

## **1.1 Introduction**

Pregnancy is characterized by extensive maternal physiological adjustments involving a variety of metabolic processes. These characteristic changes are often reflecting in the results of laboratory tests, such that value in healthy pregnant women may fall outside the normal ranges of the non- pregnant women. Failure to appreciate the effects of normal gestation can result in errors in diagnosis. The primary purpose of this study is to review the laboratory indices in blood total protein, albumin and urea, which reflect the nutritional and metabolic status during normal pregnancy, and comparing it with normal non-pregnant as control.

Because maternal physiology adjustment represent dynamic process, special care in obtaining data has been taken as far as possible, with respect to the individual location, age and duration of gestation.

Many reports indicate that serum albumin levels decrease during the first trimester In France, Yan nick Bacq, (serum albumin levels decrease during the first trimester).

In Nigeria, Ann Med Health Sei Res 2012 (decrease in serum iron and albumin ).

In American Satish Cllalhan 2000 (decreased rate of urea Synthesis during pregnant that is evident early in gestation, a lower concentration of blood urea nitrogen is apparent early in gestation ).

In Nigeria Adedeji Al 2012 (serum albumin level lower during normal pregnancy).

## 1.2 Literature Review

### 1.2.1 pregnancy:

pregnancy is the fertilization and development of one or more offspring ,known as an embryo or fetus, in a woman's uterus . It is the common name for gestation in humans . a multiple pregnancy involves more than one embryo or fetus in a single pregnancy, such as with twins .

The physiologic responsibilities of the ovary are the periodic release of gametes (eggs, oocytes) and the production of the steroid hormones estradiol and progesterone. Both activities are integrated in the continuous repetitive process of follicle maturation, ovulation, and corpus luteum formation and regression. The female gonad is a heterogeneous ever- changing tissue whose cyclicity is measured in weeks, rather than hours (Speroff *et al.*, 2001).

The ovary consists of three major portions: the outer cortex, the central medulla, and the rete ovarii (the hilum). The hilum is the point of attachment of the ovary to the mesovarium. It contains nerves, blood vessels, and hilus cells, which have the potential to become active in steroidogenesis or to form tumors. These cells are very similar to the testosterone-producing Leyden cells of the testes. The outermost portion of the cortex is called the tunica albuginea, topped on its surface by a single layer of cuboidal epithelium, referred to as the ovarian surface epithelium or the ovarian mesothelium (Speroff *et al.*, 2001).

The oocytes, enclosed in complexes called follicles, are in the inner part of the cortex, embedded in stromal tissue. The stromal tissue is composed of connective tissue and interstitial cells, which are derived from mesenchymal cells, and have the ability to respond to luteinizing hormone (LH) or human chorionic

gonadotropin (hCG) with androgen production. The central medullary area of the ovary is derived largely from mesonephric cells (Speroff *et al.*, 2001).

Pregnancy is the state of having products of conception implanted normally or abnormally in the uterus or occasionally elsewhere. Pregnancy is terminated by spontaneous or elective abortion, or delivery. A myriad of physiologic changes occur in pregnant woman, which affect every organ system (William *et al.*, 2003).

#### **1.2.1.1 Diagnosis of pregnancy:**

In a female who has regular menstrual cycles and is sexually active, a period delayed by more than a few days to a week is suggestive of pregnancy. Even at this early stage, patients may exhibit signs and symptoms of pregnancy. The classic finding of ‘morning sickness’ can begin this early and often continues through 12 to 16 weeks of gestation. On physical examination, a variety of findings indicate pregnancy .

Many over-the-counter (OTC) urine pregnancy tests have a high sensitivity and will be positive around the time of the missed menstrual cycle. These urine tests and the hospital laboratory serum assays test for the beta subunit of human chorionic gonadotropin ( $\beta$  -hCG). This hormone produced by the placenta will rise to a peak of 100,000 mIU/mL by 10 weeks of gestation, decrease throughout the second trimester, and then level off at approximately 20,000 to 30,000 mIU/mL in the third trimester. A viable pregnancy can be confirmed by ultra sound, which may show the Gestational sac as early as 5 weeks, or at a  $\beta$  -hCG of 1500 to 2000 mIU/mL, and the feta’ heart as soon as 6 weeks, or a  $\beta$ -hCG of 3000 to 6000 mIU/mL.

From the time of fertilization until the pregnancy is 8 weeks along (10 weeks gestational age [GA]), the conceptus is called an embryo. After 8 weeks until the time of birth, it is designated a fetus. The term infant is used for the period between delivery and 1 year of age .

Pregnancy is divided into trimesters. The first trimester lasts until 12 weeks but is also defined as up to 14 weeks gestational age (GA), the second trimester from 12 to 14 until 24 to 28 weeks gestational age (GA), and the third trimester from 24 to 28 weeks until delivery.

An infant delivered prior to 24 weeks is considered to be pre-viable, from 24 to 37 weeks is considered preterm, and from 37 to 42 weeks is considered term (Berek *et al.* ,1994).

The GA of a fetus is the age in weeks and days measured from the last menstrual period (LMP). Developmental age (DA) is the number of weeks and days since fertilization. Because fertilization usually occurs about 14 days after the first day of the prior menstrual period, the GA is 2 weeks more than the developmental age (DA). Classically, Naegele's rule for calculating the estimated date of confinement (EDC), or estimated date of delivery (EDD), is to subtract 3 months from the LMP and add 7 days. Ultrasound has a level of uncertainty that increases during the pregnancy but it is rarely off by more than 7% to 8% at any gestational age (GA) (Clark, 1997).

Other measures used to estimate gestational age include pregnancy landmarks such as auscultation of the fetal heart (FH) at 20 weeks by non electronic fetoscopy or at 10 weeks by Doppler ultrasound, as well as maternal awareness of fetal movement or "quickening," which occurs between 16 and 20 weeks (Cunningham, 2001).

### **1.2.1.2 Physiological changes in pregnancy**

During pregnancy, cardiac output increases by 30% to 50%. Most increases occur during the first trimester, with the maximum being reached between 20 and 24 weeks gestation and maintained until delivery. Systemic vascular resistance decreases during pregnancy, resulting in a fall in arterial blood pressure. This decrease is most likely due to the elevated progesterone leading to smooth muscle relaxation (Heffner 2002) .

There is an increase of 30% to 40% in tidal volume during pregnancy , increase in minute ventilation which in turn leads to an increase in alveolar (  $P_{aO_2}$  ) this change leads to an increased  $CO_2$  gradient between mother and fetus and is likely caused by elevated progesterone levels is that either increase the respiratory system's responsiveness to  $CO_2$  or act as primary stimulant. this is possibly secondary to decreased  $P_{aCO_2}$  levels, increased tidal volume, or decreased total lung capacity(Heffner 2002) .

Nausea and vomiting occur mainly during early pregnancy possibly due to raised estrogen or human chorionic gonadotropin ( hCG ) levels. the stomach has prolonged sphincter has decreased tone. the large bowel also decreased motility, which lead to increased water absorption and constipation. (Heffner 2002) .

Plasma volume increase in pregnancy, red blood cell volume increase which lead to decrease in the hematocrit, white blood cell count increases during pregnancy to a mean of 10. smiltion/ml, there is a slight decrease in the concentration of platelets. pregnancy is considered to be a hyper coagulable state and number of thromboembolic event increase. the increased rote of thromboembolic events in pregnancy maybe secondary to increase in venous stasis and vessel and othelial.

Pregnancy is a hyper estrogenic state, The increased estrogen is produced primarily by the placenta, the hormone hCG is composed of two dissimilar alpha and beta subunits. alpha subunits of luteinizing hormone ( LH ), follicle stimulating hormones ( FSH), and thyroid stimulating hormones (TSH), whereas the beta subunits differ. the placenta produces hCG, which to maintain progesterone production by the corpus luteum. ( Manye.,et *al* 1994).

Numerous changes in the skin occur during pregnancy, increased activity of the melanin stimulation hormones causes deeper pigmentation during pregnancy and the steroid hormones', muscles reaching maximum effect during last week of pregnancy.

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 75g/day. Recommended calcium intake is 1.5g/day. Many patients develop iron deficiency anemia because of the increased demand on hematopoiesis both by the mother and the fetus. Folate requirements increase from 0.4 to 0.5mg/day and are important in preventing neural tube defects(Gabbe et al., 2002).

Urinary system changes in the kidneys actually increase in size and the ureters dilate during pregnancy, which may lead to increased rates of pyelonephritis. The glomerular filtration rate (GFR) increases by 50% early in pregnancy and is maintained until delivery. As a result of increased GFR, blood urea nitrogen and creatinine decrease by about 25%. An increase in the renin-angiotensin system leads to increased levels of aldosterone. This ultimately results in increased sodium

resorption. However, plasma levels of sodium do not increase because of the simultaneous increase in GFR.

The glomerular filtration rate (GFR) increases during pregnancy by about 50%. The renal plasma flow rate increases by as much as 25-50%. Urinary flow and sodium excretion rates in late pregnancy can be altered by posture, being twice as great in the lateral recumbent position as in the supine position. Even though the glomerular filtration rate GFR increased dramatically during pregnancy, the volume of the urine passed each day is not increased. Thus, the urinary system appears to be even more efficient during pregnancy.

With the increase in GFR, there is an increase in endogenous clearance of creatinine. In serum it reduces in proportion to increase in GFR, and concentration of blood urea nitrogen is similarly reduced (Decherney, *et al* 2009).

Glucosuria during pregnancy is not necessarily abnormal, may be explained by the increase in GFR with impairment of tubular reabsorption capacity for filtered glucose. Increased levels of urinary glucose also contribute to increased susceptibility women to urinary tract infection.

Proteinuria changes little during pregnancy and if more than 500mg/24h is lost, a disease process should be suspected.

Levels of the enzyme rennin, which is produced in kidney, increased early in the first trimester, and continue to arise until term. This enzyme acts on its substrate angiotensinogen, to first form angiotensin1 and then angiotensin2, which acts as vasoconstrictor. Normal pregnant are resistant to the pressure effect of elevated levels of angiotensin2 those suffering from preeclampsia are not resistant, this is one of the some theories to explain this disease.

As the uterus enlarges, the urinary bladder is displaced upward and flattened in the anterior-posterior or diameter. Pressure from the uterus leads to increase in urinary frequency. Bladder vascularity increases and muscle tone decreases. Increasing capacity up to 1500ml.

Women with albuminuria are several times more likely to give birth prematurely. These findings suggest that a simple, inexpensive test for urinary albumin may help doctors target their efforts towards women most at risk of delivering early(American journal of kidney diseases. et al,. 2005).

### **1.2.2 Protein During Pregnancy:**

Proteins are composed of amino acids that are joined to form linear chains .in addition to carbon, hydrogen, and oxygen, proteins contain approximately 16%nitrogen by weight. The digestive process breaks down proteins to their constituentaminoacids, which enter the blood. The complete oxidation of proteins to  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , and  $\text{NH}_4$  in the body yields approximately 4 kcal/g (Dawn.D. et al 2009).

When amino acids are linked together by acid—amide bonds, linear macromolecules (peptides) are produced. Those containing more than 100 amino acid residues are described as proteins (polypeptides). Every organism contains thousands of different proteins, which have a variety of functions (Koolman. *et al*,. 2005).

-Establishment and maintenance of structure, Structural proteins are responsible for the shape and stability of cells and tissues e.g. collagen molecule and Histones.

-Transport protein, as moglohin in the erythrocytes. It is responsible for the transport of oxygen and carbon dioxide between the lungs and tissues. The blood plasma also contains many other roteins with transport functions. Pre albumin, for



example, transports the thyroid hormones thyroxine and triiodothyronine. Ion channels and other integral membrane proteins facilitate the transport of ions and metabolites across biological membranes.

- Protection and defense. The immune system protects the body from pathogens and foreign substances. An important component of this system is immunoglobulin

- Control and regulation. In biochemical signal chains, proteins function as signaling substances (hormones) and as hormone receptors. The small peptide hormone insulin is involved in regulating the metabolism and in differentiation processes of carbohydrates.

- Catalysis. Enzymes, with more than 2000 known representatives, are the largest group of proteins in terms of numbers.

- Movement. The interaction between actinide myosin is responsible for muscle contraction and cell movement.

- Storage. Plants contain special storage proteins, which are also important for human nutrition. In animals, muscle proteins constitute a nutrient reserve that can be mobilized in emergencies(Koolman.*et al.*, 2005).

#### **1.2.2.1 Causes of Increase Total Protein :**

- Hyper gamma globulinaemia.
- Para proteinaemia ( due to increase protein synthesis ).
- Artifactual ( haeme concentration due to stasis of blood during venipuncture ).
- Dehydration ( decrease volume of distribution )

#### **1.2.2.2 Causes of Decrease Total Protein:**

- Malnutrition and Malabsorption .
- Liver disease .
- Humeral immunodeficiency ( due to decrease protein synthesis ).

- Over hydration .
- Increase capillary permeability (due to increase volume of distribution).
- Protein losing states .
- Catabolic states ( due to increase excretion catabolism (William,. *etal* 2004).

### **1.2.3 Albumin**

Albumin is the major plasma protein, in human plasma is made of 585 amino acids linked in sequence, one of the major roles of albumin ( along with total protein) is the part of plays in osmotic or oncotic pressure is simply measure of the number of particles that are in a specific volume. any disease that decreases the production of albumin by the liver can potentially drop the oncotic pressure of plasma. Because protein is required for growth deficiency of it is more apparent in children than in adults who are maintaining an adult body mass. Dietary protein deficiency means a poor supply of essential amino acids needed for the assembly of protein . contain amino acids can be synthesized from other molecules there are so called essential amino acids that must be supplied by proteins in the diet albumin is also a part of a complex butyrate system. ( Frances, *et al* 2003).

#### **1.2.3.1 Reference Values:**

Normal Children : 2.9—5.5 g/dL or 29-55 g/L Adults : 3.5-4.8 g/dL or 35-48 g/L After age 40 years and in persons living in subtropics and tropics (secondary to parasitic infections), level slowly declines (Frances *et al*,2003).

**Table (1.1) reference value of albumin in pregnant:**

Units	Non pregnant Female	First Trimester	Second Trimester	Third Trimester
g/dL	4.1 - 5.3	3.1 - 5.1	2.6 - 4.5	2.3 - 4.2
g/L	41 - 53	31 - 51	26 - 45	23- 42

Albumin may be elevated in congestive heart failure, glucocorticoid excess, congenitally.

Albumin may be decreased with kidney disease, hypothyroidism, debilitating disease, malnutrition, burns, polydipsia, protein losing enteropathy, liver disease, insufficient anabolic hormones.( Fischbach., *et al* 2007 ) .

#### **1.2.3.2 Causes of Albumin Increased:**

Increased albumin is not associated with any naturally occurring condition. When albumin is increased, the only cause is decreased plasma water that increases the albumin proportionally (dehydration).

#### **1.2.3.3 Causes of albumin decreased:**

- A low plasma albumin concentration may be due to ( Dilution or redistribution.)
- Decrease rate of synthesis .
- Increase rate of catabolism.
- Loss from body .
- Administration of an excess of protein free fluid .
- Fluid retention usually in edematous states or during late pregnancy.

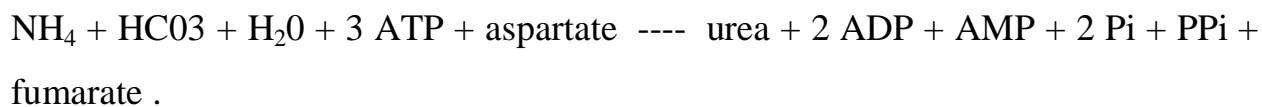
- Redistribution of albumin from plasma into the interstitial fluid space (Philip, *et al* 1994 ).

#### **1.2.4 Blood Urea:**

Urea forms in the liver and, along with CO<sub>2</sub>, constitute the final product of protein metabolism. The amount of excreted urea varies directly with dietary protein intake, increased excretion in fever, diabetes, and increased adrenal gland activity. The test for urea, is used as an index of glomerular function the production and excretion of urea. Rapid protein catabolism and impairment of kidney function will result in an elevated urea level. The rate at which the urea level rises is influenced by the degree of tissue necrosis, protein catabolism, and the rate at which the kidneys excrete the urea. A markedly increased urea is conclusive evidence of severe impaired glomerular function. In chronic renal disease, the urea level correlates with symptoms of uremia than dose the serum creatinine (Frances *et al*,. 2003).

##### **1.2.4.1 Urea cycle:**

Urea is synthesized in the liver by the urea cycle. It is then secreted into the bloodstream and taken up by the kidneys for excretion in the urine. The urea cycle was the first cyclic metabolic pathway to be discovered by Hans Krebs and Kurt Henseleit in 1932. The overall reaction of the pathway is:



One of the nitrogen atoms of urea comes from ammonia; the other is transferred from the amino acid aspartate, while the carbon atom comes from CO<sub>2</sub> .

Ornithine, an amino acid that is not the standard set of 20 amino acids and is not found in proteins, is the Carrier of these nitrogen and carbon atoms. Five

enzymatic reactions are involved in the urea cycle the first two of which take place in mitochondria other three in the cytosol:

1. Carbamoyl phosphate synthetase, which is technically not a member of the urea cycle, catalyzes the condensation and activation of ammonia (from the oxidative deamination of glutamate by glutamate dehydrogenase) and  $\text{CO}_2$  (in the form of bicarbonate,  $\text{HCO}_3^-$ ) to form Carbamoyl phosphate. The hydrolysis of two ATP molecules makes this reaction essentially irreversible.

2. The second reaction also occurs in the mitochondria and involves the transfer of the Carbamoyl group from Carbamoyl phosphate to Ornithine by Ornithine trans carbamoylase. This reaction forms another non standard amino acid citrulline which then has to be transported out of the mitochondrion into the cytosol where the remaining reactions of the cycle take place.

3. The citrulline is then condensed with aspartate, the source of the second nitrogen atom in urea, by the enzyme arginin osuccinate synthetase to form arginin osuccinate. This reaction is driven by the hydrolysis of ATP to AMP and  $\text{PPi}$ , with subsequent hydrolysis of the pyrophosphate. Thus both of the high-energy bonds in ATP are ultimately cleaved.

4. Argininosuccinase then removes the carbon skeleton of aspartate from argininosuccinate in the form of fumarate, leaving the nitrogen atom on the other product arginine. As the urea cycle also produces arginine, this amino acid is classified as non essential in ureotelic organisms. Arginine is the immediate precursor of urea.

5. The urea is then formed from arginine by the action of arginase with the regeneration of Ornithine. The Ornithine is then transported back into the mitochondrion ready to be combined with another molecule of Carbamoyl phosphate .

A block in any of the urea cycle enzyme leads to an increase in the amount of ammonia in the blood, so-called hyper ammonemia. The most common cause of such a block is a genetic defect that becomes apparent soon after birth, when the afflicted baby becomes lethargic and vomits periodically. If left untreated, coma and irreversible brain damage will follow. The reasons for this are not entirely clear but may be because the excess ammonia leads to the increased formation of glutamate and glutamine. These reactions result via depletion of the citric acid cycle intermediate  $\alpha$ -keto glutarate which may then compromise energy production, especially in the brain. It also leads to an increase in the acidic amino acids glutamate and glutamine which may directly cause damage to the brain(Howard *et al.*, 1987).

The demand for nitrogen is not distributed evenly throughout pregnancy. Demand increases in relation to the accretion of placenta and fetal tissues as well as to deposition of maternal tissue. These maternal tissues might be utilized subsequently to meet needs in later pregnancy and lactation. The quantity of protein or nitrogen needed to satisfy net tissue deposition during pregnancy has been assessed by the factorial approach as well as with balance methods.

The average weight gain for normal pregnancy is 12.5 kg, of which 0.9 kg is protein. Balance methods give a positive daily balance of 1-3 g N/d or 6-8 g protein/d in the last half of pregnancy, This is more than the estimates by the factorial method. Results of balance studies have to be placed in context, given the difficulty of carrying out such measurements with reliability, the tendency to obtain positive balances, and the need to extrapolate a measurement made over a limited number of days to the entire period of pregnancy.

Protein turnover using [ $^{15}\text{N}$ ] glycine indicate an initial high rate of synthesis in trimester 1 followed by a decline to no pregnant concentrations in trimester 3 which runs counter to the demands related to progressive fetoplacental growth.

In the non-pregnant state, the adaptive response to a low protein intake is a reduction in the rate of excretion of urea in the urine. However, a reduction in excretion does not necessarily reflect a reduction in the rate of urea production. Once the minimal requirement for protein has been satisfied, the rate of urea production changes only modestly over a wide range of protein intakes (Langran.M. et al., 1992).

At low protein intakes, urea production changes little, urea excretion falls, and the rate of urea salvage through the metabolic activity of the colonic microflora is enhanced.

Pregnancy is characterized by a state of positive nitrogen balance, and the excretion of urea falls. This fall has been interpreted as a decrease in urea production (Jackson *et al.*, 1990).

Quantitative analysis estimates of the rates of urea synthesis and excretion have been performed to assess the irreversible nitrogen loss or protein catabolism and oxidation. Previous studies in humans, either during fasting or in response to exogenously administered amino acids, showed an attenuated rate of urea synthesis during pregnancy (Koolman *et al.*, 1998).

**Table (1.2) blood urea nitrogen:**

<b>Blood Urea nitrogen , BUN (serum)</b>				
<b>Units</b>	<b>Non pregnant Adult</b>	<b>First Trimester</b>	<b>Second Trimester</b>	<b>Third Trimester</b>
mg/dL	7 -20	7 -12	3 - 13	3 -11
mmol/L	2.5 - 7.1	2.5 - 4.3	1.1 - 4.6	1.1 - 3.9

(Cunningham, *et al* 2009).

#### **1.2.4.2 Increased urea levels (azotemia):**

Increased urea levels occur in the following conditions:

- Impaired renal function caused by the following condition:
  - Congestive heart failure
  - Salt and water depletion
  - Shock
  - Stress
  - Acute myocardial infarction
  - Chronic renal disease such as glomerulonephritis and pyelonephritis
  - Urinary tract obstruction
  - Hemorrhage into gastric tract
  - Diabetes mellitus with keto acidosis
  - Excessive protein intake or protein catabolism as occurs in burns or cancer
  - Steroid use (Frances *et al.*, 2003).

#### **1.2.4.3 Decreased urea levels:**

Decreased urea levels are associated with the following conditions:

- Liver failure (severe liver disease), such as that resulting from hepatitis, drugs, or poisoning
- Acromegaly
- Malnutrition, low-protein diets
- Impaired absorption (celiac disease)
- Nephrotic syndrome (occasional)
- Syndrome of inappropriate anti diuretic hormone (SIADH) (Frances *et al.*, 2003).



- A combination of a low-protein and high-carbohydrate diet can cause a decreased urea level.
- The urea is normally lower in children and women because they have less muscle mass than adult men.
- Decreased urea values normally occur in late pregnancy because of increased plasma volume (physiologic high uremia).
- Older persons may have an increased urea when their kidneys are not able to concentrate urine adequately.
- Interfering feedings only may result in over hydration and decreased urea levels.
- Many drugs may cause increased or decreased urea levels (Frances *et al.*, 2003).

There is no store for nitrogen-containing compounds as there is for carbohydrate (glycogen) or lipids (triacylglycerol). Thus nitrogen ingested in excess of what is required by the organism has to be excreted.

#### **1.2.4.4 Reference value:**

Normal adults: 6-20 mg/dl or 2.1-7.1 mmol/L Elderly patients (<60 years):  
8-23 mg/dL or 2.9-8.2 mmol/L children: -18/mg/dL or 1.8-6.4 mmol/Ln).

### **1.3 Rationale:**

pregnancy is the fertilization and development of one or more offspring, known as an embryo or fetus, in a woman's uterus.

Physiological and anatomical alterations develop in many organ systems during the course of pregnancy and delivery. Early changes are due, in part, to the metabolic demands brought on by the fetus, placenta and uterus and, in part, to the increasing levels of pregnancy hormones, particularly those of progesterone and estrogens. Later changes, starting in mid-pregnancy, are anatomical in nature and are caused by mechanical pressure from the expanding uterus. These alterations create unique requirements for the anesthetic management of the pregnant woman.

- Although many biochemical changes occur during pregnancy, data are scarce for total Protein Albumin and Urea changes such as diet and physiological.

This result of the study may be valuable to detect the effect of pregnancy on plasma levels of total Protein, Albumin and Urea.

## **1.4 Objectives of the research**

### **1.4.1 General objective:**

To assess the concentration plasma level of total Protein, Albumin, and blood Urea in Sudanese pregnant women .

### **1.4.2 Specific objectives**

- To measure plasma levels of total Protein, Albumin and Urea in healthy pregnant women compared to control (non pregnant women).
- To compare, total Protein, Albumin and Urea concentration in different trimesters of pregnancy.