HORMONAL BEHAVIOUR AND ELECTROLYTES DISTURBANCES IN PREGNANT WOMEN WITH HYPEREMESIS GRAVIDARUM

A thesis

Submitted to the committee of the College of Medicine- Babylon University in partial fulfillment of the requirements for the degree of Master of Science in clinical biochemistry

BY

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بسم الله الرحمن الرحيم

اللَّهُ يَعْلَمُ مَا تَحْمِلُ كُلُّ أُنثَى وَمَا تَغِيضُ الْأَرْحَامُ وَمَا
تَزْدَادُ وَكُلُّ شَيْءٍ عِندَهُ بِمِقْدَارٍ

صدق الله العلي العظيم

سورة الرعد (آية 8)

Certification

We certify that this thesis was prepared under our supervision at the Biochemistry Department- College of Medicine - Babylon University as a partial requirements for the degree of Master of Science in Clinical Biochemistry.

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***We, the Examining committee ,certify that we have read this thesis entitled: (*HORMONAL BEHAVIOUR AND ELECTROLYTES DISTURBANCES IN PREGNANT WOMEN WITH HYPEREMESIS GRAVIDARUM)**

And have examined the student Maiada Muhmood H. Al-Sharbati in its contents ,and that in our opinion it is accepted as an thesis in partial fulfillment of the requirement for the degree of Master of Science in Clinical Biochemistry.

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DEDICATION

TO

MY DEVOTING HUSBAND…

MY PARENTS AND FAMILY…

MY Kid

~ Mustafa ~

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Praise worthy be to god for the accomplishment of this thesis.

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List of Abbreviation

Abbreviation	Meaning
ADH	Anti diuretic hormone
CTP	C-terminal peptide
DFE	Dietary folate equivalents
EDD	Expected Date of Delivery
EGGs	Electrogastrograms
ECF	Extracellular fluid
ELISA	Enzyme-linked immunosorbent assays
FSH	Follicle-stimulating hormone
GPH	Glycoprotein hormone
HCG	Human chorionic gonadotropin
HEG	Hyperemesis gravidarum
IFCC	International Federation of Clinical Chemistry
ITA	Invasive trophoblast antigen
ICF	Intracellular fluid
IS	International standards
KDa	kilodalton
Kg	kilograme
LMP	Last Menstrual Period
LH	Luteinizing hormone
MMPI	Minnesota Multiphasic Personality Inventory
NVP	Nausea and vomiting in pregnancy
OD	Optical density
TPN	Total parenteral nutrition
TSHR	Thyrotropin receptor
TBG	Thyroxine-binding globulin
T4	Thyroxine
T3	Triiodothyronine
TSH	Thyrotropin stimulating hormone
TMB	Ttetramethylbenzidine
US	United state
WHO	World Health Orgnaizatrion

Abstract

This study was done to explain the hormonal changes of (human chorionic gonadotrophin hormone and thyroid stimulating hormone) and the electrolytes disturbances (sodium and potassium) in pregnant women suffering from hyperemesis gravidarum.

The study has been conducted on (100) pregnant women suffering from hyperemesis gravidarm and their age ranged between (17-35) years old in the first trimester with the mean gestational age (8) weeks gestation, while the control group consist of 50 healthy pregnant women in the first trimester and the same mean gestational age .

The results revealed that there were significant difference in the mean serum level of human chorionic gonadotrophin hormone between the the patient group and the control group ($p < 0.05$) (human chorionic gonadotrophin hormone in control group higher than in patient group .

Regarding serum thyroid stimulation hormone and electrolytes level there was no significant difference between patient and control group ($p > 0.05$).

The study showed that the highest percentage of hyperemesis gravidarum depending on the age of pregnant women was (57%) in the age group (21-29) years old , while depending on maternal age was 51% in the 12 weeks of gestation while depending on gravidity 49% in third pregnancy and more that. Hyperemesis gravidarum percentage was 75% in urban area and 25 % rural area .

Human chorionic gonadotrophin hormone increased as the maternal age ,gestational age and gravidity increase, within the first trimester ,while there was no significant difference between serum thyroid stimulation hormone and electrolytes level with maternal age ,gestational age and gravidity.

The plotting of serum human chorionic gonadotrophin hormone against serum thyroid stimulation hormone revealed no significant relation between them ($p > 0.05$) and the coefficient correlation $r = 0.115$, .

On the basis of these results ,it was concluded that human chorionic gonadotrophin hormone is not the main cause of hyperemesis gravidarum and hyperemesis gravidarum is a multifactorial condition .

السلوك الهورموني والتغيرات الالكتروليتية لدى النساء الحوامل اللواتي يعانين من حالة الوحام

رسالة مقدمة الى
كلية الطب – جامعة بابل كجزء من متطلبات نيل درجة الماجستير العام في علم الكيمياء الحياتية السريرية

من قبل الطالبة
ميادة محمود حسين الشربتي
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1429 هـ 2008 م

بسم الله الرحمن الرحيم

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صدق الله العلي العظيم

سورة الرعد (آية 8)

الخلاصة

تتناول هذه الرسالة دراسة التغيرات الهورمونية والتي تتضمن الهورمونين (الهورمون الموجه للقند المشيمائية البشرية والهرمون المحفز للغدة الدرقية) والتغيرات الالكتروليتية التي يتضمنها عنصري الصوديوم والبوتاسيوم لدى الحوامل اللواتي يعانين من حالة الوحام. اشتملت الدراسة على (100) امرأة حامل تتراوح أعمارهن بين (17-35) سنه ضمن الفترة الاولى للحمل وبمعدل عمر حمل(8) أسابيع اما مجموعة السيطرة والتي تتضمن (50) امرأة حامل وبنفس فترة الحمل ونفس عمر الحمل بدون تعرضهن الى حالة وحام . تضمنت الرسالة قياس التغيرات الهرمونية والالكتروليتية بشكل عام لدى النساء الحوامل , حيث تم تقسيم الحوامل الى مجاميع حسب الفئة العمرية للمرأة الحامل وعمر الحمل وعدد مرات الحمل وحسب مناطق سكناهم . لقد اظهرت نتائج الدراسة ان هنالك فرق معنوي في مستوى الهورمون الموجه للقند المشيمائية البشرية بين مجموعة الحوامل اللواتي يعانين من حالة الوحام ومجموعة السيطرة حيث ان معدل مستوى الهورمون يكون أعلى في مجموعة السيطرة منه في مجموعة الحوامل اللواتي يعانين من حالة الوحام. ($p<0.05$)

اما بالنسبة للهورمون المحفز للغدة الدرقية والالكتروليتات فليس هناك أي فرق معنوي بين المجموعتين($p>0.05$) .كما أظهرت النتائج بان أعلى نسبة لحالة الوحام اعتماداً على الفئة العمرية للمرأة الحامل هي (57%) للفئة (21-29) واعلى نسبة لهذه الحالة اعتماداً على عمر الحمل هي (51%) في الاسبوع (12) للحمل وأعلى نسبة لهذة الحالة اعتماداً على عدد مرات الحمل (49%) من الحمل الثالث فصاعدا . كما بينت الدراسة ان النسبة الاكبر لحدوث حالة الوحام لدى النساء تكون في المدينة (75%) بينما تكون نسبة حدوثها بصورة اقل في الريف (25%).

وايضاً بينت النتائج ان نسبة الهورمون الموجه لقند المشيمائية البشرية تزداد بزيادة عمر المراة الحامل و عمر الحمل وعدد مرات الحمل . اما بالنسبة للهورمون المحفز للغدة الدرقية والالكتروليتات فليس هنالك أي فرق معنوي بينهما وبين عمر المراة الحامل وعمر الحمل وعدد مرات الحمل($P>0.05$). كما بينت الدراسة بانه ليس هنالك علاقة بين الهورمون الموجه للقند المشيمائية

والهرمون المحفز للغدة الدرقية(معامل الترابط للمرضى = 115,.) و (لمجموعة السيطرة= 107,.)

من ذلك نستنتج بان الهورمون الموجه للقند المشيمائية البشرية هو ليس المسبب الرئيسي لحالة الوحام وهذا يدل على ان حالة الوحام هي حالة متعددة الاسباب .

CHAPTER 1

Introduction

Section one

1. Pregnancy

Pregnancy is the carrying of one or more offspring, known as a fetus or embryo, inside the body of a female mammal such as a human, between conception and birth. In a pregnancy, there can be multiple gestations (for example, in the case of twins or triplets). Human pregnancy is the most studied of all mammalian pregnancies, and the medical field that deals with pregnancy is called obstetrics (1).

Pregnancy is typically divided into three trimesters (first trimester up to 14 weeks of gestation ,seconed trimester (14- 28) weeks of gestation and third trimester 28 weeks of gestation until delivery .

1.1 First Trimester

In medicine, pregnancy is often defined as beginning when the developing embryo becomes implanted into the endometrial lining of a woman's uterus. In some cases where complications may have arisen, the fertilized egg might implant itself in the fallopian tubes or the cervix, causing an ectopic pregnancy. Most pregnant women do not have any specific signs or symptoms of implantation, although it is not uncommon to experience light bleeding at implantation. Some women will also experience cramping during their first trimester. This is usually of no concern unless there is spotting or bleeding as well. The outer layers of the embryo grow and form a placenta, for the purpose of receiving essential nutrients through the uterine wall, or endometrium. The umbilical cord in a newborn child consists of the remnants of the connection to the placenta. The developing embryo undergoes tremendous growth and changes during the process of embryonic and fetal development.Morning sickness afflicts about seventy percent of all pregnant women, typically only in the first trimester, most miscarriages occur during this period (2).

1.2 Second 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. Although the fetus begins moving and takes a recognizable human shape during the first trimester, it is not until the second trimester that movement of the fetus, often referred to as "quickening", can be felt. This typically happens by the fourth month. The placenta is now fully functioning and the fetus is making insulin and urinating. The teeth are now formed inside the fetus' gums and the

reproductive organs can be recognized, and can distinguish the fetus as male or female (3).

1.3 Third Trimester

Final weight gain takes place, and the fetus begins to move regularly(4). The mother's belly button will sometimes "pop" out due to her growing belly. This period of her pregnancy can be uncomfortable, causing symptoms like bad bladder control and back-ache. Movement of the fetus becomes stronger and more frequent and the fetus prepares for viability outside the womb through improved brain, eye, and muscle function. The mother can feel the fetus "rolling" and it may cause pain or discomfort when the fetus become larger in size (3).

2. Signs of Pregnancy

A number of medical signs are associated with pregnancy

2.1 Early Signs

These signs typically appear, if at all, within the first few weeks after conception. Although not all of these signs are universally present, nor all of them are diagnostic by themselves,when taken together they may be useful to make a presumptive diagnosis of pregnancy.

- Presence of human chorionic gonadotropin (HCG) in the blood and urine, detectable by laboratory or home testing; this is the most reliable early sign of pregnancy (4) .

- Missed menstrual period . Implantation bleeding, light spotting that occurs at implantation of the embryo in the uterus, in the third or fourth week after LMP (5) .
- Increased basal body temperature sustained for over two weeks after ovulation Chadwick's sign, a darkening of the cervix, vagina, and vulva.

2.2 Later Signs

Linea nigra, a darkening of the skin in a vertical line on the abdomen, caused by hyperpigmentation resulting from hormonal changes; it usually appears around the middle of pregnancy .Steadily increasing abdominal swelling, the most visible sign of pregnancy (6) .

3. Pregnancy Symptoms

Symptoms of pregnancy vary. Of the symptoms listed, not all will occur for every woman and individuals may well experience different symptoms during different pregnancies (5) .

- There may be some twinge associated with implantation. (Generally 7-10 days after fertilization, (but taken alone such 'twinges' are not a reliable sign of pregnancy as it would be easy to mistake Mittelschmerz (mid-cycle pain at ovulation) for such a 'twinge' especially if the woman is not closely monitoring her cycle
- Absence of menstruation at expected time is usually the first clear symptom, but a pregnancy test is necessary to distinguish delay of menstruation from absence.

- Some women experience minor vaginal bleeding (spotting) especially at the expected time of menstruation right after conception (5,6).
- Many women experience swollen or tender breasts, similar, for some women, to PMS sensations. Minor lactation in third trimester.
- Some women experience fatigue and or sleeplessness during pregnancy, also absent mindedness and/or temporary loss of short-term memory.
- During the hormone surge following conception, some women can experience intense emotional surges from euphoria through anxiety and melancholy (7) .
- Nausea, sometimes accompanied by vomiting, esp. the first trimester, most likely to start at around 7 weeks. (morning sickness or hyperemesis gravidarum that well be show next section (7) .
- Some women report headaches of varying degrees of intensity, up to and including migraine-like headaches.
- Increased frequency of urination; occasionally urinary incontinence later in the pregnancy.
- Constipation, even from a very early stage. More rarely, in later trimester some women may experience involuntary defecation .
- Food cravings and/or increased appetite (6) .
- Heartburn or upset stomach, and sometimes vomiting. This can happen in the third trimester due to gravide uterus against the stomach (5) .

Other symptoms may be experienced specifically during the later trimester such as:

- Lower backache. Balance and ease of walking may be affected.
- Many women will get flatulent and gassy.
- Some may have difficulty in walking and balance.

- Some women may experience haemorrhoids and rectal irritation.
- A women few report hair loss, others have more body or "facial" hair.
- Sensitivity in teeth, higher risk for gum disease (7) .

4. Determining The Start of Pregnancy and Detection

Before pregnancy begins, a female oocyte (egg) must join, by spermatozoon in a process referred to in medicine as "fertilization", or commonly known as "conception". Though pregnancy begins at implantation, it is often convenien date from the first day of a woman's Last Menstrual Period (LMP). This is used to calculate the Expected Date of Delivery (EDD) (8) . The early detection is often discovered by using a pregnancy test, as soon as 48 hours after fertilization using sophisticated testing methods, but not until six to twelve days after fertilization using more typical methods. In the post-implantation phase the blastocyst secretes a hormone named human chorionic gonadotropin which in turn, stimulates the corpus luteum in the woman's ovary to continue producing progesterone. This acts to maintain the lining of the uterus so that the embryo will continue to be nourished. The glands in the lining of the uterus will swell in response to the blastocyst, and capillaries will be stimulated to grow in that region. This allows the blastocyst to receive vital nutrients from the woman. Pregnancy tests typically detect the presence of human chorionic gonadotropin (3) .Clinical blood and urine tests can detect pregnancy soon after implantation, which is as early as 6-8 days after fertilization. Home pregnancy tests are personal urine tests, which normally cannot detect a pregnancy until at least 12-15 days after fertilization. Both clinical and home tests can only detect the state of pregnancy, and cannot detect its age (2) .

5. Food and Nutrition During Pregnancy

Nutritional requirements increase during pregnancy and breastfeeding. The average woman requires 2000 to 2500 kcal/day. The caloric requirement is increased by 300 kcal/day during pregnancy and by 500kcal/day when breast-feeding. Most patients should gain between 20 and 30 pounds during pregnancy.

Obese women are advised to gain less, between 15 and 20 pounds; thin women are advised to gain slightly more, 25 to 35 pounds.In addition to the increased caloric requirements,there are increased nutritional requirements for protein, iron, folate, calcium, and other vitamins and minerals. The protein requirement increases from 60g/day to 70 or 75 g/day. Many patients develop iron deficiency anemia because of the increased demand on hematopoiesis both by the mother and the fetus (9) .

All patients are advised to take prenatal vitamins during pregnancy. These are designed to compensate for the increased nutritional demands of pregnancy. Furthermore, any patient whose hematocrit falls during pregnancy is advised to increase iron intake with oral supplementation (Table 1) (10)

Table (1). Recommended Dietary Allowances for Nonpregnent, Pregnant, and Lactating women (10).

Recommended Daily Dietary Allowances for Nonpregnant, Pregnant, and Lactating Women

	Nonpregnant Women by Age						
	11–14	15–18	19–22	23–50	51+	Pregnant Women	Lactating Women
Energy (kcal)	2400	2100	2100	2000	1800	+300	+500
Protein (g)	44	48	46	46	46	+30	+20
Fat-soluble vitamins							
Vitamin A activity (RE)	800	800	800	800	800	1000	1200
(IU)	4000	4000	4000	4000	4000	5000	6000
Vitamin D (IU)	400	400	400	—	—	400	400
Vitamin E activity (IU)	12	12	12	12	12	15	15
Water-soluble vitamins							
Ascorbic acid (mg)	45	45	45	45	45	60	80
Folacin (µg)	400	400	400	400	400	800	600
Niacin (mg)	16	14	14	13	12	+2	+4
Riboflavin (mg)	1.3	1.4	1.4	1.2	1.1	+0.3	+0.5
Thiamin (mg)	1.2	1.1	1.1	1	1	+0.3	+0.3
Vitamin B_6 (mg)	1.6	2	2	2	2	2.5	2.5
Vitamin B_{12} (µg)	3	3	3	3	3	4	4
Minerals							
Calcium (mg)	1200	1200	800	800	800	1200	1200
Iodine (µg)	115	115	100	100	80	125	150
Iron (mg)	18	18	18	18	10	+18	18
Magnesium (mg)	300	300	300	300	300	450	450
Phosphorus (mg)	1200	1200	800	800	800	1200	1200
Zinc (mg)	15	15	15	15	15	20	25

Source: From Gabbe SG, Niebyl JR, and Simpsen JL, Obstetrics: Normal and Problem Pregnancies. 4th ed. New York: Churchill Livingstone, 2002:196.
Note: IU = International Unit.

5.1 Folate Intake During Pregnancy

The new recommended intake for folate during pregnancy is 600 µg/day of dietary folate equivalents (DFE*) . Folate requirements increase from 0.4 to 0.8 mg/day and are important in preventing neural tube defects (10).

This recommended intake is higher than the amount of folate usually consumed through food. This means that most pregnant women should try to eat a well-balanced diet including foods naturally rich in folate, such as orange juice, strawberries, cantaloupe, dark green leafy vegetables, asparagus, broccoli, and cooked dried peas and beans. Pregnant women are also likely to need greater amounts of foods fortified with folic acid, such as breakfast cereals or enriched bread, rice, or pasta, or they should take a vitamin supplement (11).

5.2 Calcium

The 1998 recommended intake for calcium is 1,000 mg/day (increased from 800 mg/day) for women ages 19 years and older, regardless of whether they are non-pregnant, pregnant, or lactating. To reach this goal, women need three servings of calcium-rich milk products, such as low-fat milk, cheese, or yogurt, or calcium-fortified orange juice. Adolescent females through age 18 years need four servings of calcium-rich foods daily to reach the recommended intake of 1,300 mg/day of calcium. (12) .

Section two

1. Nausea and Vomiting of Pregnancy (NVP)

Nausea: An unpleasant feeling in the throat or epigastric region alerting one that vomiting is imminent (13).

Vomiting: The forceful expulsion of gastric contents through the mouth (14).

Nausea and vomiting of pregnancy is a multifactorial condition with significant adverse effects on quality of life and health of mother and fetus that warrants recognition, investigation, and treatment. (15).Nausea and vomiting in pregnancy is commonly known as morning sickness and like all forms of nausea and vomiting it can be very distressing to the women who suffer from the condition. It is generally treated conservatively (16).

NVP is a common phenomenon in early pregnancy, affecting between 50% and 90% of pregnant women. The symptoms exhibit in the first trimester and peak during the ninth week of gestation (17).

Morning sickness is generally not deleterious to either the fetus or the mother. There is some support for the hypothesis that morning sickness serves to protect the embryo by causing pregnant women to avoid potentially toxic foods (18, 19).

2. Onset, Peak and Cessation of NVP

The mean day of onset of NVP is day 39 from LMP.13% of pregnant women start NVP before day 28, which can be the first symptom of their pregnancy. 90% start NVP before day 56, that is the end of week 8 .The weeks of peak incidence, that is the weeks when the maximum number of women were experiencing these symptoms, were weeks 7-9. The symptoms rise sharply from week 6 and tail off gradually after week 10 (20). NVP is more common in primigravidae, younger women, women with fewer that 12 years education, non-smokers and women weighing more than 170 pounds and multiple gestation (21). The nausea of pregnancy ranges from mild and to severe disturbing and unremitting, with severe vomiting, dehydration, and weight loss (hyperemesis gravidarum) (22).

These noxious symptoms may lead to depression, poor nutrition, absenteeism, and hospitalization. Patient who suffer from NVP have normal upper endoscopy examination and normal computed tomographic scans of the abdomomen. No structural abnormalities are found, and yet nausea symptoms continue (23).Dysfunction of the neuromuscular apparatus of the stomach is associated with nausea and vomiting and functional dyspepsia, which also includes upper abdominal discomfort, early satiety, and bloating. These patients do not have gastric mucosal abnormalities or structural defects (24).They have disorders of neuromuscular function of the stomach, including gastric dysrhythmias, gastric tone abnormalities, and in severe cases gastroparesis (24).

Neuro-muscular abnormalities of the stomach associated with nausea and vomiting have been described in women with nausea and vomiting of pregnancy (25).

3. The Vomiting Reflex

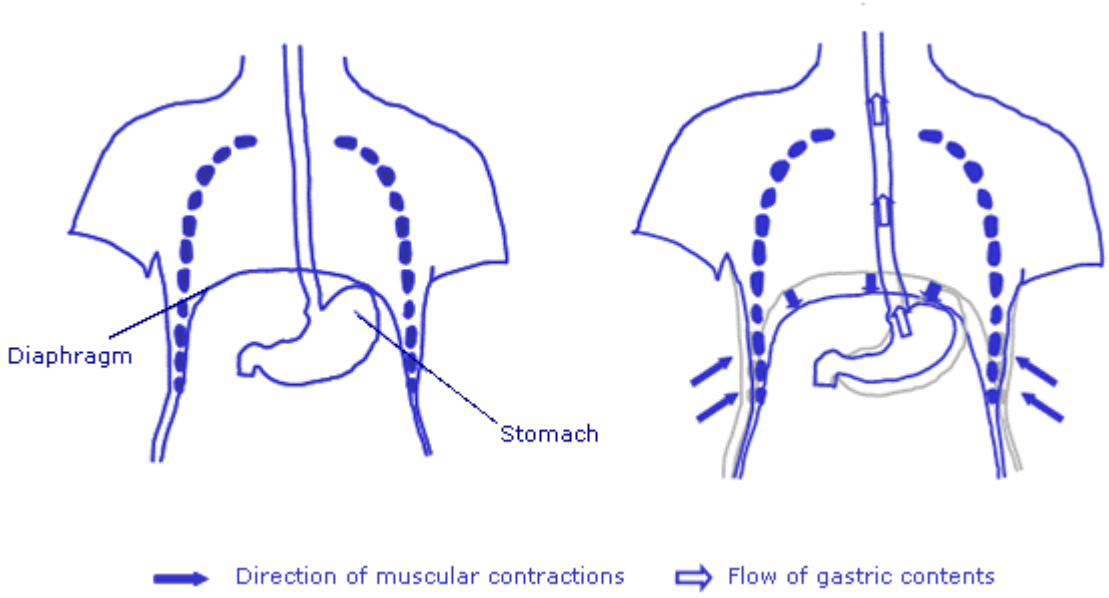
The process of emesis can be classified into three phases, nausea, retching and vomiting.

Nausea is described as an unpleasant sensation that immediately proceeds vomiting. A cold sweat, pallor, salivation, a noticeable disinterest in the surroundings, loss of gastric tone, duodenal contractions and the reflux of intestinal contents into the stomach often accompany nausea (26).

Retching follows nausea, and comprises laboured spasmodic respiratory movements against a closed glottis with contractions of the abdominal muscles, chest wall and diaphragm without any expulsion of gastric contents. Retching can occur without vomiting but normally it generates the pressure gradient that leads to vomiting (26).

Vomiting is caused by the powerful sustained contraction of the abdominal and chest wall musculature, which is accompanied by the descent of the diaphragm and the opening of the gastric cardia.

This is a reflex activity that is not under voluntary control. It results in the rapid and forceful evacuation of stomach contents up to and out of the mouth (25) .(figure 1).



Fig(1). Effect of Muscular Contractions in Stomach on Gastric Contents (27).

4. Gastrointestinal Neuromuscular Abnormalities and Nausea and Vomiting of Pregnancy

Gastric dysrhythmias were first described in women with morning sickness in 1989 by Koch et al (27). An important observation was that some of women had no nausea on the morning of their electrogastrograms EGG study and these women had normal 3-cpm EGG activity. The presence of a dysrhythmia was associated with the report of nausea, whereas the presence of normal 3-cpm EGG pattern was associated with the absence of nausea.

The symptom of free women who were studied after delivery had normal 3-cpm EGG activity, whereas they had a variety of gastric dysrhythmias during the first trimester of pregnancy when nausea was present (28,29).

Findings indicated that gastric dysrhythmias represent a patho physiologic mechanism for the nausea experienced during the first trimester of pregnancy . The acute loss of 3-cpm activity during a wave of nausea, the observation that women in the first trimester of pregnancy without nausea had normal EGG rhythms, and the return to normal 3-cpm activity after delivery supported the notion that the presence of gastric dysrhythmias is an objective finding related to stomach dysfunction and nausea symptoms. These findings were further collaborated by Jednak et

al who also found gastric dysrhythmias were present in women with nausea and vomiting of pregnancy (30).

The mechanisms underlying gastric dysrhythmias are poorly understood. The potential mechanisms range from dysfunction of the interstitial cells of Cajal to enteric neurons to malfunction of the circular or longitudinal smooth muscle layers of the stomach .Abnormalities in vagal or sympathetic neural activity involving the stomach may also be present. These gastric neuromuscular abnormalities may also result from neurohormonal and vascular changes that occur in the first trimester of pregnancy (26,31).

Section three

1. Hyperemesis Gravidarum (HEG)

Hyperemesis gravidarum (from Greek *hyper* and Latin *emesis* and *gravida*; meaning "excessive vomiting of pregnant women") is a severe form of morning sickness, with *unrelenting, excessive pregnancy-related nausea and/or vomiting that prevents adequate intake of food and fluid)*. Hyperemesis is considered a rare complication of pregnancy but, because nausea and vomiting during pregnancy exist on a continuum, there is no clear boundary between common morning sickness and hyperemesis (32).

This condition appears during the first trimester and is unassociated with other medical conditions, such as cholestasis, hepatitis, preeclampsia, viral syndrome, Meniere's disease, or influenza (32,33). In most cases the onset of symptoms is between 4 and 10 weeks gestation, and the symptoms usually subside by 20 weeks gestation (34). Severe cases of HEG can cause significant health problems for mother and for baby. HEG may cause volume depletion, electrolytes and acid-base imbalances, nutritional deficiencies, and even death (34). Severe hyperemesis requiring hospital admission occurs in 0.3-2% of pregnancies (35).

Risks to the fetus include (36).

- premature birth
- low birth weight
- a slight increase in malformation of the central nervous system .

Hyperemesis gravidarum is distinct from ordinary morning sickness with nausea and vomiting. Many pregnant women with morning sickness feel as though they are vomiting everything they ingest, but if they continue to gain weight and are not dehydrated, they do not have hyperemesis gravidarum (37). The following chart illustrates the difference in severity between morning sickness and HEG. Significant (>5% of pre-pregnancy weight) weight loss and recurrent dehydration are classic signs of HEG that most health professionals recognize (38). Table (2)

Table (2). The Comparison Between Morning Sickness & Hyperemesis Gravidarum

Morning Sickness	Hyperemesis Gravidarum
Nausea sometimes accompanied by vomiting	Nausea accompanied by severe vomiting
Nausea that subsides at 12 weeks or soon thereafter	Nausea that continues past the first trimester (13 weeks)
Vomiting that does not cause severe dehydration	Vomiting that causes severe dehydration
able to keep some food down	cannot keep any food down

Sometimes a bad case of morning sickness will progress to HEG due to ineffective or inadequate care. Delaying treatment is far too common to avoid any risks to the mother and child. Hyperemesis gravidarum may develop rapidly within a few weeks or gradually over a few months(39).Individuals with hyperemesis gravidarum experience severe and persistent nausea and vomiting that occur before the 20th week of pregnancy (gestation) and are severe enough to result in progressive weight loss of greater than five percent of their original body weight(40).

Frequent vomiting may also lead to dehydration and vitamin and mineral deficit. Hyperemesis gravidarum often leads to hospitalization to restore lost fluids and nutrients to affected women (41).

2. The Symptoms of Hyperemesis Gravidarum

The following list of symptoms are general and may be caused by other,

1 - Severe and lasting vomiting—not being able to keep liquids or any eating down .
2 - Weight loss of 5 to 10 pound or more during the first trimester.
3 - Dehydration; signs of dehydration may include:
    - Ketones in urine
    - Increased hematocrit
    - Increased pulse rate
    - Decreased blood pressure (42).

4 - Yellow skin

5 - Racing heart (tachycardia)

6 - Dizziness

7 - Feeling faint

8 - abdominal pain ,fever (42).

9 - In sever cases vomit blood or feeling throat is extremely sore (43,44).

10 - ptyalism

11 - Electrolytes imbalance (44).

12 - Malnutrition.

13 - Headaches (43).

3. Etiology and Pathophysiology

The etiology of nausea and vomiting of pregnancy remains unknown, but a number of possible causes have been investigated The etiology of HG is probably multifactorial, the prevalent theories are (45):

1- Psychological Factors

Psychological factors are responsible for nausea and vomiting of pregnancy and hyperemesis gravidarum, few data support this theory (46). In one well-known study, the Cornell Medical Index was administered to 44 pregnant women with hyperemesis and 49 pregnant women without hyperemesis; the Minnesota Multiphasic Personality Inventory (MMPI) was administered only to the pregnant women with hyperemesis. The MMPI data suggested that women with hyperemesis have hysteria,

excessive dependence on their mothers, and infantile personalities depression ,anxiety, anticipatory fear, and eating disorder have been observed among patient with HEG (47).

Theories of psychogenesis have been rooted primarily in psychoanalytic theory. psychoanalytic theory supports that a pregnant women's vomiting may represent various intrapsychic conflicts .The pregnant women vomiting has been associated with neurotic tendencies, hysteria and as a symbolic rejection an unconscious, oral attempt at abortion .An ambivalent attitude (versus a marked rejection) representing conflict between wanting and rejection the baby (48).

2- <u>Hyperolfaction</u>

Hyperacuity of the olfactory system induced by rapidly increasing estrogen concentrations during early pregnancy may be an important contributing factor. Pregnant women have long told their physicians that the smell of cooking foods, particularly meats, and especially bacon, are triggers to their nausea. They often state that the smell of coffee, perfumes, cigarette smoke, petroleum products anything volatile triggers their nausea (49,50).

3- Gastrointestinal Tract Dysfunction

This is also has been suggested as a cause of nausea and vomiting of pregnancy. In one study in which progesterone was prescribed to non pregnant women, resultant nausea and vomiting suggested that delayed gastric motility caused by progesterone may be responsible for the condition (51). Another study reviewed many potential gastrointestinal causes of nausea and vomiting of pregnancy, including abnormalities of gastric electrical rhythm (gastric dysrhythmias) (24).

4- Gestational Hormones

Many reports have suggested that some hormones may cause nausea and vomiting of pregnancy and hyperemesis gravidarum .In one comparative study, women with nausea and vomiting of pregnancy were found to have elevated levels of human chorionic gonadotropin (HCG)(52). Another study did not support this finding. Some studies have shown elevated estrogen levels in women with this condition; others have not . Many pregnant women with hyperemesis have suppressed thyrotropin-stimulating hormone (TSH) levels. Work is ongoing to elucidate the interaction of HCG and TSH in pregnant women with hyperemesis (53).

5- Vitamin B6 (Pyridoxine)

NVP has been associated with a deficiency of vitamin B6 and vitamin B complex (pyridoxine is a component of Diclectin) (54). These observations have led directly to changes in therapy, possibly through optimization of maternal nutrition. It is not known whether deficiency of vitamin B6 has a causative effect for NVP, or whether raised levels of the vitamin in pregnant women without NVP are pharmacological in alleviating symptoms (55) .

6- Immunological Factors

In the 1960s, proposed that NVP might be an allergic phenomenon, i.e., an immunological phenomenon. There has been little investigation of this possible process and no direct associations have been made. Pregnancy is associate changes, such as depressed cell-mediated immunity and primed non-specific immunity (56).

7- Bacterial Infection

A recent studies suggested that chronic infection with Helicobacter pylori may play a role in hyperemesis gravidarum (57,58).Recent reports from Europe and the Middle East suggest Helicobacter pylori (H pylori) as a causative agent for hyperemesis. The exact pathophysiologic mechanism from which H pylori is believed to cause hyperemesis is unknown (59).

Kocak hypothesized that pH changes caused by increased steroid hormone levels provide an environment from which a latent H pylori infection could be evoked (60). Other investigators fail conclusively to correlate H pylori with gastric dysmotility. Case reports from both Europe and the US demonstrated the alleviation of hyperemesis in mothers were serologically H pylori–positive and were treated with a pharmacologic regimen aimed at the eradication of the bacteria (61).

4. Differential Diagnosis and Evaluation

A thorough history and a complete physical examination are important in the evaluation of pregnant women who present with persistent vomiting. Nausea and vomiting in early pregnancy is usually a self-limited condition.

- **The history includes :**

  History of progression of this pregnancy and the present illness

  Presence of multiple fetuses

  Complications

  Diet history, with special attention to current fluid intake

  Current medications

  Past gynecological and obstetric history

  Past surgical history, particularly abdominal surgeries.

- **Social History:**

  Exposure to communicable diseases

  Environmental exposures

  Depression

physical exam

weight measurement- to determine the patient had lost weight

- **Blood electrolytes**- this test identifies disturbances in salts and other minerals in the blood due to extreme vomiting (36).
- **Blood pressure**- high blood pressure can indicate a condition called preeclampsia that can also cause nausea and vomiting.
- **Urine dip**: looks for ketones. When there is not enough sugar in the blood because of vomiting, the body produces ketones (15).

5. Risk Factors

The following factors increase pregnant chances of developing hyperemesis gravidarum.

1- Mother or sister with hyperemesis gravidarum
2- Primigravida (62).
3- Hyperemesis gravidarum in prior pregnancies (63).
4- Being pregnant with more than one fetus (multiple pregnancy) (63).
5- Emotional problems (43).
6- History of an eating disorder (64).

7- Infection with the bacterium *Helicobacter pylori* (58).
8- Carrying a fetus with Down Syndrome
9- History of motion sickness
10- History of migraines
11- Carrying a fetus that is female (65).
12- Gestational trophoblastic disease
13- Increased body weight (42).
14- Nulliparity (45).

6. Treatment of Hyperemesis Gravidarum

The management of nausea and vomiting of pregnancy depends on the severity of the symptoms. Treatment measures range from dietary changes to more aggressive approaches involving antiemetic medications, hospitalization, or even total parenteral nutrition (TPN). Firstly prefer to start with dietary changes and then add medications as necessary (66,67).

The treatment of HEG divided into three groups :

6.1 Non pharmacologic Treatment By :

6.1.1 Dietary Measures :

Initial treatment of women with mild nausea and vomiting of pregnancy (i.e., morning sickness) should include

1. dietary changes.

2. Affected pregnant women should be instructed to eat frequent, small meals and to avoid smells and food textures that cause nausea.

3. Solid foods should be bland tasting, high in carbohydrates, and low in fat.

4. Salty foods (e.g., salted crackers, potato chips) usually can be tolerated early in the morning, and sour and tart liquids (e.g., lemonade) often are tolerated better than water (68).

6.1.2 Emotional Support :

Although nausea and vomiting of pregnancy and hyperemesis gravidarum are not strongly associated with psychologic illness, some women may become depressed or exhibit other affective changes. It is important that these women receive appropriate support from family members and medical and nursing staff. Consultation is indicated if a pregnant woman is depressed, domestic violence is suspected, or evidence of substance abuse or psychiatric illness exists (67).

6.1.3 Acupressure and Acupuncture

Several studies have suggested acupressure as a treatment for nausea (69,70). The most common location for acupressure is the pericardium 6 or Neiguan point, which is located three fingerbreadths above the wrist on the volar surface. Various commercial products for relieving motion sickness (e.g., Sea-Band, Relief Band) apply pressure to this area (71).One review of data from seven trials involving Neiguan point acupressure indicated that these products are helpful for controlling morning sickness in early pregnancy; a recent study demonstrated no benefit for acupressure in pregnant women. Further data are necessary to determine whether acupressure is a viable treatment for nausea and vomiting of pregnancy (72).

6.1.4 Ginger:

A popular alternative treatment for morning sickness, ginger has been used in teas, preserves, ginger ale, and capsule form. One European study demonstrated that ginger powder (1 g per day) was more effective than placebo in reducing the symptoms of hyperemesis gravid arum (73).There have been no published reports of fetal anomalies associated with the use of ginger.

One investigator warned that ginger root contains thromboxane synthetase inhibitor, which may interfere with testosterone receptor binding in the fetus .Other investigators noted that although safety data are lacking, people in many cultures use ginger as a spice; the amounts

used are similar to those commonly prescribed for the treatment of nausea and vomiting of pregnancy (74).

6.2 Pharmacologic Treatment

Pharmacologic treatments for nausea and vomiting of pregnancy and hyperemesis gravidarum are summarized in *table3* (75, 76).

6.3 Other Treatments

6.3.1 Intravenous Fluids

Pregnant women who, despite the previously discussed treatments, are unable to keep down liquids will probably require intravenous fluids. Normal saline or lactated Ringer's solution is the mainstay of intravenous fluid therapy. Many physicians use solutions that contain dextrose;, it may be advisable to give thiamine (vitamin B1) first, because of the theoretic risk of Wernicke's encephalopathy. Intravenous fluid may provide relief from nausea and vomiting, but many pregnant women also require an antiemetic administered orally, rectally, or by infusion with the fluid. Depending on the severity of the symptoms, intravenous fluid therapy may be given in the hospital or at home by a visiting nurse (64) .

6.3.2 Enteral or Parenteral Nutrition

Enteral tube feeding and TPN are last-resort treatments for pregnant women who continue to vomit and lose weight despite aggressive treatment with any or all of the previously discussed modalities. TPN is administered through a central venous catheter. Its content is determined by the pregnant woman's daily caloric requirements and any existing electrolyte abnormalities (67).

Table (3) Pharmacologic treatment for Nausea and Vomiting of Pregnancy

Medication	*Dosage**	*Pregnancy category*
Pyridoxine (Vitamin B_6)†	25 mg orally three times daily	A‡
Doxylamine (Unisom)†	25 mg orally once daily	§
Antiemetics		
Chlorpromazine (Thorazine)	10 to 25 mg orally two to four times daily	C
Prochlorperazine (Compazine)	5 to 10 mg orally three or four times daily	C
Promethazine (Phenergan)	12.5 to 25 mg orally every four to six hours	C
Trimethobenzamide (Tigan)	250 mg orally three or four times daily	C
Ondansetron (Zofran)	8 mg orally two or three times daily	B
Droperidol (Inapsine)	0.5 to 2 mg IV or IM every three or four hours	C
Antihistamines and anticholinergics		
Diphenhydramine (Benadryl)	25 to 50 mg orally every four to eight hours	B
Meclizine (Antivert)	25 mg orally every four to six hours	B
Dimenhydrinate (Dramamine)	50 to 100 mg orally every four to six hours	B
Motility drug		
Metoclopramide (Reglan)	5 to 10 mg orally three times daily	B
Corticosteroid		
Methylprednisolone (Medrol)	16 mg orally three times daily; then taper	C

IV = intravenously; IM = intramuscularly.

A = can be use , B = use with caution , C = better but not be use

Section four

1. Human Chorionic Gonadotrophin (HCG)

Human chorionic gonadotophin (HCG) is aglycoprotein hormone that is produced by the placenta of a pregnant woman. It is detectable in the blood and urine within 10 days of fertilization and hence forms the basis of all pregnancy tests (1). Because human chorionic gonadotropin (HCG) is produced by the placenta, the presence of HCG in a woman's blood indicates that she is most probably pregnant. A pregnancy blood test or a pregnancy serum test measures the exact amount of the pregnancy hormone, human chorionic gonadotropin (HCG), in the bloodstream (77,78).

The first assay for HCG described in 1927 was based on the biological activity of HCG partially purified from urine .Various modifications and improvements of this assay were used to diagnose pregnancy until immunoassays gradually replaced bioassays (79,80).

Thanks to the high concentrations of HCG in urine during pregnancy, urine has been used for the preparation of HCG for clinical use, and partially purified urinary HCG plays an important role in assisted reproduction treatment (ART). This use prompted establishment of standards for HCG, which were assigned values based on bioactivity (81). All the international standards (ISs) for HCG have been calibrated by bioassay (82) , these standards have also been used for standardization of immunoassays (83).

The International Federation of Clinical Chemistry (IFCC) established a working group in 1995 with the aim of improving standardization of HCG determinations; the task included establishment of a uniform nomenclature and preparation of new standards. The clinically important variants were HCG and its subunits HCGα and HCGβ, the partially degraded or nicked forms of HCG (HCGn) and HCGβ (HCGβn), and the beta-core fragment (HCGβcf) (83).

2. Biochemistry of HCG

HCG is a member of the glycoprotein hormone (GPH) family, which also comprises luteinizing hormone LH, follicle-stimulating hormone FSH and thyroid-stimulating hormone TSH. All GPHs are heterodimers consisting of an α-subunit (GPHα) and β-subunit (84).

HCG composed of 244 amino acids with a molecular mass of 36.7 kDa. Its total dimensions are 75x 35x 30 angstroms .It is heterodimeric, with an α (alpha) subunit identical to that of (LH) , (FSH), and (TSH) and β (beta) subunit that is unique to HCG.See (figure.2) (85).

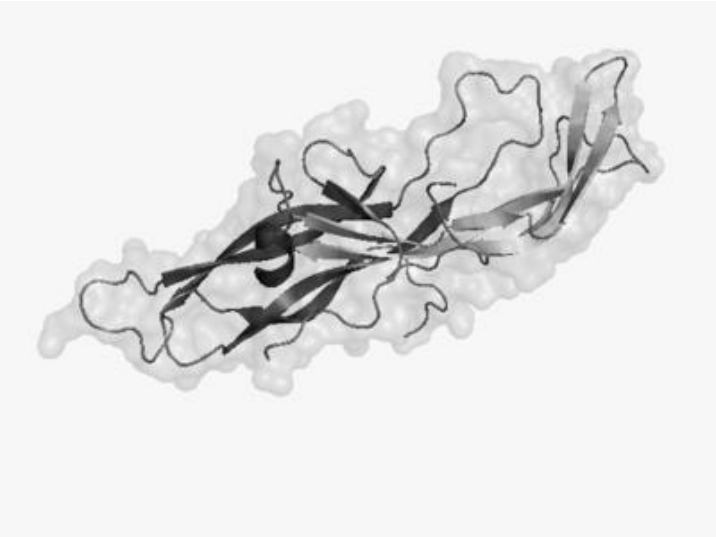


Fig (2). Structure of human chorionic gonadotrophin Hormone

- The α (alpha) subunit is 92 amino acids long and has dimensions 60x25x15 angstroms .
- βHCG is encoded by six highly homologous genes

The two subunits create a small hydrophobic core surrounded by a high surface area to volume ratio 2.8 times that of a sphere. The vast majority of the outer amino acids are hydrophilic. LHβ contains 121 amino acids whereas HCGβ contains 145 amino acids, the difference being due to a 24-amino-acid extension, the so-called C-terminal peptide (CTP) (86).

HCGβ is more acidic (pI range 3–5) than HCGα (pI range 5–8) (87). In pregnancy, the N-linked carbohydrates on HCGα are mainly monoantennary and biantennary and those on HCGβ are biantennary and to a lesser extent triantennary.

The carbohydrates on the CTP are mainly monoantennary type 1 *o*-core oligosaccharides. In HCG produced by cancerous tissues, most of the N-linked carbohydrates are complex containing more triantennary moieties on HCGβ (88), and biantennary on HCGα, whereas type 2 *o*-core oligosaccharides occur on CTP .This so-called hyperglycosylated HCG. (HCG) is also a major form in early pregnancy .It is produced by cytotrophoblasts, which dominate in the early placenta, whereas syncytiotrophoblasts, which are the main trophoblasts later in pregnancy, produce 'normally glycosylated' HCG (89,90).

In addition to these differences, HCG produced by trophoblastic disease occasionally displays reduced content of sialic acid, but variants with unusually low pI values indicating increased content of sialic acid have also been described.. Thus various forms of aberrant glycosylation are common in tumor-derived HCG (91,92) .The molecular weight (MW) of HCG produced by trophoblastic disease is higher than that of pregnancy HCG which is explained by larger carbohydrate chains (90).

3. Function

HCG mediates its action through the LH/HCG receptor, and its major function is to maintain the progesterone production of corpus luteum during early pregnancy .Progesterone enriches the uterus with a thick lining of blood vessels and capillaries so that it can sustain the growing fetus (93).

Due to its highly negative charge HCG may repel the immune cells of the mother, protecting the fetus during the first trimester (93).Because of its similarity to LH, HCG can also be used clinically to induce ovulation in the ovaries as well as testosterone production in the testes (94) .

As the most abundant biological source is women who are presently pregnant, some organizations collect urine from gravidae to extract HCG for use in fertility treatment(88).HCG is produced by cytotrophoblast during early pregnancy ,because these cells display invasive properties , HCGh has also been called invasive trophoblast antigen (ITA) (95,96). HCGα (GPHα) also does not exert HCG activity, but it has been shown to stimulate prolactin production in decidual cells (97,98).

The clearance of HCG from circulation has been studied both after injection of purified HCG and after pregnancy. The half-life of injected HCG is biphasic :

- The rapid phase has a half-life of 5–6 h
- The slower phase is 24–33 h (99,100).

The half-life of HCGα is shorter than that of HCGβ, and after term pregnancy half-lives of 0.6, 6 and 22 h have been observed (101). These half-lives are longer than those observed after injection of purified HCGα, i.e. 0.1–0.22 and 1.2–1.3 h (102).

The discrepancy between half-lives determined for injected and naturally occurring subunits may indicate that the purified forms have been partially denatured during purification, and they are therefore metabolized more rapidly. It is also possible that the slower metabolism of endogenous free subunits is explained by differences in glycosylation (91).

Most of the HCG in circulation is metabolized by the liver, whereas about 20% is excreted by the kidneys(103). During excretion, a major part of HCG is degraded to subunits, nicked forms and especially HCGβcf (104). The proportion of HCGβcf in urine is low in early pregnancy and starts to exceed that of HCG at <5 weeks of pregnancy. In the second trimester, about 80% of the HCG immunoreactivity in urine consists of HCG β(105).

HCG can also be detected in the pituitary (105), and in follicular fluid and trophoblast culture fluid (106), and some HCGβ is present in the placenta (107,108), some of the HCGβ in urine can be derived from metabolism in these tissues, but studies on the metabolic clearance rate of HCGβ show that >99% is formed in the kidneys during renal excretion (109).

4. Pregnancy Tests:

The pregnancy test is considered one of the most useful and reliable laboratory tests available. Pregnancy tests measure the levels of HCG in the blood or urine to indicate the presence or absence of an implanted embryo.The most common use of HCG determinations is the detection of pregnancy with a semi-quantitative pregnancy test, which mostly is performed on a urine sample, but serum, plasma or whole blood can also be used with some tests (109,110).

- The urine test may be a *chromatographic immunoassay* or any of several other test formats, home-, physician's office-, or laboratory-based. Published detection thresholds range from 20 to 100 mIU/ml (milli International Units per milli-liter), depending on the brand of test. The urine should be the first urine of the morning when HCG levels are highest. If the specific gravity of the urine is above 1.015, the urine should be diluted.
- The serum test, using 2-4 mL of venous blood, is typically a chemiluminescent or fluorimetric immunoassay that can detect βHCG levels as low as 5 mIU/ml and allows quantitation of the βHCG concentration. The ability to quantitate the βHCG level is useful in the evaluation of ectopic pregnancy and in monitoring germ cell and trophoblastic tumors (110).

The first immunological pregnancy test based on haemagglutination inhibition had a detection limit of 500 IU/l and took 1.5 h to perform (111). The agglutination assays were gradually replaced by more sensitive and rapid enzyme immunoassays, which facilitated detection of HCG at concentrations down to 25 IU/l in 5–10 min (112).

Presently, most pregnancy tests are based on immunochromatography and have a claimed sensitivity of 25–50 IU/l. Some are actually more sensitive, detecting HCG at concentrations <10 IU/l while the detection limit of other pregnancy tests is in the range 100–200 IU/l (113).

The optimal sensitivity of a pregnancy test is debated; the more sensitive test is, the earlier it detects a pregnancy, but because HCG may occur in serum and urine at concentrations up to 10–15 IU/l in non-pregnant women, a detection limit around 25 IU/l is considered optimal (113).

Hydatiform moles ("molar pregnancy") may produce high levels of βHCG, despite the absence of an embryo. This, as well as several other conditions, can lead to false positive readings of pregnancy tests (114).

5. Reference Values of HCG During Pregnancy

The serum concentrations of HCG start increasing 7–10 days after the LH peak or 4–7 days after implantation (115, 116) .During early pregnancy, the HCG concentrations increase exponentially doubling on average every 1.5–2 days, but the rate of increase varies individually and maximum concentrations ranging from 20 000 to 100 000 IU/l are reached at 7–10 weeks of pregnancy. After this, the levels decrease leveling out at 13–15 weeks and increase moderately again until weeks 30–33, after which there is a moderate decrease towards term (117).

In early pregnancy, it may reach 4% but it drops rapidly to <1% . Accurate reference ranges for HCG and HCGβ during pregnancy are especially important for the diagnosis of Down's syndrome, and owing to the rapid changes during pregnancy, the values are expressed as multiples of the median for each week (or even day) of pregnancy .

The HCG immunoreactivity in pregnancy urine is more heterogeneous than that in serum, and therefore the results are strongly dependent on the specificity of the assay used.

When measured by specific assays, the HCG concentrations in urine correlate strongly with those in serum. In early pregnancy, the average urine concentrations are 50–70% of those in serum, but after the fifth week, the urine-to-serum ratio of HCG decreases and HCGβ becomes the dominant form in urine. In most studies, HCGβ has been found to be a minor component in urine (117,118).

Section five

1. Thyroid-Stimulating Hormone (TSH)

Thyroid-stimulating hormone (also known as **TSH** or **thyrotropin**) is a hormone synthesized and secreted by thyrotrope cells in the anterior pituitary gland which regulates the endocrine function of the thyroid gland .

Thyroid-stimulating hormone (TSH; thyrotropin) and TSH receptor (TSHR) are key proteins in the control of thyroid function (119).

Physiological roles of TSH include stimulation of differentiated thyroid functions, such as iodine uptake and organification, production and release of iodothyronines from the gland, and promotion of thyroid growth. Thyroid function is known to be altered during normal pregnancy through several mechanisms. An increase in iodine clearance leads to hyper stimulation of the gland to compensate for a relative iodine deficiency. An increase in the thyroxine-binding globulin (TBG), induced by estrogens, leads to a temporary and moderate decrease in free thyroxine concentration, which, by negative feedback on the pituitary, provokes an increase in thyroid stimulating hormone (TSH) secretion and hyper stimulation of the thyroid (120,121).

2. Historical Background of Thyroid-Stimulating Hormone and Thyroid-Stimulating Hormone Receptor Research

The history of TSH began with the discovery of thyroid stimulating activity in the pituitary gland . In 1926 Eduard Uhlenhuth from the University of Maryland Medical School was the first to demonstrate that the anterior lobe of the pituitary gland secreted a thyroid stimulator . Using several species of salamanders (amphibians) he showed that injections of bovine pituitary extracts caused a clear histological stimulation of the thyroid gland (122).

In 1929, Leo Loeb and Max Aaron working independently confirmed Uhlenhuth's results using guinea pigs (mammals). In the early of 1970s the primary structure of the TSH subunits was determined .These initial findings were followed in the 1960s by the purification and in the early 1970s by the determination of the primary structure of the TSH subunits (122).

In the 1980s, the cloning of the human subunit (123), and TSH subunit genes were important milestones in studying TSH expression, regulation, and action (124,125) .Thirty-five years ago it was found that TSH exerts its biological effects by binding to a protein on the thyroid cell plasma membrane (126).

3. Thyroid-Stimulating Hormone Chemistry and Molecular Biology

TSH is a 28- to 30-kDa glycoprotein synthesized and secreted from thyrotrophs (basophile cells) of the anterior pituitary gland. It is a member of the glycoprotein hormone family that includes follicle-stimulating hormone (FSH), LH, and HCG. Glycoprotein hormones are among the largest and most complicated endocrine legands known to date. They are heterodimeric cystineknot glycoproteins consisting of a common α -subunit and a unique β-subunit, which confers biological specificity to each hormone (127). TSH is a glycoprotein and consists of two subunits, the alpha and the beta subunit.

The α (alpha) subunit is identical to that of human chorionic gonadotropin (HCG), luteinising hormone (LH), follicle-stimulating hormone (FSH). α-subunit (92 amino acids; common for other human glycoprotein hormones) and the β (beta) subunit is unique to TSH (86).

At high concentrations, HCG can stimulate the TSH receptor to induce hyperthyroidism. Thyroid function tests demonstrate suppressed TSH and elevated free T4 identical to the findings in Graves disease, but thyroid-stimulating immunoglobulin or TSH receptor antibody levels are negative (128,129).

3.1 Protein Structure

Human TSH consists of two noncovalently linked subunits, α-subunit (92 amino acids; common for other human glycoprotein hormones) and TSH β-subunit. The primary structures of TSH subunits are species specific .

The crystal structure of homologous TSH has revealed that each subunit contains amcentral cystine-knot and three loops, two β-hairpin loops (L1 and L3) (130,131). (figure 3)

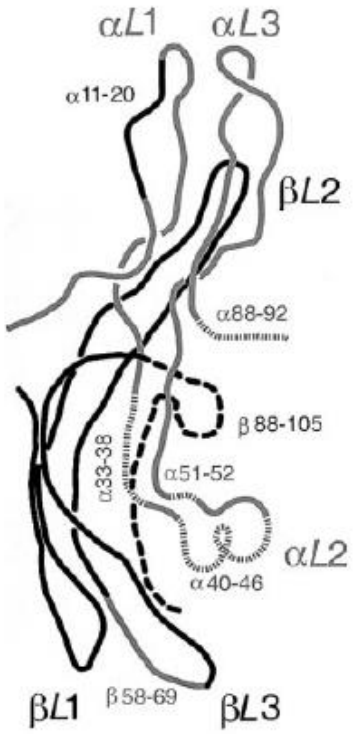


Fig (3). Protien Stracture of Thyriod Stimulation Hormone (131)

hTSH, for example, differs from bovine TSH by 28 amino acids in the α -subunit and by 12 amino acids in TSH_β-subunit(132) .

B -subunit of TSH isolated from cadaver pituitary is composed of 112 amino acids ,most likely due to proteolytic cleavage during purification. A 113- to 118-amino acid deletion does not affect bioactivity of recombinant hTSH, indicating that the COOH-terminal amino acid residues are not important in hormone function (133,134).

3.2 Carbohydrate Chains

TSH, similar to other glycoprotein hormones, is glycosylated protein(135) . The carbohydrate chains constitute 15–25% of its weight and include three asparagine (Asn;N)-linked carbohydrate chains. The human α-subunit has two carbohydrate chains linked to Asn-52 and Asn-78, respectively, and the human TSH β-subunit has one carbohydrate chain attached at the Asn-23 (136). Such N-linked oligosaccharides are complex-type structures displaying notable hormone- and species-dependent differences in their terminal residues specific functions of N-linked oligosaccharides in human thyrotropin: role of terminal residues of alpha- and beta-subunit oligosaccharides in metabolic clearance and bioactivity (137). (figure 4).

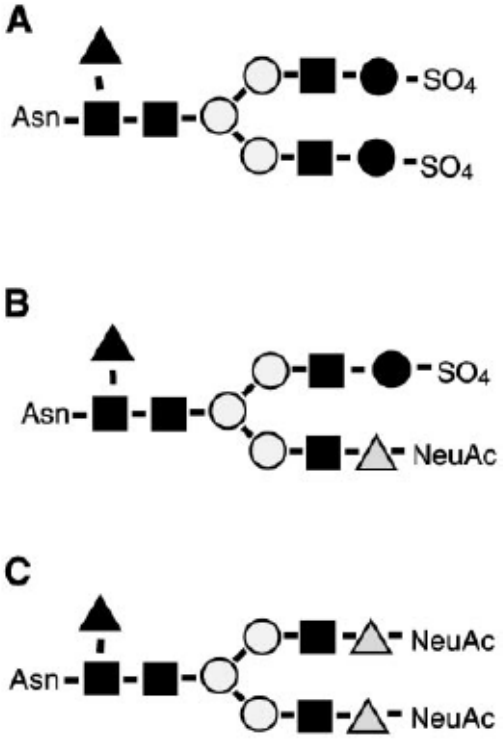


FIG. 4. *N*-linked oligosaccharides of TSH. The sulfated biantennary structure (*A*) represents that of bovine TSH and bovine luteinizing hormone (LH). The sulfated and sialylated oligosaccharide (*B*) is more typical of pituitary-derived hTSH as well as human LH. The sialylated nonsulfated structure (*C*) represents that of recombinant hTSH expressed in Chinese hamster ovary cells, pituitary human follicle-stimulating hormone, as well as placental human chorionic gonadotropin. Carbohydrate residues are marked as follows: mannose (○), *N*-acetylglucosamine (■), *N*-acetylgalactosamine (●), fucose (▲), galactose (△), and sialic acid (NeuAc). (137).

4. Physiology

TSH stimulates the thyroid gland to secrete the hormones thyroxine (T4) and triiodothyronine (T3). TSH production is controlled by aThyrotropin Releasing Hormone, (TRH), which is manufactured in the hypothalamus and transported to the anterior pituitary gland, where it increases TSH production and release (138) .Somatostatin is also produced by the hypothalamus, and has an opposite effect on the pituitary production of TSH, decreasing or inhibiting its release.

The level of thyroid hormones (triiodothyronine T3 and thyroxine T4) in the blood have an additional effect on the pituitary release of TSH; When the levels of T3 and T4 are low, the production of TSH is increased, and conversely when levels of T3 and T4 are high, then TSH production is decreased. This effect creates a regulatory negative feedback loop. Some study show that the Thyroid stimulating hormone decreased significantly during the first trimester, and the decrease was greater in twins (both $P <$ .001)(139) .A thyroid-stimulating hormone (TSH) blood test is used to check for thyroid gland problems (140).See (table.4).

Table (4). Interpretation of thyroid function results (140).

Interpretation matrix for thyroid function results*

	High T_4	Normal T_4	Low T_4
High TSH	In vivo or in vitro artefact Pituitary hyperthyroidism [TSHoma] Thyroid hormone resistance	Mild thyroid failure (primary) (also termed subclinical hypothyroidism and diminished thyroid reserve)	Primary hypothyroidism
Normal TSH	As above Sampling within 6 h of thyroxine dose	Normal (in patients taking thyroxine, TSH > 3 mU/L may indicate subtle underreplacement)	Pituitary or hypothalamic hypothyroidism Severe non-thyroidal illness
Low TSH	Hyperthyroidism (for this diagnosis, TSH must be suppressed rather than just low)	Subclinical hyperthyroidism Subtle thyroxine overreplacement Thyroid autonomy (multinodular goitre or autonomous functioning thyroid nodule) Non-thyroidal illness	Pituitary or hypothalamic hypothyroidism Severe non-thyroidal illness

The normal ranges for the results of this test may vary from laboratory to laboratory. Results are usually available within 2 to 3 days (120).

Thyroid-stimulating hormone (TSH)	
Adults:	0.4–4.5mIU/L or 0.4–4.5 mU/L (SI units)
Babies:	3–18 mIU/L or 3–18 mU/L (SI units)

High TSH leveles may caused by:

- An underactive thyroid (hypothyrodisium) .Hashimotos thyroid is the most common cause of primary hypothyroidism .
- Apituitary gland tumur that's making too much TSH .
- Not taking enough thyroid hormone medicine for treatment of an underactive thyroid gland (141,139).

Low TSH level may be caused by :

- An overactive thyroid gland (hyperthyroidism) .causes of hyperthyroidism include Graves disease,atype of goiter (toxic multinodular goiter),or anoncancerous (bengin) tumer called atpxic nodule.
- Damage to the gland that prevent it from making TSH (secondary hypothyroidism).
- Taking too much thyriod medicine for treatment of an underactive thyriod gland (138,141).

Section six

1. Electrolytes

Body fluid contain water and electrotytes. Electrolyte are substances that dissociate in solution to form charge particles ,or ions . for example sodium chloride (NaCl) molecule dissociate to form a positively charged Na and a negatively charged Cl^- ions. Particles that do not dissociate into ions such as glucose and urea called non electrolytes (142,143). Positively charged ions are called cations because they are attracted to the cathode of a wet electric cell , and negatively charged ions are called anions because they are attracted to the anode . The ions found in body fluids carry one charge (i.e.monovalent ion) or two charges (i.e.divalent ion) and more than two charge is called polyvalent (144). The distribution of electrolyte between body compartment is influenced by their electrolytes electrical charge (145).

Body fluids are distributed between the intracellular fluid (ICF) and extracellular fluid (ECF) compartments (146). The ICF compartment consists of fluid contained within all of the billions of cells in the body. It is the larger of the two compartments, with approximately two thirds of the body water in healthy adults. The remaining one third of body water is in the ECF compartment, which contains all the fluids outside the cells, including those in the interstitial or tissue spaces and blood vessels (144). The ECF, including the plasma and interstitial fluids, contains large amounts of sodium and

chloride, moderate amounts of bicarbonate, but only small quantities of potassium, magnesium, calcium, and phosphate. In contrast to the ECF,the ICF contains almost no calcium; small amounts of sodium, chloride, bicarbonate, and phosphate; moderate amounts of magnesium; and large amounts of potassium (147). (Table 5) .

Table (5). Concentrations Of Extracellular and Intacellular Electrolytes in Adults(148).

Concentrations of Extracellular and Intracellular Electrolytes in Adults		
Electrolyte	**Extracellular Concentration***	**Intracellular Concentration***
Sodium	135–145 mEq/L	10–14 mEq/L
Potassium	3.5–5.0 mEq/L	140–150 mEq/L
Chloride	98–106 mEq/L	3–4 mEq/L
Bicarbonate	24–31 mEq/L	7–10 mEq/L
Calcium	8.5–10.5 mg/dL	<1 mEq/L
Phosphate/ phosphorus	2.5–4.5 mg/dL	4 mEq/kg†
Magnesium	1.8–3.0 mg/dL	40 mEq/kg†

*Values may vary among laboratories, depending on the method of analysis used.
†Values vary among various tissues and with nutritional status.

The cell membrane serves as the primary barrier to the movement of substances between the ECF and ICF compartments. Many ions, such as sodium (Na^+) and potassium (K^+) rely on transport mechanisms such as the

Na^+/K^+ pump that is located in the cell membrane for movement across the membrane .Because the Na^+/K^+ pump relies on adenosine triphosphate (ATP) and the enzyme ATPase for energy, it is often referred to as the Na^+/K^+-ATPase membrane pump (148).

2. Electrolyte Disturbance

In physiology, an electrolyte disturbance is an abnormal change in the levels of electrolytes in the body, usually constituting a medical emergency. Severe or prolonged electrolyte disturbance can lead to cardiac problems, neuronal malfunction, organ failure, and ultimately death, such as with water intoxication (149).

Electrolytes play a vital role in maintaining homeostasis within the body. They help to regulate myocardial and neurological function, fluid balance, oxygen delivery, acid-base balance and much more. Electrolyte imbalances can develop by the following mechanisms: excessive ingestion or diminished elimination of an electrolyte or diminished ingestion or excessive elimination of an electrolyte (150).

3. Regulation of Sodium Balance

Sodium is the most abundant cation in the body, averaging approximately 60 mEq/kg of body weight . Most of the body's sodium is in the ECF compartment (135 to 145 mEq/L), with only a small amount (10 to

14 mEq/L) located in the ICF compartment. The resting cell membrane is relatively impermeable to sodium. Sodium that enters the cell is transported out of the cell against an electrochemical gradient by the energy-dependent Na^+/K^+- ATPase membrane pump. Sodium functions mainly in regulating extracellular and vascular volume. As the major cation in the ECF compartment, Na^+ and its attendant anions (Cl^- and $HCO3^-$) account for approximately 90% to 95% of the osmotic activity in the ECF. Because sodium is part of the sodium bicarbonate molecule, it is important in regulating acid-base balance (151).

4. Hyponatremia

Hyponatremia represents a decrease in plasma sodium concentration below 135 mEq/L (135 mmol/L) (152) . The main symptoms and signs include: Headache, nausea, vomiting, hallucinations, lethargy, weakness, hypoventilation, extra-pyramidal reactions, unreactive pupils, anisocoria, muscle twitching, hyperactive tendon reflexes, seizures and coma .

Unlike hypernatremia, which is always associated with hypertonicity, hyponatremia may be associated with high, normal, or low tonicity because of the effects of other osmotically activeparticles in the ECF such as glucose (153) .Hypertonic (translocational) hyponatremia results from an osmotic shift of water from ICF to the ECF as occurs with hyperglycemia. In this case, the sodium in the ECF becomes diluted as water moves out of cells in response to the osmotic effects of the elevated blood glucose level.There is

approximately a 1.7mEq/L decrease in plasma sodium for every 100 mg/dL rise in plasma glucose above the normal level (100 mg/dL)).(154) (figure 5)

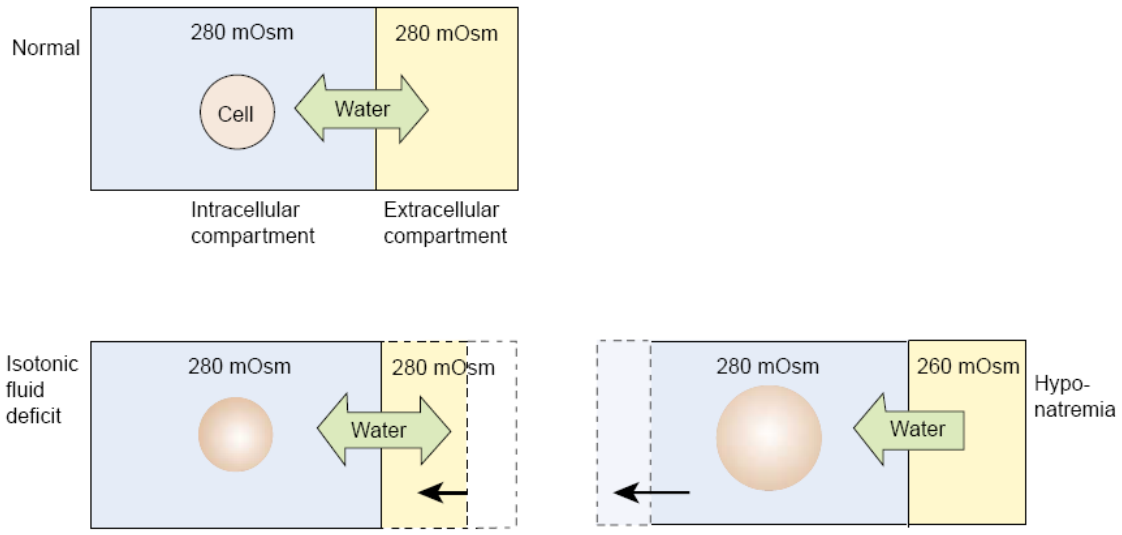


Fig (5). Clarifying of Causing hyponatremia (155)

4.1. Causes of Hyponatremia

Hypotonic, or dilutional, hyponatremia represents a decreased sodium concentration and tonicity of the ECF. The most common causes of acute dilutional hyponatremia in adults are drug therapy (diuretics and drugs that increase ADH levels), inappropriate fluid replacement during heat exposure or following heavy exercise, , and polydipsia in persons with psychotic disorder. The loss of salt and water from excessive sweating in hot among the causes of hypovolemic hyponatremia is weather, particularly during heavy exercise; hyponatremia develops when water

rather than electrolyte-containing lquids is used to replace the fluids lost in sweating (152).

4.2. Diagnosis and Treatment of Hyponatremia

Diagnosis of hyponatremia is based on laboratory reports of decreased sodium concentration, the presence of conditions that predispose to sodium loss or water retention, and signs and symptoms indicative of the disorder (153).

The treatment of hyponatremia with water excess focuses on the underlying cause. The administration of a saline solution orally or intravenously may be needed when hyponatremia is caused by sodium deficiency. There is concern about the rapidity with which plasma sodium levels are corrected, particularly in persons with chronic symptomatic hyponatremia. Cells, particularly those in the brain, tend to defend against changes in cell volume caused by increased ECF osmolality by synthesizing amino acids and other osmotically active organic solutes. Because these solutes cannot cross the cell membrane, they confine their osmotic activity to the ICF compartment (154).

5. Hypernatremia

Hypernatremia implies a plasma sodium level above 145 mEq/L and a serum osmolality greater than 295 mOsm/ kg . Because sodium is functionally an impermeable solute, it contributes to tonicity and induces movement of water across cell membranes. Hypernatremia is characterized by hypertonicity of ECF and almost always causes cellular dehydration.164) (155). (figure 6).

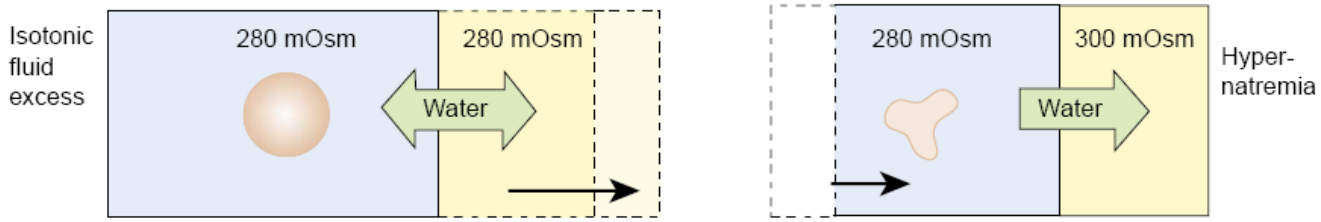


Fig (6). Clarifying of Causing Hypernatremia (165)

5.1. Causes of Hypernatremia

Hypernatremia represents a deficit of water in relation to the body's sodium stores. It can be caused by net loss of water or sodium gain. Net water loss can occur through the urine, gastrointestinal tract, lungs, or skin (156). The defect in thirst or inability to obtain or drink water can interfere with water replacement. Rapid ingestion or infusion of sodium with insufficient time or opportunity for water ingestion can produce a disproportionate gain in sodium. Hypernatremia almost always follows a

loss of body fluids that have a lower than normal concentration of sodium, so that water is lost in excess of sodium. This can result from increased losses from the respiratory tract during fever or strenuous exercise, from watery diarrhea, or when osmotically active tube feedings are given with inadequate amounts of water. With pure water loss, each body fluid compartment loses an equal percentage of its volume. Because approximately one third of the water is in the extracellular compartment, compared with the two thirds in the intracellular compartment, more actual water volume is lost from the ICF than the ECF compartment (157,158).

5.2. Diagnosis and Treatment

The diagnosis of hypernatremia is based on history, physical examination findings indicative of dehydration, and results of laboratory tests. The treatment of hypernatremia includes measures to treat the underlying cause of the disorder and fluid replacement therapy to treat the accompanying dehydration. Replacement fluids can be given orally or intravenously. The oral route is preferable .Oral glucose–electrolyte replacement solutions are available for the treatment of infants with diarrhea (158).

Until recently, these solutions were used only early in diarrheal illness or as a first step in reestablishing oral intake after parenteral replacement therapy. These solutions are now widely available in grocery stores and pharmacies for use in the treatment of diarrhea and other dehydrating disorders in infants and young children . They are particularly

important in developing countries, where the availability of intravenous fluids is limited, and diarrhea is a leading cause of death among children. The composition of the oral rehydration solution recommended by the Diarrheal Disease Control Center of the World Health Organization (WHO) contains glucose (2.0 g/L), sodium (90 mEq/L), potassium (20 mEq/L), chloride (80 mEq/L), and bicarbonate (30 mEq/L) (158).

Although cola (7up) drinks commonly are recommended as a remedy for dehydration caused by acute diarrhea, their electrolyte content often is inadequate for replacement purposes, and their high sugar content may complicate the situation by inducing an osmotic diarrhea. Sport drinks usually contain more sodium and sugar than the oral rehydration solutions. Intravenous replacement solutions continue to be the treatment of choice for severe fluid deficit (159).

6. Regulation of Potassium Balance

Potassium is the second most abundant cation in the body and the major cation in the ICF compartment. Approximately 98% of body potassium is contained within body cells, with an intracellular concentration of 140 to 150 mEq/L. The potassium content of the ECF (3.5 to 5.0 mEq/L) is considerably less. Because potassium is an intracellular ion, total body stores of potassium are related to body size and muscle mass. In adults, total body potassium ranges from 50 to 55 mmol/kg of body weight. Approximately 65% to 75% of potassium is in muscle .Thus, potassium content declines with age, mainly as a result of a decrease in muscle mass (160).

7. Alterations in Potassium Balance

Potassium do the function by regulating the resting membrane potential the opening of the sodium channels that control the flow of current during the action potential, and the rate of membrane repolarization. Changes in nerve and muscle excitability are particularly important in the heart, where alterations in serum potassium can produce serious dysrhythmias and conduction defects. Changes in plasma potassium also affect skeletal muscles and the smooth muscle in blood vessels and the gastrointestinal tract. The *resting membrane potential* is determined by the ratio of intracellular to extracellular potassium (161). (figure 7).

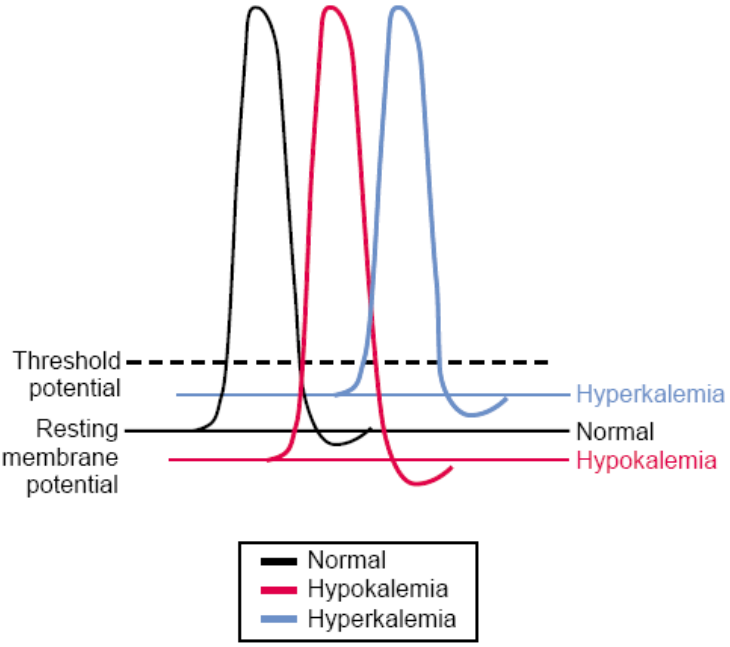


Fig (7). The resting membrane potential is determined by the ratio of intracellular to extracellular potassium **(**162).

8. Hypokalemia

Hypokalemia refers to a decrease in plasma potassium levels below 3.5 mEq/L (3.5 mmol/L). Because of transcellular shifts, temporary changes in plasma potassium may occur as the result of movement between the ICF and ECF compartments (160,161). Symptoms of hypokalemia include irregular heartbeat, which can range from mild to severe. Severe cases can result in cardiac arrest and paralysis of the lungs. Other symptoms can include muscle weakness, cramping, or flaccid paralysis (limpness); leg discomfort; extreme thirst; frequent urination; and confusion. Infants and young children with gastrointestinal illnesses that cause prolonged vomiting and diarrhea can die from cardiac arrest when potassium levels become dangerously low.

8.1. Causes of Hypokalemia

The causes of potassium deficit can be grouped into three categories:
(1) inadequate intake.
(2) excessive gastrointestinal, renal, and skin losses
(3) redistribution between the ICF and ECF compartments (161).

8.2. Treatment of Hypokalemia

Hypokalemia caused by potassium deficit is treated by increasing the intake of foods high in potassium content—meats, dried fruits, fruit juices (particularly orange juice), and bananas. Oral potassium

supplements are prescribed for persons whose intake of potassium is insufficient in relation to losses. This is particularly true of persons who are receiving diuretic therapy and those who are taking digitalis. Potassium may be given intravenously when the oral route is not tolerated or when rapid replacement is needed. Magnesium deficiency may impair potassium correction; in such cases, magnesium replacement is indicated .The rapid infusion of a concentrated potassium solution can cause death from cardiac arrest (162,163) .

9. Hyperkalemia

Hyperkalemia refers to an increase in plasma levels of potassium in excess of 5.0 mEq/L (5.0 mmol/L). It seldom occurs in healthy persons because the body is extremely effective in preventing excess potassium accumulation in the ECF. Symptoms are fairly nonspecific, and generally include malaise, palpitations and muscle weakness; mild hyperventilation may indicate metabolic acidosis, one of the possible causes of hyperkalemia (164).

9.1. Causes Of Hyperkalemia

The three major causes of potassium excess are:

1. decreased renal elimination,
2. excessively rapid administration.
3. movement of potassium from the intracellular toextracellular compartment (165).

9.2. Diagnosis and Treatment of hyperkalemia

Diagnosis of hyperkalemia is based on complete history, physical examination to detect muscle weakness and signs of volume depletion, plasma potassium levels, and ECG findings. The history should include questions about dietary intake, use of potassium sparing diuretics, history of kidney disease, and recurrent episodes of muscle weakness. The treatment of potassium excess varies with the degree of increase in plasma potassium and whether there are ECG and neuromuscular manifestations. Calcium antagonizes potassium-induced decrease in membrane excitability, restoring excitability toward normal. Plasma potassium levels affect the resting membrane potential, whereas plasma calcium levels affect the threshold potential. In persons with severe hyperkalemia, calcium gluconate (10 mL of a 10% solution) is given intravenously over a period of 2 to 3 minutes (165,166).

Aim of the study

The Research Main Guidelines Were Directed:

1. Study the level of human chorionic gonadotrophin hormone and thyroid stimulation hormone in patients with hyperemesis gravid arum and in control groups .
2. Estimation the electrolytes level (sodium and potassium) in patients with HEG and control groups.
3. Study the relation ship between (HCG , TSH) with HEG.
4. Presenting the effects of (maternal age , gestational age , gravidity ,residence area) on each parameter that mentioned above and on HEG
5. Study the relation ship between HCG and TSH in patients and control groups**.**

CHAPTER 2

Section One / Material

1. Patients

100 pregnant women with maternal age range from (17-35) years old were enrolled from Babylon Hospital of Pediatric and Maternity from 1st of July 2006 to the 1st May 2007. Subject were prospectively enrolled with diagnosis of hyperemesis gravidarum in the first trimester of pregnancy which was define by the presence of all the following criteria :

- Intractable nausea and vomiting for greater than 24 hour before admission , occruing at least 3 times per day.
- weight loss of at least 5 pound within 3 weeks of diagnosis .All subject were admitted to receive in patient treatment for hyperemesis gravidarum .All patient underwent full history and physical examination including: age , residence ,occupation ,gravidity, parity, positive family history of hyperemesis gravidarum and risk factors.

The main investigation that underwent each patient was :

- Determination the level of human chorionic gonadotrophin hormone (HCG).
- Determination the level of thyroid stimulating hormone (TSH).
- Determination electrolyte included (K^+ ,Na^+)

Healthy subjects

Fifty healthy pregnant women were taken as a control group with age range between (15-38) years old. They did not have any sign or symptoms of huperemesis gravidarum during the first trimester of pregnancy. This healthy subject also underwent all patient investigation , and also determine the level of two hormone (HCG,TSH) ,and electrolytes (K^+ ,Na^+).

Blood sampling

Venous blood samples were drawn from patients and healthy control subjects using disposable syringes in the sitting position . Five ml of blood were obtained from each subject ,and was pushed slowly into plain disposable tube without anticoagulant, blood was allowed to clot for 10-15 minutes ,the clot shrinks and serum was obtained by centrifugation 2500 xg for approximately 10-15 minutes.

2. Chemicals

Table (6). Chemical Compounds Used In The Research

NO.	Chemical Compound	Production
1-	Human Chorionic Gonadotropin (HCG) Enzyme Immunoassay Test Kit Catalog Number: BC-1027	BioCheck, Inc 323 Vintage Park Dr. Foster City, CA 94404
2-	Sensitive Thyroid Stimulating Hormone (S-Tsh) Enzyme Immunoassay Test Kit Catalog Number: Bc-1003	BioCheck, Inc 323 Vintage Park Dr. Foster City, CA 94404
3-	Standards Of Sodium & Potasium	Standards of Iraqi Instatution Of Sera & Vaccines

3. Instruments And Materials

Table (7). Instruments Used In The Research

NO.	Apparatus Or Material	Production
1-	Micropipettes Automatic (0.2-1) ml	SLAMED Made In Germany
2-	Flame analyser	GallenKomp England
3-	Centrifuge	Germany
4-	Filter papers	Scheicher & Schiill Gmbh (germany)
5-	Spectrophotomerter Elisa	Tiwan
6-	Micropipettes auto (5,50,100) µl	SLAMED Made In Germany
7-	Stop Wach	Chain

Section Two / Methods

1. Enzyme Immunoassay for the Quantitative Determination of Human Chorioni Gonadotropin (HCG) in Serum

1.1 Principle of the Assay

The BioCheck HCG ELISA test is based on a solid phase enzyme-linked immunosorbent assays (ELISA) .The assay system utilizes one mouse monoclonal anti-HCG antibody for solid phase (microtiter wells) immobilization and another mouse monoclonal anti-HCG antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test specimen (serum) is added to the HCG antibody coated microtiter wells and incubated with the Zero Buffer at room temperature for 30 minutes. If HCG is present in the specimen, it will combine with the antibody on the well. The well is then washed to remove any residual test specimen, and HCG antibody labeled with horseradish peroxidase (conjugate) is added. The conjugat will bind immunologically to the HCG on the well, resulting in the HCG molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation at room temperature for 15 minutes, the wells are washed with water to remove unbound-labeled antibodies. A solution of TMB is added and incubated at room temperature for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the color is changed to yellow and measured spectrophotometrically at 450 nm.

The concentration of HCG is directly proportional to the color intensity of the test sample (167,168).

1.2 Reagents And Materials :

- *Antibody-Coated Wells (1 plate, 96 wells)* Microtiter wells coated with sheep polyclonal anti-HCG.
- *HCG Zero Buffer (1 dropper vial, 13 mL)* contains bovine serum, green dye, NaN3.
- *Enzyme Conjugate Reagent (18 mL)*contains mouse monoclonal anti-HCG (specific to HCG, β-HCG) conjugated to horseradish peroxidase.
- *Reference Standard Set (1 mL/vial)* contains 0, 5, 20, 50, 150 and 300 mIU/mL (WHO, 1st IRP/3rd IS, 75/537) human chorionic gonadotropin in bovine serum with NaN3.
- *TMB Reagent (1 bottle, 11 mL)*contains 3, 3', 5, 5' tetramethylbenzidine (TMB) stabilized in buffer solution.
- *Stop Solution (1N HCl) (1 bottle, 11 mL)* contains diluted hydrochloric acid.

1.3 Instrumentation

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 2 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

1.4 Reagent Preparation

- All reagents should be allowed to reach room temperature (18- 25°C) before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. foaming should be avoided
- Samples with expected values greater than 300 mIU/mL should be diluted with Zero Standard or normal human male serum (1:100 initial dilution) prior to assaying.

1.5 Storage Conditions

Unopened kit should be stored at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.

1.6 Specimen Collection and Preparation

Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only. grossly hemolytic (bright red), lipemic (milky), or turbid samples were avoided .

Specimens should be capped and may be stored for up to 48 hours at 2-8°C. Specimens held for a longer time should be inverted several times prior to testing.

1.7 Assay Procedure

1. The desired number of coated wells should be Secured in the holder.
2. 50 µL of standards , samples, and controls should be dispensed in to appropriate wells.
3. Gently mixed for 30 seconds.
4. 100 µL of HCG zero buffer should be dispensed into each well.
5. It is very important that made thorough mixing for 10 seconds to ensure complete mixing
6. The mixture should be incubated at room temperature (18-25°C) for 30 minutes.
7. The incubation mixture could be removed by flicking plate contents into a suitable waste container.

8. The microtiter wells should be rinsed and flicked 5 times with distilled or de ionized water.
9. The wells droplet sharply and stroked on to absorbent paper or paper towels to remove all residual water.
10. 150 µL of enzyme conjugate reagent should be dispensed into each well and gently mixed for 5 seconds.
11. The mixture should be Incubated at room temperature for 30 minutes.
12. The incubation mixture should be removed by flicking well contents into a suitable waste container.
13. The wells should be rinsed 5 times with distilled or de-ionized water.
14. The wells droplet sharply and stroked on to absorbent paper or paper towels to remove all residual water.
15. 100 µl TMB Reagent should be dispensed into each well and gently mix for 5 seconds.
16. The mixture should be Incubated in the dark at room temperature for 20 minutes.
17. The reaction should be stopped by adding 100 µL of 1N HCl into each well.
18. Gently mixed for 5 seconds.
19. OD should be read at 450 nm with a microtiter well reader within 15 minutes.

1.8 Expected Values

Each laboratory must establish its own normal range based on patient population. HCG is not normally detected in the serum of healthy men or healthy non-pregnant women. The concentration of HCG in the serum of pregnant women increases to 5-50 mIU/mL one week after implantation and continues increasing exponentially during the first ten weeks, reaching a maximum of 100 – 200 IU/mL at the end of the first trimester.

Although normal pregnancy is usually the cause of increased HCG levels in urine and serum, elevated HCG concentrations have also been reported in patients diagnosed with molar pregnancy, chorio-carcinoma, and non-trophoblastic neoplasms (169,170,171) .

1.9 Calculation of Results

1. Calculation the mean absorbance value (OD450) for each set of reference standards, controls and samples.
2. Construct a standard curve on linear graph paper, by plotting the mean absorbance obtained for each reference standard against its concentration in mIU/mL, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.

3. Using the mean absorbance value for each sample, determine the corresponding concentration of HCG in mlU/mL from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. If patient sample has a HCG concentration greater than 300 mIU/mL, dilute 100 fold with the Zero Standard or normal male human serum and re-test (0.01 mL sample plus 0.99 mL Zero).
5. Any diluted samples must be further corrected by the appropriate dilution factor.

2. Enzyme Immunoassay for the Quantitative Determination of Thyroid Stimulating Hormone (S-TSH) in Human Serum

2.1. Principle of The Assay

The BioCheck Sensitive-TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule. Mouse monoclonal anti-TSH antibody is used fore solid phase (microtiter wells) immobilization, and goat anti-TSH antibody is used in the antibody-enzyme (horseradish peroxidase) conjugate solution. The test sample is allowed to react simultaneously with the antibodies, resulting in the TSH molecule being sandwiched between the solid phase and enzyme-linked antibodies. After a 2 hour incubation at room temperature with shaking, the solid phase is washed with distilled water to remove unbound labeled antibodies. A solution of tetramethylbenzidine (TMB) is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped with the addition of 1N HCl, and the resulting yellow color is measured spectrophotometrically at 450 nm. The concentration of TSH is directly proportional to the color intensity of the test sample (167,168).

2.2. Reagents and Materials Provided

1. Antibody-Coated Wells (1 plate, 96 wells) microtiter wells coated with mouse monoclonal anti-TSH.
2. Enzyme Conjugate Reagent (1 vial, 13 mL) contains goat anti-TSH conjugated to horseradish peroxidase.
3. Reference Standard Set (1 mL/vial) contains 0, 0.1, 0.5, 2.0, 5.0, and 10.0 μIU/mL (WHO, 2nd IRP, 80/558) TSH in equine serum with preservatives.
4. TMB Reagent (1 bottle, 11 mL) contains 3, 3', 5, 5'
5. tetramethylbenzidine (TMB) stabilized in buffer solution.
6. Stop Solution (1N HCl) (1 bottle, 11 mL) contains diluted hydrochloric acid.

2.3. Reagent Preparation

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. All reagents should be mixed by gentle inversion or swirling prior to use.
3. Reconstitute each lyophilized standard with 1.0 mL dH20.
4. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be stored sealed at 2-8°C, and are stable at 2-8°C for at least 30 days.

2.4. Storage Conditions

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

2.5. Instrumentation

Microtiter well reader with a bandwidth of 10nm or less and an optical density range of 0 to 2 OD or greater at 450 nm wavelength is acceptable for absorbance measurement. An orbital motion microtiter plate shaker is necessary for the 2 hour incubation.

2.6. Specimen Collection and Preparation

1. Serum should be prepared from a whole blood specimen obtained by acceptable medical techniques. This kit is for use with serum samples without additives only. Avoid grossly hemolytic, lipemic, or turbid samples.
2. Specimens should be capped and may be stored for up to 48 hours at 2-8°C prior to assaying. Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

2.7. Assay Procedure

1. The desired number of coated wells should be secured in the holder.
2. 100 µl of standards, specimens, and controls should be dispensed into appropriate wells.
3. 100 µl of enzyme conjugate reagent should be dispensed each well.
4. Thoroughly mix for 30 seconds. It is very important to have complete mixing.
5. The mixture should be Incubated at room temperature (18-25°C) and shake at for 120 minutes (2 hours).
6. Incubation mixture should be removed the by flicking plate contents into a waste container.
7. The microtiter wells should be rinsed and flicked 5 times with distilled or de-ionized water.
8. The wells should be stroked sharply onto absorbent paper or paper towels to remove all residual water droplets.
9. 100 µl of TMB reagent should be dispensed into each well and gently mixed for 5 seconds.
10. The mixture incubation at room temperature, for 20 minutes.
11. The reaction should be stopped by adding 100 µl of stop solution into each well.

12. Gently mixed for 30 seconds to ensure that all of the blue color changes completely to yellow.
13. OD should be read at 450 nm with a microstiter well reader within 15 minutes.

2.8. Expected Values

Each laboratory should establish its own normal range based on patient population. Differences in assay technique and the use of various standards may affect results. The results provided below are based on 43 normal and 73 hyperthyroid blood specimens. The ranges were determined from the mean ± 2SD (μIU/mL TSH).These values may differ from other published data.

	Normal	Hyperthyroid
N	43	73
Mean TSH (μIU/mL)	1.84	<0.07
Range	0.54 - 4.72	<0.07 – 0.20

2.9. Calculation of Results

1. Calculation the average absorbance value (A450) for each set of reference standards, controls and samples.
2. Using log-log graph paper, construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in μIU/mL, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of TSH in μlU/ml from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. Any diluted samples must be further corrected by the appropriate dilution factor.

3. Flam Photometer

Flame emission photometry is most commonly used for quantitative measurement of sodium and potassium in body , Li while not normally present in serum may also be measured in connection with the therapeutic use of lithium salts in the treatment of some psychiatric disorder.

Atoms of many metallic elements, when given sufficient energy such as that supplied by a hot flame will emit this energy at wave lengths characteristic for the element .A specific amount or quantum of thermal energy is absorbed by an orbital electron, the electron being unstable in this high energy (excited) state release their excess energy as photons of particular wave length as they change from the excited to their previous or ground state if the energy is dissipated as light the light may consist of one or more than one energy level and therefore of different wave lengths these line spectra are characteristic for each element .The wave length to be used for the measurement of an element depends upon the selection of a line of sufficient intensity to provide adequate of Sensitivity as wall as freedom from other interfering lines at or near the selected wave length .Alkali metals are comparatively easy to excite in the flame of an ordinary laboratory burner lithium produces a red ,sodium ayellow, potassium violet ,rubidium a red and magnesium a blue colour in aflame .

These colors are characteristic of the metal atoms that are present as cations in solution under constant and controlled condition s.the light intensity of the characteristic wave length proportional to the number of

atoms that are emitting energy ,which in turn is directly proportional to the concentration of the substance of interest in the sample .

Thus flame photometer lends itself well to direct concentration measurements of some metals other cations such as calcium are less easily excited in the ordinary flame in this cases the amont of light given off may not always provide adequate sensitivity for analysis by flame emission methods .The sensitivity can be improved slightly by using higher temperature flames.

Procedure of Determination Sodium and Potassium by Flame Photometer

1. The air motor should be operated by making the air pressure(5 bar) to ensure fluid suction.
2. The gas valve should be opened trying to make the flame in a blue cones then we pass the distilled water in the instrument trying to put the pointer on zero line at this point the flame will blue.
3. After that a standard sodium solution should be passed in a concentration of 170 mmol/l.
4. The pointer should be put on the line 100 and passed a diluted solution for the sample under testing (diluted 100 times) then read it in comparison with standard solution .

5. The same procedure should be done for K^+ (the standard K^+ solution is in concentration of 7 mmol/l and the sample diluted 100 times then read in compared with standard K^+ solution .

4. Statistical Analyses

Statistical analysis were preformed using spss program version 15 all values were expressed as mean ± standard deviation (sd) or percentage (%).multiple comparison between patient and control were made ,using t test .the simple linear regression analysis were used to assess the relationship between variable . results were considered significant when the probability (p) was less than 0.05.

CHAPTER 3

Results and Discussion

Human chorionic gonadotrophin hormone (HCG) (mlU/ml) , thyriod syimulation hormone (TSH) (µlU/ml),electrolyte include (Na^+,K^+) (m mol/l) were measured from pregnant women (n=100) in the first trimester with hyperemesis gravidarum without any pregnancy complication and in pregnant (n=50) whom are not severing from (HEG) in the first trimester whom was regarding control group.

The hormonal behaviour and electrolytes level were studied in the patient and control generally then we studied this parameter according to the following categories :

1. Age of patient and control that classified in to 3 group (≥ 20, 21-29, ≤ 30) years old
2. Gestational age (4 weeks of gestation , 8 weeks of gestationa , 14 weeks of gestationa)
3. gravidity (G1, G2, G≥3)
4. Residence (rurally, civil)

1. Human Chorionic Gonadotrophin (HCG) and Thyroid Stimulating Hormone (TSH) in Hyperemesis Gravidarum Patient

The means (± SD) of serum (HCG) levels in patient with hyperemesis gravidarum were: 520.405(± 171.497)

while in control were :

674.259(± 131.895) in healthy pregnant women

The unit of measurement used in the study for HCG is (mIU/ml)

The expected value is 250,000-500,000 mIU/ml in the first trimester.

The mean serum level of human chorionic gonadotrophin hormone in patient with HEG had shown a decreased level in comparison to that of the control group and it revealed a significant difference in relation to serum (β HCG) in control group ($P < 0.001$)

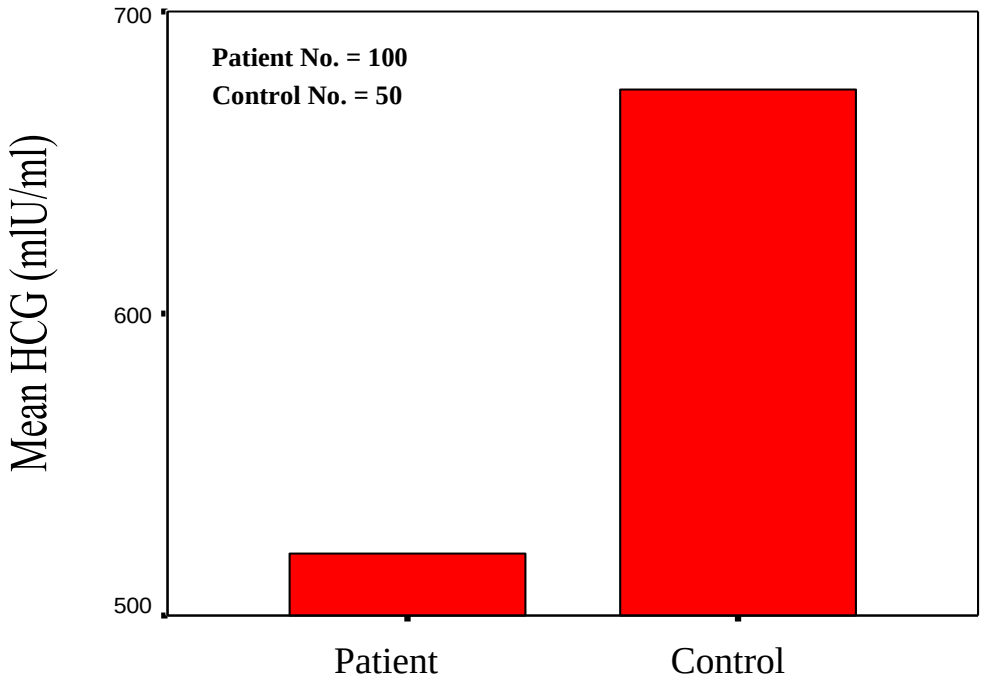


Figure (8): The mean of Human chorionic gondotrophine hormone in pregnant women with HEG group and in control group

To explain the results in figure (10) it was found , number of studies have found that women with HEG have increased concentrations of HCG compared with non- hyperemetic women,other studies have failed to show a difference in overall HCG concentration . The mechanisms of hyperemesis gravidarum are poorly understood , although normal concentrations of HCG have also been reported .If HCG levels are higher in a group of women with hyperemesis gravidarum, there is no clear threshold, since the normal range of HCG concentration is extremely large. Some pregnant women with extremely high HCG concentrations will not experience any hyperemesis whereas some with normal HCG will have hyperemesis gravidarum so the mechanisms of hyperemesis gravid arum are poorly understood .(172,173,174)

These conflicting results may be related to the heterogeneity of HCG. Total serum HCG during pregnancy is a mixture of intact HCG ,nicked HCG ,intact free α and β- subunits ,nicked β- subunits and traces of β--core fragment. Each of these HCG components displays a number of variant isoforms, which can differ in the extent of glycosylation, the degree of terminal sialylation, or in protein backbone amino acid sequence. Analogous to other glycoproteins such as LH and FSH, these isoforms may differ in biological activity. (175,176)

Changes in isoform profile may therefore work in concert with absolute HCG levels to cause HEG . Different isoform patterns of HCG can be the result of either long-term environmental influences or genetic factors.

This might be the explanation for the difference in incidence of HEG that has been observed between populations.

A higher incidence of HEG has been observed in New Zealand Pacific Island women, United Kingdom Indian and Pakistani, Asian and African American women compared with ethnic European women and lower incidences in American Indian and Eskimo populations.(176,177).

On other hand the mean serum thyroid stimulating hormone (TSH) level statistically had shown no difference between patients and control group ($p > 0.05$) in spite of the results presence little difference in mean serum of TSH of patient in comparison to control group as shown in table (8)

Table (8): The mean serum level of thyroid stimulating hormone in patients with HEG in Comparison to control groups

TSH	***No. of Cases***	***Mean TSH (µlU/ml)***	***S. D. (µlU/ml)***	***P. Value***
Patient	100	0.824	0.421	> 0.05
Control	50	0.944	0.433	

Through the current study it found that TSH level was in normal rang in patient with HEG and in control group that means the TSH, Free T4 and Free T3 are all normal throughout pregnancy so the thyroid is functioning normally during this duration of pregnancy and that does not correspondes with the studies which show that the high HCG level in the 1st trimester may result in a slightly low TSH level (179) .

We conclude that not all pregnant women suffering from hyperemesis gravid arum must have transient hyperthyriodism . The exact role of hyperthyroidism in hyperemesis gravidarum is, however, obscure. The suppressed TSH was found in 70%, at most, of patients with hyperemesis gravid arum (180) whereas in other studies (181,182,183), thyroid parameters were not significantly different between patients with hyperemesis gravidarum and control pregnant women and that agreed with our results.

2. Electrolye Disturbance in Hyperemesis Gravidarum

The electrolyte that determined in this study included (sodium and potassium).The results showed no difference in mean serum levels of K^+ and Na^+ in patient with HEG and control group($p > 0.05$).See (table .9).

Table (9) : The mean serum level of K^+ and Na^+ in patients with HHG in comparison to control groups

Electrolyte		*No. of Cases*	*Mean (mmol/L)*	*S. D. (mmol/L)*	*P. Value*
K^+	***Patient***	100	4.166	0.682	>0.05
	Control	50	4.290	0.709	
Na^+	***Patient***	100	138.420	7.179	>0.05
	Control	50	139.140	2.365	

Generally there is no change in the levels of electrolyte in pregnant women with and with out hyperemesis gravid arum we can explain this result by the following:

Some of patient had been treated immediately when entered to the hospital by intravenously infusion of fluid and supplement them by drinking juice that nutritional fluid and iv infusion can return this electrolyte level to the normal range .Or may be some of the patients were in the mild or moderate stage of hyperemesis gravid arum and did not reach to the sever stage which can causes electrolytes disturbance. while in few cases we found the level of the electrolytes was below the normal level because those patient do not take any type of treatment neither iv infusion of fluid nor oral intake of juice .

3. Maternal Age Effect on Hyperemesis Gravidarum Patients

The maternal age group distribution in hyperemesis gravidarum patients had shown a highest percentage in the age between (21-29) years old because these results belongs to this study and the selection of the patients was done randomly , as shown in (figure.9).

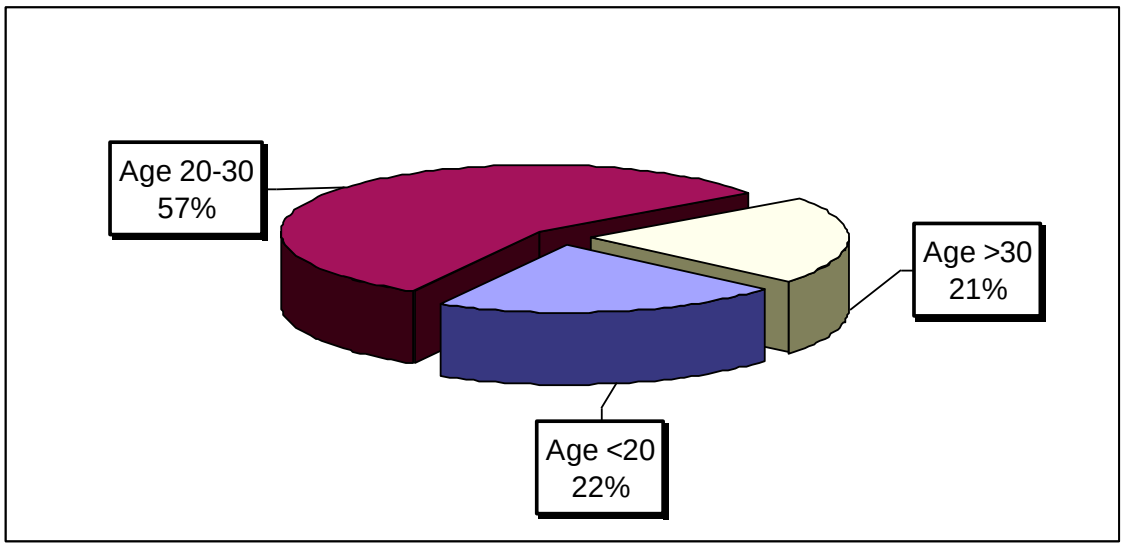


Figure (9) : The percentage of HEG patients according to their age

Hyperemesis is most common in first pregnancies and tends to recur in subsequent pregnancies the incidence of hyperemesis and the use of medication decreased with maternal age **.** It is less common with advancing maternal age**.**

This age distribution is explained by the population who differ from each other around the world ,so each population has its own age

group distribution depending on their environmental factors and life style habits that affect their activities of the hormonal behaviour which are considered essential substance in side the human body. Also the differential in ethnic population is very important factor that is strongly associated with this condition (177) .

4. The Relationship Between Maternal Age and Hormonal and Electrolyte Behaviour

The results showed no significant difference in mean serum level of HCG in patients and control group in the age <20 while there is significant difference in mean serum level of HCG in age group >20 years old .as shown in figure (10)

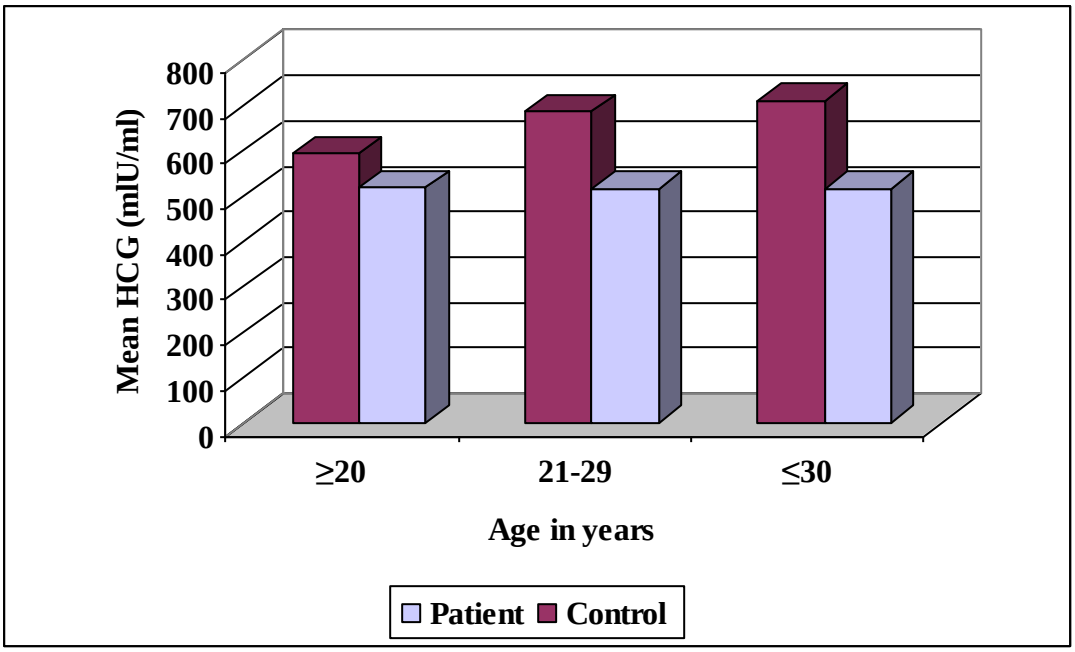


Fig.(10) :The distribution maternal age of patients and control groups.

Statistically we found that the mean serum level of HCG increased with maternal age progression for healthy pregnant women (control). In non pregnant women it has long been known that the gonadotrope cells of the pituitary produce LH and FSH under the control of hypothalamic GnRH. GnRH is regulated by ovarian steroids. With age the ovary starts to fail, limiting progesterone and estrogen feedback to control GnRH. Starting in peri-menopause and then intensifying in menopause, GnRH production looses steroidal control. As a result, continuous GnRH stimulation of gonadotrope cells then occurs, leading to elevate LH and FSH production. Under these hyper-stimulation conditions, the pituitary may secrete an HCG-like molecule (184) (this may also occur in pregnant women).

While the results showed their is no significant difference in mean serum level of TSH between the patients and control groups in all maternal age groups as shown in table (10).

Table (10): The mean serum level of TSH in patients with HEG in comparison to control groups according to their maternal age

Age (years)	***Patient***	***Control***	***P. Value***
	Mean ± S.D. (µlU/ml)	***Mean ± S.D. (µlU/ml))***	
≥20	0.876 ± 0.350	1.116 ± 0.467	>0.05
21-29	0.847 ± 0.226	0.736 ± .294	>0.05
≤30	0.702 ± 0.126	1.419 ± 0.455	>0.05

This results coincide with many studies that show there is no relation between TSH and age, from another side some study show that the differences in maternal age and thyroid function are highly discriminatory with regard to hyperemesis gravidarum (185,186) .

Also the reference intervals for TSH determined in this study differed from those reported from other countries using the same analytical platform that is coincide with a study showed that there is considerable differences in reference intervals for TSH have been reported between countries (187).

The results showed their is no significant difference in mean serum level of K^+ and Na^+ in all age group patient and control groups as shown in table (11),(12) .

Table (11): The mean serum level of K^+ in patients with HEG in comparison to control groups according to their maternal age.

Age (years)	***Patient***	***Control***	***P. Value***
	Mean ± S.D. (mmol/L)	***Mean ± S.D. (mmol/L)***	
≥20	4.448 ± 0.661	4.246 ± 0.731	>0.05
21-29	4.046 ± 0.683	4.362 ± 0.765	<0.05
≥30	4.175 ± 0.638	4.100 ± 0.441	>0.05

Table (12): The mean serum level of Na^+ in patients with HEG in comparison to control groups according to their maternal age

Age (years)	*Patient*	*Control*	*P. Value*
	Mean ± S.D. (mmol/L)	*Mean ± S.D. (mmol/L)*	
≥20	138.652 ± 8.716	139.231 ± 1.691	>0.05
21-29	137.719 ± 7.608	139.345 ± 2.567	>0.05
≥30	139.200 ± 2.764	138.250 ± 2.605	>0.05

5. Gestational Age Effect in Hyperemesis Gravidarum Patients

The results showed that the highest percentage of patients suffering from hyperemesis gravidarum is in the 12 weeks gestation while it is less in the 8 weeks gestation and the percentage is least in the 4 weeks gestation as shown in(figure 11).

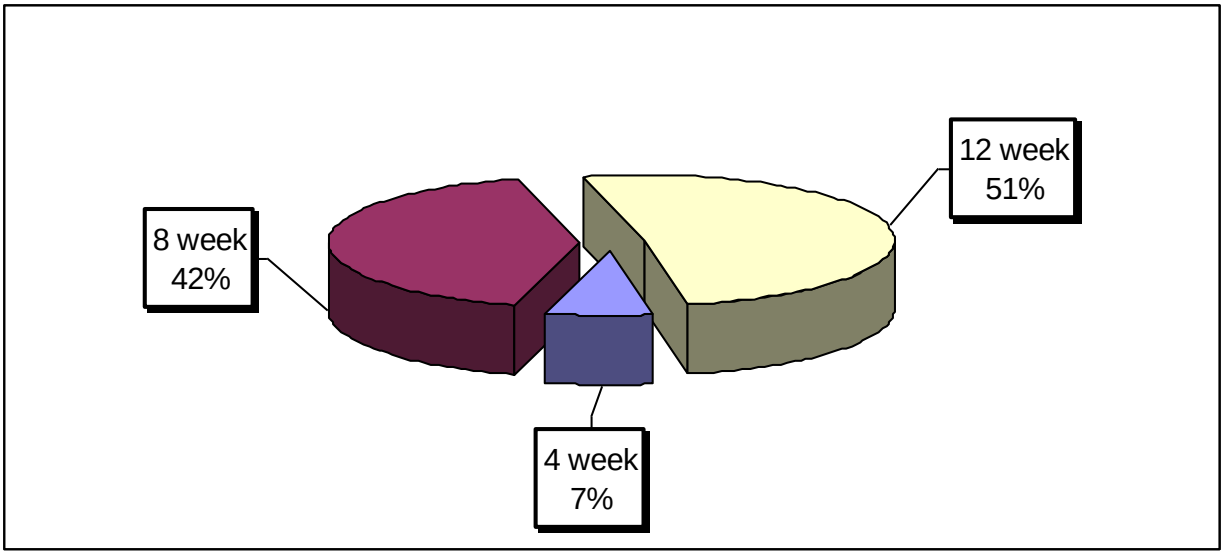


Figure (11) : The percentage of HEG patients according to their gestational age

This condition appears during the first trimester and is unassociated with other medical conditions.This results of our study are agreed with many studies that show symptoms usually start between 4 and 7 weeks gestation and resolve by 16 weeks in about 90% of women (53).

6. The Relationship Between Gestational Age and Hormonal and Electrolyte Behaviour

The results show there is a significant difference in mean serum level of HCG between patient and control group in 2 miss period and 3 miss period of gestation as shown in figure (12).

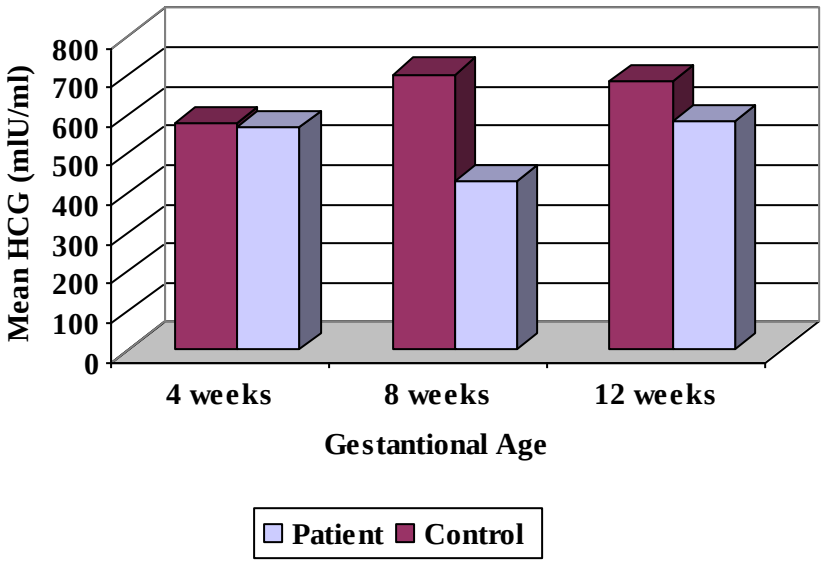


Fig.(12) :The distribution gestational age group of patients and control groups.

We can conclude that the serum level of HCG will increase as the gestational age increase that's belong to the appearance of HCG in urine or serum soon after conception and its rapid rise in concentration makes it an ideal indicator for the detection and confirmation of pregnancy .

The concentration of HCG in the serum of pregnant women increases to 5-50 mIU/mL one week after implantation and continues increasing exponentially during the first ten weeks, reaching a maximum of 100 – 200 IU/mL at the end of the first trimester so that coincinde with our results so that when we progrrise from tenth weeks of pregnancy the serum level of HCG will increase (77).

while the results show there is no significant difference in mean serum level of TSH in patient and control according to the gestational age as show in table (13)

Table (13) :The mean serum level of TSH in patients and control groups according to the gestational age

Gestational Age (L.M.P.)	***Patient***	***Control***	***P. Value***
	Mean ± S.D. (µlU/ml)	***Mean ± S.D. (µlU/ml)***	
4 weeks	0.998 ± 0.358	0.843 ± 0.407	>0.05
8 weeks	0.637± 0.358	0.983 ± 0.861	>0.05
12 weeks	0.903 ± 0.451	0.968 ±0 .974	>0.05

We can explain our results by that in spite of the variations of thyroid function during gestation have been studied (185), reference values for thyroid hormones according to age of pregnancy are still frequently unavailable (188,189,190). As a consequence, diagnosis of true hyperthyroidism is frequently based on multiple sequential measurements of TSH and thyroxine to distinguish it from moderate transient self-resuming hyperthyroxinaemia with decreased TSH ,also free thyroid hormone assays may be disturbed by changes that occur during pregnancy, such as an increase in binding proteins, haemodilution, increased free fatty acid concentration and a decrease in albumin concentration (185).Reference ranges should therefore be established for these assays according to age of pregnancy as proposed in several studies .According to Glinoer , close to 20% of

pregnant women will have a suppressed TSH, 2% will have a suppressed TSH with increased thyroxine concentration, and in 1% of pregnant women, suppressed TSH and hyperthyroxinaemia will be associated with clinical hyperthyroidism. Also the differences in assays and methodology, in the time of sampling, as well as population characteristics may be responsible for these discrepancies (185,190).

For electrolyte levels the results showed that there is non significant difference in then mean serum levels of (K^+ , Na^+) between patients and control groups according to the gestational age as shown in tables (14,15) respectively.

Table (14): The mean serum level of K^+ in patients and control groups according to the gestational age

Gestational Age	***Patient***	***Control***	***P. Value***
	Mean ± S.D. (mmol /L)	***Mean ± S.D. (mmol /L)***	
4 weeks	3.650 ± 0 .854	4.264 ± 0.616	>0.05
8 weeks	4.198 ± 0.646	3.909 ± 0 .577	>0.05
12 weeks	4.264 ± 0 .616	4.677 ± 0 .908	>0.05

Table (15) :The mean serum level of Na^+ in patients and control groups according to the gestational age

Gestational Age	*Patient*	*Control*	*P. Value*
	Mean ± S.D. (mmol /L)	*Mean ± S.D. (mmol /L)*	
4 weeks	141.250 ± 6.798	139.727 ± 1.421	>0.05
8 weeks	136.950 ± 9.243	138.462 ± 2.259	>0.05
12 weekss	139.365 ± 5.006	139.231 ± 2.703	>0.05

We measure the electrolyte level in limited period of pregnancy (1^{st}) trimester only with out making follow up to the pregnant (2^{nd} ,3^{rd}) trimester and may have changes in electrolyte level during the subsequent period.

7. The Relationship Between Gravidity and Hyperemesis Gravidarum

The results in this figure below showed that the highest percentage of patients suffering from hyperemesis gravidarum is in the G≥ 3 while it is less in the G1 and the percentage is the least in the G2 as shown in (figure 13)

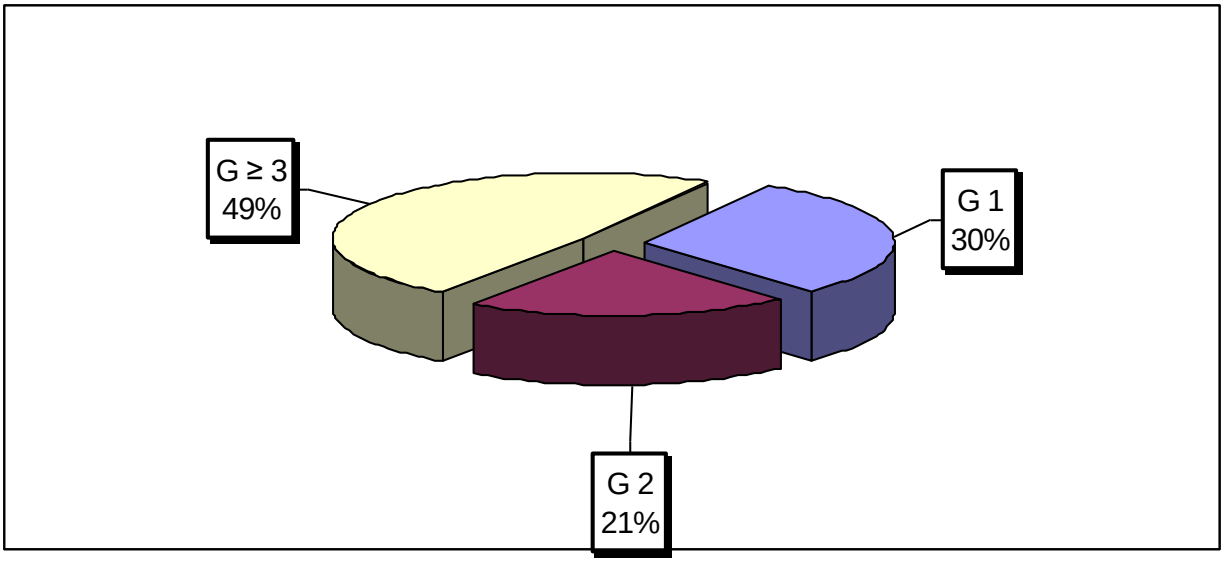


Figure (13) : The percentage of HEG patients according to their gravidity

Our results agreed with many studies which show that the occurrence of hyperemesis gravidarum is more common in prior pregnancy and first birth but this is not a rule because we may find a woman suffering from HEG in her first pregnancy while in her second pregnancy dose not suffer from HEG because this condition is effected by many factores like (anplaning pregnancy, familial history , psycological factor ,diatery factor , social factor , multiple pregnancies ,etc.) (61) .

8. The Relationship Between Gravidity With Hormonal and Electrolye Behaviour

The results showed that their is a significant difference in the mean serum levels of HCG in all group according to the gravidity as shown in table (16).

Table (16): The mean serum level of HCG in patients and control groups according to the gravidity.

gravidity	***Patient***	***Control***	***P. Value***
	Mean ± S.D. (mlU/ml)	***Mean ± S.D. (mlU/ml)***	
G1	520.118±182.4375	679.002 ±158.218	< 0.05
G2	533.448±184.791	686.917± 116.122	< 0.05
G≥3	546.599±151.3678	694.507± 102.046	<0.05

The results showed that the level of HCG will increase as the gravidity increase in patient and control group but in control group is more than patient group . We can not consider that as a general rule because this results that we have been got it came from random selected pregnant women with different gravidity and parity that mean we did not check HCG level for pregnant woman in her 1^{st} , 2^{nd} and 3^{rd} pregnencies in the same duration of gestation for same women . This results which we have got it and futural studies may explain the exact relation ship between gravidity and HCG.

In TSH the result showed there is no significant difference in the mean serum levels of TSH in patient and control group according to the gravidity as shown in table (17)

Table (17): The mean serum level of TSH in patients and control groups according to the gravidity.

gravidity	***Patient***	***Control***	***P. Value***
	Mean ± S.D. (µlU/ml)	***Mean ± S.D. (µlU/ml)***	
G1	0.6443 ± 0. 380	0.919 ±0.653	>0.05
G2	0.7423 ± 0.103	1.124 ± 0.327	>0.05
G≥3	0.9707 ± 0.228	0.804 ± 0.326	>0.05

Also the results showed their is no significant difference in mean serum levels of K^+ and Na^+ in patient and control group according to the gravidity as shown in tables (18, 19)

Table (18): The mean serum level of K^+ in patients and control groups according to the gravidity

gravidity	***Patient***	***Control***	***P. Value***
	Mean ± S.D. (mmol /L)	***Mean ± S.D. (mmol /L)***	
G1	4.097±.747	4.477 ± 0.833	>0.05
G2	4.276 ±.771	3.921 ± 0.554	>0.05
G≥3	4.159 ± 0.600	4.364 ± 0.506	>0.05

Table (19): The mean serum level of Na^+ in patients and control groups according to the gravidity

gravidity	*Patient*	*Control*	*P. Value*
	Mean ± S.D. (mmol /L)	*Mean ± S.D. (mmol /L)*	
G1	137.600 ± 7.486	139.364 ± 2.128	>0.05
G2	137.381 ± 9.831	138.857 ± 2.797	>0.05
G≥3	139.367 ± 5.510	139.071 ± 2.401	>0.05

9. The Relationship Between Residence Area and Hyperemsis Gravidarum

The results showed that the HEG is more common in urban women than in rural women as show in figure (14)

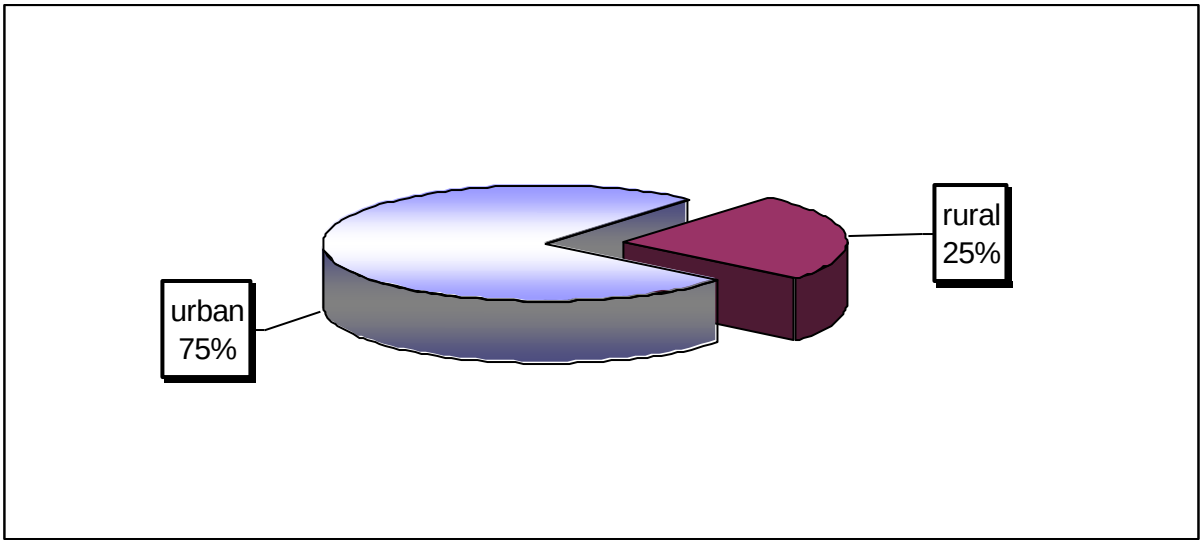


Figure (14) : The percentage of HEG patients according to their residence area

Our results agreed with many studies which showed that this condition is more common in urban women than in rural women . One study identified increased risk in housewives and decreased risk in "white collar" or professional white women who consumed alcohol before conception, and in women over 35 years of age with a history of infertility (191).

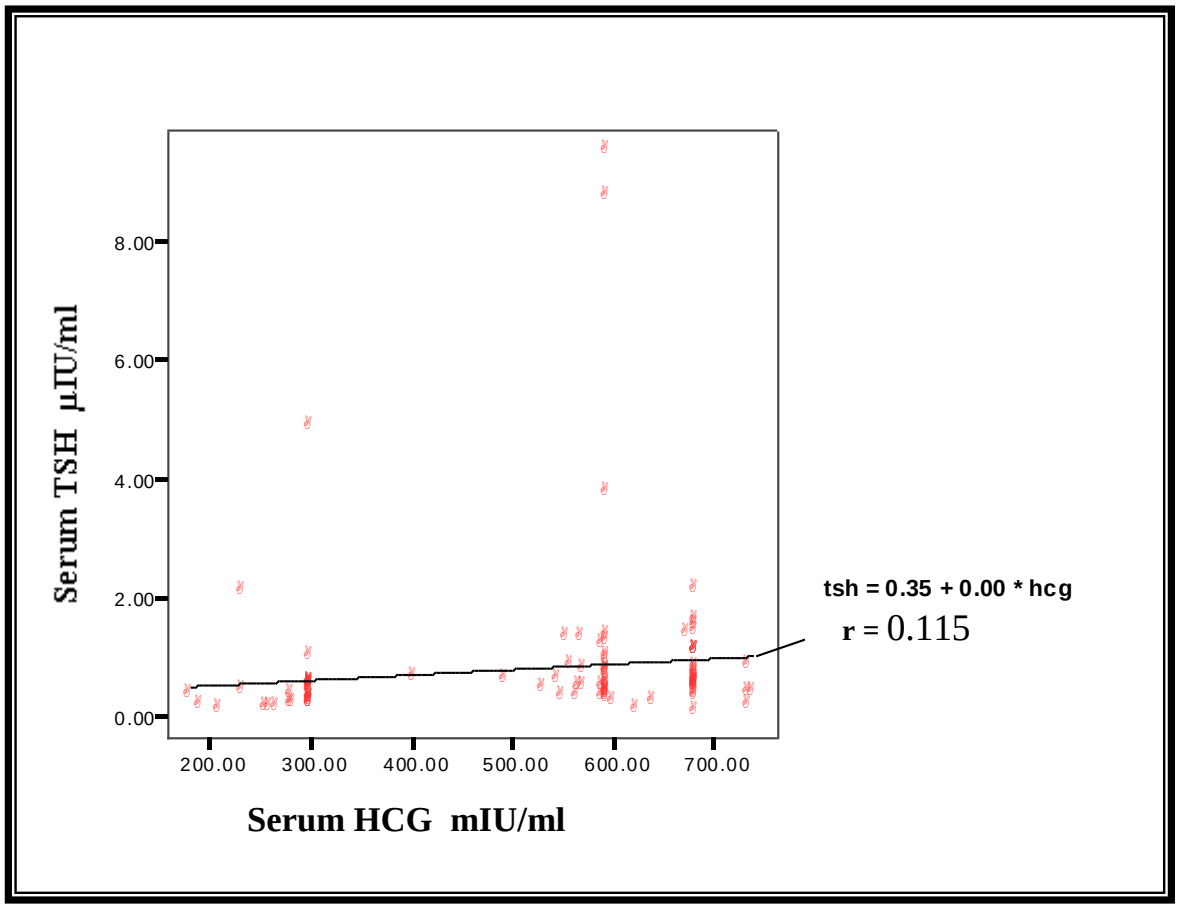
We can explain this result by many factors that are strongly assosiated with this condition firstly nutrition in rural area differs from city , in rural area there is more natural food like(milk ,chees, yogarout ,vegetable) all this food are rich in minerals and vitamins that is essential for oplimal nutrition and give the body more resistance for any condition therefore depending for this healthy.

Second factor is psycological effect that is strongly associated with HEG and many studies show that this effect promots this condition , In one well-known study, the Cornell Medical Index was administered to 44 pregnant women with hyperemesis and 49 pregnant women without hyperemesis; the Minnesota Multiphasic Personality Inventory (MMPI) was administered only to the pregnant women with hyperemesis. The MMPI data suggested that women with hyperemesis have hysteria, excessive dependence on their mothers, and infantile personalities

The women who lived in rural area had experienced violence more than women who lived in urban area and that hard life style of rural area makes pregnant woman more tolerable for such condition (192) .

10. The Plotting of Serum Thyroid Stimulation Hormone Against Serum Human Chorionic Gonadotrophin Hormone in Patient and Control Group

According to our results there is no significant relation ship between HCG and TSH in patient ($p > 0.05$) that mean there is no correlation between HCG and TSH with correlation coefficient $r = 0.115$ as shown in figure (15)



Fig(15):The correlation between serum TSH and HCG in patients

In the control group the results showed there is no significant correlation between HCG and TSH in HEG with correlation coefficient r=0.107 , p>0.05 as show in figure (16)

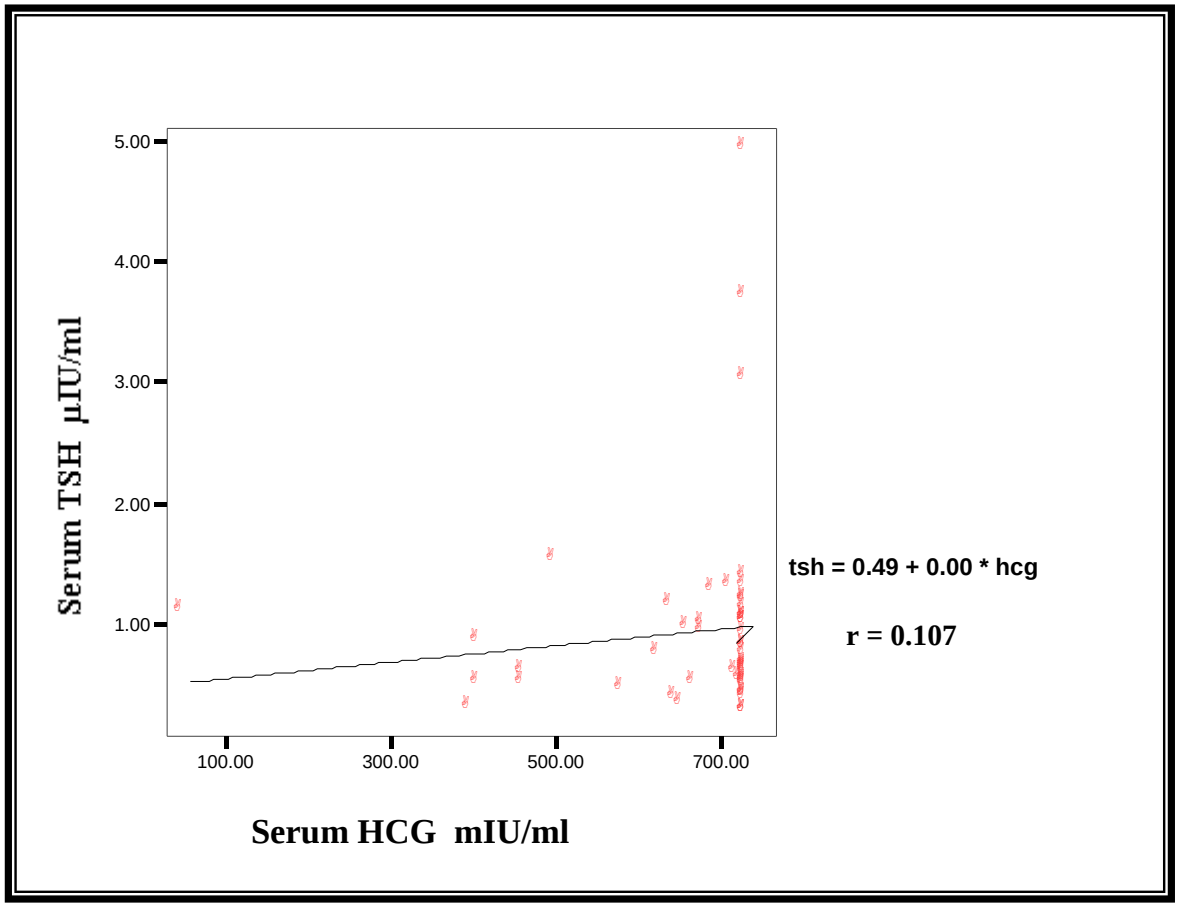


Fig (16): the correlation between serum TSH and HCG in control group.

Some studies showed that even there is high concentration of HCG in patient with HEG but there is a weak correlation between HCG and TSH (192) .

Conclusion:

1. Hyperemesis gravidarum is a multifactorial condition (familial history , emotional problems, bacterial infection ,history of eating problems ,history of motion sickness, being pregnant with more than one fetus.
2. HCG elevates in all pregnant women and it may not be the main cause of hyperemesis gravid arum .
3. It is not necessary to have a depression in TSH level and having transient hyper thyroidism in patient with hyperemesis gravidarum in the 1st trimester of pregnancy.
4. Only in severe cases of hyperemesis gravid arum there is electrolytes disturbances

Recommendations:

1. Further studies dealing with the relationship between hyperemesis gravid arumwith other hormones for ex: estrogen and progesterone is very important.
2. Advanced lab devices like automatic chemistry analyzer ,Roche Integra 800 and Cobas 6000 is very useful in hormonal and electrolytes analyses.

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