigator

Signature of participant

Holmes - Rahe stress scale

Event	Life Change Units (LCU)	My Score
Death of spouse	100	
Divorce	73	
Marital Separation	65	
Jail Term	63	
Death of close family member	63	
Personal injury or illness	53	
Marriage	50	
Fired at work	47	
Marital reconciliation	45	
Retirement	45	
Change in health of family member	44	
Pregnancy	40	
Sex difficulties	39	
Gain of a new family member	39	
Business readjustment	39	
Change in financial state	38	
Death of a close friend	37	
Change to a different line of work	36	
Change in number of arguments with spouse	35	
Mortgage over $20,000	31	
Foreclosure of mortgage or loan	30	
Change in responsibilities at work	29	
Son or daughter leaving home	29	
Trouble with in laws	29	
Outstanding personal achievement	28	
Spouse begins or stop work	26	
Begin or end school	26	
Change in living conditions	25	
Revisions of personal habits	24	
Trouble with boss	23	
Change in work hours or conditions	20	
Change in residence	20	
Change in schools	20	
Change in recreations	19	
Change in church activities	19	
Change in social activities	19	
Mortgage or loan less than $20,000	17	
Change in sleeping habits	16	
Change in number of family get-togethers	15	
Change in eating habits	15	
Vacation	13	
Christmas approaching	12	
Minor violation of the law	11	
Total		

Directions: If an event mentioned above has occurred in the past year, or is expected in the near future, copy the number in the score column. If the event has occurred or is expected to occur more than once, multiply this number by the frequency of the event.

Life Change Units	**Likelihood Of Illness In Near Future**
< 150 → Mild stress	about 30 percent
150-299 → Moderate stress	about 50 percent
> 300 → Severe stress	about 80 percent

Baby weighing scale

Electrochemiluminescence

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Ethical Committee
BIJAPUR-586103
OUT WARD
No 042/2012
Date 26/07/12
Shri B. M. PATIL Medical College

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 19-07-12 *at* 03-30pm *to scrutinize the Synopsis/Research projects of postgraduate/undergraduate student/Faculty members of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis/Research project has been accorded Ethical Clearance.*

Title "Effect of Chronic stress on Lactogenesis in human"

—x—x—x—

Name of P.G./U.G. student/Faculty member Mrs. Vandali Jyothi
Dept of Physiology

Name of Guide/Co-investigator Dr. G.B. Dhanalakshmi. Prof. Physiology

DR.M.S.BIRADAR,
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

Inward. No. 252 Date 27/7/12
Department of Physiology
Circulation / Action / for
taken, reply

Original Research Article **Allied science**

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STUDY OF CHRONIC STRESS IN PREGNANT WOMEN

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ABSTRACT

Chronic stress is one of the biopsychosocial factors that contributes to adverse pregnancy outcomes such as preterm labor, aborted fetus, delayed fetal growth, low birth weight baby etc. Chronic stress can be either due to many stressors or due to same stressor continuously for a prolonged period, which repeatedly activates autonomic nervous system and hypothalamic-pituitary-adrenal (HPA) axis without relaxation response, resulting in persistent physiologic effects. Physiologic response in turn causes malfunctioning of HPA axis to release excess cortisol, the principle stress hormone. The present study was conducted to assess the relation between the chronic stress and cortisol during pregnancy. Pregnant women were assessed for the level of stress with the help of Holmes and Rahe stress scale. 96 pregnant women were selected for the study. Equal number of non-pregnant women were included in the study as a control group. Objective measurement was done by analysing the serum cortisol levels by electrochemiluminescence immunoassay. Among 96 subjects 36 (37.5%) were mildly stressed, 34 (35.41%) were moderately stressed and 26 (27.08%) were severely stressed. There was significant increase in serum cortisol levels in stressed pregnant women when compared with the cortisol levels of nonpregnant women. Level of significance was with $p< 0.05$.

KEYWORDS：Serum cortisol, chronic stress, HPA axis, pregnancy.

VANDALI JYOTHI
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INTRODUCTION

Stress is an integral part of our lives. Stress is defined as an internal process that occurs when a person is faced with a demand that is perceived to exceed the resources available to effectively respond to it and where failure to effectively deal with the demand has important undesirable consequences.[1] Stress can be acute or chronic. Acute stress is short term stress and is experienced in response to immediate perceived threat. While chronic stress is long term stress, generally lasting weeks to months. The concept of chronic stress is based on how frequently the stressors appear.[2] So chronic stress is a state of on-going physiological arousal. This occurs when the body experiences several stressors or a single stressor continuously, that it does not have the ability or opportunity to activate the relaxation response. It can develop in response to everyday stressors which are ignored or poorly managed or in response to traumatic events.[3] Studies indicate that prevalence of stress during pregnancy has been found to range from 6% to as high as 52.9% in developing countries.[4,5] Neilsen's study of chronic stress in Indian women reveals that 87% of women are stressed severely. Short term stress is not detrimental to health as it can actually be beneficial in certain circumstances (as it can increase alertness and performance), but prolonged periods of stress (chronic stress) have been linked to negative health consequences and stress hormones are to blame.[6] Pregnancy is recognized as a stressful event in woman's life, as it is a time of physiological change that needs huge psychological adjustment.[7] Studies indicate that pregnant women with high stress and anxiety levels are at increased risk for preeclampsia, spontaneous abortion, preterm labor, for having a malformed or growth-retarded baby and may also affect the lactogenesis.[8,9] Maternal stress has been defined as a potential predictor and the causes include mostly social support, quality of life, socioeconomic status.[10] Identification of the affecting factor is essential for improved pregnancy outcomes. Physiologic stress response involves activation of autonomic nervous system and HPA axis, which originate in brain.[11] With chronic stress both systems are repetitively activated, thus resulting in persistent physiologic effects.[12] Severe stress alters HPA axis, malfunctions negative feedback loop resulting in excess production of CRH from hypothalamus, which stimulates anterior pituitary for the systemic release of ACTH (adrenocorticotrophic hormone), which subsequently signals the adrenal glands to release glucocorticoids predominantly cortisol.[13,14] Serum cortisol levels were gradually increased from 6 weeks to 40 weeks of pregnancy and a sharp rise was noted two weeks before the onset of labor. Cortisol levels may be a more objective measure of chronic stress during pregnancy. High cortisol levels in pregnancy has been associated with adverse pregnancy outcomes like aborted fetus, delayed fetal growth etc.[15,16] The study was conducted to assess the level of stress in 1st, 2nd and 3rd trimesters of pregnancy. Also to correlate the interaction between chronic stress and cortisol in all the three trimesters of pregnancy.

MATERIALS AND METHODS

The study was conducted in Karimnagar, which is one of the fast developing part of Telangana state. 96 pregnant women in the reproductive age (21-45 yrs) attending the antenatal clinic of Prathima institute of medical sciences were enrolled for the study. Equal number of non pregnant females were included in the study as controls. The study was designed and got approved by the ethical committee of the institution. Participants were informed about the purpose of study, their role in the study and were asked to sign the consent form if were willing to take a part. A proforma was obtained consisting of clinical and other details of the pregnant women. They were assured that despite entering in the study they could withdraw any time if they wished and their information would be kept confidential. Participants included were non-smokers, with no history of diabetes mellitus, with no h/o major endocrine abnormalities. Pregnant women at different age groups were assessed for the level of stress by asking questionnaire as per Holmes and Rahe stress scale and categorised into mildly, moderately, severely stressed groups based on LCU. The number of Life Change Units (LCU) apply to events in the past year of an individual's life. The final score had shown the estimate of level of stress. Mild stress; LCU<150, moderate stress; LUC150-299, severe stress; LCU≥ 300. Objective measurement was done by analyzing the serum cortisol levels in all the three trimesters of pregnancy. Blood samples (5-7ml) were collected by venipuncture between 8.30am – 10.30am with all sterile precautions, placed in evacuated tubes and then taken to laboratory in ice box, centrifuged at 1300 x g for 20 min at 4ºC in a refrigerated table top centrifuge. Serum samples for hormone assays were frozen at -20ºC until analyzed. Elecsys cortisol reagent kit,Cat. No. 11875116 was used for Serum cortisol quantitative determination by electrochemiluminescence immunoassay (Roche Elecsys 1010/2010). Results were recorded and data analysis was done. Cortisol levels of study groups were compared with the cortisol levels of controls. The software SPSS16.0 was used for data analysis.

RESULTS

Table I

Age wise distribution of pregnant women with educational status

Sl no	Age groups (yrs)	Under graduates	Graduates	Post graduates	Total
1	21-25	09	18	01	28
2	26-30	10	10	02	22
3	31-35	08	14	01	23
4	36-40	02	09	03	14
5	41-45	03	05	01	09
		32 (33.3%)	56 (58.3%)	08 (8.3%)	96

The study group of 96 pregnant women were divided into five age groups and their qualification levels were noted to predict the mostly affecting factor of stress. The age group 21-25 yrs included 28women, 26-30 yrs included 22 women, 31-35 yrs included 23 women, 36-40 yrs included 14 women and 41-45 yrs included 9 women in the study. As the area is still developing, only 8 (8.3%) women were postgraduates (Table-I).

Table II
Stress levels in different age groups

Age groups (yrs)	Mildly stressed (<150)	Moderately stressed (150-299)	Severely stressed (>300)	Total
20 – 25	14	7	7	28
26 – 30	8	9	5	22
31 – 35	8	8	7	23
36 – 40	4	7	3	14
41 – 45	2	3	4	9
	36(37.5%)	34(35.41%)	26(27.08%)	96

Among 96 pregnant women 37.5% were with mild stress, 35.41% were with moderate stress and 27.08% were with severe stress.

Table III
Cortisols at different stress levels during pregnancy

	1st trimester	2nd trimester	3rd trimester
Mean cortisol in µg/dl with SD at stress<150, n=36	14.15±1.92	22.58±2.00	27.40±2.54
Mean cortisol in µg/dl with SD at stress 150-299, n=34	20.44±1.33	26.26±1.48	30.40±2.46
Mean cortisol in µg/dl with SD at stress >300, n=26	23.10±2.53	27.64±3.11	33.02±2.95

The cortisol levels were highly significant with p≤ 0.05 when compared with controls (9.6-14.0 µg/dl).

The mean and SD of serum cortisol levels in nonpregnant women (controls) were 12.3±3.8 There were 96 pregnant women in the study group, in the age range of 21-45 yrs. It was observed that 36 (37.5%) women were in mild stress, 34 (35.41%) women were moderately stressed and 26 (27.08%) women were severely stressed (Table-II). It was observed that mean cortisol levels were higher in moderately and severely stressed women than in the controls. It was observed that women of age group 21-25 yrs with <150 LCU were having almost normal or more than normal levels of cortisol. Women of age group 36 -40 yrs were having very high levels of serum cortisol because of their declining reproductive age. It was observed that serum cortisol levels of stressed subjects were increased in 2nd trimester than that in 1st trimester, also reached to peak levels in 3rd trimester (Table-III).

DISCUSSION

In the study of Harvaline *etal* in chronically stressed subjects there was increased cortisol, which is similar to the present study. [17] Similar observations were seen by Obel etal that, higher cortisol levels were there in subjects with chronic stress.[18] Similar results were observed in the study of Dallman *etal*, ie, chronic mild stress leads to increased trough levels of plasma cortisol while chronic severe stress results in around the clock elevation in both plasma ACTH and cortisol. [14] In our study women with mild stress were having almost normal levels of serum cortisol. Most of them were newly married and their economic condition was good. Moderately and severely stressed women were having very high levels of serum cortisol when they reached 3rd trimester. Few were in elderly maternal age, few had bad obstretic history, few women were highly in need of male child, few had large number of persons in the family but common factor was poor socioeconomic status. Maternal anxiety is one of the predictors of chronic stress.

CONCLUSION

Chronic stress may lead to negative consequences like preeclampsia, spontaneous abortion, preterm labor, for having a malformed or growth-retarded baby etc. Therefore, it is necessary to bring awareness in the population and further studies are required to observe chronic stress status during pregnancy in larger population.

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INTERACTION BETWEEN CHRONIC STRESS AND LACTATION

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ABSTRACT

Chronic stress can be either due to many stressors or due to same stressor continuously for a prolonged period, which repeatedly activates autonomic nervous system and hypothalamic-pituitary-adrenal axis without relaxation response, resulting in persistent physiologic effects. Physiologic response causes malfunctioning of HPA axis to release excess cortisol, the principle stress hormone. Studies have shown that acute physical and mental stress can impair milk ejection reflex. If this occurs repeatedly during prolonged stress, it could reduce milk production by preventing full emptying of the breast at each feed. As the effects of chronic emotional stress on lactation are not known, this study was conducted to assess the relation between maternal chronic stress and lactation. Pregnant women were assessed for the level of stress with the help of Holmes and Rahe stress scale, 96 were selected for the study. Objective measurement was done by analyzing serum cortisol levels by electrochemiluminescence immunoassay. It was observed that among 96 subjects 36 (37.5%) were mildly stressed, 34 (35.41%) were moderately stressed and 26 (27.08%) were severely stressed. Serum cortisol levels were high in moderately stressed subjects when compared to mildly stressed subjects with $p< 0.05$. Also high levels of serum cortisol were observed in subjects with severe stress when compared to moderately stressed subjects with $p< 0.05$. Milk volumes were measured upto 5 days postpartum by test weighing method using digital weighing machine. Daily milk volumes were compared between mildly, moderately and severely stressed subjects. Gradual decrease in the mean milk volumes were recorded with $p< 0.05$. So chronic stress may hamper the lactation.

KEY WORDS : **Lactation, chronic stress, serum cortisol, milk volume.**

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INTRODUCTION

Human milk is a carefully engineered substance, has unique composition ideal for the growth and development of infants. Availability of mother's milk is conceivable only if lactation is sustained. To safeguard lactation, it is necessary to know the physiology of lactation, how to initiate and maintain maternal interest in breastfeeding particularly at the time of stress. The initiation of lactation/milk production is based on research conducted over the past 20 to 25 years.[1] Daly and Hartmann separated the onset of milk production into two phases, lactogenesis I (L-I) and lactogenesis II (L-II).[2,3] During mid-pregnancy the mammary gland gains the ability to secrete milk; L-I, but full milk secretion is inhibited by the high levels of oestrogen and progesterone yet the gland remains quiet and prepared to initiate lactation after birth.[4] L-II is the onset of copious milk production after parturition with a severe decline in the milk inhibiting hormones progesterone (in particular), oestrogen and placental lactogen, also simultaneous increase in concentration of prolactin from the anterior pituitary.[5] The initiation and maintenance of lactation require appropriate hormonal changes and maternal behavior. When lactation insufficiency cannot be explained with inadequate mammary development, it is routinely blamed on maternal stress. Depending on the duration of the stress, suppression of lactation may result from a decrease in milk synthesis or ejection. The prevalence of stress during pregnancy has been found to range from 6% to as high as 52.9% in developing countries.[6,7] Two potential mechanisms can be hypothesized for the relationship between stress and lactogenesis. First, maternal stress seems to interfere with the release of oxytocin, that is responsible for the milk ejection reflex. If the milk ejection reflex is impaired often, then the incomplete removal of milk from the breast eventually will lead to down-regulation of milk synthesis. Although milk removal is not necessary to trigger lactogenesis II, it may be related to the timing of onset of full milk production or the volume of milk produced.[8,9] Maternal stress affects levels of prolactin involved in lactation, but there is little evidence about this in humans. Second, a newborn who experienced stress during labor and delivery may be too weak or too sleepy to latch on and suckle effectively at the breast. Even if the lactational capacity of the mother is not compromised, this could lead to impaired lactogenesis if milk removal is not adequate. It may happen that the causal pathway between maternal stress and lactogenesis could be reversed i.e. mothers who experience delayed onset of milk production are likely to become stressed as a result. In observational studies it is often difficult to determine the relationship between cause and effect.[9] General references vary in their descriptions of the onset of L-II from two to three days, to four days up to even eight days postpartum.[4,8] Then the onset of L-II is categorised into the early onset of lactogenesis as less than 72 hours and delayed onset as more than 72 hours.[10] Animal studies have demonstrated suppression of lactation after exposure to certain types of stressful stimuli.[11] Most of these studies were performed during established lactation, not during lactogenesis. In humans the studies have examined whether maternal stress affects the milk ejection reflex or the amount of milk transferred during a feed. The first was a unique experiment by Newton and Newton in 1948, which involved three different types of distractions imposed during the first morning feed on a mother. Immersion of her feet in ice water for 10 sec of every 30 sec; verbal math problems, accompanied by mild electric shocks if the mother got the wrong answer or took too long in answering and intermittent pulling of the mother's big toes, causing sharp pain. Despite the fact that the study included only one woman, the novel design of the experiment provided useful information. Milk intake by the infant at the first morning feed was measured on 8 control days and 12 distraction days ie, 4 days for each of the three treatments. Milk intake on control days was quite consistent, ranging from 142 to 209 g (mean, 168 g).[12] These findings indicate the effect of acute stress on lactation. The effect of chronic stress on lactation is not known. As chronic stress is a state of on-going physiological arousal. This occurs when the body experiences several stressors or a single stressor continuously, that it does not have the ability or opportunity to activate the relaxation response.[13] Physiologic stress response involves activation of autonomic nervous system and HPA axis, which originate in brain.[14] With chronic stress both systems are repetitively activated, thus resulting in persistent physiologic effects.[15] Severe stress alters HPA axis, malfunctions negative feedback loop resulting in excess production of CRH from hypothalamus, which stimulates anterior pituitary for the systemic release of ACTH (adrenocorticotrophic hormone), which subsequently signals the adrenal glands to release glucocorticoids predominantly cortisol.[16,17] Serum cortisol levels gradually increase from 6 weeks to 40 weeks of pregnancy and a sharp rise may be noted two weeks before the onset of labor. Cortisol levels indicate the objective measure of chronic stress during pregnancy. High cortisol levels in pregnancy has been associated with adverse pregnancy outcomes like aborted fetus, delayed fetal growth [18,19] and may be on lactogenesis also. The study was conducted to assess the level of stress in 1st, 2nd and 3rd trimesters of pregnancy to correlate the interaction between chronic stress and cortisol in all the three trimesters of pregnancy, also relation between chronic stress and milk volume output.

MATERIAL AND METHODS

The study was conducted during the period 2012 - 2015 in Karimnagar, Telangana state. 96 pregnant women in the reproductive age (21-45 yrs) attending the antenatal clinic of Prathima institute of medical sciences were enrolled for the study. The study was designed and got approved by the ethical committee. The research protocol was explained to the participants, also their role in the study and were asked to sign the consent form if were willing to take part. A proforma was obtained consisting of clinical and other details of the pregnant women. Participants were assured that despite entering in the study they could withdraw any time they wished and their information would be kept confidential.

Inclusion criteria

Participants included were non-smokers, with no history of diabetes mellitus, with no h/o major endocrinal abnormalities.

Exclusion criteria

Pregnant women with the history of nonlactogenesis, with no intention to breastfeed were excluded from the study.

To measure stress according to the Holmes and Rahe Stress Scale, the number of "Life Change Units"(LCU) that apply to events in the past year of an individual's life were added and the final score gave a estimate of how stress affects health. Pregnant women at different age groups were assessed for the level of stress by asking questionnaire as per Holmes and Rahe stress scale and categorised into mildly, moderately, severely stressed groups.Mild stress; LCU<150, moderate stress; LUC150-299, severe stress; LCU≥ 300.Objective measurement was done by analyzing the serum cortisol levels in all the three trimesters of pregnancy. Blood samples(5-7ml) were collected by venipuncture between 8.30am – 10.30am with all sterile precautions, placed in evacuated tubes and then taken to laboratory in ice box, centrifuged at 1300xg for 20 min at 4ºC in a refrigerated table top centrifuge. Serum samples for hormone assays were frozen at -20ºC until analyzed. Elecsys cortisol reagent kit, Cat. No. 11875116 was used for Serum cortisol quantitative determination by electrochemiluminescence immunoassay (Roche Elecsys 1010/2010). Women were encouraged to express breast milk 8 times per day from day 1 to day 5 postpartum. Milk volumes(ml) were measured by test weighing method on digital weighing machine. Results were recorded and data analysis was done. The software SPSS16.0 was used for data analysis.

RESULTS

Table I

Stress levels in different age groups

Age groups (yrs)	Mildly stressed (<150)	Moderately stressed(150-299)	Severely stressed (>300)	Total
20 – 25	14	7	7	28
26 – 30	8	9	5	22
31 – 35	8	8	7	23
36 – 40	4	7	3	14
41 – 45	2	3	4	9
	36(37.5%)	34(35.41%)	26(27.08%)	96

Among 96 pregnant women 37.5% were with mild stress, 35.41% were with moderate stress and 27.08% were with severe stress.

Table II

Cortisols at different stress levels during pregnancy

	1st trimester	2nd trimester	3rd trimester
Mean cortisol in µg/dl with SD at stress<150, n=36	14.68±1.30	23.28±1.79	29.49±3.63
Mean cortisol in µg/dl with SD at stress 150-299, n=34	20.62±0.29	25.16±1.35	29.42±1.11
Mean cortisol in µg/dl with SD at stress >300, n=26	23.09±1.95	27.34±1.38	32.69 ± 0.47

The cortisol levels were significant with p≤ 0.05 when compared with controls (9.6 - 14.0 µg/dl).

Table III

Milk volumes (ml) from day1 to day 5 postpartum at different stress levels

	Mean, SD of milk vol in mild stress(< 150)	Mean, SD of milk vol in moderate stress(150-299)	Mean, SD of milk vol in severe stress(> 300)
Day 1	47.63 ± 3.33	43.60 ± 3.28	38.82 ± 4.89
Day 2	95.72 ± 5.38	85.89 ± 5.15	78.26 ± 7.51
Day 3	261.77 ± 17.57	172.81 ± 18.59	134.44 ± 16.13
Day 4	379.27 ± 14.29	294.54 ± 20.53	217.70 ± 49.22
Day 5	596.06 ± 16.81	409.83 ± 42.80	349.53 ± 39.38

Total of 96 pregnant women were included in the study group, in the age range of 21-45 yrs. It was observed that 36 (37.5%) women were in mild stress, 34 (35.41%) women were moderately stressed and 26 (27.08%) women were severely stressed (Table-I). The mean and SD of serum cortisol levels in nonpregnant women (controls) were 12.3±3.8 µg/dl. It was observed that mean cortisol levels were higher in moderately and severely stressed women than in the controls. It was observed that women of age group 21-25 yrs with <150 LCU were having almost normal or more than normal levels of cortisol. Women of age group 36-40 yrs were having very high levels of serum cortisol because of their declining reproductive age. It was observed that serum cortisol levels of stressed subjects were increased in 2nd trimester than that in 1st trimester, also reached to peak levels in 3rd trimester (Table-II). Milk volumes were reduced gradually (with p<0.05) in moderately and severely stressed women when compared with the milk volumes of mildly stressed women(Table-III). Lower milk volumes were observed in moderately stressed subjects when compared with milk volumes of mildly stressed subjects. Similarly milk volumes were still lower in severely stressed subjects when compared with the milk volumes of moderately stressed subjects. The milk volumes were significant with p≤ 0.05.

DISCUSSION

In our study it was observed that the milk volumes were significantly reduced in severely stressed women. The first study conducted by Newton and Newton in 1948, indicated that, the maternal acute stress suppresses the lactation.[12] Similar study by Feher, Berger etal, estimated the milk vol output from the mothers who gave birth prematurely.[20] They observed that the mothers were in stress and the milk volume was reduced when compared with the milk volumes of controls. So, lactation insufficiency can be because of maternal stress. These studies were conducted in women with acute stress. Our study is conducted in women with chronic stress. Chronic stress is a state of on-going physiological arousal. This occurs when the body experiences several stressors or a single stressor continuously for prolonged period, that it does not have the ability or opportunity to activate the relaxation response that leads to malfunctioning of HPA axis and so excess cortisol. This type of chronic stress response occurs may be because of our modern lifestyle, when everything from high-pressured jobs to loneliness to busy traffic can keep the body in a state of perceived threat and chronic stress ie, due to everyday stressors which are ignored or poorly managed. [13]

CONCLUSION

Human milk is the ideal food for infants because of its unique nutritional characteristics and with the benefits of human milk well recognized, it is necessary to focus attention on how to optimize lactation for the benefit of newborns and it is essential to bring awareness in population. Further research is required to gain a better understanding of the differential effects of stress on lactation.

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