Introduction:

Preeclampsia is one of the most common medical complications of pregnancy and it is characterized by hypertension, proteinuria and/or edema, usually occurring after 20 weeks of gestation. It is an important cause of maternal and perinatal morbidity and mortality worldwide, especially in developing countries. In India, the incidence of preeclampsia amongst the hospital patients is about 7-10% of all antenatal admissions, although the precise etiology of preeclampsia is not clear, defective placentation and endothelial dysfunction are considered the core features of preeclampsia (John, et al, .1999).

It is a multisystem disorder that affects the maternal kidneys, liver, brain, clotting system and primarily the placenta .Hepatic dysfunction with preeclampsia has long been recognized. Several studies have suggested that liver involvement in preeclampsia is serious and frequently accompanied by evidence of other organs involvement, especially the kidney and brain along with hemolysis and thrombocytopenia. This is commonly referred to as HELLP syndrome (hemolysis, elevated liver enzymes and low platelets). (Var, etal,. 2003).

The enzyme γ-glutamyltransferase (GGT) is widely distributed throughout the body in many tissues, particularly the liver. At the cellular level, significant activity occurs in both endothelium and epithelium. Association between serum GGT concentration and blood pressure in non-pregnant hypertensive patients have been reported in some population surveys. Also raised levels of serum GGT have been reported in stroke patients, which were assumed to be due to vascular endothelial damage (Yamada,et- al,.1990). Lactate dehydrogenase (LDH) is an intracellular enzyme which converts lactic acid to pyruvic acid and its elevated levels indicates cellular death and leakage of enzyme from the cell .Increased levels of LDH were found in association with preeclampsia in a limited numbers of studies. This is the first study that

examines the frequency symptoms occurring in severe preeclamptic patients according to the levels of GGT and LDH, indicating multiorgan involvement and severity of the disease. As severe preeclampsia causes numerous multisystem complications, we hypothesize that elevated levels of serum GGT and LDH may reflect the severity of preeclampsia and the occurrence of complications (Beyer,. 2004).

1.2 Rationale

Studies have consistently shown association between the preeclampsia and gamma glutamyletransferase and lactate dehydrogenase. There is an extremely high maternal mortality in Sudan with preeclampsia accounting for 4.2% of the obstetric complications and 18.1% of maternal deaths (Leiberman, et al,.1991) (Ali,et al., 2012).

Use of more investigations as biochemical markers which reflects the severity of preeclampsia for management of preeclampsia will decrease maternal and fetal morbidity and mortality.

No published data of gamma glutamyletransferase and lactate dehydrogenase in preeclampsia in Sudan. So This study was conducted to evaluate gamma glutamyletransferase and lactate dehydrogenase as a marker for severity of preeclampsia.

1.3 Objectives

1.3.1 General objective:

To assess the effect of preeclampsia on level of gamma glutamyletransferase and lactate dehydrogenase among Sudanese preeclamptic pregnant women Khartoum state.

1.3.2 Specific objectives:

-To determine the level of gamma glutamyletransferase and lactate dehydrogenase in Sudanese pregnant women with preeclampsia.

-To determine the association of specific type of preeclampsia (mild and severe) with gamma glutamyletransferase and lactate dehydrogenase

2. Literature review

2.1 Preeclampsia:

Preeclampsia is defined as hypertension plus significant proteinuria, specifically gestational hypertension plus new onset proteinuria, or chronic (preexisting) hypertension with new or worsening proteinuria. When preeclampsia develops in women with chronic (preexisting) hypertension, the classification of disease is chronic (preexisting) hypertension with superimposed preeclampsia. Preeclampsia can also occur without proteinuria, with hepatic, hematopoietic, or other manifestations. Edema is no longer considered a specific diagnostic criterion for preeclampsia. Pregnant women with hypertension plus other adverse conditions but no proteinuria should have further evaluation for preeclampsia (McMaster, et al,. 2008).

Early identification and management is essential for preeclampsia, which is characterized by a complex group of multi-organ processes and variable presentation. Traditionally, preeclampsia has been defined as hypertension plus significant proteinuria. Women with gestational hypertension plus new onset of 300 mg or more of urinary protein in a 24 hour period are classified as having preeclampsia. Women with chronic (preexisting) hypertension with new or worsening proteinuria are classified as having chronic (preexisting) hypertension with superimposed preeclampsia. This definition, which is cited in ACOG and JNC documents based on the NHBPEP Working Group definition, is typically used in research protocols, and therefore reflects the characteristics of pregnant women who comprise the study population in most published studies of preeclampsia. In addition, this traditional definition of preeclampsia is based on the most common maternal manifestations of preeclampsia: hypertension and proteinuria. Up to 30% or more of women with chronic (preexisting) hypertension or gestational hypertension also develop preeclampsia. Women with mild chronic (preexisting) hypertension

have a 20% risk of developing superimposed preeclampsia, and those with severe chronic (preexisting) hypertension have a 50% risk of superimposed preeclampsia. Women with chronic (preexisting) hypertension and end-organ disease, severe hypertension or secondary hypertension are at greatest risk for superimposed preeclampsia (Steegers, 2010).

Preeclampsia can also occur without proteinuria, and manifest as hypertension plus other adverse conditions, reflecting the multi-organ processes that characterize this disorder. SOGC and SOMANZ guidelines define preeclampsia as hypertension with proteinuria or other adverse conditions, since there is evidence that end-organ complications can occur without proteinuria (Al-Jameil, et al,. 2014).

(i) Signs and causes: Swelling (especially in the hands and face) was originally considered an important sign for a diagnosis of preeclampsia. However, because swelling is a common occurrence in pregnancy, its utility as a distinguishing factor in preeclampsia is not high. Pitting edema (unusual swelling, particularly of the hands, feet, or face, notable by leaving an indentation when pressed on) can be significant, and should be reported to a health care provider.

In general, none of the signs of preeclampsia are specific, and even convulsions in pregnancy are more likely to have causes other than eclampsia in modern practice. Further, a symptom such as epigastric pain may be misinterpreted as heartburn. Diagnosis, therefore, depends on finding a coincidence of several preeclamptic features, the final proof being their regression after delivery (Redman , et al,. 2005).

Causes: There is no definitive known cause of preeclampsia, though it is likely related to a number of factors. Some of these factors include: abnormal placentation (formation and development of the placenta) Immunologic

factors. Prior or existing maternal pathology preeclampsia is seen more at a higher incidence in individuals with preexisting hypertension, obesity, antiphospholipid antibody syndrome, and those with history of preeclampsia. A dietary factor, e.g. calcium supplementation in areas where dietary calcium intake is low has been shown to reduce the risk of preeclampsia. Environmental factors, e.g. air pollution (Jun ,et al,. 2009) (Al-Jameil, et al ,.2006).

Those with long term high blood pressure have a risk 7 to 8 times higher than those without (Bramham, et al,. 2014).

Physiologically, research has linked preeclampsia to the following physiologic changes: alterations in the interaction between the maternal immune response and the placenta, placental injury, endothelial cell injury, altered vascular reactivity, oxidative stress, imbalance among vasoactive substances, decreased intravascular volume, and disseminated intravascular coagulation.(Mustafa, et al,. 2012).

While the exact cause of preeclampsia remains unclear, there is strong evidence that a major cause predisposing a susceptible woman to preeclampsia is an abnormally implanted placenta (Steegers, et al,. 2010). This abnormally implanted placenta is thought to result in poor uterine and placental perfusion, yielding a state of hypoxia and increased oxidative stress and the release of anti-angiogenic proteins into the maternal plasma along with inflammatory mediators. A major consequence of this sequence of events is generalized endothelial dysfunction The abnormal implantation is thought to stem from the maternal immune system's response to the placenta and refers to evidence suggesting a lack of established immunological tolerance in pregnancy. Endothelial dysfunction results in hypertension and

many of the other symptoms and complications associated with preeclampsia (Al-Jameil, et al,. 2014).

(ii)Pathogenesis: Although much research into mechanism of preeclampsia has taken place, its exact pathogenesis remains uncertain. Preeclampsia is thought to result from an abnormal placenta, the removal of which ends the disease in most cases. During normal pregnancy, the placenta undergoes process of vascularization to allow for blood flow between the mother and fetus abnormal development of the placenta leads to poor placental perfusion. The placenta of women with preeclampsia is abnormal and characterized by poor trophoblastic invasion. It is thought that this results in oxidative stress, hypoxia, and release of factors that promote endothelial dysfunction, inflammation, and other possible reactions (Eiland, et al,.2012) (Mustafa, et al ,.2012).

The clinical manifestations of preeclampsia are associated with general endothelial dysfunction, including vasoconstriction and end-organ ischemia Implicit in this generalized endothelial dysfunction may be an imbalance of angiogenic and anti-angiogenic factors (Al-Jameil, et al,. 2014).

Both circulating and placental levels of soluble fms-like tyrosine kinase-1 (sFlt-1) are higher in women with preeclampsia than in women with normal pregnancy (Mustafa, et al ,.2012).

sFlt-1 is an anti-angiogenic protein that antagonizes vascular endothelial growth factor (VEGF) and placental growth factor (PIGF), both of which are proangiogenic factors Soluble endoglin (sEng) has also been shown to be elevated in women with preeclampsia and has anti-angiogenic properties, much like sFlt-1 does.

Both sFlt-1 and sEng are upregulated in all pregnant women to some extent, supporting the idea that hypertensive disease in pregnancy is a normal pregnancy adaptation gone awry. As natural killer cells are intimately involved in placentation and as placentation involves a degree of maternal immune tolerance for a foreign placenta which requires maternal resources for its support, it is not surprising that the maternal immune system might respond more negatively to the arrival of some placentae under certain circumstances, such as a placenta which is more invasive than normal. Initial maternal rejection of the placental cytotrophoblasts may be the cause of the inadequately remodeled spiral arteries in those cases of pre-eclampsia associated with shallow implantation, leading to downstream hypoxia and the appearance of maternal symptoms in response to up regulated sFlt-1 and sEng.

Oxidative stress is thought to play an important part in the pathogenesis of pre-eclampsia. The main source of reactive oxygen species (ROS) is the enzyme xanthine oxidase (XO) and this enzyme mainly occurs in the liver. One hypothesis is that the increased purine catabolism from placental hypoxia results in increased ROS production in the maternal liver and release into the maternal circulation causing endothelial cell damage (McMaster, et al,. 2008).

Abnormalities in the maternal immune system and insufficiency of gestational immune tolerance seem to play major roles in pre-eclampsia. One of the main differences found in pre-eclampsia is a shift toward Th1 responses and the production of IFN-γ. The origin of IFN-γ is not clearly identified and could be the natural killer cells of the uterus, the placental dendritic cells modulating responses of T helper cells, alterations in synthesis of or response to regulatory molecules, or changes in the function of regulatory T cells in pregnancy aberrant immune responses promoting pre-eclampsia may also be

due to an altered fetal all recognition or to inflammatory triggers. It has been documented that fetal cells such as fetal erythroblasts as well as cell-free fetal DNA are increased in the maternal circulation in women who develop pre-eclampsia. These findings have given rise to the hypothesis that pre-eclampsia is a disease process by which a placental lesion such as hypoxia allows increased fetal material into maternal circulation that leads to an immune response and endothelial damage ultimately resulting in preeclampsia and eclampsia (Laresgoiti, etal,. 2010).

2.1.1 Risk factors for pre-eclampsia:

Pre-eclampsia develops only during pregnancy. The risk factors include (i) A family history of pre-eclampsia, (ii) usually in first pregnancy, (iii) higher for pregnant women younger than 20 and older than 40 yr, (iv) obesity, (v) common in women who are carrying twins, triplets or more, (vi) prolonged interval between pregnancies, (vii) women who develop gestational diabetes face a greater risk of developing pre-eclampsia as the pregnancy progresses, and (viii) history of certain medical conditions such as chronic high blood pressure, migraine headaches, diabetes, kidney disease, rheumatoid arthritis, urinary tract infections, and periodontal disease during pregnancy increases the risk of pre-eclampsia (Arulkumaran, et al,. 2013)(Gary,. 2010).

2.1.2 Current clinical tools for management of pre-eclampsia:

Until recently most work on pre-eclampsia has focused on abnormal placentation, genetic and epidemiologic factors, as well as treatments aimed at slowing the progression of the disease. An elevated uric acid level seems to help in diagnosis of PE.

Doppler studies of brachial artery reactivity in women who have had pre-eclampsia show abnormal endothelial dependent flow-mediated arterial dilation three years after pregnancy10. A relation between high resistance

uterine artery waveforms in the second half of pregnancy and pre-eclampsia has already been established and persistent notching of the uterine artery Doppler waveform has shown promise as ascreening test at 20 and 24 wk14. With the introduction of transvaginal ultrasound probes it has been possible to investigate the uterine circulation in early pregnancy with Doppler ultrasound but so far this approach has not been shown to be useful for prediction of utero-placental complications such as pre-eclampsia or the delivery of a small for gestational age baby. However, with the recent advances in 3D Power Doppler ultrasound qualitative as well as quantitative assessments have become feasible predicting adverse pregnancy outcomes (Lambert, et al,. 2014).

2.1.3 Proposed laboratory tools for predicting/monitoring pre-eclampsia:

An ideal biomarker of pre-eclampsia is the one that would allow an accurate prediction during the first trimester as it offers a wide window of opportunity for effective treatment that may help in complete recovery or reduce the severity.

Several groups have been investigating biomarkers associated with the pathophysiology of pre-eclampsia, like endothelial dysfunction, general inflammatory response, proteinuria, placental dysfunction as potential diagnostic tools. This review mainly focuses on the various biomarkers available and the recent advances in the utility of these biomarkers in predicting preeclampsia (Steegers,. 2010).

2.2 Gamma glutamyltransferase (GGT):

The name glutamyltransferase was preferred by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology for this enzyme, E.C.2.3.2.2, (5-L-Glutamyl)-peptide: amino-acid 5-glutamyl

transferase. The expert .Panel on Enzymes of the International Federation of Clinical Chemistry, which developed the Recommended Method, also used this name. Some authors have continued to use the older name gamma-glutamyletranspeptidase. In order to conform to clinical practice and to avoid use of the Greek character, the abbreviation GGT is used throughout this review.

Structural studies in prokaryotes and eukaryotes, it is an enzyme that consists of two polypeptide chains, a heavy and a light subunit, processed from a single chain precursor by an autocatalytic cleavage. The active site of GGT is known to be located in the light subunit (Whitfield,. 2001).

2.2.1 Activity of the gamma glutamyltransferase (GGT):

In serum or plasma is commonly measured in clinical laboratories as a sensitive but not very specific liver function test. The measurement of GGT using automated analyzers is quick, cheap, and precise. However, the application of the test is based mostly on empirical evidence and on evaluations of its clinical characteristics, rather than on any deep understanding of the pathophysiological basis of the abnormalities of GGT in liver disease or in other conditions. GGT measurement was introduced into clinical laboratories some 35 years ago, and over that time a large amount of information on factors influencing its activity in serum has accumulated. Theories have been put forward about its normal function within the body and its role in numerous pathological conditions (Shiozawa, et al,. 1990).

Within the last few years there have been significant advances in understanding of GGT's physiological roles and their consequences at a cellular level. In addition, prospective epidemiological studies have shown that variation in GGT in the general population is associated with variation in mortality and morbidity. General reviews from a clinical perspective were undertaken by Rosalki3 in 1975 and by Goldberg4 in 1980, and material from these is not repeated in detail. Nevertheless, some older work is mentioned

where it is relevant to the main theme of this review. Reviews on selected aspects of GGT have been written by Nemesanszky and Lott5 on isoenzymes (Lieberman et al 1989). On gene expression, Wolf and Gassen on GGT activity at the blood-brain barrier, Taniguchi and Ikeda on the catalytic mechanism of GGT and gene expression, Hanigan on GGT in carcinogenesis, and Ristoff and Larsson on enzyme defects in the gammaglutamyle cycle. Reviews in languages other than English have also appeared, but, unfortunately, they are less accessible to most readers. The aim of the current review is to collect and connect experimental, epidemiological, and clinical facts and opinions on GGT. In particular, it attempts to assess and explain some of the epidemiological and clinical results in light of our knowledge of the enzyme's physiological actions and pathological changes (Garcion, et al,. 1990). With this knowledge, the interpretation of results in either the clinical or epidemiological context can be improved, and some directions for further research can be identified. The topic has been divided into three main sections: first, the physiological and pathophysiological significance of GGT; second, the clinical uses of GGT; and third, the epidemiological evidence that increased serum GGT is a prospective risk factor for death or ill health in humans (Novogrodsky, et al,. 1988).

2.2.2 Normal Function:

As mentioned previously, the most abundant substrate for GGT is glutathione, and its physiological roles relating to glutathione have been investigated extensively. Some other compounds found in vivo have the necessary gammaglutamyle structure, including glutathione conjugates of xenobiotic such as the antitumor agent cisplatin, and leukotriene's, and GGT participates in their metabolism. One of the early observations about GGT was that its activity was greatest in tissues with a transport function, such as the kidneys and in the biliary system. This led to the suggestion that GGT played an important role in the transport of amino acids, through a sequence of reactions

forming a “gammaglutamyle cycle”. Amino acid transport is probably not a significant function of GGT, because humans or animals with GGT deficiency do not show generalized disturbances of amino acid transport, but it is clear that GGT is important for the availability of the amino acid cysteine (Delanghe, et al,. 1989). However, GGT does form a key part of a cyclic process whereby glutathione in extracellular fluids can be broken down at the cell membrane to its constituent amino acids that can readily be taken up by cells possessing GGT (because the amino acids are released in close proximity) and used for synthesis of glutathione. Evidence of the normal function of GGT has come from multiple sources, some circumstantial and some direct. These include the location of the enzyme at membrane transport sites, where it has been detected by histochemical or immune histochemical methods; experiments using GGT inhibitors such as acivicin and serine-borate, cell transfection, knockout animals and human subjects with GGT deficiency; and study of the response of GGT enzyme, protein, or mRNA concentrations to the manipulation of glutathione status. The relationship between GGT and glutathione has been investigated extensively at the cellular level, and the results and implications of such studies are considered below. Specific interactions between alcohol and glutathione and their implications for the response of GGT to excessive alcohol consumption are considered in the section on GGT as a marker of alcohol use or abuse. Three general papers illustrate aspects of the interactions between GGT and its natural substrate — glutathione. One of the three amino acids in glutathione is cysteine, and it is cysteine that is most likely to be undersupplied. When diets low in protein, and particularly in sulfur-containing amino acids, were fed to rats, there were significant changes in hepatic glutathione and GGT activity (Rhone, etal,. 1976).

2.2.3 Effects of GGT Deficiency:

A small number of patients with GGT deficiency have been described, and mice with modifications to the GGT gene have been produced. As with many other gene defects, the occurrence of GGT deficiency provides information on the normal functions of the gene product. Human GGT deficiency is rare and only five patients have been reported (O'Daley, et al,. 1971). The family histories suggest recessive inheritance. The deficiency generally has been associated with intellectual impairment, but this may be because such patients tend to be screened for inborn errors of metabolism; in one case50 no intellectual problems were present. In humans, GGT deficiency is compatible with life. In the GGT-deficient mice, on the other hand, growth retardation and early death are among the symptoms in the homozygous-deficient animals. It is not clear why the effects of GGT deficiency are milder in the human patients than in the genetically engineered mice. The biochemical features in both humans and mice are consistent with the major role for GGT being in glutathione metabolism. Urine of the patients contains abnormally large amounts of glutathione-related peptides, showing that GGT is involved in the reabsorption of glutathione and/or its component amino acids from the glomerular filtrate. Tubular reabsorption of amino acids was found to be normal in two subjects, which is against the hypothesis that GGT plays a general role in amino acid transport at cell membranes through the operation of the gammaglutamyle cycle .In GGT-deficient mice there was evidence of high extracellular (and urinary) glutathione but low levels within some tissues, including the liver and pancreas. Plasma cyst(e)ine concentrations were also low, consistent with glutathione and GGT normally being a major source of this amino acid in extracellular fluids (Harding, et al,. 1997).

2.3 Lactate dehydrogenase (LDH, EC 1.1.1.27):

Lactate dehydrogenase is a hydrogen transfer enzyme that catalyses the oxidation of

L-lactate to pyruvate with nicotinamide-adenine dinucleotide (NAD^+) as hydrogen acceptor, the final step in the metabolic chain of anaerobic glycolysis. The reaction is reversible and the reaction equilibrium strongly favours the reverse reaction namely the reduction of pyruvate (P) to lactate (L) (Chapman, et al,. 1999).

$$\text{L-lactate} + \text{NAD} \xleftrightarrow[\text{PH 7.4–7.8}]{\text{LDH, pH 8.8–9.8}} \text{Pyruvate} + \text{NADH} + H^+$$

2.3.1 Enzyme isoforms:

Functional lactate dehydrogenase are homo or hetero tetramers composed of M and H protein subunits encoded by the LDHA and LDHB genes, respectively:

LDH-1 (4H)—in the heart and in RBC (red blood cells), as well as the brain.

LDH-2 (3H1M)—in the reticuloendothelial system

LDH-3 (2H2M)—in the lungs

LDH-4 (1H3M)—in the kidneys, placenta, and pancreas

LDH-5 (4M)—in the liver and striated muscle.

The five isoenzymes that are usually described in the literature each contain four subunits. The major isoenzymes of skeletal muscle and liver, M4, has four muscle (M) subunits, while H4 is the main isoenzymes for heart muscle in most species, containing four heart (H) subunits. The other variants contain both types of subunits (VanEerd, et al,. 1996).

Usually LDH-2 is the predominant form in the serum. A LDH-1 level higher than the LDH-2 level (a "flipped pattern") suggests myocardial infarction (damage to heart tissues releases heart LDH, which is rich in LDH-1, into the bloodstream). The use of this phenomenon to diagnose infarction has been largely superseded by the use of Troponin I or T measurement (Summermatter, et al,. 2013).

2.3.2 Role in muscular fatigue:

The onset of acidosis during periods of intense exercise is commonly been attributed to accumulation of lactic acid. From this reasoning, the idea of lactate production being a primary cause of muscle fatigue during exercise has been widely adopted. A closer, mechanistic analysis of lactate production under anaerobic conditions shows that there is no biochemical evidence for the production of lactate through LDH contributing to acidosis. While LDH activity is correlated to muscle fatigue the production of lactate by means of the LDH complex works as a system to delay the onset of muscle fatigue (Tesch, et al,. 1978).

LDH works to prevent muscular failure and fatigue in multiple ways. The lactate-forming reaction generates cytosolic NAD+, which feeds into the glyceraldehyde 3-phosphate dehydrogenase reaction to help maintain cytosolic redox potential and promote substrate flux through the second phase of glycolysis to promote ATP generation. This, in effect, provides more

energy to contracting muscles under heavy workloads. The production and removal of lactate from the cell also ejects a proton consumed in the LDH reaction- the removal of excess protons produced in the wake of this fermentation reaction serves to act as a buffer system for muscle acidosis. Once proton accumulation exceeds the rate of uptake in lactate production and removal through the LDH symport, muscular acidosis occurs (Juel, et al,. 2004).

2.3.3 Medical relevance:

LDH is a protein that normally appears throughout the body in small amounts. Many cancers can raise LDH levels, so LDH may be used as a tumor marker, but at the same time, it is not useful in identifying a specific kind of cancer. Measuring LDH levels can be helpful in monitoring treatment for cancer. Noncancerous conditions that can raise LDH levels include heart failure, hypothyroidism, anemia, pre-eclampsia, meningitis, encephalitis, acute pancreatitis, HIV and lung or liver disease (Spriet, et -al,.2000).

Tissue breakdown releases LDH, and therefore LDH can be measured as a surrogate for tissue breakdown, e.g. hemolysis. LDH is measured by the lactate dehydrogenase (LDH) test (also known as the LDH test or Lactic acid dehydrogenase test). Comparison of the measured LDH values with the normal range helps guide diagnosis (Irani, et al,. 1997)**.**

(i) Cancer cells: LDH is involved in tumor initiation and metabolism. Cancer cells rely on anaerobic respiration for the conversion of glucose to lactate even under oxygen-sufficient conditions (a process known as the Warburg effect (Warburg O 1956). This state of fermentative glycolysis is catalyzed by the A form of LDH. This mechanism allows tumorous cells to convert the majority of their glucose stores into lactate regardless of oxygen availability,

shifting use of glucose metabolites from simple energy production to the promotion of accelerated cell growth and replication. For this reason, LDH A and the possibility of inhibiting its activity has been identified as a promising target in cancer treatments focused on preventing carcinogenic cells from proliferating. Chemical inhibition of LDH A has demonstrated marked changes in metabolic processes and overall survival carcinoma cells. Oxamate is a cytosolic inhibitor of LDH A that significantly decreases ATP production in tumorous cells as well as increasing production of reactive oxygen species (ROS). These ROS drive cancer cell proliferation by activating kinases that drive cell cycle progression growth factors at low concentrations, but can damage DNA through oxidative stress at higher concentrations. Secondary lipid oxidation products can also inactivate LDH and impact its ability to regenerate NADH directly disrupting the enzymes ability to convert lactate to pyruvate. (Ramanathan, et al,. 2014). While recent studies have shown that LDH activity is not necessarily an indicator of metastatic risk, LDH expression can act as a general marker in the prognosis of cancers. Expression of LDH5 and VEGF in tumors and the stroma has been found to be a strong prognostic factor for diffuse or mixed-type gastric cancers (Kim, et al,. 2014).

(ii)Hemolysis: In medicine, LDH is often used as a marker of tissue breakdown as LDH is abundant in red blood cells and can function as a marker for hemolysis. A blood sample that has been handled incorrectly can show false-positively high levels of LDH due to erythrocyte damage. It can also be used as a marker of myocardial infarction. Following a myocardial infarction, levels of LDH peak at 3–4 days and remain elevated for up to 10 days. In this way, elevated levels of LDH (where the level of LDH1 is higher than that of LDH2, i.e. the LDH Flip, as normally, in serum, LDH2 is higher than LDH1) can be useful for determining whether a patient has had a

myocardial infarction if they come to doctors several days after an episode of chest pain (Heffner, et al,. 1997).

(iii)Tissue turnover: Other uses are assessment of tissue breakdown in general; this is possible when there are no other indicators of hemolysis. It is used to follow-up cancer (especially lymphoma) patients, as cancer cells have a high rate of turnover with destroyed cells leading to an elevated LDH activity (Light, et al,. 1972).

(iv)Meningitis and encephalitis: High levels of lactate dehydrogenase in cerebrospinal fluid are often associated with bacterialmeningitis. In the case of viral meningitis, high LDH, in general, indicates the presence of encephalitis and poor prognosis (Stein,. 2013).

(v)HIV: LDH is often measured in HIV patients as a non-specific marker for pneumonia due to Pneumocystis jiroveci (PCP). Elevated LDH in the setting of upper respiratory symptoms in an HIV patient suggests, but is not diagnostic for, PCP. However, in HIV-positive patients with respiratory symptoms, a very high LDH level (>600 IU/L) indicated histoplasmosis (9.33 more likely) in a study of 120 PCP and 30 histoplasmosis patients (Butt, et al,. 2002).

3. Materials and Methods

3.1 Materials:

3.1.1 Study design:

This is case control hospitalize base study.

3.1.2 Study area:

The study was conducted in preeclamptic pregnant women in Omdurman maternity hospital and medical military hospital in Khartoum state.

3.1.3 Study period:

During March to June 2015.

3.1.4 Study population:

In this study100 pregnant women were chosen for determination of plasma gamma glutamyltransferase and lactate dehydrogenase,50 of them were diagnose with preeclampsia 27 from this is severe preeclampsia and 23 is mild preeclampsiawas matched in age. Classify severe and mild preeclampsia according to blood pressure Mild preeclampsia was defined as onset of hypertension after 20 weeks of gestation with diastolic blood pressure (DBP) >90 and ≤ 110 mmHg with or without proteinuria. When diastolic blood pressure (DBP)>110 mmHg with significant proteinuria Preeclampsia was considered as severe. And 50 are normal pregnancy women in third trimester in Omdurman city hospital during March to June 2015.

3.1.5 Inclusion Criteria:

Preeclampsia pregnant women were included.

3.1.6 Exclusion criteria:

Pregnant women with kidney diseases, heart diseases, diabetes mellitus, gestational diabetes, liver diseases, muscle diseases and hemolytic anemia were excluded.

3.1.7 Data collection:

The clinical data were obtained from history; clinical examination and hospital follow up records and were recorded on a questionnaire sheet.

3.1.8 Samples:

About 3ml of venous blood were collected from each pregnant woman's in heparinized container .The samples collected under aseptic conditions. Centrifuged for 3 minutes at 3000 RPM to obtain plasma, and analyzed.

3.1.9 Equipment's:

- Spectrophotometer, model Model=BTS.310 Serial NO. =801560278
- Centrifuge
- Lithium heparins containers
- Disposable syringes
- 70% alcohol
- Tourniquets
- Cotton
- automatic pipette

3.1.10 Ethical consideration:

- Approval of the hospital administration conducts scientific research within the hospital.
- Pregnant women are who voluntarily accepted to participate in the study were included.

3.2 Methodology:

Estimation of gamma glutamyletransferase concentration method:

Principle of method:(appendix II)

Gamma-glutamyltransferase (g-GT) catalyzes the transfer of the g-glutamyl group from g-glutamyl-3-carboxy-4-nitroanilide to glycylglycine, liberating 3-carboxy-4 nitroaniline. The catalytic concentration is determined from the rate of 3-carboxy-4-nitroaniline formation.

g – Glutamyl – 3 – carboxy – 4 – nitroanilide + Glycylglycine $\xrightarrow{\text{g -GT}}$

g – Glutamyl – glycylglycine + 3 – carboxy – 4 –nitroanilin.

Normal Values

Assay temp.	Men	Women
25◦ C	6-28 U/L	4-18 IU/L
30◦ C	8-38 U/L	5-25 IU/L
37 ◦C	11-50 U/L	7-32 IU/L

Estimation of lactate dehydrogenase concentration method:

Principle of method:(appendix III)

Lactate dehydrogenase (LD or LDH) catalyzes the reduction of pyruvate by NADH, to form lactateand NAD+. The catalytic concentration is determined from the rate of decrease of NADH, measured at 340 nm

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow[\text{PH 7.4–7.8}]{\text{LDH}} \text{Lactate} + NAD^+$$

Normal value:

Reaction Temperature	Adults
25ºC	105-210IU/L
30ºC	140-280 IU/L
37ºC	207-414 IU/L

3.3 Quality control:

The precision and accuracy of all method used in this study were checked each time a batch was analyzed by including commercially prepared control sera.

3.4 Statistical analysis:

The data was recorded and analyzed using statistical package for social sciences (SPS –version 16) on programmed computer.

The mean standard deviations of variable were calculated for both the test group and the control group and P value for comparison was obtained.

P value$\leq$ 0.05 was considered significant.

Pearson's correlation and linear regression were used to access the relationship between different variable.

4. Result

In this study100 pregnant women were chosen for determination of plasma gamma glutamyltransferase and lactate dehydrogenase,50 of them were diagnose with preeclampsia 27 from this are severe preeclampsia and 23 are mild preeclampsiawas matched in age. And 50 are normal pregnancy women in third trimester. During the period from March to June 2015. The results obtained were statistically analyzed, using SPSS T. test. The level of significance was expressed as P value ≤ 0.05 for significant result.

Table 4.1 showsthe mean values of plasma gamma glutamyltransferase in mild preeclamptic pregnant women when compared to control **(14.50±7.83) (11.64±2.335) IU/L** respectively with **P value=0.346** .

Table 4.1 shows the mean values of plasma gamma glutamyltransferase in severe preeclamptic pregnant women when compared to control **(16.25±8.28) (11.64±2.335) IU/L** respectively with **P value=0.089**.

Table 4.1 shows the mean values of plasma lactate dehydrogenase in mild preeclamptic pregnant women when compared to control **(458.25±157.29) (254.09±35.02) IU/L** respectively with **P value =0.008**.

Table 4.1 shows the mean values of plasma lactate dehydrogenase in severe preeclamptic pregnant women when compared to control **(615.83±278.92) (254.09±35.02) IU/L** respectively with **P value =0.001**.

Figure4.1 shows positive moderate correlation between lactate dehydrogenase and diastolic blood pressure of Sudanese pregnant women in sever hypertensive preeclampsia **p value = 0.006**and **Pearson correlation = .851**.

Figure 4.2 shows positive moderatecorrelation between lactate dehydrogenase and diastolic blood pressure of Sudanese pregnant women in mild hypertensive preeclampsia **p value = 0.013**and **Pearson correlation = .782**.

Figure 4.3 shows **insignificant correlation** between gamma glutamyltransferase and diastolic blood pressure in sever preeclampsia **p value = 0.359** and**Pearson correlation = .215**.

Figure 4.4 shows **insignificant correlation** between gamma glutamyltransferase and diastolic blood pressure in mild preeclampsia **p value = 0.652** and **Pearson correlation = - .189**.

Table 4.1: The mean and SD of plasma gamma glutamyltransferase and lactate dehydrogenase between cases and control groups**:**

parameter	mild preeclampsia Mean ± SD	Severe preeclampsia Mean ± SD	Control Mean ± SD	P value
GGT	14.50±7.83*	16.25±8.28**	11.64±2.335	P=0.346* / P=0.089**
LDH	458.25±157.2*	615.83±278.92**	254.09±35.02	P=0.008* / P=0.001**

Independent T. test the mean difference is significant at the ≤0.05 level

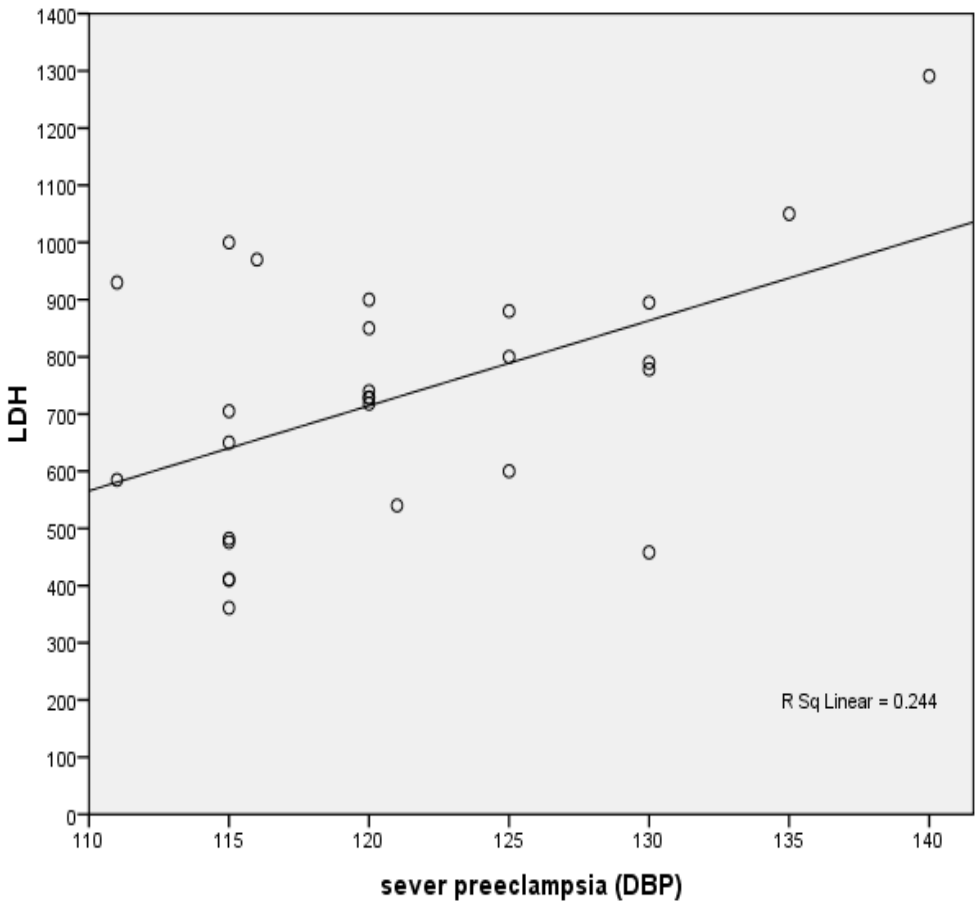

Figure 4.1: Scatter plot show significant positive moderate correlation between lactate dehydrogenase and diastolic blood pressure insever preeclampsia **p value = 0.006**and **Pearson correlation r = .851.**

Correlation is significant at the 0.01 level (2-tailed).

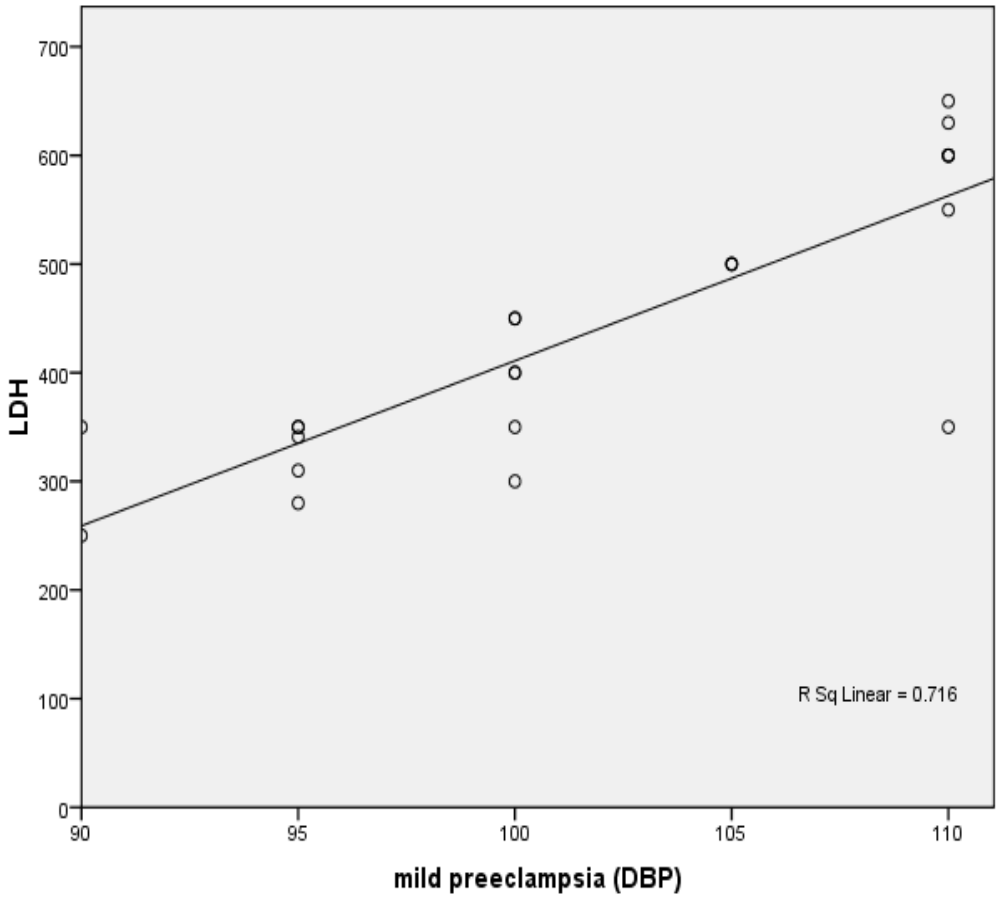

Figure 4.2: Scatter plot show significant positive moderate correlation between lactate dehydrogenase anddiastolic blood pressure in mild preeclampsia **p value = 0.013**and **Pearson correlation r= .782.**

Correlation is significant at the 0.05 level (2-tailed).

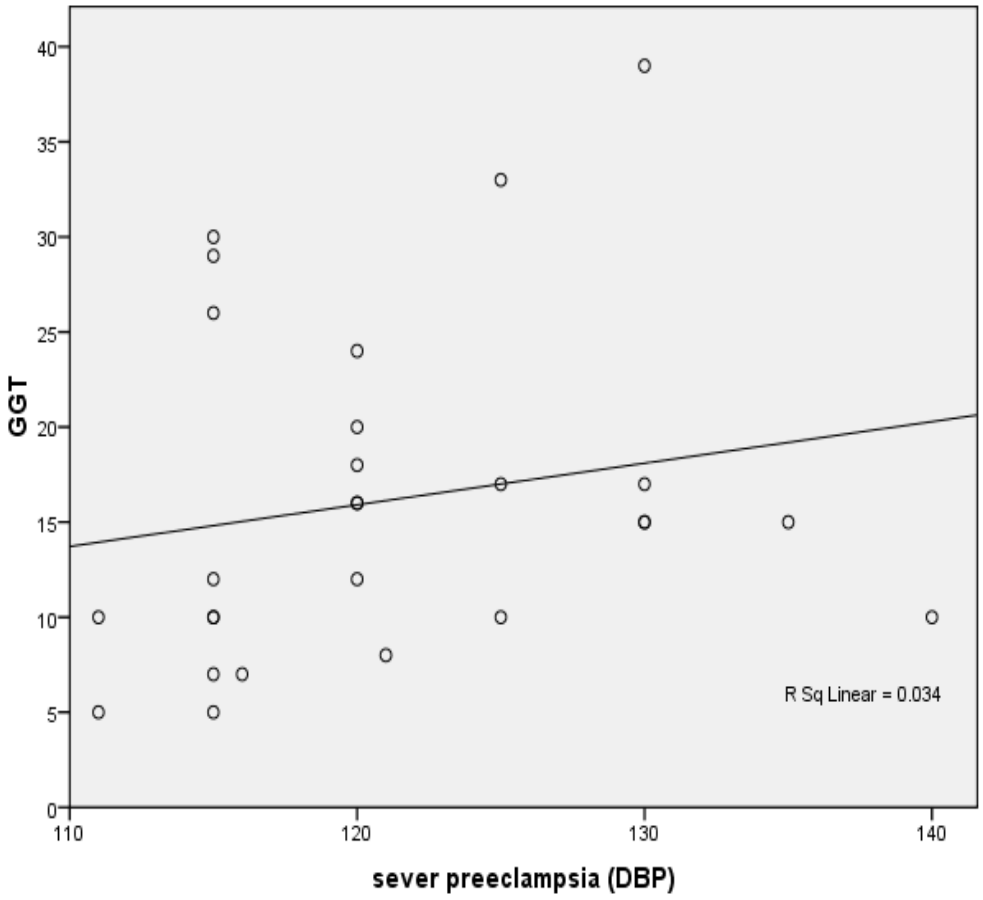

Figure 4.3: Scatter plot showed **insignificant correlation** between gamma glutamyltransferase and diastolic blood pressure in sever preeclampsia **p value = 0.359** and **Pearson correlation r= .215**

Correlation is significant at the 0.05 level (2-tailed).

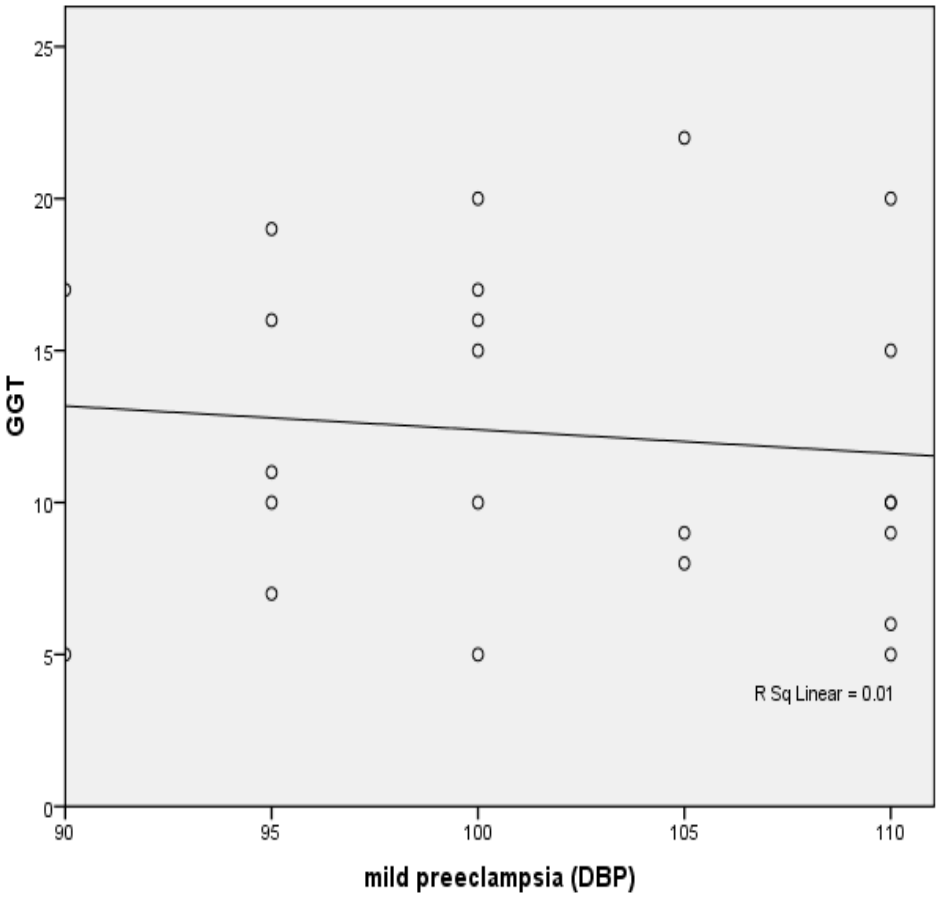

Figure 4.4: Scatter plot showed **insignificant correlation** between gamma glutamyltransferase and diastolic blood pressure in mild preeclampsia **p value = 0.652**

And **Pearson correlation r = - .189**

Correlation is significant at the 0.05 level (2-tailed).

5. Discussions, Conclusion and Recommendation

5.1 Discussions:

Preeclampsia is a pregnancy-specific disease with multisystem complications. Several potential markers have been proposed to predict the severity of preeclampsia. Most usefulamong these are GGT and LDH. There are very limitedstudies with conflicting data on GGT and LDH inpreeclampsia. This study was undertaken to investigate the possible role of GGT and LDH in theprediction of severity of preeclampsia to prevent furthercomplications.

Our data support this hypothesis suggesting an association between serum LDH levels and preeclampsia it was found that 80% of preeclamptic women had abnormal levels of **LDH > 600 IU/L**. In agreement withprospective study was conducted in the department Of Biochemistry, S.R.T.R. Medical College and Hospital, Ambajogai during the period of 2006 -2007. A total of 40 preeclampsia women (22 with mild and 18 with severe Preeclampsia) and 40 healthy normotensive pregnant women (controls) were enrolled in the study the mean level of**LDH >800 IU**/L (Munde, et- al,. 2014).

We propose that the multiorgan dysfunction in severe preeclampsia caused by vascular endothelial damage, including maternal liver, kidney, lungs and coagulation system; will lead to excessive LDH leakage and elevated levels in serum due to cellular dysfunction (Peralta Pedrero,et al,. 2004)(Beyer, et al,. 1991)(Makkonen, et- al ,.1980).

level of LDH increase in severe preeclampsia **615.83 IU/L ,P value .001** rather than mild preeclampsia **458.25 IU/L** , **P value .008** that is agreement with previous findings.

In our study it was found that 90% of preeclamptic women had normal levels **of GGT<25 IU/L**.In not agreement with previous findings mean level of**GGT > 70** IU/L (Munde, et al,. 2014)

level of GGT increase in severe preeclampsia **16.25 IU/L,P value .089**rather than mild preeclampsia **14.50 IU/L, P value .346** but this different of increase within normal value that is no agreement with previous findings (Munde, et al,. 2014).

Also our result there is showed significantpositive moderate correlation between lactate dehydrogenase and blood pressure of Sudanese pregnant women with sever and mild hypertensive preeclampsia disease.

5.2 Conclusion:

From the result of this study it is concluded that:

1- Levels of LDH are raised in Sudanese pregnant women with hypertensive preeclampsia disease.
2- Levels of GGT are normal in Sudanese pregnant women with hypertensive preeclampsia disease.
3- Show positive moderate correlation between LDH and diastolic blood pressure of Sudanese pregnant women in sever or mild hypertensive preeclampsia disease.
4- Show insignificant correlation between gamma glutamyltransferase and diastolic blood pressure in Sudanese pregnant women with hypertensive preeclampsia disease.

5.3 Recommendations:

- Use of LDH as biochemical markers which reflects the severity of preeclampsia and useful for the management of preeclampsia to decrease maternal and fetal morbidity and mortality
- Further explorations of the effect of preeclampsia on other parameters.
-More studies to determine the specific cause of increase level of Lactate dehydrogenase and how to manage it.
- Periodically check the lactate dehydrogenase and blood pressure for pregnant women infected with preeclampsia so recommended to be done.
-Comparison of the result with more data collected from pregnant women such as larger size of sample to determine the exactly association of preeclampsia and level of Lactate dehydrogenase should be carefully analyzed and interpreted.

- **Ali,A.A,andAdam,I.(2011).**Lack of antenatal care ,education,andhighmaternalmortalityinKassalahospi-tal, easternSudanduring2005-2009. J. Matern.Fetal.Neonatal.Med, p: **1077–1078**.
- **Ali,A.A and Okud,A.(2012).**High incidence of obstetric complications in Kassala hospital, easternSudan. J. Obstet. Gynaecol**,p: 148–149**.
- **Al-Jameil, N, Aziz Khan, F Fareed Khan, and M; Tabassum,(2014).** "A brief overview of preeclampsia.". Journal of clinical medicine research 6 (1): 1–7
and Female Non-Drinkers. J.HumHypertens, **P:4:609-14**.
- **Arulkumaran, N., and Lightstone, L. (2013).** "Severe pre-eclampsia and hypertensive crises". Best Practice & Research Clinical Obstetrics &Gynaecology,P:**(6): 877–884**.
- **Butt AA, Michaels S, Greer D, Clark R, Kissinger P and Martin DH (2002).** "Serum LDH level as a clue to the diagnosis of histoplasmosis". AIDS Read 12 P: (7): **317–21.**
- **Davis, J. A. and Gallup, G. G. J. (2006).**Platek, Steven M; Shackelford, Todd K, eds. "Female Infidelity and Paternal Uncertainty". **p. 191**.
- **De-Oliveira IM, Fujimori E and Pereira VG (1999).** DL-methionine supplementation of rice-andbean diets affects gamma glutamyltranspeptidase activity and glutathione content in livers of growing rats. Braz J Med Biol Res; **32 p: 483–8**.
- **F. Gary Cunningham.etal (2010).**Williams obstetrics. (23rd ed. ed.). New York: McGraw-Hill Medical.
- **Garcion E, Sindji L and LeblondelG(1999).** 1,25–dihydroxyvitamin D3 regulates the synthesis of gamma-glutamyltranspeptidase and glutathione levels in rat primary astrocytes. J Neurochem, P:**73: 859–66**.
- **Harding CO, Williams P and Wagner E(1997).** Mice with genetic gamma-glutamyltranspeptidase deficiency exhibit glutathionuria, severe growth failure, reduced life spans, and infertility. J BiolChem P:**272: 607.**

- **Heffner JE, Brown LK and Barbieri CA (1997).** "Diagnostic value of tests that discriminate between exudative and transudative pleural effusions. Primary Study Investigators". Chest 111 P:**(4): 970–80**.
- **Irani K, Xia Y, Zweier JL, Sollott SJ, Der CJ and Fearon ER (1997**). "Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts.". Science 275 (5306) P: **1649–52**.
- **J. B. Whitfield (2001)**. Critical Reviews in Clinical Laboratory Sciences, 38(4) P:**263–355**
- **Jean JC, Harding CO and Oakes SM (1999).**gamma-Glutamyltransferase (GGT) deficiency in the GGTenu1 mouse results from a single point mutation that leads to a stop codon in the first coding exon of GGT mRNA. Mutagenesis, P:**14: 31–6.**
- **John R. Barton and Baha M. Sibai (2000).** Management of Mild):**263–355.**
- **Juel C, Klarskov C, Nielsen JJ, Krustrup P, Mohr M andBangsbo J (2004).** "Effect of high-intensity intermittent training on lactate and H+ release from human skeletal muscle.". Am J PhysiolEndocrinolMetab 286 (2) P: **E245–51.**
- **Kim HS, Lee HE, Yang HK and Kim WH (2014**). "High lactate dehydrogenase 5 expression correlates with high tumoral and stromal vascular endothelial growth factor expression in gastric cancer.". Pathobiology 81 **(2) P: 78–85.**
- **Lambert, G; Brichant, JF; Hartstein, G; Bonhomme, V; Dewandre, PY (2014).** "Preeclampsia: an update.".ActaanaesthesiologicaBelgica**65 (4) P: 137–49.**
- **Laresgoiti-Servitje E, Gómez-López N and Olson DM (2010**).gamma-Glutamyltransferase (GGT) deficiency in the GGTenu1 mouse results from a single point mutation that leads to a stop codon in the first coding exon of GGT mRNA. Mutagenesis, P:**14: 31–6.**

- **Leiberman, J.R., Fraser, D.,Kasis, A., and Mazor,M.(1991).**Reducedfrequencyofhypertensivedisorders in placentapreviaObstet. Obstet. Gynecol**,P: 83–86.**
- **Light RW, Macgregor MI, Luchsinger PC and Ball WC (1972).** "Pleural effusions: the diagnostic separation of transudates and exudates". Ann. Intern. Med. P:77**(4): 507–13.**
- **McMaster-Fay RA (2008).** "Pre-eclampsia: a disease of oxidative stress resulting from the catabolism of DNA (primarily fetal) to uric acid by xanthine oxidase in the maternal liver; a hypothesis.". Bioscience Hypotheses **P: 35–43.**
- **Mustafa, Reem, Ahmed, Sana, Gupta, Anu, Venuto and Rocco C. (2012).** "A Comprehensive Review of Hypertension in Pregnancy". Journal of Pregnancy 2012**P: 1–19.**
- **Novogrodsky A, Tate SSand Meister A(1976).**gamma-Glutamyltranspeptidase, a lymphoid cellsurface marker: relationship to blastogenesis, differentiation, and neoplasia. ProcNatlAcadSci U S A, P:**73: 2414–8.**
- **O'Daley(1968).** An abnormal sulphydryl compound in urine. Irish J Med Sci, p:**7: 578–579 .**
- **Ramanathan R, Mancini RA, Suman SP and Beach CM (2014**). "Covalent Binding of 4-Hydroxy-2-nonenal to Lactate Dehydrogenase Decreases NADH Formation and Metmyoglobin Reducing Activity.". J Agric Food Chem**62, P: 2112–7.**
- **Redman CW and Sargent IL (2005).** "Latest Advances in Understanding Preeclampsia". Science **308 (5728) P: 1592–1594.**
- **Rhone DP and White FM (1976).** Effects of storage in the cold on activity of gamma-glutamyltransferase in serum. ClinChem,**22 P: 103–4.**
- **Rojas E, Valverde M and Kala SV (2000).** Accumulation of DNA damage in the organs of mice deficient in gamma-glutamyltranspeptidase. Mutat Res **14; 447 P: 305–16.**

- **Shiozawa M, Hiraoka Y and Yasuda K (1990).** Synthesis of human gamma-glutamyltranspeptidase (GGT) during the fetal development of liver. Gene; **87 P: 299–303.**
- **Spriet LL, Howlett RA and Heigenhauser GJ (2000).** "An enzymatic approach to lactate production in human skeletal muscle during exercise.". Med Sci Sports Exerc**32 (4)P: 756–63.**
- **Steegers, Eric AP, von Dadelszen, Peter, Duvekot, Johannes J, Pijnenborg, and Robert (2010).** "Pre-eclampsia". The Lancet **376 (9741) P: 631–644**.
- **Stein JH (1998).** Internal Medicine. Elsevier Health Sciences. **p. 1408**.
- **Summermatter S, Santos G, Pérez-Schindler J andHandschin C (2013)**ProcNatlAcadSci U S A**110 (21) p: 8738–43.**
- **Tesch P, Sjödin B, Thorstensson A andKarlsson J (1978**). "Muscle fatigue and its relation to lactate accumulation and LDH activity in man.".ActaPhysiolScand**103 (4) p: 413–20.**
- **Van Eerd, J. P. F. M and Kreutzer (1996).**KlinischeChemievoorAnalistendeel**p. 138–139.**
- **Var A, Yildirim Y andWarburg O "OnurE(1956**). Endothelial Dysfunction in

On the origin of cancer cells.".Science **123 (3191) P: 309–14.**
- **Xu HN, Kadlececk S, Profka H, Glickson JD andRizi R, Li LZ (2014).** "Is Higher Lactate an Indicator of Tumor Metastatic Risk? A Pilot MRS Study Using Hyperpolarized (13)C-Pyruvate.". AcadRadiol**21 (2) p: 223–31.**