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BIOCHEMICAL CHANGES OF NEUROCHEMICAL HORMONES IN PREGNANT AND NON-PREGNANT SUDANESE WOMEN IN KHARTOUM STATE - SUDAN

By

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صدق الله العظيم

Declaration and Statement

I Mutasem Mahmoud Abdallah Ahmed under signed, I declare and affirm that this Thesis is our own original work. We have followed all ethical and technical principles in the preparation, data collection, data analysis and compilation of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation. I solemnly declare that this Thesis has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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Dedication

This work is dedicated to my

wonderful children

The symbol of love and giving

To my Parents

Who gave me Light

To my Teachers

Who taught me wrong from Right

To my brother, sisters,

Who always with me

To Those Who Have,

And Always Will,

Stand Beside me

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Abstract

Background:-

Multipara was basically understood to be a parity of three or higher and has stable relationship with obstetric troubles. It could be seen as a reason for unhealthy maternal and neonatal outcome. This study aimed to compare the levels of neurochemical hormones in multiparity, with primiparity and nulliparity

Materials and methods:-

This is a prospective hospital- laboratory based study. It was conducted in Jabal Awlia Hospital, Khartoum State-Sudan, during the period of September 2016 to April 2019. A total of (400) women aged (20-48 years) were enrolled in this study regularly visiting this hospital for routine follow up. (200) of participated women were multiparity while(100) of them were primiparity and others (100) were nulliparity. Moreover, all participants were in their fertility period and/or premenopausal period. After provided oral consent, questionnaires were administered to the women and venous blood samples (5mLs) were taken from each participant by standard procedures. Then serum for neurochemical hormones was measured using full automated ELISA [Elisys Duo (6234000096), human-Germany]. Furthermore, the precision and accuracy of all methods used in this study were checked each time; a batch was analyzed by including commercially prepared control sera.

Results: it was found that the Serotonin , Oxytocin and Ghrelin levels showed significant changes in multiparty group when compared to nulliparity and also primiparity. β-endorphin, adrenalin and dopamine levels showed insignificant changes in multiparity group when compared to nulliparity .In multiparty, serotonin and oxytocin levels showed significant changes when classified according to age (P- value < 0.05). in which most certainly these levels of

serotonin as well as oxytocin in multiparity pregnant women increased by getting older. Nevertheless, body mass index (BMI) of multiparty women exhibited insignificant changes on both serotonin and oxytocin levels (P- value > 0.05). In multiparity, oxytocin levels showed significant changes when classified according to the delivery type (P- value <0.05). In multiparity group, ghrelin levels revealed insignificant changes when classified according to age and BMI, (P- value>0.05). but elucidates significant change when classified according to parity number, type of delivery and number of miscarriage(P- value<0.05).

Conclusion:-

The results of this current study proved that the multiparity pregnancy has significant effect to levels of serotonin, oxytocin and ghrelin hormones ,but has insignificant effect on the levels of β-endorphin, adrenalin and dopamine hormones. Also the results adopted that, the levels of oxytocin and ghrelin hormones in multiparity pregnant women are affected significantly by women age, number of miscarriage and delivery type, knowing that BMI and gender of childbirth have insignificant effect on hormones. But yet needless to say that the levels of ghrelin hormone in multiparity group are significantly affected by the number of parity.

Key Words: Neurochemical Hormones, Pregnant and non Pregnant Women.

ملخص الدراسة

خلفية الدراسة :- يعتبر تعدد الولادة في النساء على أنه حمل مكتمل لثلاثة أو أعلى وله علاقة مستقرة مع مشاكل التوليد. يمكن أن يكون السبب في وجود نتائج غير صحية للأمهات والأطفال حديثي الولادة. هدفت هذه الدراسة إلى مقارنة مستويات الهرمونات الكيميائية العصبية في التعددية ، مع ذوات الحمل الواحد و الغير حوامل

طرق الدراسة:- في هذه الدراسة تم جمع (400) عينه من مستشفى جبل أولياء بولاية الخرطوم - السودان ، خلال الفترة من سبتمبر 2016 إلى أبريل 2019. تم تسجيل ما مجموعه أربعمائة امرأة تتراوح أعمارهن ما بين(20-48 سنة) .من خلال زياراتهم المنتظمة للمستشفى من أجل المتابعة الروتينية. كانت هناك (200) من النساء المشاركات متعددات الولادة، في حين كان (100) منها من ذوات الحمل الواحد ، بينما كانت (100) أخرى غير حوامل. جميع المشاركين في فترة الخصوبة و / أو فترة ما قبل انقطاع الطمث. كانت جميع النساء الحوامل في الأسبوع (26) إلي الأسبوع (34) من الحمل .عندما قدمت موافقة شفهية ، تم إعطاء الاستبيانات إلى النساء وتم أخذ عينات دم وريديه (mL5) من كل مشاركه من خلال إجراءات قياسية. ثم تم قياس مصل الهرمونات الكيميائية العصبية باستخدام جهاز (ELISA) الآلي الكامل. Elisys] Duo (6234000096), human-Germany]

النتائج :- تشير النتائج أن مستويات هرمون السيروتونين , الأوكسيتوسين والغريلين أظهرت تغيرات مهمة في مجموعة متعددات الولادة عند مقارنتها بذوات الحمل الواحد وكذلك بالنساء الغير حوامل . أما بالنسبة لمستويات هرمون البيتا- اندورفين , الادرينالين و الدوبامين لم تظهر أي تغيرات مهمة في مجموعه متعددات الولادة بالمقارنة مع ذوات الحمل الواحد وكذلك بالنساء الغير حوامل.
في مجموعه متعددات الولادة ، أظهرت مستويات هرمون السيروتونين و الأوكسيتوسين تغييرات كبيرة عند تصنيفها وفقًا للعمر حيث كان مستوي الدلالة (P- value < 0.05) . حيث من المؤكد أن مستويات السيروتونين وكذلك الأوكسيتوسين في النساء الحوامل المضاعفات ازدادت مع تقدمهن في السن.ومع ذلك ، فإن مؤشر كتلة الجسم (BMI) من متعددات الولادة أدى إلى تغيرات غير مهمة على مستويات هرمون السيروتونين والأوكسيتوسين حيث كان مستوي الدلالة (P- value > 0.05) . أيضا ، أظهرت مستويات هرمون الأوكسيتوسين تغيرات هامة عند تصنيفها وفقًا لنوع الولادة حيث كان مستوي الدلالة (P- value .< 0.05)
في مجموعة متعددات الولادة ، أظهرت مستويات هرمون الغريلين تغيرات ضئيلة عند تصنيفها حسب العمر ومؤشر كتلة الجسم ، حيث كان مستوي الدلالة(P- value > 0.05) . ولكن أظهرت تغير كبير عند تصنيفها وفقا لعدد الولادات ، ونوع الولادة وعدد مرات الإجهاض حيث كان مستوي الدلالة (P- value .< 0.05)

الخلاصة:- أثبتت نتائج الدراسة الحالية أن الحمل متعدد الولادة له تأثير كبير على مستويات السيروتونين والأوكسيتوسين وهرمون الغريلين ، ولكن له تأثير ضئيل على مستويات بيتا- إندورفين ، الأدرينالين وهرمونات الدوبامين.أظهرت النتائج أيضا أن مستويات الأوكسيتوسين والغريلين تتأثر في النساء الحوامل التعدديات بشكل كبير بسن النساء ، وعدد الإجهاض ونوع الولادة ، مع العلم أن مؤشر كتلة الجسم ونوع الجنس في الولادة لهن تأثير ضئيل على الهرمونات. ولكن حتى الآن لا داعي للقول أن مستويات هرمون الغريلين في مجموعة متعددات الولادة إلى حد كبير بعدد مرات الحمل.

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ACTH	Adrenocorticotropic hormone
AG	Arachidonoyl-glycerol
AgRP	Agouti-related protein
Ala	Alanine
NH2	Amino group
ALP	Alkaline phosphatase
ALT	Alanine amino transferase
APH	Ante partum hemorrhage
Arg	Arginine
Asn	Asparagines
AST	Aspartate amino transferase
ATD	Acute tryptophan depletion
ADC	Amino acid decarboxylase
α	Alpha
β	Beta
BDNF	Brain-derived neurotropic factor
BFT	Benign familial tremor
BMI	Body mass index
CNS	Central nervous system
Cys	Cysteine
Da	Dalton
DBH	Dopamine beta Hydroxylase

DHEA	Dehydroepiandrosterone
DM	Diabetes Mellitus
DOPA	Di hydroxy phenethyl amine
ECL	Electro Chemical Luminescence
EDD	Expected date of delivery
ELISA	Enzyme-linked immune sorbent assay
GABA	Gamma-amino butyric acid
GH	Growth hormone
GHSR	Growth hormone secretagogue receptor
GI	Gastrointestinal
Gln	Glutamine
Gly	Glycine
GMP	Grand multiparty
GNRH	Gonadotropin-releasing hormone
γ	Gamma
HIAA	Hydroxyl indole acetic acid
HPLC	High performance liquid chromatography
HRP	Horseradish Peroxidase
HT	Hydroxyl tryptamine
Ile	Isoleucine
IU	International unit
INN	Lenomorelin
LFTs	Liver function tests

LMP	Last menstrual period
MAO	Monoamine oxidase
μg	Micro gram
MDMA	3,4-Methylene dioxy methamphet amine
ng	Nanogram
NGF	Nerve growth factor
NPY	Neuropeptide Y
σ	Neo
OSHA	Occupational Safety and Health Administration
Oxt	Oxytocin
OXTR	Oxytocin receptor
PAM	Peptidylglycine alpha-amidating mono oxygenase
PEA	Phenyl ethyl amine
pg	Pico gram
Phe	Phenylalanine
PNMT	Phenyl ethanol amine N-methyl transferase
PPH	Post -partum hemorrhage
Pro	Proline
PUPPP	Pruritic urticarial papules and plaques of pregnancy
QA	Quality Assurance
REA	Radio-enzymatic assays
RIA	Radio- immune assay
SAMe	S-adenosyl methionine

SSRIs	Selective serotonin reuptake inhibitors
TAAR	Trace amine-associated receptor
TMB	Tetra methyl benzidine
TPH	Tryptophan hydroxylase
Tyr	Tyrosine
Val	Valine
VMAT	Vesicular monoamine transporter
VTA	Ventral Tegmental Area

CHAPTER ONE

(Introduction, Rationale and Objective)

CHAPTER ONE

1. INTRODUCTION

1.1 Neurochemical Hormones :-

1.1.1Definition:-

Neurohormones are chemical messenger molecules that are released by neurons, but enter the blood stream where they travel to distant target sites within the body. Therefore, neurohormones share characteristics with both neurotransmitters and hormones. Similar to neurotransmitters, neurohormones are released by neurons. Similar to hormones, neurohormones travel in the bloodstream. The endocrinology of human pregnancy involves endocrine and metabolic changes that result from physiological alterations at the boundary between mother and fetus [1].So that we expect the hormones special neurochemical hormones has many alterations during pregnancy of multiparas.

1.2 Pregnancy:-

Pregnancy alters the physiology of the woman and affects the cardiovascular, and other systems specially the endocrine system since it displays hypertrophy of functions, altered hemostasis of some substances as well as exaggerated response to posture [2]. On the other hand, multiparity has always been associated with poor pregnancy outcomes for both mother and baby. The term grand multipara was introduced by Solomon who called grand multipars as dangerous multipara. Complications like hypertension, diabetes mellitus, malpresentation, anemia, hypertension, difficult labor and postpartum hemorrhage, increased risk of operative delivery with higher rates of cesarean section and incidence of obstructed labor and ruptured uterus. All these complications have often been associated with multiparity [3]. Pregnancy and the postpartum period are considered

to be relatively high-risk times for women with pre-existing psychiatric illnesses, specially for depressive episodes in women. The prevalence of depression has been reported to be between (10 and 16%) during pregnancy. Pregnancy and the postpartum periods appear to confer an even greater risk for women with bipolar disorder. Rates of relapse are estimated at (30-50%) during the postpartum period. Mood and anxiety disorders are common in women during their childbearing years during pregnancy. The course of panic disorder can be variable. Miller *et al* reports suggested an initial onset of obsessive-compulsive disorder (OCD), symptoms, and it is typically worsening. When psychotic disorders occur during pregnancy special considerations are needed [4] . Pregnancy either induces or exacerbates pre-existing stress and in turn, stress seems to have a negative effect on pregnancy, especially in the first trimester which is the period of greatest stress, and highest rate of pregnancy loss.

Major depression is twice as common in women than in men and frequently clusters during the childbearing years. Although pregnancy has traditionally been considered a time of emotional well-being for women conferring protection against psychiatric disorders. Sharma *et al*, India 2005 study describes rates of major and minor depression as approximating (10%)Other studies also note clinically significant depressive symptoms during pregnancy, particularly in the setting of antidepressant discontinuation The risk increases with a past history of mood disorder . However, it has also been observed in about (1/3) of depressed pregnant women, this represents the first episode of major depression other risk factors for antenatal depression include marital-discord or dissatisfaction, inadequate psychosocial supports, recent adverse life events, lower socioeconomic status, and unwanted pregnancy [5]. Kemppainen *et al*, North Finland 2000 studies have shown that maternal grand multiparity may predict an increased risk of

psychopathology in adult offspring. These studies have demonstrated that among offspring born to grand multiparous mothers increased risks which are evident for mood disorders, schizophrenia, suicides and other psychotic disorders. Furthermore, this studies have shown that offspring born as a 4th or later born - child have higher risks of psychiatric hospitalizations for any reason and that offspring born at 3 rd or later-born child has an increased risk of personality and behavioral disorders in adult life [6].

1.2 Rationale:

The Islamic religion is urged to encourage marriage and high number births. Since this study will be conducted in Khartoum State Sudan, so multiparity of and grand-multiparity are considered common in the community. In Saudi Arabia, multiple child- birth is valuable for the transfer of inherited cultural backgrounds; therefore, a high incidence of grand multiparity is expected. In addition, early age of marriage (before 20 years) might be one of the reasons for this high incidence of grand multiparas. On the other hand, the health status of grand-multiparity become of less interest in Europe and USA, and that because the percentage of grand-multiparity is reduced remarkably in the last few decades as a result of the birth control policy adopted in those countries. In the united states in 2014 (2.8%) of live births is the 5th child in the family, (1.7%) are the 6th or 7th child, and (0.5%) are the 8^{ht} child and over[7]. Maternal mental health significantly influences Maternal–fetal attachment (MFA) [11]. Perceived social support predicted MFA to a greater extent when compared with other predictors such as anxiety, self-esteem and depression. Researchers opened but despite of that there is quite notable information on the development and moderation of psychological features of the maternal–fetal relationship, relatively little is known about the manner in which maternal psychological functioning influences the fetus [8] . Alaraisanen *et al* studies showed that grand multiparity may predict a psychopathology in both of mother and adult children [9]. These studies disclosed that grand multiparous mothers increase risks of mood and psychotic disorders. Likewise, regardless of parity, a study showed that birth at late ages may expose the pregnant lady to personality and behavioral disorders. There are some disorders that almost more attached to pregnant women, such as eating disorders, depression, mood swings and disorders of the body. A systematic review in developed countries showed that of the (18%) of women reporting depressed

mood during pregnancy, (13%) met the diagnostic criteria for a major depressive episode according to American Psychiatric Association (DSM-IV) [10]. In a Japanese study on both antenatal and postnatal, about (12%) of the women were suffering from psychiatric disorders as depressive disorder, manic episode, generalized anxiety disorder, social phobia, specific phobia and obsessive compulsive disorder [11].

1.3 Objectives:-

1.3.1General Objective:-

Biochemical changes of neurochemical hormones in pregnant and non-pregnant Sudanese women in Khartoum State - Sudan

1.3.2Specific Objectives:-

- To compare the levels of neurochemical hormones between (3) groups
- To assess the levels of neurochemical hormones in (3) groups according to women age
- To assess the levels of neurochemical hormones in multiparity according to number of miscarriage
- To assess the levels of neurochemical hormones in multiparity according to number of parity
- To compare the levels of neurochemical hormones in multiparity between Caesarean section birth and normal vaginal delivery

Chapter Two

Literature Review

Chapter Two

Literature Review

2.1.Pregnancy

Pregnancy is also known as gestation, is the time during which one or more offspring develops inside a woman. A multiple pregnancy involves more than one offspring, such as with twins. It can occur by sexual intercourse or assisted reproductive technology. Childbirth typically occurs around (40) weeks from the last menstrual period (LMP). This is just over nine months, where each month averages (31days). When measured from fertilization it is about (38) weeks [12]. Pregnancy is typically divided into (3) trimesters. The 1st trimester is from week one through (12) and includes conception, which is when the sperm fertilizes the egg. The fertilized egg then travels down the fallopian tube and attaches to the inside of the uterus, where it begins to form the embryo and placenta. During the 1st trimester, the possibility of miscarriage (natural death of embryo or fetus) is at its highest. The 2nd trimester is from week (13) through (28). Around the middle of the 2nd trimester, movement of the fetus may be felt. At (28) weeks, more than (90%) of babies can survive outside of the uterus if provided with high-quality medical care.The 3rd trimester is from (29) weeks through (40) weeks [13] . About (213) million pregnancies occurred in 2012, of which, (190) million were in the developing world and (23) million were in the developed world. The number of pregnancies in women ages (15 to 44 is 133 per 1,000) women [14]. Globally, (44%) of pregnancies are unplanned. Over half (56%) of unplanned pregnancies are aborted [15]. Among unintended pregnancies in the United States, (60%) of the

women used birth control to some extent during the month at which pregnancy occurred.

2.1.1 Signs and symptoms of pregnancy

The usual symptoms and discomforts of pregnancy do not significantly interfere with activities of daily living or pose a health-threat to the mother or baby. However, pregnancy complications can cause other more severe symptoms, such as those associated with anemia [16].

Common symptoms and discomforts of pregnancy include:

- Tiredness
- Morning sickness
- Constipation
- Pelvic girdle pain
- Back pain
- Braxton Hicks contractions. Occasional, irregular, and often painless contractions that occur several times per day.
- Peripheral edema swelling of the lower limbs. Common complaint in advancing pregnancy. Can be caused by inferior vena cava syndrome resulting from compression of the inferior vena cava and pelvic veins by the uterus leading to increased hydrostatic pressure in lower extremities.
- Low blood pressure often caused by compression of both the inferior vena cava and the abdominal aorta (aortocaval compression syndrome).
- Increased urinary frequency. A common complaint, caused by increased intravascular volume, elevated glomerular filtration rate, and compression of the bladder by the expanding uterus.
- Urinary tract infection [20] .

- Varicose veins. Common complaint caused by relaxation of the venous smooth muscle and increased intravascular pressure.
- Hemorrhoids (piles). Swollen veins at or inside the anal area. Caused by impaired venous return, straining associated with constipation, or increased intra-abdominal pressure in later pregnancy [17] .
- Regurgitation, heartburn, and nausea.
- Stretch marks
- Breast tenderness is common during the 1st trimester, and is more common in women who are pregnant at a young age[18] .

2.1.2 Start of gestational age

According to American Congress of Obstetricians and Gynecologists, the main methods to calculate gestational age are:

Directly calculating the days since the beginning of the LMP.

Early obstetric ultrasound, comparing the size of an embryo or fetus to that of a reference group of pregnancies of known gestational age (such as calculated from last menstrual periods), and using the mean gestational age of other embryos or fetuses of the same size. If the gestational age as calculated from an early ultrasound is contradictory to the one calculated directly from the LMP, it is still the one from the early ultrasound that is used for the rest of the pregnancy [19] .

In case of in vitro fertilization, calculating days since oocyte retrieval or co-incubation and adding (14 days) [20] .

Estimated date of confinement

Due date estimation basically follows two steps:

- Determination of which time point is to be used as origin for gestational age, as described in section above.

- Adding the estimated gestational age at childbirth to the above time point. Childbirth on average occurs at a gestational age of (280 days) (40 weeks), which is therefore often used as a standard estimation for individual pregnancies[21] . However, alternative durations as well as more individualized methods have also been suggested.

Naegele's rule is a standard way of calculating the due date for a pregnancy when assuming a gestational age of (280 days) at childbirth. The rule estimates the expected date of delivery (EDD) by adding a year, subtracting (3) months, and adding (7) days to the origin of gestational age. Alternatively there are mobile apps, which essentially always give consistent estimations compared to each other and correct for leap year, while pregnancy wheels made of paper can differ from each other by (7) days and generally do not correct for leap year [22] . Furthermore, actual childbirth has only a certain probability of occurring within the limits of the estimated due date. Hoffman *et al*, USA 2008 study of singleton live births came to the result that childbirth has a standard deviation of (14 days) when gestational age is estimated by 1st trimester ultrasound, and (16 days) when estimated directly by last menstrual period [23] .

2.1.3 Physiology

2.1.3.1 Initiation

Human fertilization

Through an interplay of hormones that includes follicle stimulating hormone (FSH) that stimulates folliculogenesis and oogenesis creates a mature egg cell, the female gamete. Fertilization is the event where the egg cell fuses with the male gamete, spermatozoon. After the point of fertilization, the fused product of the female and male gamete is referred to as a zygote or fertilized egg. The fusion of male and female gametes usually occurs following the act of sexual

intercourse. Pregnancy rates for sexual intercourse are highest during the menstrual cycle time from some (5 days) before until (1 to 2 days) after ovulation. Fertilization can also occur by assisted reproductive technology such as artificial insemination and in vitro fertilization. Fertilization (conception) is sometimes used as the initiation of pregnancy, with the derived age being termed fertilization age. Fertilization usually occurs about (2) weeks before the next expected menstrual period. A third point in time is also considered by some people to be the true beginning of a pregnancy: This is time of implantation, when the future fetus attaches to the lining of the uterus. This is about a week to (10 days) after fertilization. In this model, during the time between conception and implantation, the future fetus exists, but the woman is not considered pregnant [24].

2.1.3.2 Development of embryo and fetus

Prenatal development, Human embryogenesis, and Fetus

An embryo is the developing offspring during the first (8) weeks following fertilization, after which, the term fetus is used until birth. Symptoms of early pregnancy may include missed periods, tender breasts, nausea and vomiting, hunger, and frequent urination [25]. The sperm and the egg cell, which has been released from one of the female's two ovaries, unite in one of the two fallopian tubes. The fertilized egg, known as a zygote, then moves toward the uterus, a journey that can take up to a week to complete. Cell division begins approximately (24 to 36) hours after the male and female cells unite. Cell division continues at a rapid rate and the cells then develop into what is known as a blastocyst. The blastocyst arrives at the uterus and attaches to the uterine wall, a process known as implantation. The development of the mass of cells that will become the infant is called embryogenesis during the first approximately (10 weeks) of gestation. During this time, cells begin to differentiate into the various body systems. The

basic outlines of the organ, body, and nervous systems are established. By the end of the embryonic stage, the beginning of features such as fingers, eyes, mouth, and ears become visible. Also during this time, there is development of structures important to the support of the embryo, including the placenta and umbilical cord. The placenta connects the developing embryo to the uterine wall to allow nutrient uptake, waste elimination, and gas exchange via the mother's blood supply. The umbilical cord is the connecting cord from the embryo or fetus to the placenta. After about (10 weeks) of gestational age, the embryo becomes known as a fetus. At the beginning of the fetal stage, the risk of miscarriage decreases sharply. At this stage, a fetus is about 30 mm (1.2 inches) in length, the heartbeat is seen via ultrasound, and the fetus makes involuntary motions. During continued fetal development, the early body systems, and structures that were established in the embryonic stage continue to develop. Sex organs begin to appear during the 3^{rd} month of gestation. The fetus continues to grow in both weight and length, although the majority of the physical growth occurs in the last weeks of pregnancy Electrical brain activity is first detected between the (5^{th} and 6^{th}) week of gestation. It is considered primitive neural activity rather than the beginning of conscious thought. Synapses begin forming at (17) weeks, and begin to multiply quickly at week 28 until (3 to 4 months) after birth [25].

2.1.3.3 Maternal physiological changes

Breast changes as seen during pregnancy .The areola are larger and darker during pregnancy, the woman undergoes many physiological changes, which are entirely normal,including behavioral, cardiovascular, hematologic, metabolic, renal,and res -piratory changes. Increases in blood sugar, breathing, and cardiac output

are all required. Levels of progesterone and estrogens rise continually throughout pregnancy, suppressing the hypothalamic axis and therefore also the menstrual cycle. The fetus is genetically different from the woman and can be viewed as an unusually successful allograft. The main reason for this success is increased immune tolerance during pregnancy. Immune tolerance is the concept that the body is able to not mount an immune system response against certain triggers. Pregnancy is typically broken into (3) periods, or trimesters, each of about (3) months. Each trimester is defined as (14 weeks), for a total duration of (42 weeks), although the average duration of pregnancy is (40 weeks). While there are no hard and fast rules, these distinctions are useful in describing the changes that take place over time [26].

2.1.3.4 First trimester

Minute ventilation increases by (40) in the 1st trimester. The womb will grow to the size of a lemon by (8) weeks. Many symptoms and discomforts of pregnancy like nausea and tender breasts appear in the 1st trimester [27].

2.1.3.5 Second trimester

By the end of the 2nd trimester, the expanding uterus has created a visible "babybump". Although the breasts have been developing internally since the beginning of the pregnancy, most of the visible changes appear after this point.Weeks (13 to 28) of the pregnancy are called the 2nd trimester. Most women feel more energized in this period, and begin to put on weight as the symptoms of morning sickness subside and eventually fade away. The uterus, the muscular organ that holds the developing fetus, can expand up to (20 times) its normal size during pregnancy. Although the fetus begins to move during the 1st trimester, it is not until the 2nd trimester that movement, known as quickening, can be felt. This typically

happens in the 4th month, more specifically in the 20th to 21st week, or by the 19th week if the woman has been pregnant before. It is common for some women not to feel the fetus move until much later. During the 2nd trimester, most women begin to wear maternity clothes.

2.1.3.6 Third trimester

Final weight gain takes place, which is the most weight gain throughout the pregnancy. The woman's abdomen will transform in shape as it drops due to the fetus turning in a downward position ready for birth. During the 2nd trimester, the woman's abdomen would have been upright, whereas in the 3rd trimester it will drop down low. The fetus moves regularly, and is felt by the woman. Fetal movement can become strong and be disruptive to the woman. The woman's navel will sometimes become convex, "popping" out, due to the expanding abdomen.

Head engagement, where the fetal head descends into cephalic presentation, relieves pressure on the upper abdomen with renewed ease in breathing. It also severely reduces bladder capacity, and increases pressure on the pelvic floor and the rectum.

It is also during the 3rd trimester that maternal activity and sleep positions may affect fetal development due to restricted blood flow. For instance, the enlarged uterus may impede blood flow by compressing the vena cava when lying flat, which is relieved by lying on the left side [28].

2.1.3.7 Childbirth

In the ideal childbirth labor begins on its own when a woman is "at term". Pregnancy is considered at full term when gestation has lasted (39 to 41 weeks). After (41) weeks, it is known as late term and after (42) weeks post term. Babies born before (39) weeks are considered early term while those before (37) weeks are preterm. Preterm babies are at higher risk of health problems such as cerebral palsy. Delivery before (39)

weeks by labor induction or caesarean section is not recommended unless required for other medical reasons .Referred to as labor and delivery in the medical field, is the process whereby an infant is born. A woman is considered to be in labor when she begins experiencing regular uterine contractions, accompanied by changes of her cervix – primarily effacement and dilation. While childbirth is widely experienced as painful, some women do report painless labors, while others find that concentrating on the birth helps to quicken labor and lessen the sensations. Most births are successful vaginal births, but sometimes complications arise and a woman may undergo a cesarean section. During the time immediately after birth, both the mother and the baby are hormonally cued to bond, the mother through the release of oxytocin, a hormone also released during breastfeeding. Cooijmans KHM , *et al* , Radboud University Netherlands 2017 Studies showed that skin-to-skin contact between a mother and her newborn immediately after birth is beneficial for both the mother and baby[29]. A review done by the World Health Organization (WHO) found that skin to skin contact between mothers and babies after birth reduces crying, improves mother–infant interaction, and helps mothers to breastfeed successfully. They recommend that neonates be allowed to bond with the mother during their first two hours after birth, the period that they tend to be more alert than in the following hours of early life [30]. In the ideal childbirth labor begins on its own when a woman is "at term". Events before completion of (37) weeks are considered preterm. Preterm birth is associated with a range of complications and should be avoided if possible [31]. Sometimes if a woman's water breaks or she has contractions before (39) weeks, birth is unavoidable. However, spontaneous birth after (37) weeks is considered term and is not associated with the same risks of a pre-term birth. Planned birth before (39) weeks by caesarean section or labor induction, although

"at term", results in an increased risk of complications. This is from factors including underdeveloped lungs of newborns, infection due to underdeveloped immune system, feeding problems due to underdeveloped brain, and jaundice from underdeveloped liver [32]. Babies born between (39 and 41) weeks gestation have better outcomes than babies born either before or after this range. This special time period is called "full term". Whenever possible, waiting for labor to begin on its own in this time period is best for the health of the mother and baby.The decision to perform an induction must be made after weighing the risks and benefits, but is safer after (39) weeks [33]. Events after (42 weeks) are considered post term. When a pregnancy exceeds (42 weeks), the risk of complications for both the woman and the fetus increases significantly. Therefore, in an otherwise uncomplicated pregnancy, obstetricians usually prefer to induce labor at some stage between (41 and 42 weeks) [34].

Postnatal period

The postnatal period, also referred to as the puerperium, begins immediately after delivery and extends for about (6 weeks). During this period, the mother's body begins the return to pre-pregnancy conditions that includes changes in hormone levels and uterus size [35].

2.1.4 Diagnosis:-

The beginning of pregnancy may be detected either based on symptoms by the woman herself, or by using pregnancy tests. However, an important condition with serious health implications that is quite common is the denial of pregnancy by the pregnant woman.

2.1.4.1 Physical signs

Symptoms and discomforts of pregnancy:-

Most pregnant women experience a number of symptoms, which can signify pregnancy. A number of early medical signs are associated with pregnancy [36] . These signs include:-

- the presence of human chorionic gonadotropin (HCG) in the blood and urine
- missed menstrual period
- implantation bleeding that occurs at implantation of the embryo in the uterus during the 3rd or 4th week after last menstrual period.
- increased basal body temperature sustained for over (2 weeks) after ovulation
- Chadwick's sign (darkening of the cervix, vagina, and vulva).
- Goodell's sign (softening of the vaginal portion of the cervix)
- Hegar's sign (softening of the uterus isthmus)
- Pigmentation of the linea alba – linea nigra, (darkening of the skin in a midline of the abdomen, caused by hyperpigmentation resulting from hormonal changes, usually appearing around the middle of pregnancy).
- Darkening of the nipples and areolas due to hormonal increase [37].

2.1.4.2 Biomarkers

Pregnancy detection can be accomplished using one or more various pregnancy tests, which detect hormones generated by the newly formed placenta, serving as biomarkers of pregnancy. Blood and urine tests can detect pregnancy (12 days) after implantation. Blood pregnancy tests are more sensitive than urine tests (giving fewer false negatives). Home pregnancy tests are urine tests, and normally detect a pregnancy (12 to 15 days) after fertilization. A quantitative blood test can determine approximately the date the embryo was conceived because HCG doubles every (36 to 48 hours)[35]. A single test

of progesterone levels can also help determine how likely a fetus will survive in those with a threatened miscarriage (bleeding in early pregnancy [38].

2.1.5 Management:-

2.1.5.1 Prenatal care

improves pregnancy outcomes, by taking extra folic acid, avoiding drugs and alcohol, regular exercise, blood tests, and regular physical examinations. Pre-conception counseling is care that is provided to a woman and/ or couple to discuss conception, pregnancy, current health issues and recommendations for the period before pregnancy. Prenatal medical care is the medical and nursing care recommended for women during pregnancy, time intervals and exact goals of each visit differ by country. Women who are high risk have better outcomes if they are seen regularly and frequently by a medical professional than women who are low risk. A woman can be labeled as high risk for different reasons including previous complications in pregnancy, complications in the current pregnancy, current medical diseases, or social issues. The aim of good prenatal care is prevention, early identification, and treatment of any medical complications. A basic prenatal visit consists of measurement of blood pressure, fundal height, weight and fetal heart rate, checking for symptoms of labor, and guidance for what to expect next [39].

2.1.5.2 Nutrition

Important to ensure healthy growth of the fetus. There are increased energy requirements, protein intake and specific micronutrient requirements [40]. Adequate folic acid intake has been shown to decrease the risk of fetal neural tube defects, such as spina bifida. The neural tube develops during the first (28 days) of pregnancy, folate is abundant in green leafy vegetables, legumes, and citrus [41]. For the pregnant

woman consume adequate amounts of DHA omega-3 during pregnancy and while nursing to support her well-being and the health of her infant. Developing infants cannot produce DHA efficiently, and must receive this vital nutrient from the woman through the placenta and in breast milk after birth [42]. Certain nutrients such as Vitamin D and calcium, required for bone development, may also require supplementation [43]. Vitamin E supplementation has not been shown to improve birth outcomes [44] . Zinc supplementation has been associated with a decrease in preterm birth, but it is unclear whether it is causative [45] . Daily iron supplementation reduces the risk of maternal anemia [46]. The nutritional needs for women carrying twins or triplets. are higher than those of women carrying one baby [46].

2.1.5.3 Weight gain

The amount of healthy weight gain during a pregnancy varies. Weight gain is related to the weight of the baby, the placenta, extra - circulatory fluid, larger tissues, and fat and protein stores. Most needed weight gain occurs later in pregnancy [47]. The Institute of Medicine in America recommends an overall pregnancy weight gain for those of normal weight (body mass index of 18.5–24.9), of 11.3–15.9 kg (25–35 pounds) having a singleton pregnancy [48]. Women who are underweight (BMI of less than 18.5), should gain between 12.7–18 kg (28–40 lbs), while those who are overweight (BMI of 25–29.9) are advised to gain between 6.8–11.3 kg (15–25 lbs) and those who are obese (BMI>30) should gain between 5–9 kg (11–20 lbs) [49]. These values are references for expectations of a term pregnancy. During pregnancy, insufficient or excessive weight gain can compromise the health of the mother and fetus. The most effective intervention for weight gain in underweight women is not clear. Being or becoming overweight in pregnancy increases the risk of complications for mother and fetus, including cesarean section,

gestational hypertension, pre-eclampsia, macrosomia and shoulder dystocia.

Around (50%) of women of childbearing age in developed countries like the United Kingdom(UK) are overweight or obese before pregnancy. Diet modification is the most effective way to reduce weight gain and associated risks in pregnancy. A diet that has foods with a low glycemic index may help prevent the onset of gestational diabetes [50].

2.1.5.4 Sexual activity

Sex during pregnancy is a low-risk behavior except when the healthcare provider advises to be avoided for particular medical reasons [51]. Most research suggested that during pregnancy both sexual desire and frequency of sexual relations decrease, indicated a 2nd-trimester increase, preceding a decrease during the 3rd trimester.

2.1.5.5 Exercise

Regular exercise during pregnancy improve physical fitness and decrease the need for C-section. Bed rest, outside of research studies, is not recommended as there is no evidence of benefit and potential harm. Although an upper level of safe exercise intensity has not been established, women who were regular exercisers before pregnancy and who have uncomplicated pregnancies should be able to engage in high intensity exercise programs [52].

2.1.5.6 Sleep

It has been suggested that shift work and exposure to bright light at night should be avoided at least during the last trimester of pregnancy to decrease the risk of psychological and behavioral problems in the newborn [53].

2.1.6. Complications of pregnancy:-

About (10% to 15%) of recognized pregnancies end in miscarriage [56].

The following are some examples of pregnancy complications, pregnancy induced hypertension, Anemia [55], postpartum depression, postpartum psychosis, thromboembolic disorders. These are the leading cause of death in pregnant women in the US [56][57], pruritic urticarial papules and plaques of pregnancy (PUPPP), a skin disease that develops around the 32nd week. Signs are red plaques, papules, and itchiness around the belly button that then spreads all over the body except for the inside of hands and face, ectopic pregnancy, implantation of the embryo outside the uterus, hyperemesis gravidarum, excessive nausea and vomiting that is more severe than normal morning sickness and pulmonary embolism, a blood clot that forms in the legs and migrates to the lungs [58] and there is also an increased susceptibility and severity of certain infections in pregnancy.

2.2. gravidity and parity

2.2.1. Defenitions

In biology and human medicine, gravidity and parity are the number of times a female has been pregnant (gravidity) and carried the pregnancies to a viable gestational age (parity) [59]. These terms are usually coupled, sometimes with additional terms, to indicate more details of the woman's obstetric history, the gravida indicates the number of times the woman has been pregnant, regardless of whether these pregnancies were carried to term. A current pregnancy, if any, is included in this count. Twin pregnancy is counted as 1. Parity, or "para" indicates the number of (>28-week in the US) births (including viable and non-viable; i.e., stillbirths) (>24 in UK). Pregnancies consisting of multiples, such as twins or triplets, counts as (2 or 3) respectively in terms of parity. Abortus is the number of pregnancies that were lost for any reason, including induced

abortions or miscarriages. The abortus term is sometimes dropped when no pregnancies have been lost. Stillbirths are not included.

In human medicine, "gravidity" refers to the number of times a woman has been pregnant,[59] regardless of whether the pregnancies were interrupted or resulted in a live birth. The term "gravida" can be used to refer to a pregnant woman. A "nulligravida" is a woman who has never been pregnant. A "primigravida" is a woman who is pregnant for the first time or has been pregnant one time. A "multigravida" or "secundigravida" is a woman who has been pregnant more than one time. Terms such as "gravida 0", referring to a nulligravida, "gravida 1" for a primigravida, and so on, can also be used. The term "elderly primigravida" has also been used to refer to a woman in her first pregnancy, who is at least (35 years) old, In biology, the term "gravid" (Latin: gravidus "burdened, heavy") is used to describe the condition of an animal (most commonly fish or reptiles) when carrying eggs internally. For example, Astatotilapiaburtoni females can transform between reproductive states, one of which is gravid, and the other non-gravid. In entomology it describes a mated female insect. Parity is the number of pregnancies carried to viable gestational age. A woman who has never carried a pregnancy beyond (20 weeks) is nulliparous, and is called a nullipara or para 0. A woman who has given birth once before is primiparous, and is referred to as a primipara or primip; moreover, a woman who has given birth two or more times is multiparous and is called a multip. Finally, grand multipara describes the condition of having given birth (3) or more times. Like gravidity, parity may also be counted. A woman who has given birth one or more times can also be referred to as para 1, para 2, para 3 and so on. Viable gestational age varies from region to region, for example in the UK it is considered to be (24 weeks) whilst in the USA (23) weeks is considered viable. Prolonged nulliparity

is a risk factor for breast cancer. For instance, a meta-analysis of (8) population-based on Marianne E, *et al* studies in the Nordic countries found that nulliparity was associated with a (30%) increase in risk of breast cancer compared with parous women, and for every (2) births, the risk was reduced by about (16%). Women having their 1st birth after the age of (35 years) had a (40%) increased risk compared to those with a first birth before the age of (20 years). Criticism in humans, it can lead to some ambiguity for events occurring between (20 and 24) weeks, and for multiple pregnancies [60].

2.2.2 Complications multiparity

Grand multiparity has been considered an independent risk factor for increasing adverse outcome for fetus and mother, specially diabetes mellitus, antepartum hemorrhage, malpresentations, cesarean section rate, postpartum hemorrhage, iron deficiency anemia, and a high perinatal mortality rate. The relationship between parity and pregnancy complications continues to be of interest for most obstetricians. Parity has been used as a risk marker with nulliparous and grand multiparous women classified as high risk population for pregnancy complications. There is no universally accepted definition for grand multiparity. The term “grand multipara” was introduced in 1934 by Solomon, who called grand multipara the “the dangerous multipara”. (WHO) classifies women having (6) previous deliveries after (24 weeks) gestation as grand multipara, where as international federation of gynaecologist and obstetrician(FIGO) defines (5) or more deliveries as grand multiparity. The terms great grand multipara and extreme grand multipara have also been used to describe (10) or more and (18-20) pregnancies respectively. Women of low parity are defined as those having (1-3) deliveries. The prevalence of grand multiparity is very low in developed countries (3-4%) of deliveries as a result of increasing awareness of associated health

problems, health promotion and effective methods of contraception, but in most of developing countries like Pakistan grand multiparity is still seen quite oftenly. Population in this country has now crossed (160) millions and fertility rate is (5-6 children) per women . Grand multiparity is particularly common in low income group and those marrying at an early age. Other factors which influence the occurrence of grand multiparity in society include social and cultural factors often intermingled with religious factors [61].

The risk to mother and child is relatively high in first pregnancy and then the risk declines during second and third pregnancy, but again rises slowly with increasing parity. By the 6th pregnancy the risk exceeds those of first pregnancy and after that it rises steeply with each subsequent pregnancy. Grand multiparity is likely to be associated with complications during pregnancy, labor and postpartum which should alert obstetrician to provide extra care to these patients. Anemia is one of most common problem associated with multiparity. These women do not get enough time to recover the hemoglobin level to normal as the interval between successive pregnancies is short. They also lactate numerous children at the expense of their own nutrition. However, there is conflicting evidence on occurrence of anemia. There are reports showing increase incidence and other study showed no difference and even some studies showing decreased risk. The risk of miscarriages is also high in grand multipara . The grand multipara are usually older than (30) years, therefore medical disorders i.e., diabetes, hypertension, cardiac and renal disease are also common in these women. Uterine laxity in multiparity results in malpresentations and malpositions. If un recognized these may end up in obstructed labor. Each successive pregnancy results in increasing weight of baby which may consequently result in cephalo-pelvic disproportion. Increasing inclination of pelvic brim and occasional

forward subluxation of sacrum upon sacro-iliac joint reduces true conjugate which may result in cephalopelvic disproportion, therefore pelvic assessment is also important in grand multipara. Uterine atonicity after delivery leads to postpartum hemorrhage in grand multipara often aggravated by preexisting anemia in majority of women .Advanced maternal age of grand multipara has been reported to be an independent risk factor of gestational DM, ante_ partum hemorrhage, fetal distress, prematurity, low birth weight, perinatal mortality and chromosomal abnormalities particularly Down Syndrome. In this regard, consideration of the confounding effect of advanced age of the grand multipara is pivotal when analyzing the maternal and neonatal outcome of grand multiparity. In the absence of clear and consistent evidence of the association of grand multiparity with adverse pregnancy outcomes, classifying grand multipara as a high-risk group could increase the cost burden to families and health systems as well as physical and psychological stress to the mother and family. The present study intended to know the association of obstetric complication with grand multiparity and to compare it with women of low parity. We it is to estimated to compare the specified maternal and perinatal complications among grand multipara and women of low parity delivered at civil hospital Karachi and identify their associated risk factors for poor maternal and perinatal outcome and convey it to community which might influence and create wareness in those local population regarding complications of grand multiparity. Deeba F *et al* ,Civil Hospital Karachi 2016 study concluded that grand multipara are at a greater risk during pregnancy and labor. In view of the results obtained in this study, who feel that It can be concluded that in comparison with the other patients, grand multiparity continue to pose major threats to women life. The results of this study showed that grand multiparity is associated with increased risk of

miscarriage, anemia, APH, malpresentations, instrumental deliveries and PPH. It is also strongly associated with high rate of neonatal death. Majority of local population is ignorant of the fact that through proper antenatal care many of their problems can be identified and managed. As in low health-resource settings all pregnancies are prone to adverse outcomes, so adequate management of labor, a good referral system as well as the practice of basic and comprehensive obstetric emergency care should be mandatory. Awareness regarding proper antenatal care and family planning should be developed in society to reduce complications associated with grand multiparity [62].

In Saudi Arabia, large family is desirable for cultural reasons; consequently, a high incidence of grand multiparity is expected. The Fertility rate in Saudi Arabia was last reported at (2.81) in 2010, according to a World Bank report published in 2012 . In addition, early age of marriage might be one of the reasons for this high incidence of grand multiparas.

The Saudi population has one of the highest fertility rates in the world. Multiparity, grand multiparity, and above, are seen frequently in Saudi populations, with (12) children in a family not being uncommon. However, in Bondagji's, Bahrain 2005 study a significant factor for negative obstetric outcomes were related to unbooked deliveries (usually referring to a pregnant mother who receives no antenatal care). Yet, when the study controlled for this variable by including a similar number of un booked deliveries in both its multiparous and GMP groups, the results "showed a significant increase in the perinatal mortality, the rate of caesarean section, medical complications and postpartum hemorrhage among (GMP) compared to multiparous women in a population with high rate of un booked deliveries" . The trend in many recent papers is toward a conclusion that the outcome of GMP is much improved when compared

to previous years, specially when mothers receive adequate antenatal However, anemia is still a common problem even in developed countries. This could be due to some of the factors already noted, including late- or even non-booking of deliveries and low socio-economic status, or even poor spacing of children [63].

Mgaya *et al,* Muhimbili National Hospital Tanzania 2013 reports defined grand multiparity as parity starts from (5) because the threshold of risks of any pregnancy problems, neonatal disturbances, and perinatal death increase clearly at parity equal to or more than five . High parity and reduced inter-pregnancy interval are reported to be risk factors for poor maternal and perinatal outcome. These factors together or separately may predispose the pregnant women to many diseases .Pregnancy, itself, alters the levels of many circulating hormones, enzymes, and proteins produced and released from many organs . Grand multiparas area high-risk obstetric group of patients liable to develop a number of ante partum and intra-partum complications with adverse neonatal outcome. Little is known about the cumulative effects of numerous childbearing on long-term hepatic dysfunctions. The main findings of the previous study were that mortality from cancers of the liver and stomach among the GM women were(18%) . Liver function tests (LFTs) are blood tests used to assess the general state of the liver or biliary system. LFTs are used with the history and physical examination to guide to diagnose and manage a number of liver disorders. Perhaps the most commonly used indicators of liver (hepatocellular) damage are the alanine aminotransferase (ALT) and aspartate aminotransferase (AST). These are enzymes normally found in liver cells that leak out of these cells and make their way to the blood when liver cells are injured. The level of the ALT and AST may be used as a general measure of the degree of liver inflammation or damage. The alkaline phosphatase is the most frequently used test to detect obstruction

in the biliary system. Increased level of this enzyme may be found in a large number of disorders as common as gallstones, alcoholism, and toxic hepatitis. Bilirubin is the main bile pigment in humans which, when elevated causes jaundice. Bilirubin is formed primarily from the breakdown of heme found in red blood cells and processed and secreted into the bile by the liver. It is, however, generally useful as a real test for liver function, for it is reflecting ability of the liver to seize, intoxicate, and secrete bilirubin into the bile. Albumin is a major protein which is formed by the liver. So, liver disease causes a decrease in the amount of albumin formation, which leads to clear reduction in plasma albumin concentration . Kamal Eldin A, *et al*, Al-Ajyal hospital Sudan 2015 studies is designed to determine if there would be any changes in serum activities of liver enzymes [AST, ALT and ALP] as well as levels of total protein, albumin and bilirubin in grand multiparas pregnant Sudanese women and to compare the results of these parameters with the results from age- and sex matched primiparas [first time pregnancy] and apparently healthy individuals non-pregnant females [nulliparas, control] significance and differences from control and test values were evaluated by student t-test, at which the P value of less than 0.05 considers the significance. Who concluded from study that Grand multiparas are a high-risk obstetric group of patients liable to develop a number of antepartum and intrapartum complications with adverse neonatal outcome. The liver showed great functioning impairment in multiparity. Women need to be informed of the dangers of multiparity and advised to prevent it [64].

Labor carries a significant concern not only for the mother but also for the fetus. The impact is even more in the under developed nations of the world where most of the women deliver at home or at places where even the minimum health care amenities are not available. Under these

situations, the women and their babies may encounter a variety of complications which at their extreme may lead to fetal, neonatal or maternal mortality. Therefore, there is a huge need to identify women whose pregnancy is at increased risk of complications which is a pivotal part of antenatal screening. It is a real obligation to provide them necessary health care services to rescue and save their lives. Factors contributing to pregnancy complications are diverse including young age, lack of cognizance regarding provision of antenatal care, health education deficiency, negligence, monetary limitations, ecological & traditional biases, involvement of male members in maternal health care, deprived nutritional status of young pregnant women like high prevalence of anemia, conveyance issues, lesser focus on patient counseling before decision of mode of delivery particularly in primiparous are the important explanations behind high frequency of these complications. Moreover, Women facing these problems are by all means bound to receive care in insufficient facilities. The impact of first delivery on forth coming obstetric record and delivery decision by patient and the attending obstetrician is unmatched. Cesarean section rates fluctuate among obstetricians due to various indications in low risk pregnant population. Since decision for labor and mode of delivery has a great bearing on future obstetrics of a woman, therefore cautious monitoring and a sensible decision for labor and mode of delivery is essential from the attending doctor. A primigravida's labor is way different from that of a multigravida. The distinguishing feature in a primigravida is lengthier duration when compared with multigravida. Low birth weight infants are more frequent in primigravida group which is responsible for higher morbidity and mortality risks. Furthermore, the likelihood of perineal trauma is higher in primigravida as a consequence of episiotomy or spontaneous tears. Another important aspect is the fact that intrapartum

risk assessment usually depends upon past obstetrical performance which is obviously not pertinent to primigravida. Besides all these facts, the risk of adverse outcome with parity does not show a steady pattern. The objective of this study was to compare the obstetric outcome between primigravida and multigravida presenting at term in labor. Nulliparous women are at greater risk of labor abnormalities, fetal distress, instrumental deliveries, cesarean section, postpartum hemorrhage and neonatal morbidity. Such risk factors should be sought after and dealt well in time [65].

2.3. Neurochemical hormones :-

In chemistry, neurochemistry is the study of the chemical function and operation of the nervous system. Neurochemistry includes the study of cellular neurochemistry and neural membranes; intercellular signaling; growth, development, and differentiations; metabolism; inherited and neurodegenerative diseases; sensory transduction; and neural processing and behavior. In human chemistry, research efforts over the last few decades has focused on how various neurochemicals, in particular: Oxt, vasopressin, endorphin, dopamine, serotonin, norepinephrine, testosterone, estrogen, epinephrine, phenylethylamine (PEA), dehydroepiandrosterone (DHEA), brain-derived neurotropic factor (BDNF), monoamine oxidase (MAO), gamma-aminobutyric acid (GABA), prolactin, cortisol, progesterone, estradiol, nerve growth factor (NGF), gonadotropin-releasing hormone (GNRH), among others, related to various moods, e.g. happiness, and states of human existence, e.g. being in love, in relation to changes in human bonding . The human body produces hundreds of neurochemicals. Only a small fraction of these have been identified by scientists. Lifetime, it will not be known how cell of these molecules[66] .

2.3.1 Vasopressin

monogamy molecule (hormone); responsible for creating intense loving memories during passionate situations; responsible for clarity of thought and alertness during passionate situations. Associated with male-female attachment. Ejaculation increases level in the brain, triggering spousal and parenting zeal. When injected into the brain it causes males to engage in territorial defense behaviors from other males and to become extremely possessive of the female[s] in his territory. When the production in the brain is artificially blocked, males will copulate with a female and then abandon her for another mating opportunity. Is made in the hypothalamus, ovaries, and testes. At orgasm, levels increase dramatically in men. Released during stimulation of the genitals and nipples. Works with testosterone, modulating male sexual behavior, keeping it from reaching extremes or becoming too hot, i.e. it has a tempering influence. It turns a person's attention from the abstract to the concrete, away from the past and future to the here and now, and appears to improve memory, cognitive powers, and concentration. Secreted from the posterior pituitary gland. It increases attention and alertness while reducing emotionalism. A deficiency of causes a reduction of REM sleep. Artificially increased levels of induces flank-marking behaviors, thus communicating status and instilling dominant-subordinate hierarchies [67].

2.3.2 Nor epinephrine

[C8H11NO3] also called nor adrenaline or noradrenalin, is an organic chemical in the catecholamine family that functions in the brain and body as a hormone and neurotransmitter. The name "nor adrenaline", derived from Latin roots meaning "at/alongside the kidneys", is more commonly used in the United Kingdom; in the United States, "nor epinephrine", derived from Greek roots having that same meaning, is usually preferred. It is elevated levels are associated with romantic love.

Is a precursor of epinephrine (adrenaline) in its major biosynthetic pathway. Increased levels associated with sexual arousal and heightened motivation. The smell of male urine causes levels to increase in the female brain. Levels spike as an estrus female looks at slides of a male's face. Heightened levels associated with lordosis. Increasing levels produce exhilaration, excessive energy, sleeplessness, and loss of appetite. Is associated with increased memory for new stimuli, especially social memory [68].

2.3.3 Testosterone

[C19H28O2] – is the primary male sex hormone and an anabolic steroid. In male humans, testosterone plays a key role in the development of male reproductive tissues such as testes and prostate, as well as promoting secondary sexual characteristics such as increased muscle and bone mass, and the growth of body hair. In addition, testosterone is involved in health and well-being, and the prevention of osteoporosis. Insufficient levels of testosterone in men may lead to abnormalities including frailty and bone loss [69].

2.3.4 Estrogen

It is s the primary female sex hormone. It is responsible for the development and regulation of the female reproductive system and secondary sex characteristics. There are three major endogenous estrogens in females that have estrogenic hormonal activity: estrone, estradiol, and estriol. The estrane steroid estradiol is the most potent and prevalent of these. Estrogens are synthesized in all vertebrates as well as some insects. Their presence in both vertebrates and insects suggests that estrogenic sex hormones have an ancient evolutionary history. The three major naturally occurring forms of estrogen in females are estrone (E1), estradiol(E2), and estriol (E3).

Another type of estrogen called estetrol (E4) is produced only during pregnancy. Quantitatively, estrogens circulate at lower levels than androgens in both men and women. While estrogen levels are significantly lower in males compared to females, estrogens nevertheless also have important physiological roles in males [70].

2.3.5 Phenethylamine

(PEA) [C8H11N] functions as a monoaminergic neuromodulator, and to a lesser extent, a neurotransmitter in the human central nervous system. It is biosynthesized from the amino acid L-phenylalanine by enzymatic decarboxylation via the enzyme aromatic L-amino acid decarboxylase. Amphetamine-like molecule (neurotransmitter) often known as the 'molecule of love.' It speeds up the flow of information between nerve cells. Keeps one alert, confident, and ready to try something new. Causes people to feel optimistic of the future, sleep less, and be more socially out-going. Low levels have been correlated with love sickness. Is considered the visual component of the chemistry of love, in that its levels surge in the sight of someone desirable or even a picture, art, or something one reads. Levels are typically higher in women, particularly at ovulation, than in men. Levels are high in schizophrenics and during a divorce [71].

2.3.6 Dehydroepiandrosterone

(DHEA), [C19H28O2] also known as androstenolone, is an endogenous steroid hormone. Most abundant hormone in the body; increases sex drive and influences who one finds attractive; levels increase to (3) to (5) times that of baseline before and during orgasm. Because most of the other sex hormones are derived from it, it thus unconsciously tells a person when they can or cannot have sex. Pheromones are derived from it. Responds directly to both emotional and

environmental stimuli and drops drastically under stress. It excites the septum and medial preoptic areas, which are known to promote active and pleasurable sexual behavior and reactions. When males are exposed to the scent of receptive females for (7) days, levels are increased in the amygdala and hypothalamus. Vigorous exercise, for about (30) minutes per day, for one month, causes a measurable increase in levels of DHEA [72].

2.3.7 Brain-Derived Neurotropic Factor

[BDNF] is a protein that, in humans, is encoded by the BDNF Gene. Is a member of the neurotrophin family of growth factors, which are related to the canonical nerve growth factor. Neurotrophic factors are found in the brain and the periphery [73].

2.3.8 Monoamine oxidase (MOA)

are a family of enzymes that catalyze the oxidation of monoamines, employing oxygen to clip off their amine group. They are found bound to the outer membrane of mitochondria in most cell types of the body. The first such enzyme was discovered in 1928 by Mary Bernheim in the liver and was named tyramine oxidase. The MAOs belong to the protein family of flavin-containing amine oxidoreductases. MAOs are important in the breakdown of monoamines ingested in food, and also serve to inactivate monoamine neurotransmitters. Because of the latter, they are involved in a number of psychiatric and neurological diseases, some of which can be treated with monoamine oxidase inhibitors(MAOIs) which block the action of MAOs [74].

2.3.9 Gamma-amino butyricacid

(GABA)plays and inhibitory role in the brain. GABA receptors induce calm by inhibiting or modulating over-reactive neurons. Substances such as alcohol and valium have a potentiating effect on GABA synapses, thus

instilling a state of calmness. Its principal role is reducing neuronal excitability throughout the nervous system. In humans, GABA is also directly responsible for the regulation of muscle tone [75].

2.3.10 Prolactin

Motherly hormone (stops female and male sex drive). Is secreted by the pituitary gland and stimulates the growth of mammary tissue and triggers the production of milk. When a baby suckles, increase more (10) times their normal level. Women who suckle their infants on a regular basis have a severely reduced sex drive. Men with prolactin-producing pituitary tumors often lose their libido completely along with their erections. Dopamine inhibits prolactin, consequently boosting sex drive indirectly. Estrogen gradually increase prolactin secretion, thus diminishing the aggressive sex drive. In both men and women, prolactin secretion is increased by exercise, psychological stress, stimulation of the nipples, and sleep.It was discovered in non-human animals around 1930 by Oscar Riddle [76].

2.3.11 Cortisol

The primary hormone product of the adrenal glands, in other tissue in lower quantities , also it helps restore homeostasis after a state of stress; heightened levels are associated with those newly in love and with the establishment of new relationships. Depressed people have elevated levels beyond which under stress can be explained. The scent of and rostadienone, a volatile component of male sweat, causes levels to rise in females[77].

2.3.12Progesterone

[C21H30O2] Is an endogenous steroid and progesterone sex hormone involved in the menstrual cycle, pregnancy and embryogenesis of humans. Reverse sex drive hormone; one of the (2) female sex hormones along with estradiol that during pregnancy begin to rise causing an

intensification of a women's natural femaleness, where she becomes more mothering and nurturing, and while at the same time dampening sexual receptiveness. The rapid decrease in progesterone and estradiol after childbirth is accompanied by increased prolactin and oxytocin, which are themselves stimulated for production by the nursing infant's suckling at its mothers' breast. Hormonal treatment of induces maternal behavior towards infants, even those not of the mothers. It reduces sex drive, by lowering testosterone levels in both sexes. It decreases positive sexual scents, e.g. pheromones, and may make people smell bad to each other, reducing the likelihood of attraction. It makes women irritable towards men and aggressive in protecting their young. Has a mild sedative, anesthetic, and calming effect [78].

2.3.13 Estradiol

[C18H24O2] is an estrogen steroid hormone and the major female sex hormone. It is involved in the regulation of the estrous and menstrual female reproductive cycles. Estradiol is responsible for the development of female secondary sexual characteristics such as the breasts, widening of the hips, and a feminine pattern of fat distribution in women and is important in the development and maintenance of female reproductive tissues such as the mammary glands, uterus, and vagina during puberty, adulthood, and pregnancy [79].

2.3.14 Nerve growth factor

A neuroprotein that stimulates cell growth; higher levels are found with those newly in love as compared to those single or in long-term relationships.

2.3.15 Gonadotropin-releasing hormone

(GnRH) Is a pulsatile, rhythmic-release sexual stimulant that reliably increases mating and sexual behavior, e.g. male copulation and female

lordosis. Heightened levels in males induces them to spend more time near females sniffing, licking, nuzzling, and nipping.

2.3.16 Glutamate

Is the most common neurotransmitter. Most neuron are secreted with glutamate or GABA. Glutamate is excitatory, meaning that the release of glutamate by one cell usually causes adjacent cells to fire an action potential. (Note: Glutamate is chemically identical to the MSG commonly used to flavor food.)

2.3.17 GABA

Is an example of an inhibitory neurotransmitter.

2.3.18 Acetylcholine

assists motor function and is involved in memory.

2.3.19 Nitric oxide

Functions as a neurotransmitter, despite being a gas. It is not grouped with the other neurotransmitters because it is not released in the same way.

2.3.20 Endocannabinoids

Act in the endocannabinoid system to control neurotransmitter release in a host of neuronal tissues, including the hippocampus, amygdala, basal ganglia, and cerebellum.

2.3.21Eicosanoids

Act as neuromodulators via the arachidonic acid cascade.

2.3.22 Neurotrophic factors

Are biomolecules – nearly all of which are peptides or small proteins – that support the growth, survival, and differentiation of both developing and mature neurons.

2.3.23Oxytocin

(Oxt) is a human peptide hormone and neuropeptide that is used as a

par ventricular nucleus of the hypothalamus and released by the posterior pituitary. It plays a role in social bonding, sexual reproduction in both sexes, during and after childbirth. Oxt is released into the blood stream as a hormone in response to stretching of the cervix and uterus during labor and with stimulation of the nipples from breastfeeding.

2.3.23.1 Biochemistry

Estrogen has been found to increase the secretion of Oxt and to increase the expression of its receptor, the Oxt receptor, in the brain. In women, a single dose of estradiol has been found to be sufficient to increase circulating Oxt concentrations.[80] Oxt is a peptide of (9) amino acids (a nonapeptide) in the sequence cysteine-tyrosine-isoleucine-glutamine asparagine- cysteine-proline-leucine-glycine-amide (Cys– Tyr – Ile – Gln – Asn – Cys – Pro – Leu – Gly – NH2, or CYIQNCPLG-NH2); its C-terminus has been converted to a primary amide and a disulfide bridge joins the cysteine moieties. Oxt has a molecular mass of 1007 Da, and one international unit (IU) of Oxt is the equivalent of about (2 μg) of pure peptide. While the structure of Oxt is highly conserved in placental mammals, a novel structure of Oxt was recently reported in marmosets, tamarins, and other new world primates. Genomic sequencing of the gene for Oxt revealed a single in-frame mutation (thymine for cytosine) which results in a single amino acid substitution at the 8-position (proline for leucine).

2.3.23.2 Biosynthesis

The Oxt peptide is synthesized as an inactive precursor protein from the Oxt gene. This precursor protein also includes the Oxt carrier proteinneurophysin I. The inactive precursor protein is progressively hydrolyzed into smaller fragments (one of which is neurophysin I) via a series of enzymes. The last hydrolysis that releases the active Oxt non apeptide is catalyzed by peptidylglycine alpha-amidating mono

oxygenase (PAM).The activity of the PAM enzyme system is dependent upon vitamin C (ascorbate), which is a necessary vitamin cofactor. By chance, sodium ascorbate by itself was found to stimulate the production of Oxt from ovarian tissue over a range of concentrations in a dose dependent manner. Many of the same tissues (e.g. ovaries, testes, eyes, adrenals, placenta, thymus, pancreas) where PAM (and Oxt by default) is found are also known to store higher concentrations of vitamin C. Oxt is known to be metabolized by the oxytocinase, leucyl/cystinylaminopeptidase. Other oxytocinases are also known to exist. Amastatin, bestatin (ubenimex), leupeptin, and puromycin have been found to inhibit the enzymatic degradation of Oxt, though they also inhibit the degradation of various other peptides, such as vasopressin, met-enkephalin, and dynorphin A [81].

2.3.23.2.1 Neural sources

In the hypothalamus, Oxt is made in magnocellular neurosecretory cells of the supraoptic and paraventricular nuclei, and is stored in herring bodies at the axon terminals in the posterior pituitary. It is then released into the blood from the posterior lobe (neurohypophysis) of the pituitary gland. These axons (likely, but dendrites have not been ruled out) have collaterals that innervate neuronsin the nucleus accumbens, a brain structure where Oxt receptors are expressed. The endocrine effects of Oxt hormone and the cognitive or behavioral effects of Oxt neuropeptides are thought to be coordinated through its common release through these collaterals. Oxt is also produced by some neurons in the paraventricular nucleus that project to other parts of the brain and to the spinal cord. Depending on the species, Oxt receptor-expressing cells are located in other areas, including the amygdala and bed nucleus of the striaterminalis. In the pituitary gland, Oxt is packaged in large, dense-core vesicles, where it is bound to neurophysin (1), neurophysin is a large

peptide fragment of the larger precursor protein molecule from which oxt is derived by enzymatic cleavage. Secretion of Oxt from the neuro secretary nerve endings is regulated by the electrical activity of the Oxt cells in the hypothalamus. These cells generate action potentials that propagate down axons to the nerve endings in the pituitary; the endings contain large numbers of Oxt-containing vesicles, which are released by exocytosis when the nerve terminals are depolarized [82].

2.3.23.2.2 Non-neural sources

Endogenous Oxt concentrations in the brain have been found to be as much as (1000-fold) higher than peripheral levels. Outside the brain, Oxt-containing cells have been identified in several diverse tissues, including in females in the corpus luteum and the placenta, in males in the testicles' interstitial cells of Leydig, the retina, the adrenal medulla,] the thymus and the pancreas [83].

2.3.23.2.3 Male

The Leydig cells in some species have been shown to possess the biosynthetic machinery to manufacture testicular Oxt de novo, to be specific, in rats (which can synthesize vitamin C endogenously), and in guinea pigs, which, like humans, require an exogenous source of vitamin C (ascorbate) in their diets [84].

2.3.23.2.4 Female

Oxt is synthesized by corpora lutea of several species, including ruminants and primates. Along with estrogen, it is involved in inducing the endometrial synthesis of prostaglandin (F_2 α)to cause regression of the corpus luteum.

2.3.23.2.5 Evolution

Virtually all vertebrates have an Oxt-like nonapeptide hormone that supports reproductive functions and a vasopressin-like non apeptide hormone involved in water regulation. The (2) genes are usually located

close to each other (less than 15,000 bases apart) on the same chromosome, and are transcribed in opposite directions (however, in fugu, the homolog's are further apart and transcribed in the same direction).The (2) genes are believed to result from a gene duplication event; the ancestral gene is estimated to be about(500) million years old and is found in cyclostomata (modern members of the Agnatha). Biological function of Oxt are peripheral (hormonal) actions, and also has actions in the brain. Their actions are mediated by specific, oxytocin receptors. The Oxt receptor is a G-protein-coupled receptor that requires magnesium and cholesterol. It belongs to the rhodopsin-type (class I)group of G-protein-coupled receptors [85].

2.3.23.3 Physiological:-

The peripheral actions of Oxt mainly reflect secretion from the pituitary gland. The behavioral effects of Oxt are thought to reflect release from centrally projecting Oxt neurons, different from those that project to the pituitary gland, or that are collaterals from them. Oxt receptors are expressed by neurons in many parts of the brain and spinal cord, including the amygdala, ventromedial hypothalamus, septum, nucleus accumbens, and brainstem. Milk ejection reflex/Let down reflex: In lactating (breastfeeding) mothers, Oxt acts at the mammary glands, causing milk to be 'let down' into sub areolar sinuses, from where it can be excreted via the nipple. Suckling by the infant at the nipple is relayed by spinal nerves to the hypothalamus. The stimulation causes neurons that make Oxt to fire action potentials in intermittent bursts; these bursts result in the secretion of pulses of Oxt from the neurosecretory nerve terminals of the pituitary gland. Uterine contraction: Important for cervical dilation before birth, Oxt causes contractions during the 2nd and 3rd stages of labor. Oxt release during breastfeeding causes mild but often painful contractions during the first few weeks of lactation. This also

serves to assist the uterus in clotting the placental attachment point postpartum. However, in knockout mice lacking the Oxt receptor, reproductive behavior and parturition are normal. Due to its similarity to vasopressin, it can reduce the excretion of urine slightly. In several species, Oxt can stimulate sodium excretion from the kidneys (natriuresis), and, in humans, high doses can result in hyponatremia. Cardiac effects: Oxt and Oxt receptors are also found in the heart in some rodents, and the hormone may play a role in the embryonal development of the heart by promoting cardiomyocyte differentiation. However, the absence of either Oxt or its receptor in knockout mice has not been reported to produce cardiac insufficiencies, modulation of hypothalamic-pituitary-adrenal axis activity: Oxt, under certain circumstances, indirectly inhibits release of adrenocorticotropic hormone and cortisol and, in those situations, may be considered an antagonist of vasopressin. Preparing fetal neurons for delivery: crossing the placenta, maternal Oxt reaches the fetal brain and induces a switch in the action of neurotransmitter GABA from excitatory to inhibitory on fetal cortical neurons. This silences the fetal brain for the period of delivery and reduces its vulnerability to hypoxic damage. Atasoy *et al*, U.S 2012 paper suggested that Oxt neurons in the para-ventricular hypothalamus in the brain may play a key role in suppressing appetite under normal conditions and that other hypothalamic neurons may trigger eating via inhibition of these Oxt neurons. This population of Oxt neurons are absent in Prader-Willi syndrome, a genetic disorder that leads to uncontrollable feeding and obesity, and may play a key role in its pathophysiology [86]**.**

2.3.23.4 Psychological

Autism: Oxt has been implicated in the etiology of autism. Wermter AK, *et al*, University Hospital Marburg Germany 2010 reports suggested that

autism is correlated with genomic deletion of the gene containing the Oxt receptor gene (OXTR). Autism may also be associated with an aberrant methylation of OXTR [87].

2.3.23.4.1 Bonding

In the prairie vole, Oxt released into the brain of the female during sexual activity is important for forming a monogamous pair bond with her sexual partner. Vasopressin appears to have a similar effect in males. Oxt has a role in social behaviors in many species, so it likely also does in humans. Oxt is involved in the initiation of maternal behavior, not its maintenance; for example, it is higher in mothers after they interact with unfamiliar children rather than their own. In group bonding: Oxt can increase positive attitudes, such as bonding, toward individuals with similar characteristics, who then become classified as "in-group" members, whereas individuals who are dissimilar become classified as "out-group" members. Race can be used as an example of in group and out-group tendencies because society often categorizes individuals into groups based on race Caucasian, African American, Latino, etc.). Sheng F, *et al*, China 2013 studies that examined race and empathy found that participants receiving nasally administered Oxt had stronger reactions to pictures in group members making pained faces than to pictures of out group members with the same expression [88]. This shows that Oxt may be implicated in ability to empathize with individuals of different races and could potentially translate into willingness to help individuals in pain or stressful situations. Moreover, individuals of one race may be more inclined to help individuals of the same race than individuals of another race when they are experiencing pain. Oxt has also been implicated in lying when lying would prove beneficial to other in-group members. Shalvi S, *et al*, U.S 2014 studies was examined a relationship was, it was found that when individuals were administered Oxt, rates of dishonesty in

the participants' responses increased for their in-group members when a beneficial outcome for their group was expected. Both of these examples show the tendency to act in ways that benefit people with which one feels is part of their social group, or in group. Oxt is not only correlated with the preferences of individuals to associate with members of their own group, but it is also evident during conflicts between members of different groups. During conflict, individuals receiving nasally administered Oxt demonstrate more frequent defense motivated responses toward in-group members than out-group members. Further, Oxt was correlated with participant desire to protect vulnerable in group members, despite that individual's attachment to the conflict. Similarly, it has been demonstrated that when Oxt is administered, individuals alter their subjective preferences in order to align with in-group ideals over out-group ideals. These studies demonstrate that Oxt is associated with intergroup dynamics. Further, Oxt influences the responses of individuals in a particular group to those of another group. The in-group bias is evident in smaller groups; however, it can also be extended to groups as large as one's entire country leading toward a tendency of strong national zeal [89].

2.3.23.4.2 Drugs

Drug interaction Impact on effects of alcohol and other drugs: According to Kovács GL, *et al*, Taiwan 2001 studies in animals, Oxt inhibits the development of tolerance to various addictive drugs (opiates, cocaine, alcohol), and reduces withdrawal symptoms [90]. MDMA (ecstasy)may increase feelings of love, empathy, and connection to others by stimulating Oxt activity primarily via activation of serotonin 5-HT1A receptors, if initial studies in animals apply to humans. The anxiolytic Buspar (buspirone) may produce some of its effects via 5-HT1A receptor-induced Oxt stimulation as well. Addiction vulnerability Endogenous Oxt

can also impact on drug effects and susceptibility to develop Substance use disorder. Endogenous Oxt concentrations can directly impact on drug effects. Additionally, bilateral interactions with numerous systems, including the dopamine system, Hypothalamic–pituitary–adrenal axis and immune system, can impact on development of dependence. The status of the endogenous Oxt system might enhance or reduce susceptibility to addiction through its interaction with these systems. Individual differences in the endogenous Oxt system based on genetic predisposition, gender and environmental influences, may therefore affect addiction vulnerability [91].

2.3.23.4.3 Fear and anxiety

Oxt is typically remembered for the effect it has on prosocial behaviors, such as its role in facilitating trust and attachment between individuals. Consequently, Oxt is often referred to as the "love hormone". However, Oxt has a more complex role than solely enhancing prosocial behaviors. There is consensus that Oxt modulates fear and anxiety; that is, it does not directly elicit fear or anxiety [92] . Nasally administered Oxt has been reported to reduce fear, possibly by inhibiting the amygdala (which is thought to be responsible for fear responses) [93]. Individuals who receive an intranasal dose of Oxt identify facial expressions of disgust faster than individuals who do not receive Oxt. Facial expressions of disgust are evolution airily linked to the idea of contagion. Thus, Oxt increases the salience of cues that imply contamination, which leads to a faster response because these cues are specially relevant for survival [94].

2.3.23.4.4 Mood and depression

Oxt produces antidepressant-like effects in animal models of depression. and a deficit of it may be involved in the pathophysiology of depression in humans. The antidepressant-like effects of Oxt are not blocked by a selective antagonist of the Oxt receptor, suggesting that these effects are

not mediated by the Oxt receptor. In accordance, unlike Oxt, the selective non-peptide Oxt receptor agonistWAY-267,464 does not produce antidepressant-like effects, at least in the tail suspension test. (In contrast to WAY-267,464, carbetocin, a close analogue of Oxt and peptide oxytocin receptor agonist, notably does produce antidepressant-like effects in animals.) As such, the antidepressant-like effects of Oxt may be mediated by modulation of a different target, perhaps the vasopressin receptor where Oxt is known to weakly bind as an agonist. Sildenafil has been found to enhance electrically evoked Oxt release from the pituitary gland. In accordance, the drug showed Oxt-dependent antidepressant-like effects in animals, and it has proposed that sildenafil may hold promise as a potential antidepressant in humans [95].

2.3.23.4.5 Sex differences

It has been shown that Oxt differentially affects males and females. Females who are administered Oxt are overall faster in responding to socially relevant stimuli than males who received Oxt. Additionally, after the administration of Oxt, females show increased amygdala activity in response to threatening scenes; however, males do not show increased amygdale activation. This phenomenon can be explained by looking at the role of gonadal hormones, specifically estrogen, which modulate the enhanced threat processing seen in females. Estrogen has been shown to stimulate the release of Oxt from the hypothalamus and promote receptor binding in the amygdale. It has also been shown that testosterone directly suppresses Oxt in mice. This has been hypothesized to have evolutionary significance. With Oxt suppressed, activities such as hunting and attacking invaders would be less mentally difficult as Oxt is strongly associated with empathy [96].

2.3.23.4.6 Social

Affecting generosity by increasing empathy during perspective taking: In a neuroeconomics experiment, intranasal Oxt increased generosity in the ultimatum game by (80%), but had no effect in the dictator game that measures altruism. Perspective-taking is not required in the dictator game, but the researchers in this experiment explicitly induced perspective-taking in the ultimatum game by not identifying to participants into which role they would be placed. Serious methodological questions have arisen, however, with regard to the role of Oxt in trust and generosity. Empathy in healthy males has been shown to be increased after intranasal Oxt. This is most likely due to the effect of Oxt in enhancing eye gaze. There is some discussion about which aspect of empathy Oxt might alter for example, cognitive vs. emotional empathy. Trust is increased by Oxt. Disclosure of emotional events is a sign of trust in humans. When recounting a negative event, humans who receive intranasal Oxt share more emotional details and stories with more emotional significance. Humans also find faces more trust worthy after receiving intranasal Oxt. In a study, participants who received intranasal Oxt viewed photographs of human faces with neutral expressions and found them to be more trust worthy than those who did not receive Oxt. This may be because Oxt reduces the fear of social betrayal in humans. Even after experiencing social alienation by being excluded from a conversation, humans who received Oxt scored higher in trust on the Revised NEO Personality Inventory. Moreover, in a risky investment game, experimental subjects given nasally administered Oxt displayed "the highest level of trust" twice as often as the control group. Subjects who were told they were interacting with a computer showed no such reaction, leading to the conclusion that Oxt was not merely affecting risk aversion. When there is a reason to be distrustful, such as experiencing betrayal, differing reactions are associated with Oxt receptor

gene(OXTR) differences. Those with the CT haplotype experience a stronger reaction, in the form of anger, to betrayal [97].

2.3.23.4.7 Romantic attachment

Marazziti D, *et al*, University of Pisa Italy 2006 studies, showed that a high levels of plasma Oxt have been correlated with romantic attachment. For example, if a couple is separated for a long period of time, anxiety can increase due to the lack of physical affection. Oxt may aid romantically attached couples by decreasing their feelings of anxiety when they are separated[98] .

2.3.23.4.8 Group-serving

Dishonesty/deception: In a carefully controlled study exploring the biological roots of immoral behavior, Oxt was shown to promote dishonesty when the outcome favored the group to which an individual belonged instead of just the individual [99].

2.3.23.4.9 Sexual activity

The relationship between Oxt and human sexual response is unclear. Carmichael MS, *et al*, California 1987 studies was found increased in plasma Oxt at orgasm in both men and women. Plasma Oxt levels are notably increased around the time of self-stimulated orgasm and are still higher than baseline when measured (5 minutes) after self arousal. The authors of one of these studies speculated that Oxt effects on muscle contractibility may facilitate sperm and egg transport [100]. Blaicher W, *et al*, University of Vienna, Austria 1999 studies was measuring oxytocin serum levels in women before and after sexual stimulation, the author suggests it serves an important role in sexual arousal. Who found that the genital tract stimulation resulted in increased Oxt immediately after orgasm [101].

2.3.23.4.10 Social behavior and wound healing

Oxt is also thought to modulate inflammation by decreasing certain cytokines. Thus, the increased release in Oxt following positive social interactions has the potential to improve wound healing. Gouin JP, *et al*, Hospital Research Unit USA 2011 reports was used heterosexual couples to investigate this possibility. They found increases in plasma Oxt following a social interaction were correlated with faster wound healing. They hypothesized this was due to Oxt reducing inflammation, thus allowing the wound to heal more quickly. Also provides preliminary evidence that positive social interactions may directly influence aspects of health [102]. Oxt evokes feelings of contentment, reductions in anxiety, and feelings of calmness and security when in the company of the mate. This suggests that Oxt may be important for the inhibition of the brain regions associated with behavioral control, fear, and anxiety, thus allowing orgasm to occur. Research has also demonstrated that Oxt can decrease anxiety and protect against stress, particularly in combination with social support [103].

2.3.24 Dopamine

is an organic chemical of the catecholamine and phenethyl amine families that plays several important roles in the brain and body. It is an amine synthesized by removing a carboxyl group from a molecule of its precursor chemical L DOPA, which is synthesized in the brain and kidneys. Dopamine is also synthesized in plants and most multi cellular animals. The anticipation of most types of rewards increases the level of dopamine in the brain, and many addictive drugs increase dopamine release or block its reuptake into neurons following release [104].

2.3.24.1 Structure

A dopamine molecule consists of a catechol structure (a benzene ring with two hydroxyl side groups) amine group attached via an ethyl chain. As such, dopamine is the simplest possible catecholamine, a family that

also includes the neurotransmitters nor epinephrine and epinephrine. The presence of a benzene ring with this amine attachment makes it a substituted phenethylamine, a family that includes numerous psychoactive drugs. Like most amines, dopamine is an organic base. As a base, it is generally protonated in acidic environments (in an acid-base reaction).The protonated form is highly water-soluble and relatively stable, but can become oxidized if exposed to oxygen or other oxidants. In basic environments, dopamine is not protonated. In this free base form, it is less water-soluble and also more highly reactive. Because of the increased stability and water-solubility of the protonated form, dopamine is supplied for chemical or pharmaceutical use as dopamine hydrochloride that is, the hydrochloride salt that is created when dopamine is combined with hydrochloric acid. In dry form, dopamine hydrochloride is a fine colorless powder [105] .

2.3.24.2 Biochemistry

Biosynthetic pathways for catecholamines and trace amines in the human brain. In humans, catecholamines and phenethylaminergic trace amines are derived from the amino acid phenylalanine. It is well established that dopamine is produced from L-tyrosine via L-dopa; however, recent evidence has shown that CYP2D6 is expressed in the human brain and catalyzes the biosynthesis of dopamine from tyrosine via m-tyramine and p-tyramine [106].

2.3.24.3 Synthesis

Dopamine is synthesized in a restricted set of cell types, mainly neurons and cells in the medulla of the adrenal glands. The primary and minor metabolic pathways respectively are:.

Primary: L-Phenylalanine → L-Tyrosine → LDOPA→ Dopamine

Minor: L-Phenylalanine → L-Tyrosine → p- Tyramine → Dopamine

Minor: L-Phenylalanine → m-Tyrosine → m- Tyramine → Dopamine

The direct precursor of dopamine, L-DOPA, can be synthesized indirectly from the essential amino acid phenylalanine or directly from the non-essential amino acid tyrosine. These amino acids are found in nearly every protein and so are readily available in food, with tyrosine being the most common. Although dopamine is also found in many types of food, it is incapable of crossing the blood–brain barrier that surrounds and protects the brain. It must therefore be synthesized inside the brain to perform its neuronal activity. L-Phenylalanine is converted into L-tyrosine by the enzyme phenylalanine hydroxylase, with molecular oxygen(O_2) and tetrahydrobiopterin as cofactors. L-Tyrosineis converted into L-DOPA by the enzyme tyrosine hydroxylase, with tetra hydro biopterin, (O_2), andiron (Fe^{2+}) as cofactors. L-DOPA is converted into dopamine by the enzyme aromatic L-amino acid decarboxylase (also known as DOPA decarboxylase), with pyridoxal phosphate as the cofactor. Dopamine itself is used as precursor in the synthesis of the neurotransmitters nor epinephrine and epinephrine. Dopamine is converted into nor epinephrine by the enzyme dopamine β-hydroxylase, with ($O_{2)}$ and L-ascorbic acid as cofactors. Nor epinephrine is converted into epinephrine by the enzyme PNMT with S-adenosyl-L-methionine as the cofactor. Some of the cofactors also require their own synthesis. Deficiency in any required amino acid or cofactor canimpair the synthesis of dopamine, nor epinephrine, and epinephrine [106].

2.3.24.4 Functions

2.3.24.4.1 Cellular effects

Dopamine exerts its effects by binding to and activating cell surface receptors. In mammals, (5) subtypes of dopamine receptors have been identified, labeled from (D1 to D5). All of them function as metabotropic, G-protein-coupled receptors, meaning that they exert their effects via a complex second messenger system. These receptors can be divided into

(2) families, known as D1-like and D2-like. For receptors located on neurons in the nervous system, the ultimate effect of D1-like activation (D1 and D5) can be excitation (via opening of sodium channels) or inhibition (via opening of potassium channels); the ultimate effect of D2-like activation (D2, D3, and D4) is usually inhibition of the target neuron. Consequently, it is incorrect to describe dopamine itself as either excitatory or inhibitory: its effect on a target neuron depends on which types of receptors are present on the membrane of that neuron and on the internal responses of that neuron to the second messenger CAMP. D1 receptors are the most numerous dopamine receptors in the human nervous system; D2 receptors are next; D3, D4, and D5 receptors are present at significantly lower levels[107].

2.3.24.4.2Storage, release, and reuptake

Inside the brain, dopamine functions as aneurotransmitter and neuromodulator, and is controlled by a set of mechanisms common to all monoamine neurotransmitters. After synthesis, dopamine is transported from the cytosol into synaptic vesicles by a solute carrier—a vesicular monoamine transporter, VMAT2. Dopamine is stored in these vesicles until it is ejected into the synaptic cleft. In most cases, the release of dopamine occurs through a process called exocytosis which is caused by action potentials, but it can also be caused by the activity of an intracellular trace amine-associated receptor, TAAR1.TAAR1 is a high-affinity receptor for dopamine, trace amines, and certain substituted amphetamines that is located along membranes in the intracellular milieu of the presynaptic cell. activation of the receptor can regulate dopamine signaling by inducing dopamine reuptake inhibition and efflux as well as by inhibiting neuronal firing through adverse set of mechanisms Once in the synapse, dopamine binds to and activates dopamine receptors. These can be post synaptic dopamine receptors, which are located on dendrites

(the postsynaptic neuron), or pre synaptic auto receptors (e.g., the D2sh and pre synaptic D3 receptors), which are located on the membrane of an axon terminal (the pre synaptic neuron). After the postsynaptic neuron elicits an action potential, dopamine molecules quickly become unbound from their receptors. They are then absorbed back into the pre synaptic cell, via reuptake mediated either by the dopamine transporter or by the plasma membrane monoamine transporter. Once backi n the cytosol, dopamine can either be broken down by a monoamine oxidase or repackaged into vesicles by VMAT2, making it available for future release. In the brain the level of extracellular dopamine is modulated by (2) mechanisms: phasic and tonic transmission. Phasic dopamine release, like most neurotransmitters release in the nervous system, is driven directly by action potentials in the dopamine-containing cells. Tonic dopamine transmission occurs when small amounts of dopamine are released without being preceded by pre synaptic action potentials. Tonic transmission is regulated by a variety of factors, including the activity of other neurons and neurotransmitter reuptake [108].

2.3.24.4.3 Nervous system

Inside the brain, dopamine plays important roles in executive functions, motor control, motivation, arousal, reinforcement, and reward, as well as lower-level functions including lactation, sexual gratification, and nausea. The dopaminergic cell groups and pathways make up the dopamine system which is neuromodulatory. Dopaminergic neurons (dopamine-producing nerve cells)are comparatively few in number a total of around (400,000) in the human brain and their cell bodies are confined in groups to a few relatively small brain areas. However their axons project to many other brain areas, and they exert powerful effects on their targets. These dopaminergic cell groups were first mapped in 1964 by Annica Dahlström and KjellFuxe, who assigned the mlabels starting with the

letter “A” (for “aminergic”). The substantia nigra is a small midbrain area that forms a component of the basal ganglia. This has two parts aninput area called the pars compacta and an output area the pars reticulata. The dopaminergic neurons are found mainly in the pars compacta (cell group A8) and nearby (group A9). In humans, the projection of dopaminergic neurons from the substantia nigra pars compacta to the dorsal striatum, termed the nigrostriatal pathway, plays a significant role in the control of motor function and in learning new motor skills. These neurons are specially vulnerable to damage, and when a large number of them die, the result is a parkinsonian syndrome. The ventral tegmental area (VTA) is another midbrain area. The most prominent group of VTA dopaminergic neurons projects to the prefrontal cortex via the mesocortical pathway and another smaller group projects to the nucleus accumbens via the mesolimbic pathway. Together, these (2) pathways are collectively termed the mesocorticolimbic projection. The VTA also sends dopaminergic projections to the amygdala, cingulated gyrus, hippocampus, and olfactory bulb. Mesocorticolimbic neurons play a central role in reward and other aspects of motivation. The posterior hypothalamus has dopamine neurons that project to the spinal cord, but their function is not well established. There is some evidence that pathology in this area plays a role in restless legs syndrome, a condition in which people have difficulty sleeping due to an overwhelming compulsion to constantly move parts of the body, especially the legs. The arcuate nucleus and the periventricular nucleus of the hypothalamus have dopamine neurons that form an important projection the tuberoinfundibular pathway which goes to the pituitary gland, where it influences the secretion of the hormone prolactin. Dopamine is the primary neuroendocrine inhibitor of the secretion of prolactin from the anterior pituitary gland. Dopamine produced by neurons in the arcuate

nucleus is secreted into the hypophyseal portal system of the median eminence, which supplies the pituitary gland. The prolactin cells that produce prolactin, in the absence of dopamine, secrete prolactin continuously; dopamine inhibits this secretion. In the context of regulating prolactin secretion, dopamine is occasionally called prolactin-inhibiting factor, prolactin-inhibiting hormone, or prolactostatin. The zonaincerta, grouped between the arcuate and periventricular nuclei, projects to several areas of the hypothalamus, and participates in the control of gonadotropin-releasing hormone, which is necessary to activate the development of the male and female reproductive systems, following puberty. An additional group of dopamine-secreting neurons is found in the retina of the eye. These neurons are amacrine cells, meaning that they have no axons. They release dopamine into the extracellular medium, and are specifically active during daylight hours, becoming silent at night. This retinal dopamine acts to enhance the activity of cone cells in the retina while suppressing rod cells the result is to increase sensitivity to color and contrast during bright light conditions, at the cost of reduced sensitivity when the light is dim [109].

2.3.24.4.4 Basal ganglia

The largest and most important sources of dopamine in the vertebrate brain are the substantia nigra and ventral tegmental area. These structures are closely related to each other and functionally similar in many respects. Both are components of the basal ganglia, a complex network of structures located mainly at the base of the forebrain. The largest component of the basal gangliais the striatum. The substantia nigra sends a dopaminergic projection to the dorsal striatum, while the ventral tegmental area sends a similar type of dopaminergic projection to the ventral striatum. Progress in understanding the functions of the basal ganglia has been slow. The most popular hypotheses, broadly stated,

propose that the basal ganglia play a central role in action selection. The action selection theory in its simplest form proposes that when a person or animal is in a situation where several behaviors are possible, activity in the basal ganglia determines which of them is executed, by releasing that response from inhibition while continuing to inhibit other motor systems that if activated would generate competing behaviors. Thus the basal ganglia, in this concept, are responsible for initiating behaviors, but not for determining the details of how they are carried out. In other words, they essentially form a decision-making system. The basal ganglia can be divided into several sectors, and each is involved in controlling particular types of actions. The ventral sector of the basal ganglia (containing the ventral striatum and ventral tegmental area) operates at the highest level of the hierarchy, selecting actions at the whole-organism level.. The dorsal sectors(containing the dorsal striatum and substantia nigra) operate at lower levels, selecting the specific muscles and movements that are used to implement a given behavior pattern. Dopamine contributes to the action selection process in at least (2) important ways. First, it sets the "threshold" for initiating actions. The higher the level of dopamine activity, the lower the impetus required to evoke a given behavior. As a consequence, high levels of dopamine lead to high levels of motor activity and impulsive behavior; low levels of dopamine lead to torpor and slowed reactions. Parkinson's disease, in which dopamine levels in the substantia nigra circuit are greatly reduced, is characterized by stiffness and difficulty initiating movement however, when people with the disease are confronted with strong stimuli such as a serious threat, their reactions can be as vigorous as those of a healthy person. In the opposite direction, drugs that increase dopamine release, such as cocaine or amphetamine, can produce heightened levels of activity, including at the extreme, psychomotor agitation and stereotyped movements. The

second important effect of dopamine is as a “teaching” signal. When an action is followed by an increase in dopamine activity, the basal ganglia circuit is altered in a way that makes the same response easier to evoke when similar situations arise in the future. This is a form of operant conditioning, in which dopamine plays the role of a reward signal [110].

2.3.24.4.5 Medical uses

Dopamine as a manufactured medication is sold under the trade names Intropin, Dopastat, and Revimine, among others. It is on the WH O List of essential medicines. It is most commonly used as a stimulant drug in the treatment of severe low blood pressure, slow heart rate, and cardiac arrest. It is specially important in treating these in newborn infants [111].

2.3.24.5 Disease, disorders, and pharmacology

The dopamine system plays a central role in several significant medical conditions, including Parkinson’s disease, attention deficit hyperactivity disorder, schizophrenia, and addiction. Aside from dopamine itself, there are many other important drugs that act on dopamine systems in various parts of the brain or body. Some are used for medical or recreational purposes, but neurochemists have also developed a variety of research drugs, some of which bind with high affinity to specific types of dopamine receptors and either agonize or antagonize their effects, and many that affect other aspects of dopamine physiology, including dopamine transporter inhibitors, VMAT inhibitors, and enzyme inhibitors [112].

2.3.25 Serotonin

2.3.25.1 synthesis

5-hydroxytryptamine (5-HT) is a monoamine neurotransmitter. Biochemically derived from tryptophan. Serotonin is primarily found in the (GIT), blood platelets, and the (CNS) of animals, including humans. It is popularly thought to be a contributor to feelings of well-being and

happiness. Approximately (90%) of the human body's total serotonin is located in the enterochromaffin cells in the GIT, where it is used to regulate intestinal movements. The serotonin is secreted luminally and baso - laterally which leads to increased serotonin uptake by circulating platelets and activation after stimulation, which gives increased stimulation of mesenteric neurons and gastrointestinal motility. The remainder is synthesized in serotonergic neurons of the CNS, where it has various functions. These include the regulation of mood, appetite, and sleep. Serotonin also has some cognitive functions, including memory and learning. Modulation of serotonin at synapses is thought to be a major action of several classes of pharmacological antidepressants. Serotonin secreted from the enterochromaffin cells eventually finds its way out of tissues into the blood. There, it is actively taken up by blood platelets, where it is stored. When the platelets bind to a clot, they release serotonin, where it serves as a vasoconstrictor and helps to regulate hemostasis and blood clotting. Serotonin also is a growth factor for some types of cells, which may give it a role in wound healing. There are various serotonin receptors. Serotonin is metabolized mainly to 5-HIAA, chiefly by the liver. Metabolism involves first oxidation by MAO to the corresponding aldehyde. This is followed by oxidation by aldehyde dehydrogenase to 5- HIAA, the indole acetic acid derivative. The latter is then excreted by the kidneys. In addition to animals, serotonin is found in fungi and plants. Serotonin’s presence in insect venoms and plant spines serves to cause pain, which is a side-effect of serotonin injection. Serotonin is produced by pathogenic amoebae, and its effect on the gut causes diarrhea. Its widespread presence in many seeds and fruits may serve to stimulate the digestive tract into expelling the seeds.

In the body, serotonin is synthesized from the amino acid tryptophan by a short metabolic pathway consisting of (2) enzymes: tryptophan

hydroxylase (TPH) and amino acid decarboxylase (DDC). The TPH-mediated reaction is the rate-limiting step in the pathway. TPH has been shown to exist in two forms: TPH1, found in several tissues, and TPH2, which is a brain-specific isoform. There is evidence that genetic polymorphisms in both these subtypes influence susceptibility to anxiety and depression. There is also evidence that ovarian hormones can affect the expression of TPH in various species, suggesting a possible mechanism for postpartum depression and premenstrual stress syndrome. Serotonin taken orally does not pass into the serotonergic pathways of the CNS because it does not cross the blood-brain barrier. However, tryptophan and its metabolite 5-hydroxytryptophan (5-HTP), from which serotonin is synthesized, can and do cross the blood-brain barrier. These agents are available as dietary supplements and may be effective serotonergic agents. One product of serotonin breakdown is (5-HIAA), which is excreted in the urine. Serotonin and (5- HIAA) are sometimes produced in excess amounts by certain tumors or cancers, and levels of these substances may be measured in the urine to test for these tumors[113].

2.3.25.2 Function

In the CNS, serotonin plays an important role as a neurotransmitter in the modulation of anger, aggression, body temperature, mood, sleep, human sexuality, appetite, and metabolism, as well as stimulating vomiting. Serotonin has broad activities in the brain, and genetic variation in serotonin receptors and the serotonin transporter, which facilitates reuptake of serotonin into pre synapses, have been implicated in neurological diseases. Drugs targeting serotonin-induced pathways are being used in the treatment of many psychiatric disorders, and the focus on clinical research will revealed the influence of genetics on serotonin action and metabolism in psychiatric settings. Lesch, K, *et al*, Germany

1996 studies have revealed that the variation in the promoter region of the serotonin transporter protein accounts for nearly (10%) of total variance in anxiety-related personality, and the effect of this gene on depression was found to interact with the environment. Levels of serotonin in the brain show association with aggression , and a mutation in the gene which codes for the 5-HT2A receptor may double the risk of suicide for those with that genotype. Using the ultimatum game as model, it was shown that people whose serotonin levels have been artificially lowered will reject unfair offers more often than players with normal serotonin levels. In addition, serotonin is also a peripheral signal mediator. It is found extensively in the human GIT as about (80-90%) of the body's total serotonin is found in the enterochromaffin cells in the gut. In the blood, the major storage site is platelets, which collect serotonin for use in mediating post-injury vasoconstriction [114].

The gut is surrounded by enterochromaffin cells, which release serotonin in response to food in the lumen. This makes the gut contract around the food. Platelets in the veins draining the gut collect excess serotonin. If irritants are present in the food, the enterochromaffin cells release more serotonin to make the gut move faster, i.e., to cause diarrhea, so the gut is emptied of the noxious substance. If serotonin is released in the blood faster than the platelets can absorb it, the level of free serotonin in the blood is increased. This activates 5-HT3 receptors in the chemoreceptor trigger zone that stimulate vomiting. The enterochromaffin cells not only react to bad food but are also very sensitive to irradiation and cancer chemotherapy. Drugs that block 5HT3 are very effective in controlling the nausea and vomiting produced by cancer treatment, and are considered the golds standard for this purpose [115]. Human serotonin can also act as a growth factor directly. Liver damage increases cellular expression of 5-HT2A and 5-HT2B receptors, mediating liver

compensatory re growth serotonin present in the blood then stimulates cellular growth to repair liver damage. 5-HT2B receptors also activate osteocytes, which build up bone. However, serotonin also inhibits osteoblasts, through 5-HT1B receptors. Several classes of drugs target the 5-HT system, including some antidepressants, antipsychotics, anxiolytics, antiemetics, and antimigraine drugs, as well as the psychedelic drugs and empathogens. Serotonin is fundamentally very much like the other neurochemicals that are found in the nervous system. It is a molecule that brain cells, or neurons, release in order to communicate with other cells by stimulating their receptor molecules. The ultimate purpose of any neurochemical signal is to carry some form of information [116].

Van der Veen, F, *et al*, Netherlands 2007 studies was explored whether rapidly lowering serotonin levels makes healthy individuals feel less happy, and have consistently found that this is *not* the case. One common method used for this purpose is acute tryptophan depletion (ATD), which effectively and temporarily lowers brain serotonin levels by (50-90%) over the course of several hours. Participants of this studies are given a drink containing a variety of essential amino-acids except for tryptophan an essential molecule which is processed in the CNS to produce serotonin. These consumed amino-acids compete with the relatively fewer tryptophan molecules to access the brain from the blood stream, and naturally win. As a result of this, serotonin synthesis goes down and levels of the neurochemical drop dramatically within (5-7) hours. Using this method, was found that healthy individuals don't actually report feeling any less happy when deprived of serotonin [117].

2.3.25.3 Depressed and anxious people might simply be too good at learning about bad outcomes

Mental health issues such as depression and anxiety might fundamentally be disorders of learning, rather than outcomes of a ‘chemical imbalance’ that requires correction by a serotonin boost. Specifically, certain individuals which have atypical function of the serotonin system (which might be caused by genetic factors or stressful lives) may be at risk of developing depression or anxiety because they are too good at learning about negative outcomes, and thus are more likely to feel that the world is a bad place if they experience negative life events. Caspi, A. *et al*, London 2003 studies in the psychiatric literature supports this possibility. Itis found that individuals with a particular genotype affecting the serotonin system were more likely than others to develop depression or anxiety only if they had experienced stressful life events, such as child abuse, unemployment, or loss of a loved one. Clearly, having an atypical serotonin system alone wasn’t enough it had to be combined with negative experiences [118].

2.3.26 Ghrelin

2.3.26 synthesis

the “hunger hormone”, also known as Lenomorelin (LNN), is a peptide hormoneproduced by ghrelinergic cells in the GIT which functions as a neuropeptide in the CNS. Besides regulating appetite, ghrelin also plays a significant role in regulating the distribution and rate of use of energy. When the stomach is empty, ghrelin is secreted. When the stomach is stretched, secretion stops. acts on hypothalamic brain cells both to increase hunger, and to increase gastric acid secretion and GI motility to prepare the body for food intake. The receptor for ghrelin, the ghrelin/growth hormone secretagogue receptor (GHSR), is found on the same cells in the brain as the receptor for leptin, the satiety hormone that has opposite effects from ghrelin. Ghrelin also plays an important role in regulating reward perception in dopamine neurons that link the ventral

tegmental area to the nucleus accumbens (a site that plays a role in processing sexual desire, reward, and reinforcement, and in developing addictions) through its co-localized receptors and interaction with dopamine and acetylcholine. Ghrelin is encoded by the GHRL gene and is presumably produced from the cleavage of the prepropeptide ghrelin/obestatin. Full-length preproghrelin is homologous to promotilin and both are members of the motilin family. Unlike the case of many other endogenous peptides, ghrelin is able to cross the BBB, giving exogenously-administered ghrelin unique clinical potential [119]. Ghrelin cells are found mainly in the stomach and duodenum, but also in the jejunum, lungs, pancreatic islets. gonads, adrenal cortex, placenta, and kidney.

2.3.26.2 Function and mechanism of action

Ghrelin is a participant in regulating the complex process of energy hemeostasis which adjusts both energy input– by adjusting hunger signals and energy output by adjusting the proportion of energy going to ATP production, fat storage, glycogen storage, and short-term heat loss. The net result of these processes is reflected in body weight, and is under continuous monitoring and adjustment based on metabolic signals and needs. At any given moment in time, it may be in equilibrium or disequilibrium. Gastric-brain communication is an essential part of energy hemeostasis, and several communication pathways are probable, including the gastric intracellular pathway mediating the interaction among ghrelin, nesfatin and endocannabinoid gastric systems, and both afferent and efferent vagal signals. Ghrelin and synthetic ghrelin mimetics (growth hormone secretagogues) increase body weight and fat mass by triggering receptors in the arcuate nucleus that include the orexigenic neuropeptide Y (NPY) and agouti-related protein (AgRP) neurons. Ghrelin-responsiveness of these neurons is both leptin- and

insulin-sensitive. Ghrelin reduces the mechano-sensitivity of gastric vagal afferents, so they are less sensitive to gastric distension. In addition to its function in energy homeostasis, ghrelin also activates the cholinergic–dopaminergic reward link in inputs to the ventral tegmental area and in the mesolimbic pathway, a circuit that communicates the hedonic and reinforcing aspects of natural rewards, such as food and addictive drugs such as ethanol. Ghrelin receptors are located on neurons in this circuit. Hypothalamic ghrelin signaling is required for reward from alcohol and palatable/ rewarding foods. Ghrelin also improves endothelial function and inhibits proatherogenic changes in cell cultures. It activates the endothelial isoform of nitric oxide synthase in a pathway that depends on various kinases including Akt. Ghrelin has been linked to inducing appetite and feeding behaviors. Circulating ghrelin levels are the highest right before a meal and the lowest right after. Injections of ghrelin in both humans and rats have been shown to increase food intake in a dose-dependent manner. So the more ghrelin that is injected the more food that is consumed. However, ghrelin does not increase meal size, only meal number. Ghrelin injections also increase an animal's motivation to seek out food, behaviors including increased sniffing, for aging for food, and hoarding food. Body weight is regulated through energy balance, the amount of energy taken in versus the amount of energy expended over an extended period of time [120].

Ghrelin promotes intestinal cell proliferation and inhibits apoptosis during inflammatory states and oxidative stress. It also suppresses pro-inflammatory mechanisms and augments anti-inflammatory mechanisms, thus creating a possibility of its therapeutic use in various gastrointestinal inflammatory conditions, including colitis, ischemia reperfusion injury, and sepsis. Ghrelin promotes gastrointestinal and pancreatic malignancy [121].

Ghrelin inhibits glucose-stimulated insulin secretion from Beta- cells in the pancreatic islets. Ghrelin does this indirectly by promoting local negative feedback mediated by somatostatin from pancreatic delta cells, which selectively express the ghrelin receptor [122]. Short sleep duration is associated with high levels of ghrelin and obesity. An inverse relationship between the hours of sleep and blood plasma concentrations of ghrelin exists; as the hours of sleep increase, ghrelin levels tend to be lower and obesity is less likely [123] . Ghrelin levels in the plasma of obese individuals are lower than those in leaner individuals suggesting that ghrelin does not contribute to obesity, except in the cases of Prader-Willi syndrome-induced obesity, where high ghrelin levels are correlated with increased food intake However, it is also found that consumption of food for pleasure increased peripheral levels of both ghrelin and the endocannabinoid 2-arachidonoyl-glycerol (2-AG) in healthy humans, and this hedonic eating influences food intake and, ultimately, body mass [124] . Those with anorexia nervosa have high plasma levels of ghrelin compared to both constitutionally thin and normal-weight controls .The level of ghrelin increases during the time of day from midnight to dawn in thinner people, which suggests there is a flaw in the circadian rhythm of obese individuals. Ghrelin levels reflect release in a circadian rhythm which can be interrupted by exposure to light at night. Short sleep duration may also lead to obesity, through an increase of appetite via hormonal changes during pregnancy ,Lack of sleep increases ghrelin, and decreases leptin,both effects producing increased hunger and obesity [125]. Ghrelin levels are high in patients with cancer induced cachexia. Ghrelin and its receptors are found in the reproductive organs and in the placenta, clearly indicating a role for ghrelin in reproduction. Circulating ghrelin levels peak at mid-gestation, then with advancing gestational age declining ghrelin levels are observed. At the same time the maternal

organism increases its fat mass, becomes insulin resistant and the growth hormone (GH) axis is dominated by placental growth hormone circulating in concentrations comparable to GH levels observed in acromegaly. After delivery, normalization of ghrelin levels occurs before the maternal fat mass is restored at prepregnant levels. The physiological course of ghrelin during the (3) trimesters of human pregnancy is discussed, as are the physiological roles ghrelin may subserve. Regulation of maternal energy intake may be the prevailing effect of ghrelin in pregnancy and lactation, but several other effects of ghrelin may coexist, including local effects. Finally, ghrelin secretion in the fetus is briefly discussed [126].

2.3.27 Endorphins

2.3.27.1 Synthesis

Are endogenous opioid neuropeptides in humans and other animals. They are produced by the CNS and the pituitary gland. The term implies a pharmacological activity (analogous to the activity of the corticosteroid category of biochemicals) as opposed to a specific chemical formulation. It consists of (2) parts: endo- and -orphin; these are short forms of the words endogenous and morphine, intended to mean"a morphine-like substance originating from within the body". The class of endorphin compounds includes α-endorphin, β-endorphin, γ-endorphin, σ-endorphin, α-neo-endorphin, and β-neo-endorphin.

2.3.27.2 function

The principal function of endorphins is to inhibit the transmission of pain signals; they may also produce a feeling of euphoria very similar to that produced by other opioids [127]. Endorphins are naturally produced in response to pain, but their production can also be triggered by various human activities. Vigorous aerobic exercise can stimulate the release of endorphins in the blood stream, leading to an effect known as a "runner's

high". Endorphins are suspected to play a role in depersonalization disorder. The opioid antagonists Naloxone and naltrexone have both been proven to be successful in treating depersonalization [128].

2.3.28 Epinephrine

2.3.28.1 Synthesis and Mechanism of Actions

also known as adrenaline or adrenaline, is a hormone, neurotransmitter and medication. Epinephrine is normally produced by both the adrenal glands and certain neurons. It plays an important role in the fight-or-flight response by increasing blood flow to muscles, output of the heart, pupil dilation, and blood sugar. Epinephrine does this by its effects on α and β receptors. It is found in many animals and someone cell organisms. Jokichi Takamine first isolated epinephrine in 1901. As a medication it is used to treat a number of conditions, including anaphylaxis, cardiac arrest, and superficial bleeding. Inhaled epinephrine may be used to improve the symptoms of croup. It may also be used for asthma when other treatments are not effective. It is given intravenously, by injection into a muscle, by inhalation, or by injection just under the skin. Common side effects include shakiness, anxiety, and sweating. A fast heart rate and high blood pressure may occur. Occasionally it may result in an abnormal heart rhythm. While the safety of its use during pregnancy and breastfeeding is unclear, the benefits to the mother must be taken into account [129]. The adrenal medulla is a minor contributor to total circulating catecholamines (L-DOPA is at a higher concentration in the plasma), though it contributes over (90%) of circulating epinephrine. Little epinephrine is found in other tissues, mostly in scattered chromaffin cells. Following adrenal ectomy, epinephrine disappears below the detection limit in the blood stream. The adrenals contribute about (7%) of circulating nor epinephrine, most of which is a spillover from neurotransmission with little activity as a hormone. Pharmacological

doses of epinephrine stimulate α_1, α_2, β_1, β_2, and β_3 adrenoceptors of the sympathetic nervous system. Sympathetic nerve receptors are classified as adrenergic, based on their responsiveness to adrenaline. The term "adrenergic" is often misinterpreted in that the main sympathetic neurotransmitter is nor epinephrine (nor adrenaline), rather than epinephrine, as discovered by Ulf von Euler in 1946. Epinephrine does have a β_2 adrenoceptor-mediated effect on metabolism and the airway, there being no direct neural connection from the sympathetic ganglia to the airway. The concept of the adrenal medulla and the sympathetic nervous system being involved in the flight, fight and fright response was originally proposed by Cannon. But the adrenal medulla, in contrast to the adrenal cortex, is not required for survival. In adrenal ectomized patients hemodynamic and metabolic responses to stimuli such as hypoglycemia and exercise remain normal [130]. During exercise the epinephrine blood concentration rises partially from increased secretion from the adrenal medulla and partly from decreased metabolism because of reduced hepatic blood flow. Infusion of epinephrine to reproduce exercise circulating concentrations of epinephrine in subjects at rest has little hemodynamic effect, other than a small β2-mediated fall in diastolic blood pressure. Infusion of epinephrine well within the physiological range suppresses human airway hyper-reactivity sufficiently to antagonize the constrictor effects of inhaled histamine [131]. Increased epinephrine secretion is observed in phaeochromocytoma, hypoglycemia, myocardial infarction and to a lesser degree in benign essential familial tremor. A general increase in sympathetic neural activity is usually accompanied by increased adrenaline secretion, but there is selectivity during hypoxia and hypoglycemia, when the ratio of adrenaline to noradrenalin is considerably increased. Therefore, there must be some autonomy of the adrenal medulla from the rest of the sympathetic system.

Myocardial infarction is associated with high levels of circulating epinephrine and nor epinephrine, particularly in cardiogenic shock. Benign familial tremor (BFT) is responsive to peripheral β- adrenergic blockers and β_2- stimulation is known to cause tremor. Patients with BFT were found to have increased plasma epinephrine, but not nor epinephrine. Low, or absent, concentrations of epinephrine can be seen in autonomic neuropathy or following adrenalectomy. Failure of the adrenal cortex, as with Addison's disease, can suppress epinephrine secretion as the activity of the synthesing enzyme, PNMT, depends on the high concentration of cortisol that drains from the cortex to the medulla [132]. As a hormone, epinephrine acts on nearly all body tissues. Its actions vary by tissue type and tissue expression of adrenergic receptors. For example, high levels of epinephrine causes smooth muscle relaxation in the airways but causes contraction of the smooth muscle that lines most arterioles. Epinephrine acts by binding to a variety of adrenergic receptors. Epinephrine is a nonselective agonist of all adrenergic receptors, including the major subtypes α_1,α_2, β_1, β_2, and β_3. Epinephrine's binding to these receptors triggers a number of metabolic changes. Binding to α-adrenergic receptors inhibits insulin secretion by the pancreas, stimulates glycogenolysis in the liver and muscle, and stimulates glycolysis and inhibits insulin mediated glycogenesis in muscle. β -adrenergic receptor binding triggers glucagon secretion in the pancreas, increased adrenocorticotropic hormone (ACTH) secretion by the pituitary gland, and increased lipolysis by adipose tissue. Together, these effects lead to increased blood glucose and fatty acids, providing substrates for energy production within cells throughout the body. Their actions are to increase peripheral resistance via α_1- receptor-dependent vasoconstriction and to increase cardiac output via its binding to β_1 receptors. The goal of reducing peripheral circulation is to increase coronary and cerebral

perfusion pressures and therefore increase oxygen exchange at the cellular level. While epinephrine does increase aortic, cerebral, and carotid circulation pressure, it lowers carotid blood flow and endtidal CO_2 or $ETCO_2$ levels. It appears that epinephrine may be improving microcirculation at the expense of the capillary beds where actual perfusion is taking place[133].

In chemical terms, epinephrine is one of a group of monoamines called the catecholamine's. It is produced in some neurons of the CNS, and in the chromaffin cells of the adrenal medulla from the amino acids phenylalanine and tyrosine. Epinephrine is synthesized in the medulla of the adrenal gland in an enzymatic pathway that converts the amino acid tyrosine into a series of intermediates and, ultimately, epinephrine. Tyrosine is first oxidized to L-DOPA, which is subsequently decarboxylated to give dopamine. Oxidation gives nor epinephrine. The final step in epinephrine biosynthesis is the methylation of the primary amine of nor adrenaline. This reaction is catalyzed by the enzyme PNMT which utilizes S-adenosyl methionine (SAMe) as the methyl donor. While PNMT is found primarily in the cytosol of the endocrine cells of the adrenal medulla (also known as chromaffin cells), it has been detected at low levels in both the heart and brain [134].

The major physiologic triggers of adrenaline release center upon stresses, such as physical threat, excitement, noise, bright lights, and high ambient temperature. All of these stimuli are processed in the CNS. ACTH and the sympathetic nervous system stimulate the synthesis of adrenaline precursors by enhancing the activity of tyrosine hydroxylase and dopamine β-hydroxylase, (2) key enzymes are involved in catecholamine's synthesis. ACTH also stimulates the adrenal cortex to release cortisol, which increases the expression of PNMT in chromaffin cells, enhancing adrenaline synthesis. This is most often done in response

to stress. The sympathetic nervous system, acting via splanchnic nerves to the adrenal medulla, stimulates the release of adrenaline. Acetylcholine released by preganglionic sympathetic fibers of these nerves acts on nicotinic acetylcholine receptors, causing cell depolarization and an influx of calcium through voltage-gated calcium channels. Calcium triggers the exocytosis of chromaffin granules and, thus, the release of adrenaline (and nor adrenaline) into the bloodstream. Unlike many other hormones adrenaline (as with other catecholamine's) does not exert negative feedback to down-regulate its own synthesis. Abnormally elevated levels of adrenaline can occur in a variety of conditions, such as surreptitious epinephrine administration, pheochromocytoma, and other tumors of the sympathetic ganglia[135].

Chapter Three

Materials and Methods

Chapter Three

Materials and Methods

3. Material and methods

3.1 Study design

This study was designed to be a prospective hospital laboratory based study

3.2 Study area

This study was carried out in the Departments of Obstetrics and Gynaecology jabal awlia hospital - Khartoum state.

3.3 Study duration

The study was carried out during the period from September 2016 to April 2019.

3.4 Ethical considerations

This study was approved by the research committee – College of Medical Laboratory Sciences –Shendi University. Informed consent was obtained from each participant before taking the samples.

3.5 Sample Size and Study population

(400) women were enrolled in this study, (200) volunteers were multiparity pregnant women as study group, (100) volunteers were primiparity pregnant women as control positive group and 100 volunteers were nulliparity (non-pregnant women) as a control negative group. All participants were in their fertility period and/or premenopausal period, in age of (20-48 years) old. All pregnant women were at (26-34 weeks) gestational age.

For sample size we used this equation:

$$N = \frac{X^2\ P \times (1-P)}{D^2}$$

Where:

n Required sample size.

X confidence level at (95%),(standard value of 1.96).

P population proportion (percentage picking a choice).

D required margin of error (5%), (standard value of 0.05).

There is no prevalence for accurate rate to estimate the proportion of multiparity in Sudan thus we take the P maximum = (0.5). by substituting this figure in the formula above, the calculated sample size is about (384), were maximize the sample size to include (200)cases and (200)controls. (1:1)

3.6 Sampling technique

Venous blood was collected using antiseptic for the skin, as well as data concerning any sample from Laboratories according to inclusion criteria and exclusion criteria.

3.6.1Materials

1. Safety Needles, (22 g) or less.
2. Butterfly needles. (21 g) or less.
3. Deposable sterile syringes nontoxic pyrogen free (5ml).
4. Blood collection tube. The vacuum tubes are designed to draw a predetermined volume of blood, serum and gel tube (5 ml).
5. Tourniquets. Latex-free tourniquets were used.
6. Antiseptic. Individually packaged (70%) isopropyl alcohol wipes were used.
7. 2x2 inches Gauze or cotton balls.
8. Sharps disposal was used container An OSHA acceptable, puncture proof container marked Biohazardous was used.
9. Bandages or tape
10. Pasteur pipette 5ml
11. Plaster

12.Gloves

3.6.2 Procedure

1. The patient was identified in phlebotomy area, and has been told about the research, fill the consent form and the prepared questionnaire.
2. The patient has been told that the minimum amount of blood should be collected.
3. The necessary equipment appropriate to the patient's physical characteristics was collected.
4. Hands were washed and gloves were placed.
5. The patient's arm was positioned a straight-line form shoulder to wrist.
6. A venipuncture was attempted only one time.
7. The appropriate vein for venipuncture was selected.
8. Then tourniquet (3-4 inches) was applied to above the collection site. has Never leave the tourniquet more than 1 minute. If a tourniquet is used for preliminary vein selection, release it and reapply after (2 minutes).
9. Puncture site was cleaned by making a smooth circular pass over the site with the (70%) alcohol pad, moving in an outward spiral from the zone of penetration. Allow the skin to be dried before proceeding. The puncture site has not to be touched after being cleaned.
10. Venipuncture has to be performed.

11.3.6.3 Venipuncture procedure by using syringe

Blood collection is done by venipuncture from the straight line extended from the shoulder to wrist, after selecting the appropriate site, the tourniquet was applied (3-4 inches) above the puncture site the needle was held in the vein by a quick small thrust to penetrate the skin and vein in the same motion. After the required volume of blood was drown the tourniquet was released, the patient was asked to apply pressure for at

least (2 minutes). When bleeding stops, a fresh bandage, gauze or tape was applied, the blood sample was placed in the blood container and the syringe and needle were disposed in the sharps container [136].

3.6.4 Sample separation

All the collected blood samples were put in sample rack in room temperature for (30minutes) until clot was formed after that serum was separated by using a centrifuge for 5minutes at (5000 rpm); the serum was immediately transported to labeled Eppendorf safe–lock (5ml) tubes.

3.6.5 Sample storage

Serum samples were kept in a laboratory refrigerator at (-20°C) till time of analysis.

All the samples were analyzed in Rashid Polyclinic- Buridah City, Kingdom of Saudi Arabia (KSA).

3.7 Data collection

Personal data were obtained by structured pre-prepared questionnaire.

3.8 Laboratory tests

All tests were carried by full automated Competitive ELISA (Enzyme Linked Immune Sorbent Assay) [*Elisys Duo (6234000096), human-Germany*]. Using the control sera, level 1and level2 .

To perform sample programming:-

1- Workplace have been chosen for the test selection
2- The routine option of sample area was selected
3- Disk number was type
4- Sample ID was set
5- (Type of hormone)tests was select
6- Save menu was chosen .
7- Sample in disk position was select
8- Barcode after the last sample in disk position was placed

9- The start (global button) was chosen

10- two hour for test complete.

3.9 Methodology

ELISA is a methods where in color is produced out of an immune reaction and the color is estimated for qualitative and quantitative analysis.

3.9.1 ELISA principle

The antigens or antibodies present in patient's sample are allowed to stick to a polyvinyl plate and then plate is washed to separate antigens or antibodies (if any present) from remaining sample components. To this plate a corresponding second antibody or second antigen is added to get fixed to the already adhered first antigen in the plate. To this added second antigen or second antibody an enzyme is also tagged so that, when a suitable substrate is added, the enzyme reacts with it to produce a color. This color produced is measurable as a function or quantity of antigens or antibodies present in the given sample and there by identified. Here the enzyme's role is the key to develop color. So we use immobilized enzymes for the purpose to keep them stable[137].

3.9.2 Advantage of ELISA methods:-

- very short and one can measure very fast
- Minimal skill and knowledge required
- No special permissions required
- Disposal of waste is simple and can be done along with medical waste if used in hospital.
- has different types like direct ELISA, indirect ELISA, sandwich ELISA. Even there are multiple bindings like antigen-antibody-antigen or antibody-antigen-antibody.

- The detection of color generated due to ELISA reaction is done by simple photometer (visible spectrophotometer) where as in RIA; the measurement of the radioactive emission is done by using a γ counter.

On the other hand RIA has got limited use due to safety issues and legal binding rule. Yet it is an indispensable tool in medical research as it helps in study of drug receptor binding and other micro-structural imaging.

3.9.2 .1 . 3,4-dihydroxyphenethylamine ELISA Method

3.9.2 .1.1 Principle: This assay employs the competitive inhibition enzyme immunoassay technique. The micro-titer plate provided in this kit has been pre-coated with goat-anti-rabbit antibody. Standards or samples are added to the appropriate micro-titer plate wells with an antibody specific for DBH and Horseradish Peroxidase (HRP) conjugated DBH. The competitive inhibition reaction is launched between with HRP labeled DBH and unlabeled DBH with the antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of DBH in the sample. The color development is stopped and the intensity of the color is measured [138].

3.9.2 .2 Epinephrine ELISA Method

3.9.2 .2.1 Principle: Adrenaline is extracted by using a cis-diol-specific affinity gelacylated and then converted enzymatically. The competitive ELISA kit uses the micro-titer plate format. The antigen is bound to the solid phase of the micro-titer plate. The derivatized standards, controls and samples and the solid phase bound analytes compete for a fixed number of antibody binding sites. After the system is in equilibrium, free antigen and free antigen-antibody complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at (450nm). Quantification of unknown samples is achieved by

comparing their absorbance with a standard curve prepared with known standard concentrations [139].

3.9.2 .3 Endorphins ELISA Method

3.9.2 .3.1 Principle: Micro-plate well is pre-coated with monoclonal antibody specific to human β- endorphin. Biotin labeled β -endorphin and unlabeled beta endorphin are added simultaneously to wells which compete for monoclonal antibody coated on wells. After incubation the unbound conjugate gets washed way. In the next step Avidin conjugated to Horseradish Peroxidase (HRP) is added into the wells. Avidin binds specifically to biotin in labeled β -endorphin molecules. The amount of bound HRP conjugate is indirectly proportional to the concentration of beta endorphin in the sample. TMB substrate is added. HRP enzyme reacts on TMB substrate to give blue color. Stop solution is added to terminate the reaction. After addition of stop solution the reaction mixture changes to yellow color. The intensity of the color is indirectly proportional to the concentration of beta endorphin in the sample [140].

3.9.2.4 Oxytocin ELISA Method

3.9.2 .4.1 Principle: The Oxytocin ELISA kit is a competitive immunoassay for the quantitative determination of oxytocin in samples. The kit uses a polyclonal antibody to oxytocin to bind, in a competitive manner, the oxytocin in the standard or sample or an alkaline phosphatase molecule which has oxytocin covalently attached to it. After a simultaneous incubation at (4°C) the excess reagents are washed away and substrate is added. After a short incubation time the enzyme reaction is stopped and the yellow color generated read on a micro-plate reader at (405nm). The intensity of the bound yellow color is inversely proportional to the concentration of oxytocin in either standards or samples [141].

3.9.2.5 Lenomorelin ELISA Method

3.9.2.5.1Principle:This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to Ghrelin (GHRL) has been pre-coated onto a micro-plate. A competitive inhibition reaction is launched between biotin labeled Ghrelin (GHRL) and unlabeled Ghrelin (GHRL) (Standards or samples) with the pre-coated antibody specific to Ghrelin (GHRL). After incubation the unbound conjugate is washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) is added to each micro-plate well and incubated. The amount of bound HRP conjugate is reverse proportional to the concentration of Ghrelin (GHRL) in the sample. After addition of the substrate solution, the intensity of color developed is reverse proportional to the concentration of Ghrelin (GHRL) in the sample [142].

3.9.2.6 5-hydroxytryptamineis ELISA Method

3.9.2 .6.1 Principle: The competitive Serotonin ELISA kit uses the micro-titer plate format. Serotonin is bound to the solid phase of the micro-titer plate. Acylated serotonin and solid phase bound serotonin compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase serotonin is detected by anti-rabbit/peroxidase. The substrate TMB / peroxidase reaction is monitored at (450 nm). The amount of antibody bound to the solid phase serotonin is inversely proportional to the serotonin concentration of the sample [143].

3.10 Methods of BMI estimation:-

It calculates a value indicative of the fat content of the body by dividing the weight by the square of height [144].

$$\mathrm{BMI} = \frac{\mathrm{mass(kg)}}{(\mathrm{height(m)})^2}$$

Table 3-1: BMI Categories:[144]

Categories	BMI
Underweight	Less than 18.5
Normal weight	18.5 – 24.9
Overweight	25 – 29.9
Obese	30 or higher

3.11Quality controls and Managements:-

Blood was collected with care and adequate safety precautions to ensure test results were reliable. Quality Assurance (QA) and Standard Operating System were followed for all biological and clinical tests to achieve validity and reliability of test results.

3.12 Data analysis:-

The statistical analyses were performed using SPSS version 22. The descriptive results were expressed as mean ± standard deviation and percentage. Demographic data (non – normal distribution) of all hormones within group was analyzed using non parametric test (Kruskal-Wallis test). Independent t-test and one way anova was used to compare mean values of each parameter among the groups. (defined as 95% confidence limits) was determined and statistical significance of (P value≤0.05) was selected . Spearman's rank correlation coefficient test use to comparison of demographic data and biochemical parameters within groups. . All variables with P- value less than 0.05 were considered as statistical significance.

Chapter Four

Results

Chapter Four

Results

The results of this study were given in tables and figures. This study included 400 pregnant participants, 50% of them were multiparity pregnant women, 25% of them were primiparity pregnant women and last 25% were nulliparity women (figure 4.1).

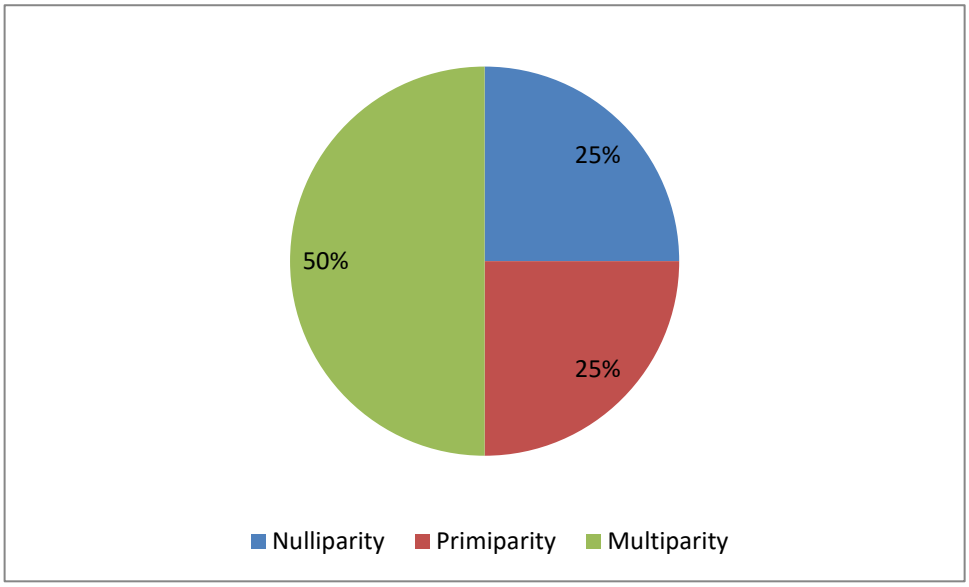

Figure (4.1): women groups

Table (4.1): Comparison between biochemical parameters within participants groups

Groups	Serotonin (ng/ml)	Oxytocin Pg/ml)(	Ghrelin (Pg/ml)
	Mean± SD	Mean± SD	Mean± SD
Multiparity a	107.98±38.06	798.69±569.5	720.89±513.14
Nulliparity b	223.45±80.15	290.03±226.3	306.05±193.13
Primiparity c	97.7±38.36	398.13±265.64	1023.45±796.53
	P- value 0.000	P- value 0.000	P- value 0.000

a= significant change in comparison between multiparity and primiparity ($p<0.05$)

b= significant change in comparison between multiparity and nulliparity ($p<0.001$)

c= significant change in compersion between primiiparity and nulliiparity ($p<0.05$)

Table (4.1) showed statistical anaylsis data for serotonin,oxytocin and ghrelin hormones computed for the three groups (nulliparity, primiparity and multiparity). The results showed that there was significant differences between the pregnant women and non pregnant women ($P < 0.001$)as will as between multiparity and primiparity ($P < 0.001$).

Table(4.2): Demographic data and qualitative biochemical parameters

Parameters	Multiparity (n=200)	Primiparity (n=100)	Nulliparity (n= 100)
Adrenaline	<50±0.00	<50±0.00	<50±0.00
Beta -endorphins	<100±0.00	<100±0.00	<100±0.00
Dopamine	<50±0.00	<50±0.00	<50±0.00

in comparison of β- endorphin , adrenalin and dopamine levels between the three groups, there was completely insignificant change between nulliparity and multiparity as well as primiparity.

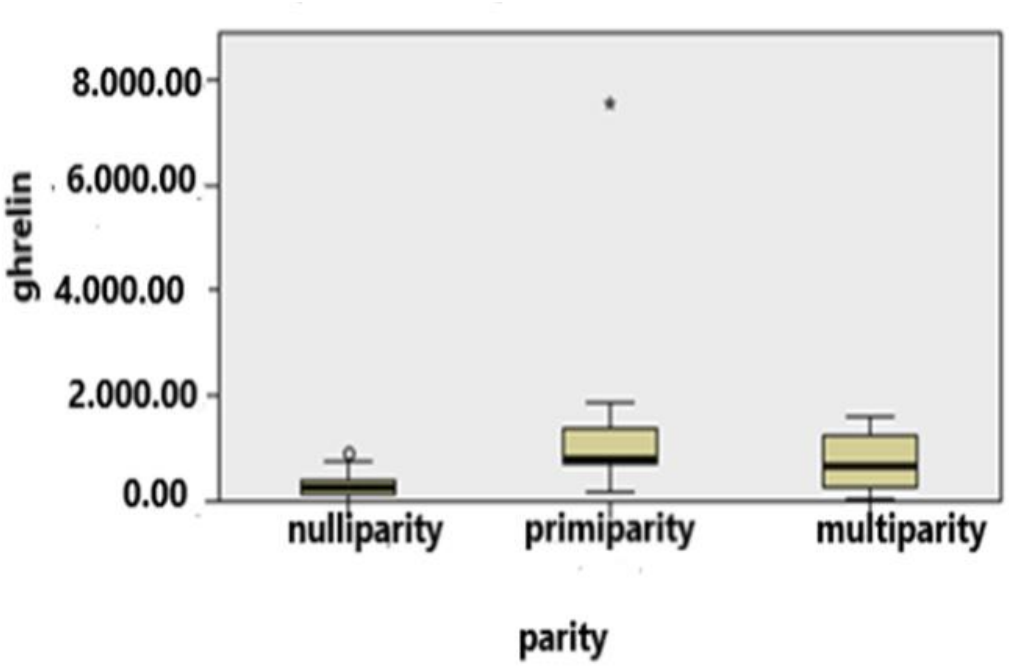

Figure (4.2): Comparison between ghrelin hormone and participants groups

In comparison between Ghrelin hormone and participants groups there was significant differences between the pregnant women and non pregnant women ($P < 0.001$)as well as between multiparity and primiparity ($P < 0.001$).

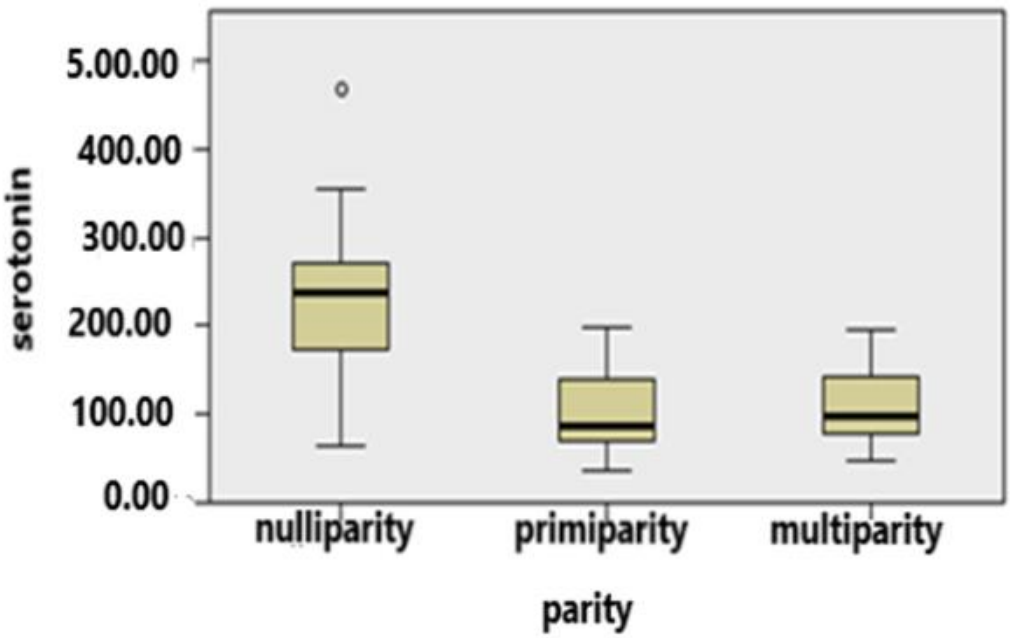

Figure (4.3): Comparison between serotonin hormone and participants groups

In comparison between Serotonin hormone and participants groups there was significant differences between the pregnant women and non pregnant women (P < 0.001)as well as between multiparity and primiparity (P < 0.001).

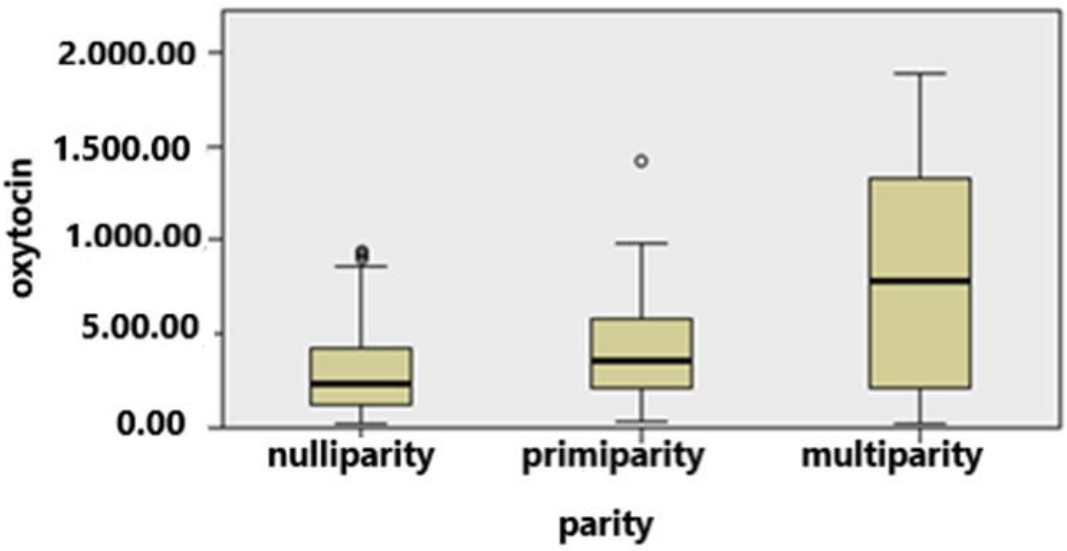

Figure (4.4): Comparison between Oxytocin hormone and participants groups

In comparison between Oxytocin hormone and participants groups there was significant differences between the pregnant women and non pregnant women ($P < 0.001$)as well as between multiparity and primiparity ($P < 0.001$).

Table (4.3): Comparison between multiparity group and hormones

Parameters	Multiparity	
	r	P- value
Age - Serotonin	0.101	0.153
Age - Oxytocin	0.220	0.002
Age -Ghrelin	0.314	0.000
BMI - Serotonin	0.041	0.565
BMI - Oxytocin	-0.003	0.964
BMI- Ghrelin	0.029	0.683

Table(4.3): showed that there were correlation between age and oxytocin hormone , and ghrelin hormone with (P-value 0.002 and less than 0.001) respectively.

Table(4.4): Comparison of hormones according to sex childbirth in multiparity groups

Parity	Hormone	Child	N	Mean	Std. Deviation	P-value
multiparity	serotonin	Male	169	108.48	38.67	
		Female	31	105.28	35.01	0.66
	oxytocin	Male	169	783.99	577.64	
		Female	31	878.75	524.43	0.39
	ghrelin	Male	169	707.37	513.30	
		Female	31	794.58	514.32	0.38

Table(4.4): The sex of childbirth showed insignificant differences in serotonin, oxytocin and ghrelin hormones.

Table (4.5): Comparison of hormones in multiparity group according to number of miscarriages

hormones	number	N	Mean	Std. Deviation	P-value
Serotonin	Two times	5	97.88	31.09	0.794
	One time	12	111.70	32.40	
	None	183	108.01	38.67	
Oxytocin	Two times	5	972.34	598.56	0.0583
	One time	12	1156.76	484.24	
	None	183	770.46	567.88	
Ghrelin	Two times	5	700.00	472.51	0.000
	One time	12	1330.16	100.84	
	None	183	681.51	506.30	

Table (4.5): in this table the only hormone significantly affected by miscarriage in multiparty pregnant group, is ghrelin hormone (P value <0.001) while the others hormones were insignificantly affected (P value >0.05).

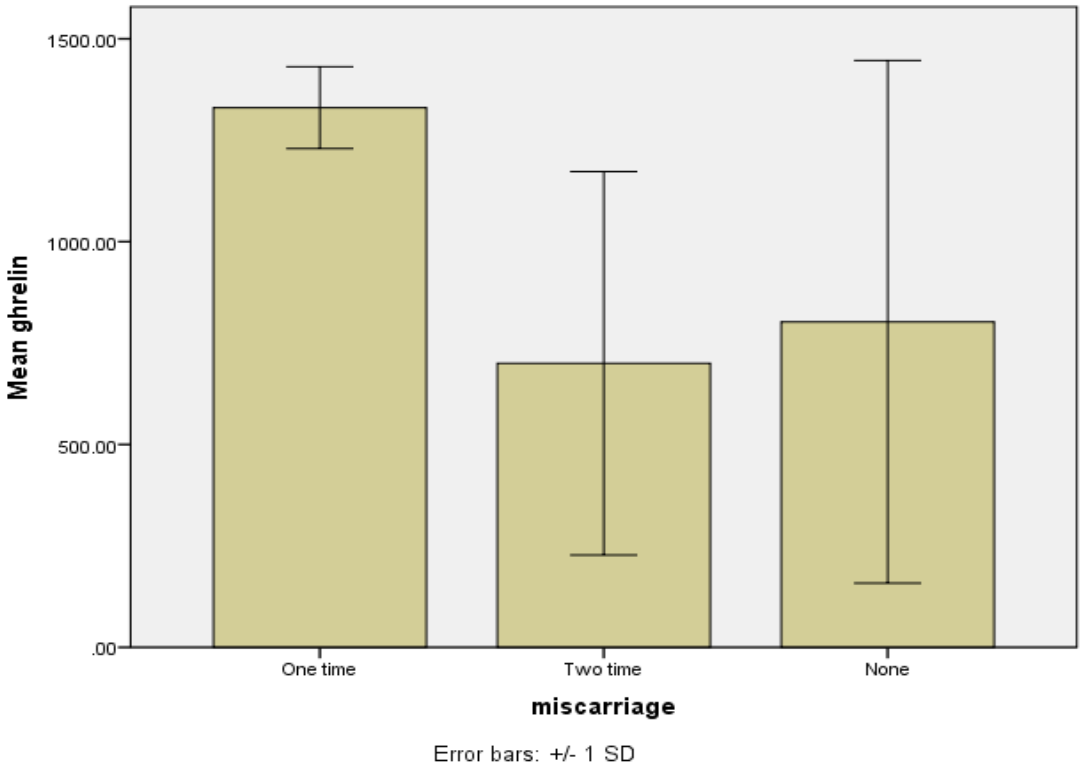

Figure (4.5): Comparison of ghrelin hormone in multiparity group according to number of miscarriage

In comparison of Ghrelin hormone in multiparity group according to number of miscarriage there was significant differences between Ghrelin hormone and miscarriage in multiparty pregnant group, is (P value <0.001) .

Table (4.6): Comparison of hormones in multiparity group according to number of parity

Hormones	Parity Number	N	Mean	Std. Deviation	P-value
Serotonin	para4	71	110.58	37.51	
	para5	50	106.58	40.74	
	para6	49	107.03	36.36	0.96
	para7	18	107.33	34.40	
	para8	12	103.28	46.78	
Oxytocin	para4	71	827.94	569.84	
	para5	50	747.70	522.69	
	para6	49	840.69	602.92	0.287
	para7	18	913.52	552.26	
	para8	12	494.27	615.35	
Ghrelin	para4	71	768.93	518.43	
	para5	50	523.83	459.13	
	para6	49	789.39	516.59	0.017
	para7	18	927.49	530.22	
	para8	12	668.17	479.67	

Table (4.6):in this table the only hormone significantly affected by parity number in multiparty pregnant group, is ghrelin hormone (P value <0.05). Showing maximum level in para7and minimum levels in para5, while the others hormones were insignificantly affected (P value >0.05).

Table (4.7):Comparison of hormones in multiparity group according to type of delivery

Hormones	Delivery type	N	Mean	Std. Deviation	P-value
Serotonin	Caesarean section	39	108.51	36.64	0.924
	Normal term	161	107.86	38.51	
Oxytocin	Caesarean section	39	1125.26	545.84	0.000
	Normal term	161	719.57	548.02	
Ghrelin	Caesarean section	39	1058.38	459.38	0.000
	Normal term	161	639.14	492.83	

Table (4.7): women who delivered in caesarean section showed significant increase result in both of oxytocin and ghrelin hormones when compared women who had normal term delivery (P value <0.001) in both hormones .

Chapter Five

(Discussion, Conclusion and Recommendations)

Chapter Five

5.1Discussion

The pregnancy is associated with many physiological and morphological changes to suit the needs of the growing fetus. These changes accompanying pregnancy are feared to have deleterious effects for mother and baby as the number of gestations [2].

In this study, a total of (400) women were selected and divided into three groups, (200) women (50%) were multiparity pregnant women, (100) women (25%) were primiparity who were in there first ever being pregnant, as control positive, and (100) women (25%) were nulliparity who were non pregnant at the time of the test as control negative .

In a comparison of hormones levels between the three groups, the results revealed that the mean concentration of the serotonin, oxytocin and ghrelin hormones in multiparity pregnant women were significantly altered when compared to nulliparity women as well as primiparity pregnant women (P.value<0.001). Serotonin hormone in multiparity showed significant decrease in levels, while ghrelin, and oxytocin hormones, showed highly significant increased levels. These results proved by report of Levine *et al* [145]. who found a strong significant correlations between Oxytocin levels in pregnant ladies and a (6) months postpartum. Also the same results in ghrelin levels were found in the report of Onur *et al* [146] . It is mentioned that in pregnancy preeclampsia can increase ghrelin levels significantly.

In a comparison of β-endorphin, adrenalin and dopamine levels between the three groups, there was insignificant change between nulliparity and multiparity as well as primiparity. This result is opposite to the report of Cahill *et al* [147].Who concluded that pregnant women had plasma levels of β-endorphin significantly higher than non pregnant women at the

midpoint of their menstrual cycle.

In the present study, the results showed that for multiparity there was a statistically significant correlation between age and oxytocin hormone (P-value = 0.002), and also there was a statistically significant correlation between age and ghrelin hormone(P-value <0.001). On the other hand, there was insignificant correlation between age and serotonin hormone (P-value >0.05). Dissimilar to these results, the study of Victoria *et al* [148] . Concluded significant age-related changes in the female hormones during reproductive years.

In the present study, according to the BMI in the three study groups, there were insignificant correlation between BMI and the mean level of serotonin, oxytocin and ghrelin hormones (P-value > 0.05). Having said that, previous studies were showed that multiparity is a risk factor for obesity in later life either before or after menopause and definitely a real association between the number of parties and obesity was noticed [149].

According to the type of childbirth delivery in multiparty pregnant group, there was an insignificant difference in the mean level of serotonin, oxytocin and ghrelin hormones. (P value >0.05) This findings proved by the report of Enninga *et al* [150] . Who showed that the maternal hormonal undergoes many changes during pregnancy that vary based on fetal sex. Enninga *et al* demonstrated that no significant differences between women carrying a male fetus versus women carrying a female fetus. The results of this study manifested that the number of miscarriage in multiparty pregnant women showed insignificant effect in the mean level concentration of serotonin and oxytocin hormones (P-value > 0.05); but the number of miscarriage effected in the mean levels of ghrelin hormone significantly (P-value < 0.001). The results exhibited that pregnant woman with one time of miscarriage has the maximum ghrelin value while the minimum value was in pregnant women with zero time of

miscarriage. Previous study Allen *et al* [151]. Measured the different hormones levels between menstrual cycle and after pregnancy loss and found insignificant low hormones levels after pregnancy loss.

In multiparity pregnant women and according to the number of parity the results of current study illustrated that there was insignificant difference in the mean levels of serotonin, oxytocin hormones (P-value > 0.05) and there was significant difference in the mean levels of ghrelin hormone (P-value < 0.05) in which the maximum value was in 7th parity and the minimum value was in 5th parity .This result proved the report of Alan *et al* [152]. Who located a profound effect of parity on maternal hormones levels.

The delivery type of multiparity women in this study showed insignificancy in the mean level of serotonin (P-value >0.05) but showed significant change in the mean levels of both oxytocin and ghrelin hormones with (P-value <0.001), were that maximum value was in the pregnant women delivered by cesarean section, and the minimum value wherein the pregnant women delivered by a normal term. This result is opposite to the report of Abdel Hakeem *et al* [153] .Who concluded that delivery mode will not affect the core ghrelin levels.

5.2Conclusions

The results of current study proved that the multiparty pregnancy has significant affect the levels of serotonin, oxytocin and ghrelin hormones, but has insignificant affect on the levels of β-endorphin, adrenalin and dopamine hormones. Also the results presented that the levels of oxytocin and ghrelin hormones in multiparty pregnant women are affected significantly by women age, number of miscarriages and delivery type, knowing that BMI and gender of childbirth have insignificant affect on hormones. But yet needless to say that the levels of ghrelin hormone in multiparity group are significantly affected by the number of parity.

5.3 Recommendations

For prospective future work we recommend the following:

6.2.1 Assessment of neurochemical hormones in pregnant Women must be conducted in all Sudan state to determine the prevalence of multiparty.

6.2.2 Further researches neurochemical hormones focus on eclampsia and preeclampsia might be important to expand our knowledge and its association with hormonal changes.

6.2.3 Measurement of neurochemical hormones in a child delivered by multiparty women.

6.2.4 Measurement of neurochemical hormones in postmenopausal women

Chapter six

(References& Appendices)

Chapter six

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6.2 Appendices

Gantt chart

Taskk	Responsible person	Month 1-2	Month 3 to 6	Month 7 to 8	Month 9-10	Month 11-13	Month 15 to 24	Month 25-30
Meeting faculty authorities and present the study proposal	Researchers							
Data collection	Patients and controls attending the study areas							
Biochemical Analysis	Researchers							
Data analysis	Researchers							
Writing of the results	Researchers							
Finalizing the study	Researchers							
Submission of the final project	Researchers							

بسم الله الرحمن الرحيم

جامعه شندي

كليه الدراسات العليا

استمارة مشاركه في بحث مقدم لنيل درجه الدكتوراه في الكيمياء ألسريريه بعنوان:- تقييم الهرمونات الكيميائية العصبية لدي النساء الحوامل

السيدة .....

إنني أقوم بدراسة لمعرفه أمكانيه ان يكون هناك علاقة للهرمونات الكيميائية العصبية لدي الحريم الحوامل ومتعددات الحمل وتأثيرها علي الحالة النفسية وتغير الطباع . لذا أمل منكم المشاركة معي في هذا البحث وذلك بالسماح لي بأخذ عينات الدم للفحص وسوف تكون بياناتكم لغرض البحث فقط وسوف أقوم باتخاذ جميع الاجراءت التي تضمن سريه وخصوصية المعلومات الشخصية المتعلقة بكم ولكم مني كل الشكر والتقدير .

موافق ( ) غير موافق ( )

SHANDI University

Assessment of neurochemical hormones in pregnant Sudanese women

QUESTIONNAIRE

1. General Information

Name:……No ……

Age:…… Years

Address:……..Tel. No:……

No of parity: ……No of miscarriage: ……

No of twins: …… Gestational age: ……

Childbirth: C-section ☐No…… Normal term☐No…..

2. Complications of pregnancy:

……..……...

……

……

3. Tall: …….m. Weight: …….kg BMI: …….

4. Statement of other health condition:

o CVD	Yes ☐	No ☐
o Inflammation or infection	Yes ☐	No ☐
o Diabetes Mellitus	Yes ☐	No ☐
o Hypertension	Yes ☐	No ☐
o Cancer	Yes ☐	No ☐
o Hormonal problems	Yes ☐	No ☐
o Paralysis	Yes ☐	No ☐
o Bone problems	Yes ☐	No ☐
o Hypothyroidism	Yes ☐	No ☐
o Liver Disease	Yes ☐	No ☐
o Autoimmune Disease	Yes ☐	No ☐

o Gout	Yes ☐	No ☐
o Kidneydisease	Yes ☐	No ☐
o Cushing's syndrome	Yes ☐	No ☐
o Smoking	Yes ☐	No ☐
o Other Hormonal disorders	Yes ☐	No ☐

6. Statement of drugs used at this moment:

……
……
……
……
……

Results

- **Serotonin :**……. …ng/ml N.R (40–200)
- **Ghrelin :**…… ……..pg/ml N. R(upto 1000)
- **endorohin:**……..... ..pg/ml N. R(<100)
- **Oxytocin:**…….…... pg/ml N. R(upto 1000)
- **Adrenaline** ……..……ng/ml N. R(<50)
- **Dopamine** ……..……..ng/ml N. R(<50)
- **BMI:** Lean ☐ Normal ☐ Overweight ☐ Obese ☐ Fatal Obesity ☐

Package Insert

EAGLE BIOSCIENCES

Serotonin ELISA Assay Kit

Catalog Number: SER39-K01
96 Wells
For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.
v. 2.0 030116

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

3. Storage and Stability

- On arrival, store the Serotonin ELISA kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.
- Do not use components beyond the expiration date shown on the kit labels.
- Do not mix various lots of any Serotonin ELISA kit component within an individual assay.

4. Contents of the Serotonin ELISA kit

4.1 **MT-Strips** — STRIPS — 12 strips
8 wells each, break apart
precoated with serotonin

4.2 **Standards 1 - 6** — CAL 1-6 — 6 vials
Each 4 ml, ready for use

Concentrations:

Standard	1	2	3	4	5	6
ng/ml	0	15	50	150	500	2,500

4.3 **Control 1 & 2** — CONTROL 1 & 2 — 2 vials
Each 4 ml, ready for use
Range: see q.c. certificate

4.4 **Acylation Buffer** — ACYL-BUFF — 1 vial
3 ml, ready for use

4.5 **Acylation Reagent** — ACYL-REAG — 1 vial
2.5 ml, ready for use

4.6 **Antiserum** — AS — 1 vial
11 ml, ready for use, colour coded yellow
Rabbit-anti-N-acylserotonin

4.7 **Enzyme Conjugate** — CONJ — 1 vial
12 ml, ready for use
Goat anti-rabbit-IgG-peroxidase

4.8 **Wash Buffer** — WASH — 1 vial
20 ml, concentrated, 50x concentrated
Dilute contents with dist. water to 1 litre total volume.

4.9 **Substrate** — SUB — 1 vial
12 ml TMB solution, ready for use

4.10	**Stop Solution** 12 ml, ready for use Contains 0.3 M sulphuric acid	STOP	1 vial
4.11	**Reaction plate** for acylation	ACYL-PLATE	1 piece
4.12	**Equalizing Reagent** lyophilized, dissolve content with 20.5 ml dist. water, dissolve carefully to minimize foam formation	EQUA-REAG	1 vial

Additional materials and equipment required but not provided:

- Pipettes (10, 25, 50, 100 and 200 µl)
- Orbital shaker
- Microplate washing device
- Microplate photometer (450 nm)

5. Sample Collection

5.1. Serum and Plasma

- The Serotonin ELISA test can be performed with serum as well as with EDTA plasma. If plasma is to be used care must be taken to get true platelet-free plasma. Otherwise, the Serotonin level has to be related to the number of thrombocytes in the sample. Since the preparation of platelet-free plasma requires special precautions, it is generally recommended to use serum instead of plasma.
- Hemolytic and lipemic samples should not be used in the Serotonin ELISA kit.
- The samples can be stored up to 6 hours at 2 - 8 °C. For a longer storage (up to 6 months) the samples must be frozen at -20 °C
- Repeated freezing and thawing should be avoided.

5.2. Urine

The total volume of urine excreted during a 24-hours period should be collected and mixed in a single bottle containing 10 - 15 ml of 6 M hydrochloric acid as preservative. Avoid exposure to direct sun light. Determine the total volume and take an aliquot for the measurement. For samples with suspected kidney disorders the creatinine

5. Incubate for 15 minutes at room temperature (approx. 20 °C) on an orbital shaker. Colour changed to green.
6. Take each 20 µl for the ELISA.

7. Test Procedure ELISA

7.1 Sample Incubation
Pipette each **20 µl prepared Standards 1 to 6**, **20 µl prepared controls** and **20 µl prepared controls and 20 µl prepared samples** into the respective wells of the coated microtiter strips (duplicates are recommended).

Pipette each **100 µl Antiserum** into all wells.

Incubate for 30 minutes at room temperature on an orbital shaker.

7.2 Washing
Discard or aspirate the contents of the wells and wash thoroughly with each **250 µl Wash Buffer**. Repeat the washing procedure 3 to 4 times. Remove residual liquid by tapping the inverted plate on clean absorbent paper.

7.3 Conjugate Incubation
Pipette each **100 µl enzyme conjugate** into all wells.
Incubate for 15 minutes at room temperature on an orbital shaker.

7.4 Washing
Repeat step 7.2.

7.5 Substrate Incubation
Pipette each **100 µl Substrate** into all wells and incubate for 15 ± 5 minutes at room temperature on an orbital shaker.

7.6 Stopping
Pipette each **100 µl Stop Solution** into all wells.

7.7 Reading
Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer.

Package Insert

EAGLE
BIOSCIENCES

Dopamine ELISA Assay Kit

Catalog Number: DOP31-K01
96 Wells
For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.
v. 1.1

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
www.EagleBio.com

3. Storage and Stability

- On arrival, store the Dopamine ELISA assay kit at 2-8 °C. Once opened the kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.
- Do not use components beyond the expiration date shown on the Dopamine ELISA assay kit labels.
- Do not mix various lots of any Dopamine ELISA assay kit component within an individual assay.

4. Contents of the Kit

Reagents for Sample Preparation:

4.1 **Extraction Plate** — **EX-PLATE** — 2 plates
48 wells
coated with boronate affinity gel

4.2. **Extraction-Buffer** — **EX-BUFF** — 1 vial
6 ml, ready for use

4.3 **HCl** — **HCL** — 1 vial
21 ml, ready for use
0.025 M HCl

4.4 **Standards (1 - 7)** — **CAL 1 - 7** — 7 vials
Each 4 ml, ready for use

Concentrations:

Standard		1	2	3	4	5	6	7
Dopamine	(ng/ml)	0	1.5	10	40	160	640	2,560
Dopamine	(nmol/l)	0	9.8	65.3	261	1,045	4,179	16,717

For only determination of urine samples: Standard 2 is not required.
For only determination of plasma samples: Standard 7 is not required.

4.5 **Control 1 & 2** — **CON 1 & 2** — 2 vials
Each 4 ml, ready for use
Concentrations: see q.c. certificate

4.6 **Acylation Reagent** ACYL-REAG 1 vial
6 ml, ready for use
Contains DMSO and DMF
(please note that solvent reacts with many plastic materials including plastic trays; it does not react with normal pipette tips and with glass devices).

4.7 **Acylation Buffer** ACYL-BUFF 1 vial
20 ml, ready for use

4.8 **Enzyme** ENZYME 3 vials
each 1.7 ml, lyophilized
Catechol-O-methyltransferase

4.9 **Coenzyme** COENZYME 1 vial
1 ml, ready for use (S-adenosyl-L-methionine)

4.10 **Enzyme Buffer** ENZYME-BUFF 1 vial
3.5 ml, ready for use

Reagents for ELISA:

4.11 **Dopamine-Antiserum** AS-DA 1 vial
5.5 ml, ready for use, rabbit
colour coded green

4.12 **MT-Strips** STRIPS-DA 1 plate
8 wells each, break apart, precoated with:
derivatized dopamine (12 strips), colour coded green

4.13 **POD Conjugate** CONJ 1 vial
Each 12 ml, ready for use,
Anti-rabbit IgG-POD conjugate/ peroxidase

4.14 **Wash Buffer** WASH 1 vial
20 ml, concentrate
Dilute content with dist. water to 500 ml total volume

4.15 **Substrate** SUB 1 vial
12 ml TMB solution, ready for use

4.16 **Stop Solution** STOP 1 vial
12 ml, ready for use. Contains 0.3 M sulphuric acid

4.17 **Adhesive Foil** FOIL 10 pieces
Ready for use

7. Test Procedure ELISA

Allow reagents to reach room temperature. Duplicates are recommended.

1. Pipette each 10 µl of freshly prepared Enzyme Mix into all wells (colour code green).
2. Pipette each 50 µl prepared Standards, Controls and Patient Samples into the respective wells (colour coded green).
3. Incubate the plate with adhesive foil for 30 minutes at room temperature (20 – 25 °C) on an orbital shaker (400 - 600 r/min).
4. Pipette each 50 µl Dopamine-Antiserum (colour coded green) into all wells.
5. Cover the plate with adhesive foil, shake for 10 seconds and incubate for 12 – 20 hours (overnight) at 2-8 °C.
6. Discard or aspirate the contents of the wells and wash thoroughly with each 250 µl Wash Buffer. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 4 times.
7. Pipette each 100 µl POD-Conjugate into all wells.
8. Incubate for 30 minutes at room temperature on an orbital shaker (400 - 600 r/min).
9. Washing: Repeat wash step 6.
10. Pipette each 100 µl Substrate into all wells.
11. Incubate 25 to 35 minutes at room temperature (20 – 25 °C) on an orbital shaker (400 - 600 r/min).
12. Pipette 100 µl Stop Solution into all wells.
13. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

Package Insert

EAGLE BIOSCIENCES

Adrenaline (Epinephrine) High Sensitive ELISA Assay Kit

Catalog Number:
ADU39-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.0

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

3. Storage and Stability

- On arrival, store the Adrenaline (Epinephrine) High Sensitive ELISA kit at 2 - 8 °C. Once opened the Adrenaline (Epinephrine) High Sensitive ELISA kit is stable until its expiry date. For stability of prepared reagents refer to Preparation of Reagents.
- Do not use components beyond the expiration date shown on the Adrenaline (Epinephrine) High Sensitive ELISA kit labels.
- Do not mix various lots of any kit component within an individual assay.

4. Contents of the Adrenaline (Epinephrine) High Sensitive ELISA Kit

Reagents for Sample Preparation:

4.1 **Extraction Plate** — **EX-PLATE** — 2 plates
48 wells
coated with boronate affinity gel

4.2. **Extraction-Buffer** — **EX-BUFF** — 2 vials
6 ml, ready for use

4.3 **HCl** — **HCL** — 1 vial
21 ml, ready for use
0.025 M HCl

4.4 **Standards (A - F)** — **CAL A - F** — 6 vials
Each 4 ml, ready for use
Concentrations:

Standard		A	B	C	D	E	F
Adrenaline	(ng/ml)	0	0.15	0.5	1.5	5	25
Adrenaline	(nmol/l)	0	0.82	2.7	8.2	27.3	137

4.5 **Control 1 & 2** — **CON 1 & 2** — 2 vials
Each 4 ml, ready for use
Concentrations: see q.c. certificate

4.6 **Acylation Reagent** — **ACYL-REAG** — 1 vial
6 ml, ready for use
Contains DMSO and DMF
(please note that solvent reacts with many plastic materials including plastic trays; it does not react with normal pipette tips and with glass devices).

4.7 **Acylation Buffer** — **ACYL-BUFF** — 1 vial
20 ml, ready for use

4.8	**Enzyme** each 2 ml, lyophilized Catechol-O-methyltransferase	ENZYME	3 vials
4.9	**Coenzyme** 1 ml, ready for use S-adenosyl-L-methionine	COENZYME	1 vial
4.10	**Enzyme Buffer** 3.5 ml, ready for use	ENZYME-BUFF	1 vial
4.11	**Enzyme Plate** 96 wells, ready for use	ENZYME-PLATE	1 piece
4.12	**Sample Stabilizer** 20 ml, ready for use	STABILIZER	1 vial
	Reagents for ELISA:		
4.13	**Adrenaline-Antiserum** 2.5 ml, ready for use, rabbit colour coded blue	AS-AD	1 vial
4.14	**MT-Strips** 8 wells each, break apart, precoated with adrenaline, colour coded blue	STRIPS-AD	12 strips
4.15	**POD Conjugate** 12 ml, ready for use, Anti-rabbit IgG-POD conjugate / peroxidase	CONJ	1 vial
4.16	**Wash Buffer** 20 ml, concentrate Dilute content with dist. water to 500 ml total volume	WASH	2 vials
4.17	**Substrate** 12 ml TMB solution, ready for use	SUB	1 vial
4.18	**Stop Solution** 12 ml, ready for use Contains 0.3 M sulphuric acid	STOP	1 vial
4.19	**Adhesive Foil** Ready for use	FOIL	10 pieces

12. Decant the plate and remove residual liquid by tapping the inverted plate on a paper towel.

13. Repeat the wash steps 11. and 12.

14. Pipette each 125 µl HCl (0.025 M) for elution into all wells.

15. Cover the plate with adhesive foil and incubate for 20 minutes at room temperature on an orbital shaker (medium shaking rate).
Caution: Do not decant the supernatant thereafter.

16. Transfer 100 µl from the extraction plate into the respective wells of the enzyme plate.

17. Pipette each 20 µl of freshly prepared Enzyme Mix (s. 6.1.2) into all wells of the enzyme plate. Colour changes to red.

18. Cover the plate with adhesive foil and incubate for 1 minute at room temperature on an orbital shaker (medium shaking rate).

19. Incubate the plate for 90 minutes at 37°C without shaking.
(Alternatively: 120 minutes at room temperature (20 - 25°C) on an orbital shaker at medium shaking rate).
Caution: Do not decant the supernatant thereafter.

Take each 100 µl of the supernatant for the Adrenaline (Epinephrine) High Sensitive ELISA.

7. Test Procedure ELISA

1. Pipette each 100 µl prepared Standards, Controls and Samples into the respective wells (colour coded blue).

2. Pipette each 20 µl Adrenaline-Antiserum (colour coded blue) into all wells.

3. Cover the plate with adhesive foil, shake briefly and incubate for 15 – 20 hours (overnight) at 2 - 6 °C.

4. Discard or aspirate the contents of the wells and wash thoroughly with each 250 µl prepared Wash Buffer. Remove residual liquid by tapping the inverted plate on clean absorbent paper. Repeat the washing procedure 3 times.

5. Pipette each 100 µl POD-Conjugate into all wells.

6. Incubate for 60 minutes at room temperature on an orbital shaker (medium shaking rate).

7. Washing: Repeat wash step 4.

8. Pipette each 100 µl Substrate into all wells.

9. Incubate for 35 to 45 minutes at room temperature (20 – 25 °C) on an orbital shaker (medium shaking rate). Avoid exposure to direct sun light.

10. Pipette 100 µl Stop Solution into all wells.

11. Read the optical density at 450 nm (reference wavelength between 570 and 650 nm) in a microplate photometer within 15 minutes.

8. Calculation of Results

On a semilogarithmic graph paper the concentration of the standards (x-axis, logarithmic) are plotted against their corresponding optical density (y-axis, linear). Alternatively, the optical density of each standard and sample can be related to the optical density of the zero standard, expressed as the ratio OD/OD_{max}, and then plotted on the y-axis.
A good fit is provided with 4 Parameter Logistic (alternatively Log-Logit or Cubic Spline).

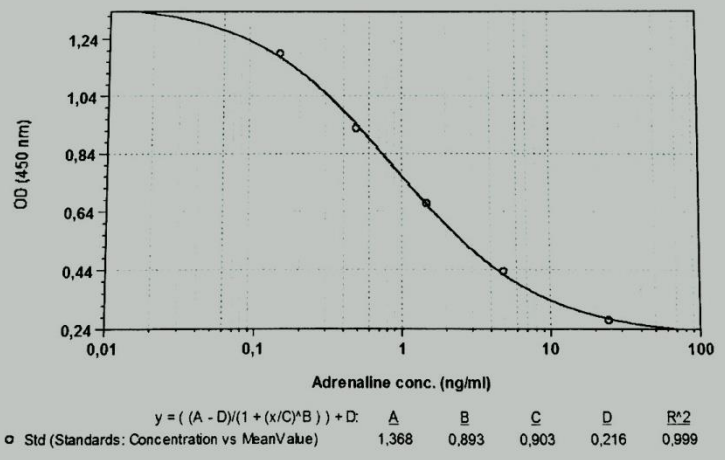
The concentration of the controls can be read off the standard curve directly without any further conversion. The read concentrations of the samples have to be divided by a correction factor due to the use of 1 µl - 300 µl sample volume in relation to 20 µl standard.

$$\text{Correction factor} = \frac{\text{Sample volume for extraction (µl)}}{\text{20 µl (Standard volume)}}$$

Example:
300 µl sample was extracted and the concentration read off from the standard curve is 0.6 ng/ml.
Correction factor = 300 µl / 20 µl = 15
Concentration of the sample = 0.6 ng/ml / 15 = 0.040 ng/ml = 40 pg/ml

Conversion into pmol/l:
Adrenaline: 1 pg / ml = 5,46 pmol / l

Typical Example
Below a typical example of a standard curve with Adrenaline (Epinephrine) High Sensitive ELISA:



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ab133050 –

Oxytocin ELISA Kit

Instructions for Use

For the quantitative measurement of Oxytocin concentrations in culture supernatants, milk, plasma and serum from any species.

This product is for research use only and is not intended for diagnostic use.

Version: 2 Last Updated: 09 May 2013

4. Kit Contents

Item	Description	Quantity
Goat anti-Rabbit IgG Microtiter Plate	A plate using break-apart strips coated with goat antibody specific to rabbit IgG.	1x 96 wells
Oxytocin Conjugate	A blue solution of alkaline phosphatase conjugated with Oxytocin.	5 ml
Oxytocin Antibody	A yellow solution of a monoclonal antibody to Oxytocin.	5 ml
Assay Buffer	Buffer containing proteins and sodium azide as preservative.	27 ml
Wash Buffer Concentrate	Tris buffered saline containing detergents.	27 ml
Oxytocin Standard	A solution of 10,000 pg/ml Oxytocin.	0.5 ml
pNpp Substrate	A solution of p-nitrophenyl phosphate in buffer. Ready to use.	20 ml
Stop Solution	A solution of trisodium phosphate in water. Keep tightly capped.	5 ml
Plate Sealer		1

2. Oxytocin Conjugate

Allow the conjugate to warm to room temperature. Any unused conjugate should be aliquoted and re-frozen at or below -20 °C.

3. Wash Buffer

Prepare the Wash Buffer by diluting 5 ml of the supplied concentrate with 95 ml of deionized water. This can be stored at room temperature for 3 months.

D. Assay Procedure

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards and samples should be run in duplicate.

1. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells with the desiccant back into the pouch and seal the ziploc. Store unused wells at 4 °C.
2. Pipette 100 µl of standard diluent (Assay Buffer or Tissue Culture Media) into the NSB and the Bo (0 pg/ml Standard) wells.
3. Pipette 100 µl of Standards #1 through #7 into the appropriate wells.
4. Pipette 100 µl of the Samples into the appropriate wells.

5. Pipette 50 µl of Assay Buffer into the NSB wells.
6. Pipette 50 µl of blue Conjugate into each well, except the Total Activity (TA) and Blank wells.
7. Pipette 50 µl of yellow Antibody into each well, except the Blank, TA and NSB wells.

NOTE: Every well used should be Green in color except the NSB wells which should be Blue. The Blank and TA wells are empty at this point and have no color.

8. Tap the plate gently to mix. Seal the plate and incubate at 4°C for 18-24 hours.
9. Empty the contents of the wells and wash by adding 400 µl of wash solution to every well. Repeat the wash 2 more times for a total of 3 Washes.
10. After the final wash, empty or aspirate the wells, and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
11. Add 5 µl of the blue Conjugate to the TA wells.
12. Add 200 µl of the pNpp Substrate solution to every well. Incubate at room temperature for 45 minutes without shaking.
13. Add 50 µl of Stop Solution to every well. This stops the reaction and the plate should be read immediately.
14. Blank the plate reader against the Blank wells, read the optical density at 405 nm, preferably with correction between 570 and 590 nm. If the plate reader is not able to be blanked against the

Human Ghrelin (Active)

96-Well Plate

Cat. # EZGRA-88K, EZGRA-88BK

III. REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well plate and contains the following reagents:

1. **Microtiter Plate**
   Coated with pre-titered anchor antibodies.
   Quantity: 1 Strip Plate
   Preparation: Ready to use.
   Note: Unused strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8 °C.

2. **Adhesive Plate Sealer**
   Quantity: 2 sheets
   Preparation: Ready to use.

3. **10X HRP Wash Buffer Concentrate**
   10X concentrate of 50 mM Tris Buffered Saline containing Tween-20.
   Quantity: 2 bottles containing 50 mL each
   Preparation: Dilute 1:10 with distilled or de-ionized water.

4. **Human Ghrelin (Active) Standard**
   Human Ghrelin (active) reference standard, lyophilized
   Quantity: 2 mL/vial upon hydration
   Preparation: Hydrate thoroughly in distilled or de-ionized water immediately before use. Please refer to the analysis sheet for exact concentration. After hydration dilute with Assay Buffer according to § VIII. A.

5. **Quality Controls 1 and 2**
   One vial each, lyophilized, containing human ghrelin (active) at two different levels.
   Quantity: 0.5 mL/vial upon hydration.
   Preparation: Reconstitute each vial with 0.5 mL de-ionized water immediately before use. Aliquot unused portion in smaller quantity and freeze at 2 - 8°C for later use. Avoid further freeze and thaw.

6. **Matrix Solution**
   Processed serum matrix containing 0.08% Sodium Azide
   Quantity: 1 mL/vial
   Preparation: Ready to use.

III. REAGENTS SUPPLIED (Continued)

7. Assay Buffer

0.05 M phosphosaline, pH 7.4, containing 0.025 M EDTA, 0.05 % Triton X-100, 0.08% sodium azide, and 0.1% BSA.

Quantity: 15 mL/vial

Preparation: Ready to use.

8. Human Ghrelin (Active) Capture Antibody

Pre-titered capture antibody solution in buffer

Quantity: 3 mL/vial

Preparation: Mix 1:1 with Human Ghrelin (Active) Detection Antibody before use according to § VIII. C.

9. Human Ghrelin (Active) Detection Antibody

Pre-titered detection antibody solution in buffer

Quantity: 3 mL/vial

Preparation: Mix 1:1 with Human Ghrelin (Active) Capture Antibody before use according to § VIII. C.

10. Enzyme Solution

Pre-titered streptavidin-horseradish peroxidase conjugate in buffer.

Quantity: 12 mL/vial

Preparation: Ready to use

11. Substrate

3, 3',5,5'-tetramethylbenzidine in buffer.

Quantity: 12 mL/vial

Preparation: Ready to use. Minimize the exposure to light.

12. Stop Solution

0.3 M HCl

Quantity: 12 mL/vial

Preparation: Ready to use.

[Caution: Corrosive Solution]

IX. HUMAN GHRELIN (ACTIVE) ELISA ASSAY PROCEDURE

Pre-warm all reagents to room temperature immediately before setting up the assay.

1. Dilute the 10X concentrated HRP wash buffer 10 fold by mixing the entire contents of both buffer bottles with 900 mL de-ionized or glass distilled water.
2. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8°C. Assemble the strips in an empty plate holder and fill each well with 300 µL diluted Wash Buffer. Decant wash buffer and remove the residual amount by inverting the plate and tapping it smartly onto absorbent towels several times. Wash assay plate using this procedure 2 additional times. **Do not let wells dry before proceeding to the next step.** If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.
3. Add 20 µL Matrix Solution to Blank, Standards and Quality Control wells (refer to § X. for suggested well orientations).
4. Add 30 µL assay buffer to each of the Blank and sample wells.
5. Add 10 µL assay buffer to each of the Standard and Quality Control wells.
6. Add in duplicate 20 µL Ghrelin Standards in the order of ascending concentrations to the appropriate wells.
7. Add in duplicate 20 µL QC1 and 20 µL QC2 to the appropriate wells.
8. Add sequentially 20 µL of the unknown samples in duplicate to the remaining wells.
9. Transfer the Antibody Solution Mixture (1:1 mixture of capture and detection antibody) to a buffer or reagent reservoir and add 50 µL to each well with a multi-channel pipette.
10. Cover the plate with plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed, about 400 to 500 rpm.
11. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in well.
12. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.
13. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 min on the micro-titer plate shaker.

IX. HUMAN GHRELIN (ACTIVE) ELISA ASSAY PROCEDURE (continued)

14. Remove sealer, decant solutions from the plate and tap plate to remove the residual fluid.

15. Wash wells 6 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

16. Add 100 µL of Substrate solution to each well, cover plate with sealer and shake in the plate shaker for approximately 5-20 minutes.

    (**Note:** Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.)

    Blue color should be formed in wells of Ghrelin standards with intensity proportional to increasing concentrations of Ghrelin. Remove sealer and add 100 µL stop solution **[CAUTION: CORROSIVE SOLUTION]** and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn into yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there is no air bubbles in any well.

FOR RESEARCH USE ONLY!

BioVision
BioVision Incorporated

QuickDetect™ beta-Endorphin (Human) ELISA Kit

rev 11/18

(Catalog # E4458-100, 100 assays, Store at 4°C)

I. Introduction:

β-Endorphin (beta-EP) is an endogenous opioid neuropeptide and peptide hormone that is produced in certain neurons within the central nervous system and peripheral nervous system. It is one of five endorphins that are produced in human, the others of which include α-endorphin, γ-endorphin, α-neoendorphin, and β-neoendorphin. Function of β-endorphin has been known to be associated with hunger, thrill, pain, maternal care, sexual behavior, and reward cognition. In the broadest sense, β-endorphin is primarily utilized in the body to reduce stress and maintain homeostasis. In behavioral research, studies have shown that β-endorphin is released via volume transmission into the ventricular system in response to a variety of stimuli, and novel stimuli in particular. BioVision's beta-EP ELISA kit is a sandwich ELISA assay for the quantitative measurement of beta-EP in human serum, plasma and cell culture supernatants in 90 minutes. The density of color is proportional to the amount of beta-EP captured from the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of beta-EP.
Detection Range: 3 - 200 pg/ml
Sensitivity: < 0.5 pg/ml
Assay Precision: Intra-Assay: CV < 10%; Inter-Assay: CV < 12% (CV (%) = SD/mean X 100)
Cross Reactivity: No significant cross-reactivity or interference between this analyte and its analogues was observed.

III. Specificity:

Human

IV. Sample Type:

Serum, plasma, urine, cell culture samples, biological fluid.

V. Kit Contents:

Components	E4458-100	Part No.
Micro ELISA strip-plate	1	E4458-100-1
Standard (270 pg/ml)	0.5 ml	E4458-100-2
Standard diluent	6 ml	E4458-100-3
HRP- Conjugate reagent	10 ml	E4458-100-4
Sample diluent	6 ml	E4458-100-5
Chromogen Solution A	6 ml	E4458-100-6
Chromogen Solution B	6 ml	E4458-100-7
Stop Solution	6 ml	E4458-100-8
Wash buffer (20X)	25 ml	E4458-100-9
Plate sealers	2	E4458-100-10

VI. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VII. Storage and Handling:

The entire kit may be stored at 4°C in dark for up to 6 months from the date of shipment. Avoid freeze-thaw cycles.

VIII. Reagent Preparation:

Note: Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer**: Dilute the concentrated washing buffer (20X) with distilled water.
2. **Standard Preparation:**

Ten wells are set for standards in a Microelisa stripplate. In Well 1 and Well 2, 50 µl Standard solution and 50 µl Standard Dilution buffer are both added and mixed in each well. In Well 3 and Well 4, 50 µl solution from Well 1 and Well 2 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. 50 µl solution is discarded from Well 3 and Well 4. In Well 5 and Well 6, 50 µl solution from Well 3 and Well 4 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. In Well 7 and Well 8, 50 µl solution from Well 5 and Well 6 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. In Well 9 and Well 10, 50 µl solution from Well 7 and Well 8 are added respectively. Then 50 µl Standard Dilution buffer are added and mixed well. 50 µl solution is discarded from Well 9 and Well 10. After dilution, the total volume in all the wells are 50 µl and the concentrations are 135 pg/ml,67.5 pg/ml · 33.75 pg/ml,16.86 pg/ml and ,8.44 pg/ml, respectively.

FOR RESEARCH USE ONLY! Not to be used on humans.

155 S. Milpitas Blvd., Milpitas, CA 95035 USA | T: (408)493-1800 F: (408)493-1801 | www.biovision.com | tech@biovision.com

3. Sample Preparation:

Note: Sample extraction and ELISA assay should be performed as soon as possible after sample collection. If ELISA assay can not be performed immediately, samples can be stored at -20°C. Avoid multiple freeze-thaw cycles. Samples with NaN_3 should be avoided for this assay.

- **Serum**: After collection of the whole blood, allow the blood to clot by leaving it undisturbed at room temperature. This usually takes 10-20 minutes. Remove the clot by centrifuging at 2,000-3,000 rpm for 20 minutes. If precipitates appear during reservation, the sample should be centrifuge again.
- **Plasma**: Collect the whole blood into tubes with anticoagulant (EDTA or citrate). After incubated at room temperature for 10-20 minutes, tubes are centrifuged for 20 min at 2,000-3,000 rpm. Collect the supernatant carefully as plasma samples. If precipitates appear during reservation, the sample should be centrifuge again.
- **Urine**: Collect urine into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuge again. The preparation procedure of cerebrospinal fluid and pleuroperitoneal fluid is the same as that of urine sample.
- **Cell Samples**: If you want to detect the secretions of cells, collect culture supernatant into aseptic tubes. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If you want to detect intracellular components, dilute the cells to 1X100/ml with PBS (pH 7.2-7.4). The cells were destroyed to release intracellular components by repeated freezing and thawing. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. If precipitates appear during reservation, the sample should be centrifuge again.
- **Tissue Samples**: Tissue samples are cut, weighed, frozen in liquid nitrogen and stored at -80°C for future use. The tissue samples were homogenized after adding PBS (pH 7.4). Samples should be operated at 4°C. Collect the supernatant carefully after centrifuging for 20 min at 2,000-3,000 rpm. Aliquot the supernatant for ELISA assay and future use.
- End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit.

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed in section VIII.
2. In sample wells, add 40 µl Sample dilution buffer and 10 µl samples are added (dilution factor is 5). Leave a well empty as blank control. Samples should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking.
3. Add HRP-Conjugate reagent 100 µl to each well, except blank well. Incubate 60 min at 37°C after sealed with plate sealer.
4. Remove plate sealer, aspirate and refill with the wash solution. Discard the wash solution after resting for 30 seconds. Repeat the washing procedure for 5 times.
5. Add 50 µl Chromogen Solution A and 50 µl Chromogen Solution B to each well, mix with gently shaking and incubate at 37°C for 15 minutes in dark.
6. Add 50 µl stop solution to each well to terminate the reaction. The color in the well should change from blue to yellow.
7. Read absorbance O.D. at 450nm within 15 minutes after adding stop solution. The OD value of the blank control well is set as zero.

X. CALCULATION:

Known concentrations of Human beta-EP Standard and its corresponding reading OD is plotted respectively. The concentration of beta-EP in sample is determined by plotting the sample's O.D. on the X-axis. The original concentration is calculated by multiplying the dilution factor.

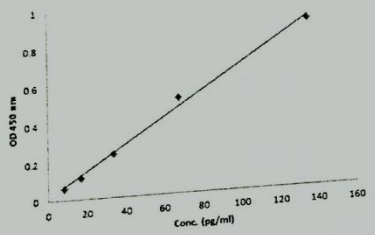

Figure: Typical Standard Curve: These standard curves are for demonstration only. A standard curve must be run with each assay.

XI. RELATED PRODUCTS:

- QuickDetect™ beta-EP (Mouse) ELISA Kit (E4459-100)
- QuickDetect™ beta-EP (Rat) ELISA Kit (E4460-100)
- QuickDetect™ Acetylcholine (ACh) (Human) ELISA Kit (E4452-100)
- QuickDetect™ Acetylcholine (ACh) (mouse) ELISA Kit (E4453-100)


Elisys Duo
Human