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Department of Chemistry & Biochemistry

Matrix Metalloproteinase -9 and IL-18 Level in Cord Blood and Tissue Matrix in Caesarean and Normal Vaginal Delivery

A thesis

Submitted to the College of Medicine/ Al-Nahrain University in Partial Fulfillment of the Requirement for the Degree of Doctor of Philosophy In

Clinical Biochemistry

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وَلَمْ يُولَدْ ﴿٣﴾ وَلَمْ يَكُن لَّهُ كُفُوًا أَحَدٌ ﴿٤﴾}

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We certify that this thesis was prepared under our supervision at College of Medicine-Al-Nahrain University, as a partial requirement for the degree of Doctor of Philosophy in Clinical Biochemistry.

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Dedication

To the spirit of my life who supports me

To my family

Brothers and sisters

And

To My Father & My Mother souls

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Abstract

Matrix metalloproteinases (MMPs) are calcium-dependent zinc-containing endopeptidases. These enzymes are capable of degrading all kinds of extracellular matrix proteins, but also can process a number of bioactive molecules. They are known to be involved in the cleavage of cell surface receptors, the release of apoptotic ligands (such as the FAS ligand), and cytokine inactivation. During pregnancy, specific physiological changes such as cervical ripening, rupture of the fetal membranes, and placental detachment require the remodeling of the extracellular matrix (ECM). Cytokine like Interleukin -18(IL-18) play a critical role in host defense, and it may participate in providing a cervical/decidual barrier against microbial invasion of the amniotic fluid and might serve as a cervical marker for intra-amniotic infection. Oxidative Stress, a state characterized by an imbalance between pro-oxidant molecules, including reactive oxygen species (ROS), with antioxidant defense. These ROS may cause tissue injury resulting in cytotoxic damage to cellular proteins, and DNA which has been implicated in early pregnancy complications and during ,labor and may effect on mode of labor. Body defense response involves enzymatic and non-enzymatic antioxidant buffering pathways which reduce the effect of these free radicals. This study aimed to assess the relationship between matrix metalloproteinase enzyme levels and delivery mode and also an attempt to assess the impact of oxidative stress on maternal antioxidant defense during labour and correlate it with mode of parturition. **Methods:** A case-control study design was employed in this study. Sixty four pregnant women were recruited 31 term pregnant women with normal vaginal delivery and 33 term pregnant women underwent cesarean section delivery. At the

time of their admission to operation theatre, blood samples were taken and a piece of tissue was collected from umbilical cord after delivery .

Blood and tissue samples were used to measure matrix metalloproteinase (MMP-9), Interleukin-18 (IL-18), Superoxide Dismutase (SOD) activity and trace elements levels was determined in the umbilical cord blood only. Placental tissue was prepared and stained for histological assessment. Anti-MMP-9 and Anti-IL-18 monoclonal antibody laboratory kits were employed to demonstrate degradation and cytokine levels in umbilical cord tissue.

Results: results showed that both blood MMP-9 and IL-18 levels were significantly higher in term women who underwent cesarean section compared with those women who underwent normal vaginal delivery. In addition, the enzymatic anti-oxidant SOD was significantly lowest among term women who underwent normal vaginal delivery when compared term women who underwent cesarean section. The present study also reported a significant decreases in level of trace elements (Zn, Cu and Mg) in blood of term women who underwent NVD compared those a with C/S labor ($P<0.01$).

The histological examination of umbilical cord tissue revealed that term women underwent normal vaginal delivery group had the lowest reactivity with both anti-MMP-9 and anti-IL-18 monoclonal antibody in comparison to term women underwent cesarean section group.

From the study it was concluded that term pregnancy women underwent normal vaginal delivery was associated with increased oxidative stress and decreased anti-oxidant activity. In contrast, term pregnancy underwent cesarean section was associated with low oxidative stress status and high enzymatic anti-oxidant activity

(SOD). In other hand, pregnancy underwent with cesarean section was significantly associated with high levels of serum MMP-9 and IL-18 and high level of enzymatic anti-oxidant activity (SOD). While the Histological examination showed high MMP-9 and IL-18 concentrations in the umbilical cord tissue of term women underwent cesarean section in comparison to term women underwent normal vaginal delivery.

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Abbreviations

Abbreviation	*Details*
AUC	*Area under curve*
BM-MSC	*Bone marrow-Mesenchymal Stem Cell*
CAT	*Catalase*
C/S	*Cesareans Section*
DNA	*Deoxyribonucleic acid*
ECM	*extracellular matrix*
GSH	*Glutathione*
GPX	*Glutathione peroxidase*
GR	*Glutathione reductase*
HSCs	*Hematopoietic stem cells*
H&E	*Hematoxylin and Eosin*
HEL	*Hexanoyl-Lysine*
H_2O_2	*Hydrogen peroxide*
IRAK	*IL-1 receptor activating kinase*
IHC	*Immunohistochemistry*
IkB	*Inhibitory kappa B*

IKK	*Inhibitory kappa B kinase*
IFN	*Interferon*
ICE	*Interleukin converting enzyme*
ILs	*Interleukins*
MHC	*Major Histocompatibility*
MMP-9	*Matrix metalloproteinase-9*
MMPs	*Matrix metalloproteinases*
MeSH	*Medical subject headings*
MSCs	*Mesenchymal stem cells*
NVD	*Normal vaginal delivery*
OS	*Oxidative Stress*
PBS	*Phosphate Buffer Solution*
PROM	*Premature Rupture of Membrane*
ROS	*Reactive oxygen species*
ROC	*Receiver Operating Characteristics*
RNA	*Ribonucleic acid*
S.Cu	*Serum Copper*
S.Cr	*Serum Creatinine*

S.Mg	*Serum Magnesium*
S.Zn	*Serum Zinc*
SSPS version 22	*Statistical Analysis Software*
SOD	*Superoxide Dismutase*
$^{\bullet}O_2$	*Superoxide radical*
TIMP	*Tissue inhibitors of metalloproteinases*
TRAF	*TNF receptor associated factor*
TGF-β,	*Transforming growth factors-β*
TNF-α	*Tumor necrosis factors-α*
UC	*Umbilical cord*
UC-MSC	*Umbilical cord- Mesenchymal Stem Cell*
VSMCs	*Vascular Smooth Muscle Cells*

Chapter one

Introduction and literature Review

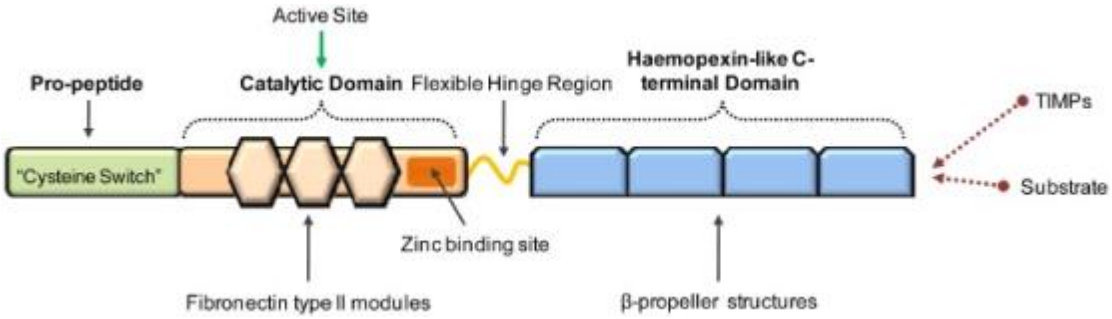
1. Matrix metalloproteinases

1.1 Human matrix metalloproteinases

Matrix metalloproteinases (MMPs) are calcium-dependent zinc-containing endopeptidases. The MMPs belong to a larger family of proteases (Sankari SL., 2016). Collectively, these enzymes are capable of degrading all kinds of extracellular matrix proteins. They are known to be involved in the cleavage of cell surface receptors, the release of apoptotic ligands (such as the (fas) ligand), and cytokine inactivation. (Sankari SL., 2016).

Matrix metalloproteinases (MMPs) consists of more than (23) types from Zn^{+2}-dependent an individual that degrades and remodels multiple components of the extracellular matrix including collagens, fibronectin, laminin, hyaluronin, proteoglycans, and elastin (Bonnans C., 2014). The biological importance of MMPs has been described in multiple cellular processes including proliferation, angiogenesis, migration, host defense, cancer invasion, and metastasis. (Antoine Dufour *etal*. 2010)

Most of the matrix metalloproteinases consist of four distinct domains, which are N-terminal pro-domain, catalytic domain, hinge region, and C-terminal hemopexin- like domain (Figure 1). This may be responsible for the macromolecular substrate recognition as well as for interaction with tissue inhibitors of metalloproteinases (TIMPs). (Wenyang Li., 2016) Figure(2).



Figure(1):Structure of gelatinases (MMP-9). The four common domains are described as the pro-peptide, catalytic domain, and the hemopexin-like c-terminal domain which is connected to the catalytic domain via a flexible hinge region domain (Sara M McCarty, et al.; 2012)·

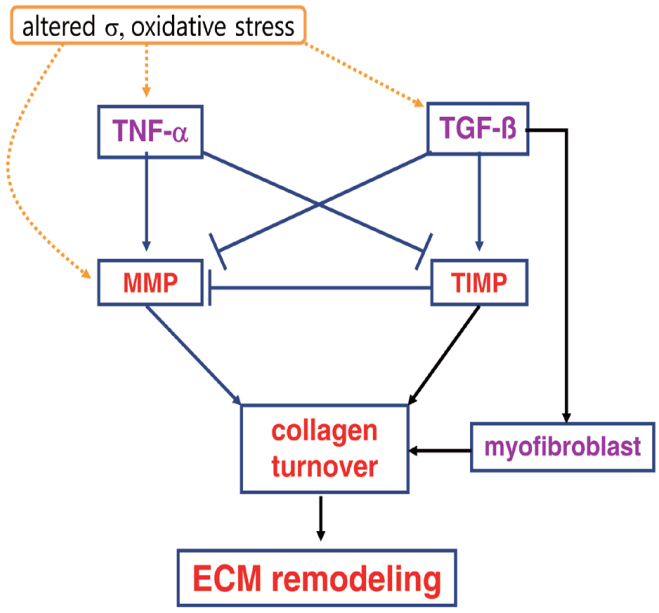


Figure (2):Collagen ECM turnover signaling in the heart. Altered mechanical stress (σ) and oxidative stress may stimulate TNF-α, TGF-β, and MMP. TNF- α may stimulate MMP and inhibit TIMP. However, TGF-β may inhibit MMP and stimulate TIMP and myofibroblast. Finally, MMP degrades collagens, but TIMP and myofibroblast inhibit collagen degradation and promote collagen synthesis, which determine collagen ECM remodeling. (Hyo-Bum Kwak,; 2013)

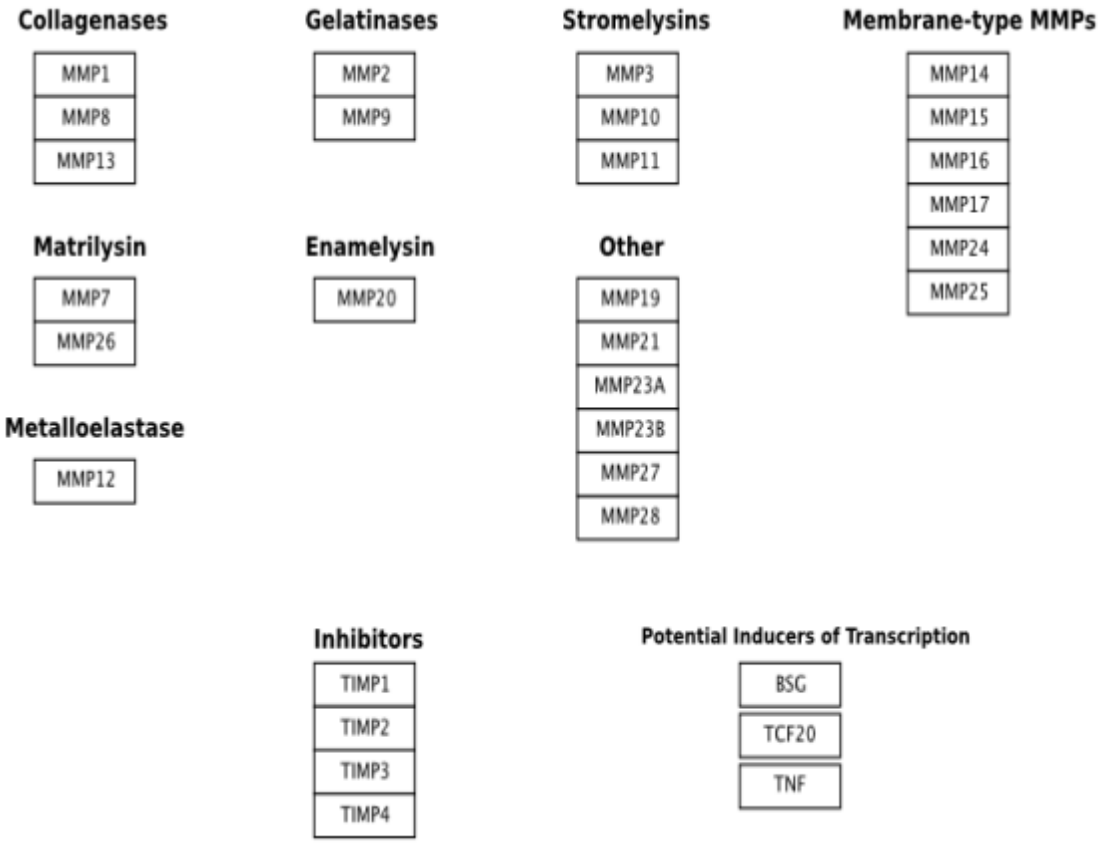
The classification of the MMPs which is most commonly used groupings (by researchers in MMP biology) are based partly on historical assessment of the substrate specificity of the MMP and partly on the cellular localization of the MMP (Figure 3). These groups are the collagenases, the gelatinases, the stromelysins, and the membrane-type MMPs (MT-MMPs)(Snoek-van Beurden; 2005).

- The collagenases are capable of degrading triple-helical fibrillar collagens into distinctive 3/4 and 1/4 fragments. These collagens are the major components of bone, cartilage and dentin, and MMPs are the only known mammalian enzymes capable of degrading them. The collagenases are 1, 8, 13, and 18. In addition, 14 has also been shown to cleave fibrillar collagen, and there is evidence that 2 is capable of collagenolysis.

- The main substrates of the gelatinases are type IV collagen and gelatin, and these enzymes are distinguished by the presence of an additional domain inserted into the catalytic domain. This gelatin-binding region is positioned immediately before the zinc-binding motif, and forms a separate folding unit that does not disrupt the structure of the catalytic domain. The gelatinases are 2 and 9.

- The stromelysins display a broad ability to cleave extracellular matrix proteins but are unable to cleave the triple-helical fibrillar collagens. The three canonical members of this group are 3, 10, and 11.

- All six membrane-type MMPs (14, 15, 16, 17, 24, and 25) have a furin cleavage site in the pro-peptide, which is a feature also shared by 11(Snoek-van Beurden; 2005).



Figure(3):The categories of matrix metalloproteinases (Snoek-van Beurden; 2005).

1.1.2. Matrix metalloproteinase-9 (MMP-9)

The MMP-9 causes degradation of extracellular matrix (ECM) which is an important feature of development, morphogenesis, tissue repair and remodeling. It is precisely regulated under normal physiological conditions, but when dysregulated it becomes a cause of many diseases such as arthritis, nephritis, cancer, encephalomyelitis, chronic ulcers, fibrosis. (Liutkeviciene R., 2014)

. Various types of proteinases are implicated in ECM degradation, but the major enzymes are considered to be matrix metalloproteinases (MMPs), also called matrixins (Reddy NR.,2012) .

The activities of most matrixins are very low or negligible in the normal steady-state tissues, but expression is transcriptionally controlled by inflammatory cytokines, growth factors, hormones, cell–cell and cell–matrix interaction (Bonnans C., 2014). Matrixin activities are also regulated by activation of the precursor zymogens and inhibition by endogenous inhibitors, tissue inhibitors of metalloproteinases (TIMPs). Thus, the balance between MMPs and TIMPs are critical for the eventual ECM remodeling in the tissue (Gong Y., 2014).

Relying on the action of Matrix metalloproteinases (MMPs), the two types of MMPs like MMP-2 and MMP-9 specifically attack type IV collagen, laminin, and fibronectin, the major components of the basal lamina around cerebral blood vessels (Sapojnikova N., 2014).

During pregnancy, specific physiological changes such as cervical ripening, rupture of the fetal membranes, and placental detachment require the remodeling of the extracellular matrix (ECM) (Pereza N., 2014). And the rupturing of the membranes is believed to be the result of the effects of physical forces which are localized in areas surrounding the membranes that are made weaker by the degradation of structural collagens (Chai M., 2013). The MMPs are enzymes allowing for the movement of cells and tissue reorganization in order to support the growing fetus (Myntti T.,2017). Several MMPs are constitutively generated by reproductive tissues, and a fluctuation in the gene expression level of certain

MMPs has been observed during the various stages of parturition (Nicole and Sandra, 2010).

Most studies have reported changes in MMP-1 and MMP-9 in serum, amniotic fluid and fetal membranes during preterm pregnancy. However, since the detachment of the placenta requires ECM degradation in which MMPs are involved, there is a need to examine the levels of MMPs in the placenta for better understanding of their role in delivery(Zhong Y., 2015). Many studies have examined the gene expression of MMP-9 from the placenta in preeclampsia and preterm deliveries but not in term placenta (Sundrani DP.,2012). Furthermore there are few studies which have examined the protein levels of MMP-1 and MMP-9 in term placenta with respect to the mode of delivery *i.e*. spontaneous vaginal delivery or caesarean section (Sundrani *et al* .,2012).

In women with normal pregnancies, elevated levels of MMP-9 have been found in the cervicovaginal fluid and are associated with cervical ripening before labor, but are not a useful predictor for labor induction at term (Zhong Y., 2015). Under certain conditions, cervical ripening can be induced by the inflammatory process which involves the catabolism of the cervical ECM by enzymes discharged from infiltrating leukocytes (Olgun and Reznik., 2010). The MMP-9 has been found to play a role in the events associated with term and preterm labor, comparable changes in MMP-2 protein levels and activity. This may suggest that MMP-2 is expressed continuously throughout labor, while MMP-9 expression is induced by various factors as previously mentioned (Olgun and Reznik., 2010).

1.2. Cytokines and labor

Cytokine is a small protein released by cells that have a specific effect on the interactions between cells, communications between cells or on the behavior of cells. The cytokines include the interleukins (ILs), lymphokines and cell signal molecules, such as tumor necrosis factor(TNF) and the interferons, which trigger inflammation and respond to infections(Dorosz SA.,2015).

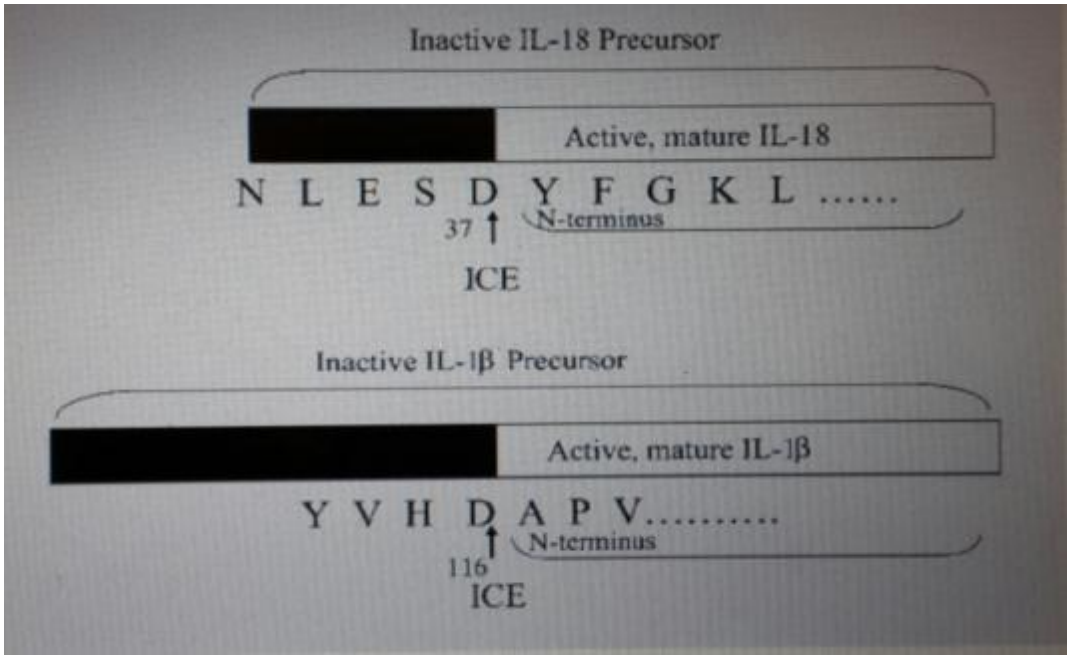
Cytokines are clustered into several classes: interleukins(ILs), tumor necrosis factors (TNF), interferons (IFN), colony stimulating factors (CSF), transforming growth factors (TGF), and chemokines. They are especially important for regulating inflammatory and immune responses and have crucial functions in controlling both innate and adaptive immunity (Dorosz SA.,2015).

Shynlova, 2011 has studied the immune cascade set in motion by the stretching of myometrial cells as the fetus increases in size. At 2013, Shynlova and his collaborators have suggested that uterine contraction signals the cells of the myometrium to secrete increasing amounts of pro-inflammatory cytokines and chemokines , which activate white blood cells associated with inflammation, such as neutrophils and macrophages, further increasing the production of cytokines and chemokines and exacerbating inflammation.(Shynlova *et al*. 2013). This cascade could aid in triggering the onset of labor by ramping up the expression of contractile-associated proteins, including receptors for hormones that serve to remodel uterine and cervical tissue to ready the mother’s body for parturition. Furthermore, they have begun to describe a proinflammatory cytokine signature that could be used to predict the onset of both preterm and term labor(Shynlova *et al*., 2011). Towards the end of pregnancy there are

changes that goon within the muscle layer and the lining of the uterus that produce and release cytokines into the mother's circulation, and subsets of those cytokines seem to be able to activate subsets of the immune cells and target the uterus (Bob Grant ., 2013)

1.2.1. Interleukin-18

Interleukin 18(IL-18) *[formerly interferon-γ (IFN-γ) inducing factor],* an interleukin converting enzyme (ICE) dependent cytokine with structural similarities to IL-1β . Precursor IL-18 (proIL-18) does not contain a signal peptide required for the removal of the precursor amino acids with subsequent secretion Figure(4). Interleukin (IL-18) was identified as cytokine in 1995 . It is considered to have pleiotropic qualities that regulate both the innate and acquired immune responses and it can stimulate both a T helper 1 and a T helper 2 response depending on the local cytokine environment (Francesconi W., 2016).



Figure(4):Caspase-1 processing of the IL-18 and IL-1β precursors. ICE = IL-1β-converting enzyme.(Charles *et al*., 2003)

IL-18 is synthesised as a pro-form and is activated through cleavage by caspase-1 (Xiao M.,2016). It is important in the host defense against severe infections via induction of other cytokines and effector cells and molecules. It is mainly synthesised by macrophages, monocytes and keratinocytes, but can also be produced by epithelial cells. IL-18 enhances the inflammatory process by stimulating the production of interferon γ (INF-γ), tumors necrosis factor (TNF)-α . IL-12 can act synergistically with IL-18 to provoke a T helper 1 response (Francesconi et al., 2016)

The IL-18-receptor in the cell membrane activates the same intracellular signal pathway as the IL-1-receptor, through activation of nuclear factor nh(Nakanishi *et al*., 2001).

IL-18 can also activate apoptosis by enhancing Fas ligand and Fas expression (Lyons ., 2015).Many studies found that IL-18 present in the amniotic fluid as well as in maternal and fetal plasma. The levels of IL-18 appear to increase with advancing gestational age (Polettini J.,2012). Menon and his collaborators have detected IL-18 mRNA in the chorion and in the interface between the decidua and the chorion, whereas the amniotic epithelium was absence of mRNA and protein for IL-18. (Polettini J.,2012)

Higher levels of IL-18 were found in women with preterm prelabour rupture of membranes with no microbial invasion of the amniotic fluid or contractions, in comparison with women with preterm labour. On the other hand, it was found that the concentration of IL-18 in the amniotic fluid increases with microbial invasion of the amniotic fluid in preterm labour and in women in labour at term.

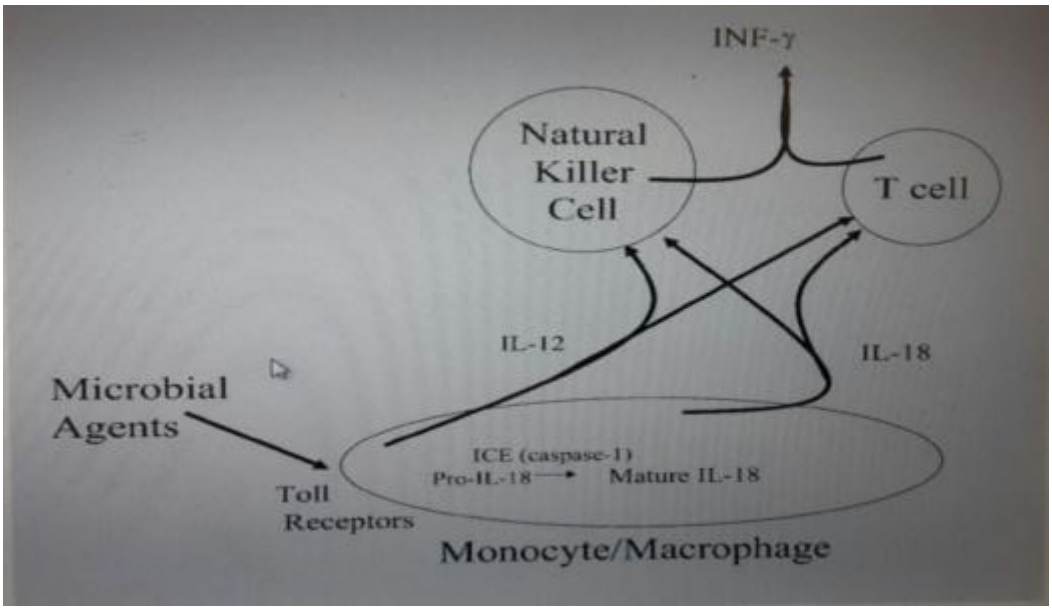
The elevated level of IL-18 in preterm prelabour rupture of membranes was suggested (Polettini J.,2012) to initiate apoptosis in the fetal membranes through the Fas–Fas ligand pathway. Thus, the role of IL-18 in preterm labour and preterm prelabour rupture of membranes remains uncertain, and no data are available on the levels of IL-18 in cervical mucus (Polettini J.,2012).

Also, its play critical role in host defense, IL-18 may participate in providing a cervical/decidual barrier against microbial invasion of the amniotic fluid and might serve as a cervical marker for intra-amniotic infection (Jacobssona *et al* .,2003).

1.2.2.Interleukin 18 Production:

1.2.2.1 Caspase-1 Processing of the IL-18 Precursor

Interleukin -18 is constitutively present in an inactive precursor form in human monocytes, keratinocytes , epithelial cells , and glial cells (Hofman M.,2015).



Figure(5): Production of IFN-γ by IL-18 plus IL-12. ICE = IL-1β-converting enzyme(Charles *et al* ; 2003)

In general, macrophage stimulators, such as lipopolysaccharide LPS, exotoxins from gram-positive bacteria, and a variety of microbial products, induce the production and secretion of macrophage cytokines via the Toll-like receptors. The stimulation of the IL-18 production by Toll-like receptors is called caspase-1 dependent (Alnakip., 2014)(figure 5). Therefore, non-Toll-like receptor activation of macrophages may be a general area of non- caspase-mediated processing of the IL-18 precursor.

Caspase-1 processing of the IL-18 precursor is similar to that of IL-1. And in the presence of specific inhibitors of caspase-1, generation and secretion of mature IL-18 and IL-1β are reduced. The precursor form of IL-1 can accumulate inside the cell, but the precursor is also found outside the cell (Tsutsui H., 2012).

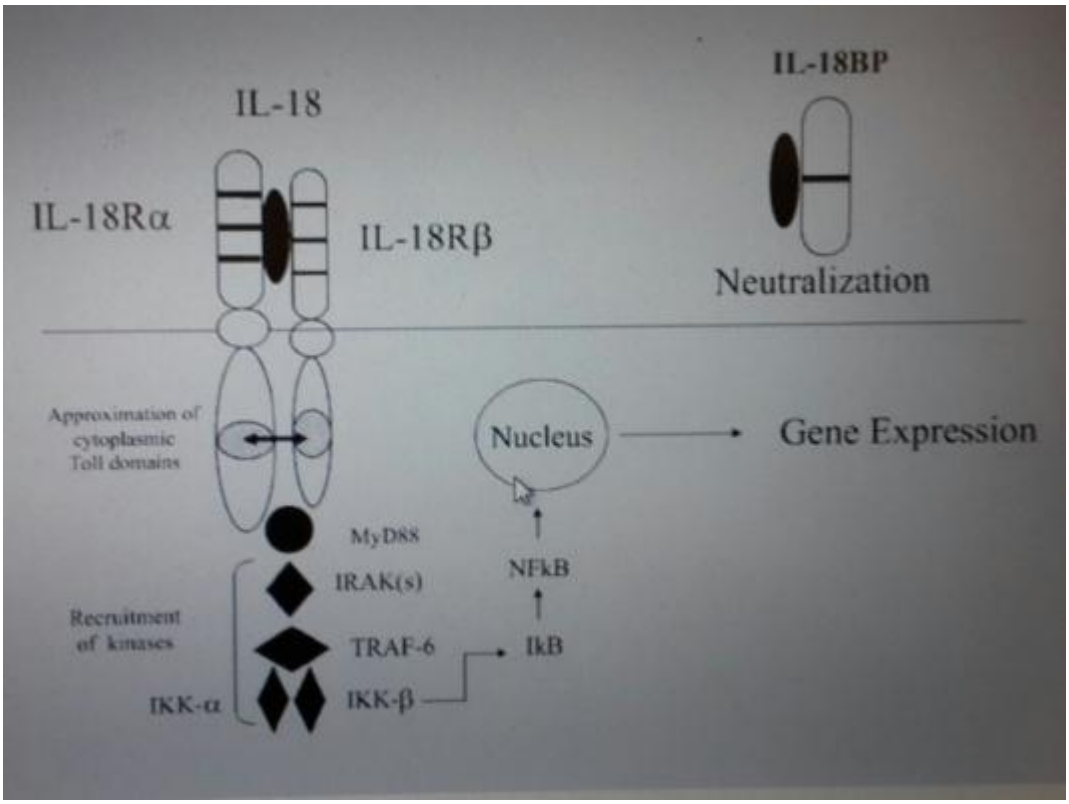
1.2.2.2 Noncaspase Processing of Interleukin-18

The enzyme that may be responsible for the extracellular processing of both the IL-18 as well as the IL-1 precursor may be (proteinase-3) PR-3. This enzyme is in the class of neutral elastase serine proteases and is commonly found in neutrophils. Although there is no evidence that recombinant PR-3 cleaves the precursors of IL-1 or IL-18, inhibitors of PR-3 and related neutral elastases reduce the processing of extracellular IL-1 precursor (Schreiber A.,2012).

The IL-18 precursor is constitutively expressed in primary human oral epithelial cells and several epithelial cell lines. When primed by IFN-α and then stimulated with PR-3 in the presence of LPS, these cells release active IL-18 into the supernatant (Sugawara et al,; 2001).The release of active IL-18 was independent of caspase-1 activity (Alnakip., 2014)

1.2.3. Interleukin -18 and Its Receptors

The activity of IL-18 begins with the formation of a heterodimeric complex comprised of two chains of the IL-18 receptor (IL-18R) complex plus IL-18 figure(6). The ligand binding chain is termed IL-18R which was described using amino acid sequencing of the purified receptor (Dias-Melicio LA., 2015).



Figure(6): Signal transduction of IL-18. IRAK(s) = IL-1 receptor activating kinase(s); TRAF-6 = TNF receptor associated factor 6;IKK = inhibitory kappa B kinase; IkB = inhibitory kappa B. (Charles *et al* ., 2003)

IL-18R is a member of the IL-1 receptor family, previously identified as the IL-1R-related protein (Alboni S., 2010), following the binding of IL-18 to the IL-18R chain, a second chain is recruited to the complex his second chain, termed IL-18R chain, is a different gene product but structurally related to IL-18R; however, the IL-18R chain does not bind to IL-18, unless IL-18 is already bound to the alpha(α) chain (figure 6). Because the IL-18R is structurally related to the IL-1 signal transducing chain, IL-1R accessory protein, the IL-18R chain was initially termed the IL-18R accessory protein-like chain (Wang D., 2010). The binding of IL-18 to the IL-18R is a low-affinity binding (20–40 nM), but

the formation of the tri-complex with the IL-18R chain forms a high-affinity complex (600 nM) (Ingram JT., 2011).

1.3. Oxidative Stress and Anti-oxidant in Labour

Oxidative stress occurs whenever the balance between the production of reactive oxidizing species (ROS) and the antioxidant defense is disrupted .

Redox imbalance is another name for oxidative stress which is based on the Nernst equation taking into account all the redox couples present in the cell or in the different cellular subcompartments (Aung-Htut, *et al.,* 2012). More practical and operational definition of oxidative stress is given by Lushchak: "Oxidative stress is a situation when steady-state ROS concentration is transiently or chronically enhanced, disturbing cellular metabolism and its regulation and damaging cellular constituents" (Lushchak, 2014; Michael Breitenbach ., 2015)

Pregnancy is a physiological stress due to hormonal, neuronal and metabolic changes occurring at various levels to meet the growing demands of the fetus. More commonly, the pregnancy-stress gets overwhelmed in the form of oxidative stress at the time of labour (Alehagen S., 2005).

Normally also transitional and mild oxidative stress occurs in non-pregnant woman. In pregnancy, as a part of respiratory adaptation, there is triggering of aerobic environment that favors oxidative stress (Chitra M., 2015)

During labour, oxidative stress increases several folds because of the repeated uterine contractions leading to ischemia; this is followed by reperfusion,

resulting in increased production of ROS. The resultant stress, during spontaneous vaginal delivery is, in turn, influenced by neural and hormonal factors in addition to pain, anxiety, fear and duration of labor (Rubens., 2014).

Oxidative stress of higher intensity may disrupt the normal functioning of the body starting from the simple cell membrane damage up to the danger of cell death by triggering apoptosis or necrosis. To counteract, body is provided with anti-oxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) which can remove the ROS efficiently and protect the body from the adverse effects of oxidative stress (Rahal., 2014) .

The imbalance between the antioxidant defense and oxidative stress in complications of pregnancy and during labour .However, the studies regarding the effect of altered antioxidant mechanisms in uncomplicated pregnancy with spontaneous vaginal delivery is scanty (Chitra .,2015).

1.3.1. Superoxide Dismutase

Superoxide dismutases (SODs) (EC.1.15.1.1) are metallo enzymes that catalyze the dismutation of the superoxide anion to molecular oxygen and hydrogen peroxide and hence form a vital part of the cellular antioxidant defense mechanism (Li *et al*., 2016).

$$2\ O_2^{\cdot -} + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

There are several enzymes have evolved in aerobic cells to overcome the damaging effects of ROS. They are significantly used to maintain the

redox balance during oxidative stress and are collectively called as endogenous ant-oxidative enzymes. Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) are the main endogenous enzymatic defense systems of all aerobic cells. They give protection by directly scavenging superoxide radicals and hydrogen peroxide, converting them to less reactive species (Krautbauer S., 2014) (Fig. 7).

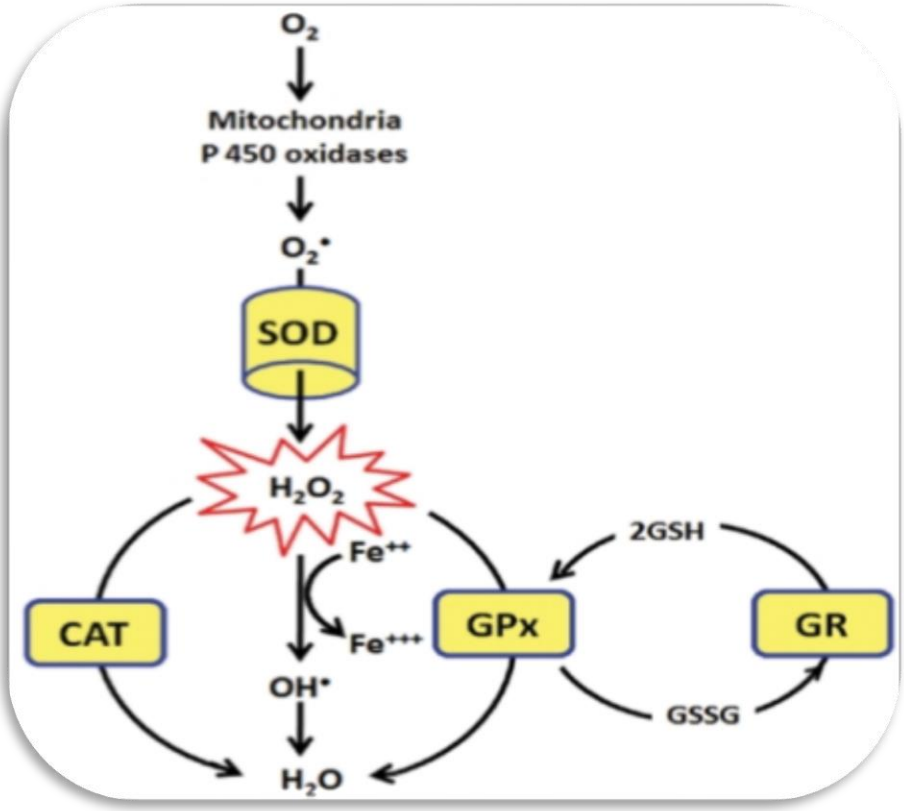


Figure (7): Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT) are the main endogenous enzymatic defense systems of all aerobic cells. They give protection by directly scavenging superoxide radicals and hydrogen peroxide, converting them to less reactive species. SOD catalyzes the dismutation of superoxide radical (•O2) to hydrogen peroxide (H2O2). Although H2O2 is not a radical, it is rapidly converted by fenton reaction into •OH radical which is very reactive. GPx neutralizes hydrogen peroxide by taking hydrogens from two GSH molecules resulting in two H2O and one GSSG. GR then regenerates GSH from GSSG. CAT the important part of enzymatic defense, neutralizes H2O2 into H2O.(Krautbauer, *et al*., 2014)

The main function of superoxide dismutase is catalyzes the dismutation of superoxide radical ($\bullet O_2$) to hydrogen peroxide (H_2O_2). Although H_2O_2 is not a radical, but it is rapidly converted by fenton reaction into •OH radical which is very reactive. Among various antioxidant mechanisms in the body, SOD is thought to be one of the major enzymes that protect cells from ROS. Cellular concentration of SOD relative to metabolic activity is a very good lifespan predictor of animal species.(Shukla V., 2011)(Figure 8).

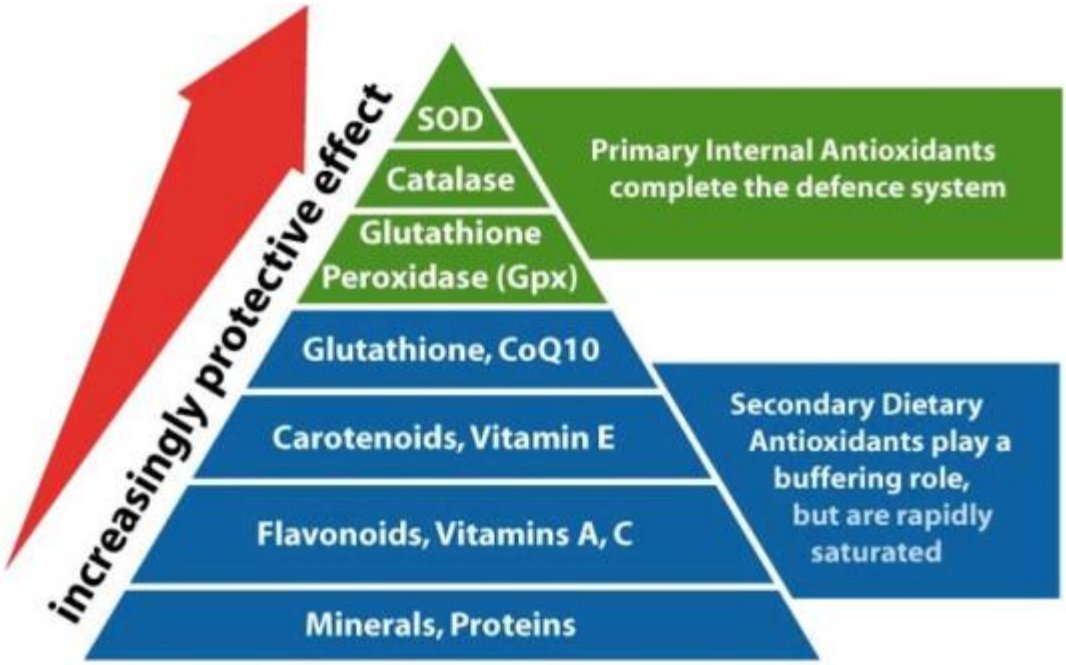


Figure (8):The most powerful antioxidant known, the body internal antioxidant defense system including superoxide dismutase SOD, Catalase and Glutathione peroxidase (Wood SJ ., 2009)

Superoxide dismutase in three isoforms (each encoded by separate genes) localized within specific cellular compartments.

**Copper-zinc SOD (CuZnSOD; SOD1) is located predominately within the cytosol, as well as in the nucleus and is thought to be expressed in all mammalian cells.

**Manganese SOD (MnSOD; SOD2) is targeted to the mitochondrial matrix and is considered to be the primary SOD isoform in relation to oxidative stress in mitochondria.

**Extracellular-SOD (EC-SOD; SOD3), also a copper-zinc containing SOD is secreted extracellular and is found primarily bound to heparin sulfate proteoglycan on cell surfaces (Petersen.,2004).

The reaction catalyzed by SOD is extremely fast, having a turnover of 2 x 10^9 M^{-1} sec^{-1} and the existence of enough amounts of SOD in cells and tissues typically maintains the level of superoxide $(O_2^{\bullet})^-$ very low (Li *et al*., 2016). The amount of SOD existing in cellular and extracellular environments is vital for the prevention of diseases linked to oxidative stress. (Shukla V., 2011).

In women with normal, uncomplicated pregnancies antioxidant levels like (superoxide dismutase) are elevated as compared to non-pregnant women; this increase is proportional to gestational length. In normal pregnancy, placental oxidative stress is believed to be kept under control by placental antioxidant enzymes. When compared to women with normal pregnancies, women with complicating factors such as diabetes mellitus, preeclampsia, and preterm labor experience ever-increasing levels of oxidative stress and reduced antioxidant capacity (Clerici G .,2012).

The increase in oxidative stress during complicated pregnancy has an effect on neonatal outcome, leading to various short- and long-term problems in neonates such as retinopathy, bronchopulmonary dysplasia, intraventricular hemorrhage, and necrotizing enterocolitis, especially in premature babies (Mondal .,2010 ; Eun., 2014).

In 2012 a study on Indian population found that mean cord blood SOD values is lower in preterm deliveries than full term deliveries. Increase in red cell superoxide dismutase activity in response to hypoxic stress may prevent toxic effects of ($\bullet O_2$) - radicals and oxygen toxicity to the lung (Soumitra .,2012 ; Soumika .,2014).

1.4. Trace elements

Trace elements are required in very small amounts to maintain health and them often from essential parts of enzymes and hormones, which regulate metabolism(Prashanth L.,2015).

Trace elements such as zinc, copper and magnesium are essential nutrients with regulatory, immunologic and oxidative stress functions resulting from their actions as essential components or cofactors of enzymes throughout metabolism (Prashanth L.,2015).

They are also essential cofactors for metalloproteinases and the enzymes catalase, superoxide dismutase (SOD) and cytochrome oxidase (Rodrigo R.,2013).

The increased metabolic demands, and deficiency of one or more of the trace elements during the period of Pregnancy can lead to adverse maternal and perinatal outcomes. (Engle-Stone 2014) . The transport of the trace element to the fetus is not fully understood; while some elements have active, the others have passive placental transport (Al-Saleh., 2004). Although the exact mechanism initiating labor is not fully understood, during labor, the metabolic

requirements increase sharply for both the mother and the fetus(Cunningham.,2010 ; Elhadi *et al*.,2015).

Therefore, researches on the mechanism/initiation of labor are of paramount importance for both academicians as well as managing clinicians. There are few published data on the role of trace elements in the mechanism of labor (Lazer *et al*., 2012). Although most of the studies were mainly focused on maternal iron and zinc and their correlation with poor pregnancy outcome, others were concerned with copper and its correlation with pregnancy and birth defects (Awadallah *et al*., 2004).

1.4.1 Copper (Cu)

Copper is a mineral found in trace amounts in all tissues and widely distributed in the body and occurs in liver, muscle and bone. It is transported in the bloodstream on a plasma protein called ceruloplasmin. When copper is first absorbed in the gut it is transported to the liver bound to albumin. Copper metabolism and excretion is controlled through delivery of copper to the liver by ceruloplasmin, where it is excreted in bile. (Bhattacharya PT., 2016).

It is found in a variety of enzymes including the copper centers of cytochrome oxidase which is involved in mitochondria for reduction of O_2 to water and produce energy in form of ATP. Also the enzyme superoxide dismutase (containing copper and zinc) (Bost M., 2016).

1.4.1.1. Biological Role of copper

Although only a small amount is needed, copper is an essential element that play a role in:- the production of hemoglobin (the main iron component of red blood cells), in the production of myelin (the substance that surrounds nerve fibers), and the production of collagen (a key component of bones and connective tissue). Copper also works with vitamin C to help make a component of connective tissue known as elastin (Stucki P., 2010).

It can act as both an antioxidant and a pro-oxidant. As an antioxidant, it scavenges damaging particles in the body known as free radicals. Free radicals occur naturally in the body and can damage cell walls, interact with genetic material, and possibly contribute to the aging process as well as the development of a number of health conditions. Antioxidants can neutralize free radicals and may reduce or even help prevent some of the damage they cause. (Persson T.,2014).

When copper acts as a pro-oxidant at times, it promotes free radical damage and may contribute to the development of Alzheimer's disease and, possibly, cervical dysplasia (precancerous lesions of the cervix which forms the opening to the uterus). Maintaining the proper dietary balance of copper (along with other minerals such as zinc and manganese) is important (Awadallah *et al*.,2004).

The importance of copper for prenatal development was first demonstrated by studies of diseases in lambs and other animals called enzootic ataxia, which is characterized by various neurological, skeletal, and connective tissue abnormalities (Galbat S.A., 2015). In human adults, severe copper deficiency is

relatively rare, whereas signs of moderate copper deficiency were observed in human infants under a variety of conditions (Tabrizi FM.,2011). Copper deficiency caused by inadequate maternal dietary intake is very rare, whereas moderate copper deficiency attributed to secondary causes, such as disease states, drug interactions, and nutritional genetic factors, are more common and may result in pregnancy complications (Awadallah *et al*., 2004).

A number of studies have shown that serum copper increases during pregnancy. Also, the lower levels of serum copper during pregnancy are correlated with some pathological conditions.(Vukelic., 2012).In 1979 , Artal *et al* have found no relationship between copper level in PROM and controls (Artal R., 1979).while Fu 1989 have reported that serum copper levels significantly lower in women with PROM than in control (Fu 1989).In other hand , Zhang found no difference in the serum zinc and copper concentration levels in mothers with delivery complications and those with normal pregnancies and deliveries (Kashanian M., 2016).

1.4.2. Zinc (Zn)

Zinc is an essential trace element which is necessary for the plant and animal life, including microorganisms (Prasad ., 2008).The human body has 2-4 grams of zinc distributed throughout the body. Most zinc is in the brain, muscle, bones, kidney, and liver, with the highest concentrations in the prostate and parts of the eye (Jurowski K., 2014).

Zinc is present in all body tissues and fluids. The total body zinc content has been estimated to be 30 mmol (2g). Skeletal muscle accounts for approximately 60 percent of the total body content and bone mass, with a zinc concentration of 1.5-3 μmol/g (100-200 μg/g), for approximately 30 percent. Zinc concentration of lean body mass is approximately 0.46 μmol/g (30 μg/g) (Bertinato J., 2016).

Serum zinc has a rapid turnover rate and it represents only about 0.l percent of total body zinc content. This level appears to be under close homeostatic control. High concentrations of zinc are found in the choroid of the eye 4.2 μmol/g (274 μg /g) and in prostatic fluids 4.6-7.7 mmol/l (300-500 mg/l).

1.4.2.1 Biological Role of zinc

In human zinc plays "ubiquitous biological roles". It interacts with "a wide range of organic ligands and has a role in the metabolism of RNA and DNA, signal transduction ,and gene expression (Abolurin OO., 2016).

It plays a central role in the immune system, affecting a number of aspects of cellular and Humoral immunity. It can help us by strengthening the natural immunity, helping prevent blindness, regulating levels of vitamin E in the blood, in fighting bacteria [zinc lactate is known as a bacteria seeded that used in toothpaste to prevent halitosis] (Dubrovinsky L.,2015) .

Also zinc is used to speed up the process of healing, helps the body to absorb vitamin A and B, and it is also important for growth hormone, testosterone, and estrogen.

It is plays an important role in normal embryogenesis, intrauterine growth and helps the mother during labour.(Uriu-Adams., 2010) Previous studies have shown that the maternal zinc level in women with PROM and preterm labour is significantly lower than in women without such complications.

There are numerous animal experiments and observational studies suggest the potential role of zinc deficiency in labour and delivery related complications such as PROM. Supplementation studies, however, do not confirm these associations (Rahmanian., 2014).

The importance of zinc and other micronutrients in relation to pregnancy outcomes and fetal health have been the concern of many studies(Alison D., 2016). Severe maternal zinc deficiency has been associated with spontaneous abortion and congenital malformation, whereas milder forms of zinc deficiency have been associated with low birth weight, intrauterine growth retardation, and preterm delivery. Additionally, low plasma zinc has also been reported to correlate with pregnancy complications, such as prolonged labor, hypertension, and postpartum hemorrhage (Awadallah *et al*., 2004).

The pregnant women are facing zinc and iron deficiency more than other groups (Ota E., 2015), Accordingly, it is critical to identify the status of maternal serum zinc level during pregnancy and also its relationship with prolonged labor. (Bakouei., 2015).

1.4.3. Magnesium (Mg)

Magnesium (Mg) is the fourth most abundant extracellular cation found in the body, and the second most abundant intracellular cation. Most is sequestered in bone and soft tissue cells, with only about 1% in the extracellular fluid. Normal plasma Mg concentration ranges from 0.70 to 1.05 mmol/L.

Magnesium ions Mg^{2+} homeostasis depends on the collaborative actions of the intestine, responsible for Mg^{2+} uptake from food, the bone, which stores Mg^{2+} in its hydroxyapatite form, and the kidneys, regulating urinary Mg^{2+} excretion (Jeroen *et al*., 2015) (Figure 9).

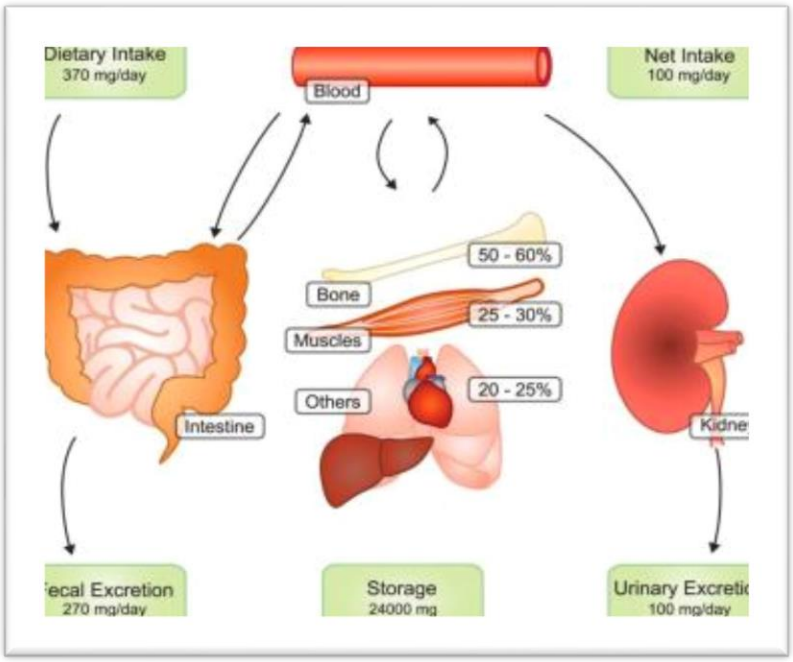


Figure (9): Magnesium homeostasis.

Daily the intestines absorb 120 mg and secrete 20 mg of Mg^{2+}, resulting in a net absorption of 100 mg. In the kidney daily 2,400 mg Mg^{2+} is filtered by the glomerulus, of which 2,300 mg is reabsorbed along the kidney tubule. This results in a net excretion of 100 mg, which matches the intestinal absorption. Bone and muscle provide the most important Mg^{2+} stores.(Jeroen H. F. , 2015)

Magnesium (Mg) is involved in a wide range of functions in human physiology. It is essential for all enzyme processes involving adenosine triphosphate (ATP) and many enzymes involved in nucleic acid metabolism. It is a cofactor for DNA, RNA and protein synthesis. It is involved in neuromuscular excitability, cell permeability, regulation of calcium [transport and accumulation] and potassium ion channels, mitochondrial function, cellular proliferation, apoptosis, regulation of parathyroid hormone secretion, muscle contraction, vasomotor tone, blood pressure, cardiac excitability, glucose metabolism and a host of other physiological functions. Changes in normal levels can thus have sweeping effects on body function (Volpe., 2013).

Plasma concentration is a reflection of the dietary intake of Mg and of the ability of the kidneys and gastrointestinal tract to retain it. Because most Mg is found intracellularly, the relationship between total body deficiency and plasma concentration is poor. However, in cases of severe deficiency, a reduction in plasma concentration can be seen (Volpe., 2013).

Almonte and colleague findings have implicated magnesium as being an essential element for fetal well-being and supplementation of magnesium may be benefited to fetal outcome (Parizadeh SM.,2013).

Magnesium supplementation during pregnancy was associated with significantly fewer maternal hospitalizations, a reduction in preterm delivery, and less frequent referral of the newborn to the neonatal intensive care unit. The results suggest that magnesium supplementation during pregnancy has a significant influence on fetal and maternal morbidity both before and after deliver (Parizadeh *et al*., 2013).

It is known that serum magnesium levels fall during pregnancy with gestational age. This decrease of magnesium plays an important role in the physiology of parturition. Decrease of magnesium in plasma may be responsible for decrease of same in myometrium leading to initiation of uterine contractions and labour (Kamal S., 2003).

Since magnesium has an inhibitory role on myometrial contractions, attention has been paid to the role of magnesium deficiency in causing preterm labour. The inhibitory effect of magnesium on preterm labour contractions is attributed to antagonism of calcium mediated uterine contractions(Bhat S., 2012).

Hence, hypomagnesemia leads to neuromuscular hyperexcitability resulting in muscle cramps and uterine hyperactivity. The hyperexcitability of uterine musculature induced by hypomagnesemia leads to increased cervical dilatation which in turn facilitates approach of vaginal micro-organisms into cervical canal and changes quality and quantity of vaginal discharge while uterine passage is being colonized by pathogenic micro-organisms (Vasavi, 2014).

Shakura study goal is to determine the relationship between serum magnesium level and the mode of parturition, so that the high morbidity and related to prematurity could be reduced by early diagnosis of this deficiency and its correction.(Shakura ., 2012).

1.5.Labor

Labor, delivery, or parturition is defined as the onset of regular contractions and cervical change. It is traditionally divided into three stages. The first stage encompasses the onset of labor to the complete dilatation of the cervix, and is subdivided into latent and active phases. The active phase begins when the rate of cervical dilatation accelerates, which occurs at 4 cm on average. The second stage consists of the time from complete dilatation of the cervix to delivery of the infant. The third stage is complete at the delivery of the placenta. Subsequent studies suggests that more time should be allowed than previously recommended before intervening to hasten labor.(González MS.,2015).

There are many types of delivery, each type of baby delivery holds certain risks for both the mother and the child, and should be performed only by a doctor or other delivery expert. (Curtin SC.,2015).

Vaginal. The typical way to deliver a baby, vaginal births are the basic type of delivery in which a mother pushes the child out through the birth canal. A mother may choose to get drugs to help numb the pain of a vaginal birth, or she can have a completely natural delivery. (Curtin SC.,2015)

Caesarean Section. A C-section can be planned or unplanned, but it is normally a fairly quick surgery where the doctors slice you open underneath your belly button and take the baby out. Sometimes, a C-section is necessary if a mother's hips are too narrow to allow for a vaginal birth, so it can be planned for in advance. Other times, fetal distress or problems with the cord may cause doctors to perform an emergency C-section. (Curtin SC.,2015)

Forceps. Sometimes, the doctor may have to insert forceps into the birth canal. They are like giant spoons that cup the baby's head, allowing the doctor to guide the baby or reposition him. They are most often used in breech births, or if the baby's heartbeat slows while the head is in the birth canal. (Curtin SC.,2015)

Vacuum. The use of vacuums in births are supplanting forceps because they are gentler. A doctor will take a suction device and place it on your baby's head. A small vacuum force is applied, which gives a doctor the chance to rotate the baby's head and also pull while the mother pushes.(Curtin SC.,2015).

Vaginal delivery is a natural process that usually does not require significant medical intervention. Management guided by current knowledge of the relevant screening tests and normal labor process can greatly increase the probability of an uncomplicated delivery and postpartum course.(González MS.,2015)

Spontaneous vaginal delivery at term has long been considered the preferred outcome for pregnancy. Because of the perceived health, economic, and societal benefits derived from vaginal deliveries, lowering the cesarean delivery rate has been a goal in the United States for more than 25 years(Hew-Butler T.,2015).

Gestation in singleton pregnancies lasts an average of 40 weeks (280 days) from the first day of the last menstrual period to the estimated date of delivery (WHO ; 2013).

Early births scheduled without a medical reason, also known as early elective deliveries, occur between 37 and 39 weeks of pregnancy. Elective deliveries may occur either by induction or cesarean section (C-section), and are associated with

an increased risk of maternal and neonatal morbidity and longer hospital stays for both mothers and newborns, as compared to deliveries occurring between 39 and 40 completed weeks gestation.(Ashton ., 2010)

The cesarean rate has increased rapidly even among women considered to be at low risk like women with a full-term singleton infant in vertex presentation (Degani N.,2015). Much of the overall increase is due to a substantial rise in primary cesarean section rates, with the main contributing factors being maternal request, the effect of malpractice litigation, convenience for the clinician, and repeat CS1 with no attempt at vaginal birth after a previous cesarean (Derbent *et al*., 2011). Medical indications for planned elective cesareans have also broadened over the years and have influenced medical practice. For example, the recommendation for CS whenever there is breech presentation in a singleton term baby (Riskin *et al* ., 2014)

Other factors contributing to the high C-section rate can be divided into the following four categories:

a) Common labor interventions (continuous fetal monitoring, labor induction when cervix is not ready)

b) Uninformed choice of vaginal delivery

c) Economical reasons (hospital work organization, financial incentives)

d) Limited awareness of adverse effects related to C-section.

Taken together, these data suggest that the environment surrounding the expectant mother shapes the choice of the mode of delivery and may contribute to the current high C-section rate in some countries, despite evidence

demonstrating that offspring are more susceptible to diseases.(Khafipour & Ghia, .,2013)

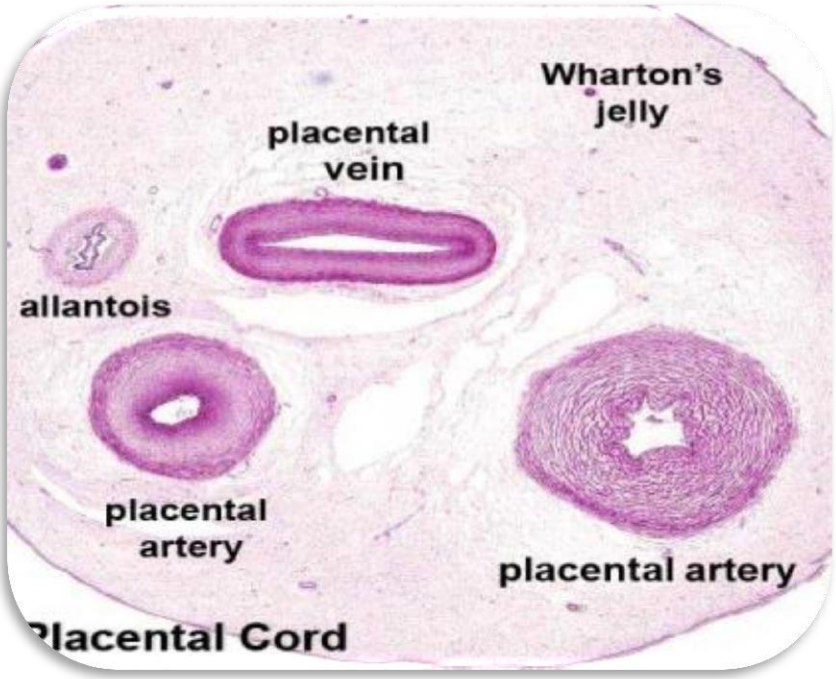
In Babylon, Hassain G.S has found The percentage of C-section in Babylon Province (34.4%) to total deliveries which is high in comparison to World Health Organization (WHO) (15%) with poor registration.(Hassan ., 2015)

A cesarean section is a major abdominal surgery carrying risks to both the mother (including thrombosis, excess bleeding, and bladder damage) and infant (including breathing difficulties), and may also lead to increased cost due to extended hospital stays (Fairley et al.,2011). Labor induction at any gestational age has also been associated with a two-fold increase in the rate of cesarean delivery (Lee HR., 2015)

1.6. Structure of the Umbilical Cord

The umbilical cord is long, usually around 55 centimeters or two feet, this offers a lot of slack so that a baby can move quite freely without danger. Adding to the safety is the sheer volume of amniotic fluid, which keeps the cord from being pushed up against the wall of the uterus (Gharibi B.,2014).

The cord has a specialized membrane that plays a role in fluid exchange and other important interchanges. Contained within it are two arteries and a vein (Figure10). These are the main channels for funneling oxygen and nutrition to the baby. Protecting the blood vessels from being crushed by twisting or compression of the cord is a substance called Wharton's Jelly (Rafah H.,2013).



Figure(10)This Histological section of the a Cord overview unlabeled(Dr Mark Hill 2016)

The human umbilical cord forms a connection between the placenta and the fetus. It is composed of three blood vessels of different structure and function, one vein, which transports oxygenated and nutrition-rich blood from placenta to fetus, and two arteries, which transport deoxygenated blood and metabolic waste products from fetus to placenta. All these vessels are surrounded by Wharton's jelly (Rafah H.,2013), which constitutes the major part of human umbilical cord and provides a thick protective mantle around vessels. Wharton's jelly plays also an important role as a storage for some compounds, such as growth factors (Rafah H.,2013).

The extracellular matrix (ECM) in the vascular wall contains many macromolecules (collagen, elastin, proteoglycans, and glycoproteins) necessary for the structural and functional properties of vessel wall (Abdeen AA.,2016).

Proteolysis is a major process leading to changes in the ECM (Mott and Werb ;2004)and study from (Romanowicz & Galewska., 2010)that they found the umbilical cord arteries of newborns delivered by mothers with preeclampsia contain more than twice the amount of collagen in comparison to corresponding arteries of newborns from normal pregnancies (Manisha SB.,2015). In the same time a decrease in elastin content was observed. Furthermore a decrease in hyaluronate content and its replacement by sulphated proteoglycans were found (Rostamzadeh S., 2015). These phenomena correspond to an early ageing of these tissues and may reduce the elasticity of arterial wall and decrease the blood flow in the fetus of woman with preeclampsia.

Matrix metalloproteinases and their tissue inhibitors (TIMPs) are engaged in metabolism of extracellular proteins (Galewska., 2010). Human umbilical cord tissues contain several matrix metalloproteinases (Deng CL.,2015). Among them MMP-2 exerts the highest content in umbilical cord vessels (Deng CL.,2015). Its amount is several times higher than other MMPs. Gelatinase A is the main collagenolytic enzyme of both kind of the vessel wall (Galewska., 2010).

1.6.1. Wharton's Jelly

Wharton's Jelly is the primitive mucous, connective tissue of the umbilical cord lying between the amniotic epithelium and the umbilical vessels. First observed by Thomas Wharton in 1656, this gelatinous substance is comprised of proteoglycans and various isoforms of collagen. The main role of the Wharton's Jelly is to prevent the compression, torsion, and bending of the umbilical vessels which provide the bi-directions flow of oxygen, glucose and amino acids to the developing fetus, while also depleting the fetus and placenta of carbon dioxide

and other waste products (Ma S.,2014). Cells found in Wharton's Jelly are a primitive mesenchymal stem cell (MSC), likely trapped in the connective tissue matrix as they migrated to the AGM (aorta-gonad-mesonephros) region through the developing cord, during embryogenesis (Ting CH.,2014)

During early embryogenesis, hematopoiesis takes place in the yolk sac and later in the AGM region. Colonies of early hematopoietic cells and mesenchymal cells migrate through the early umbilical cord to the placenta between embryonic day 4 and 12 of embryogenesis (Ma S.,2014).

There is a second migration from the placenta again through the early umbilical cord to the fetal liver and then finally to the fetal bone marrow where hematopoietic stem cells (HSCs) and mesenchymal stem cells engraft and predominantly reside for the duration of life. Included in these migrating hematopoietic colonies are early precursors of HSCs, as well as primitive mesenchymal stromal (stem) cells. Researchers have postulated that during this migration to and from the placenta through the umbilical cord, mesenchymal stromal cells become embedded in the Wharton's Jelly early in embryogenesis and remain there for the duration of gestation (Taghizadeh et al., 2011)**.**

1.6.2 Umbilical Cord Blood Contains Different Types of Stem Cells

The increasing interest in umbilical cord blood emanated from its utilization in hematological applications in the past couple of decades. Previous and current clinical efforts, focused on analyzing and characterizing the constituents of umbilical cord blood. Beside the blood cells, that include erythrocytes, leukocytes and thrombocytes, the umbilical cord blood was found to contain

different populations of stem cells, a unique feature not shared with peripheral blood. Scientists and researchers have characterized the following stem cell populations from umbilical cord blood; hematopoietic stem cells (HSCs), multipotent non-hematopoietic stem cells and Mesenchymal stem cells (MSCs) (Ali., 2012).

1.6.2.1 Hematopoietic Stem Cells (HSCs)

Haematopoiesis is the process by which blood cells are formed. All blood cellular components are derived from a multipotent stem cell population called hematopoietic stem cells through a series of complex proliferation and differentiation events (Felli., 2010). Umbilical cord blood has been shown to contain a population of hematopoietic stem cells (HSCs) at different stages of hematopoietic commitment, characterized by their differential expression of hematopoietic antigens CD133, CD34 and CD45 according to a model previously described. It has been shown that cord blood hematopoietic stem cells can be selectively induced into specific hematopoietic line-ages *in-vitro* including erythroid, megakaryocytic and monocytic lineages(Felli., 2010).

1.6.2.2 Umbilical Cord-derived Mesenchymal Stem Cells (UC-MSCs) as a universal source

UC-MSCs, like bone marrow mesenchymal stem cells, are immunologically privileged. MSCs invoke only minimal immune reactivity, and, furthermore, may possess anti-inflammatory and immuno-modulatory effects (Kim GJ.,2015).

UC-MSCs express MHC class I antigens and express low levels of MHC class II antigens, relatively less than BM-MSCs. As several studies currently

suggest, UC-MSCs, like BM-MSCs, do not require tissue matching, thus, allowing for an allogeneic cell therapy source, as any donor can give cells to any other person without rejection or need of immunosuppressant drugs (Carbone A.,2014)

1.6.2.3 Multipotent Non-Hematopoietic Stem Cells

A unique population of multipotent non-hematopoietic stem cells has been identified in umbilical cord blood. These stem cells are small in size and exist at low density in cord blood and are negative for the major hematopoietic marker CD45 . This population of cells has been shown to express transcription factors normally expressed by embryonic stem cells including pluripotency key players OCT4, SOX2 and NANOG. In addition, they expressed specific surface markers, which have been used previously to characterize human embryonic stem cell lines.(Ali H., 2011)

1.7 THE AIM OF STUDY

1- Estimate the concentration of MMP-9 and IL-18 levels in umbilical cord blood in both normal vaginal delivery and caesarean section and compare between them.

2- Determined Activity and localization of MMP-9 and IL-18 in umbilical cord tissue matrix of patients with normal vaginal delivery and caesarean section and assessment of these changes using Aperio software.

3- Find correlation between MMP-9 and IL-18 concentration in cord blood with their activities in tissue .

4- Study the concentration of some trace elements like Zn, Cu, and Mg as they are related to the activity of MMP-9 and SOD enzyme in the umbilical cord of both normal vaginal delivery and caesarean section.

Chapter two

Subjects, Materials & Methods

2.1 Subjects and study design

A case-control study has been conducted between January 2015 and May 2016. The current study recruited sixty four pregnant women at term gestation (37–41 completed weeks) whom were approached from Al-Imamayn Al-Khademyiayn Medical City Hospital in Baghdad.

The 64 women involved in this study were divided into two groups based on mode of delivery. The first group included 31 normal non-assisted vaginal delivery (NVD) with mean age 26.44 ± 1.1 years, while the second group included 33 women with cesarean section delivery(CS) with age mean 29.1 ± 1.21 years. The primigravida cases were excluded.

All women participating in this study were not suffering from any serious systemic illnesses and were excluded by the physician and this study excluded women with :

- Hypertension
- Diabetic Mellitus
- Thyroid disease
- Smoking
- Preeclampsia
- Renal disease
- No history of infection for the last month
- Primi , first child delivery .

These conditions were excluded by obstetrician, because these conditions may result in abnormally high oxidative stress status, which may overestimate its effect on pregnancy duration.

After taking concent from those qualified women to participate in this study, where the women were asked who accepted to register succumb to draw blood and collect umbilical cord tissue samples.

2.2 Materials

Table (2.1) below shows kits that used to measure the enzymes and the cytokine biomarkers:

Table (2-1) :biomarker kits in this study.

biomarkers	Laboratory Kit	Manufacturer	Country
Serum Human MMP-9	Human MMP-9/Gelatinase B (Matrix Metalloproteinase 9/Gelatinase B) ELISA Kit	Elabscience Biotechnology Co.	USA
Serum Human IL-18	Human IL-18/IL-1F4 (Interleukin 18) ELISA Kit	Elabscience Biotechnology Co.	USA
Superoxide Dimutase	Human SOD (Superoxide Dismutase,Soluble) ELISA kit	Elabscience Biotechnology Co.	USA

2.3 Sample collection

At operation theater, umbilical cord blood samples and umbilical cord tissue were collected from umbilical cord for each participant after delivery.

Part one :Biochemistry Study

2.3.1 Serum Collection

Five to ten milliliters of venous blood was aspirated from all umbilical cords, allowed to clot at room temperature and then centrifuged for 10 minute at 3000 rpm to obtain serum. The separated serum was then immediately stored at −20 °C in capped labeled aliquots to analyze them later using Ohkawa method (Ohkawa et al 1979). The serum samples were used to measure MMP-9 , cytokine (IL-18) and enzymatic antioxidant levels (SOD) by Enzyme Liked Immune-Sorbent Assay (ELISA) technique . In addition, serum was used to determine, glucose, urea, creatinine, zinc, copper and magnesium by colorimetric enzymatic method using Biomaghreb, Sa, France kits.

2.3.1.1 Determination of Serum Human MMP-9/Gelatinase B (Matrix Metalloproteinase 9/Gelatinase B) by ELISA Technique

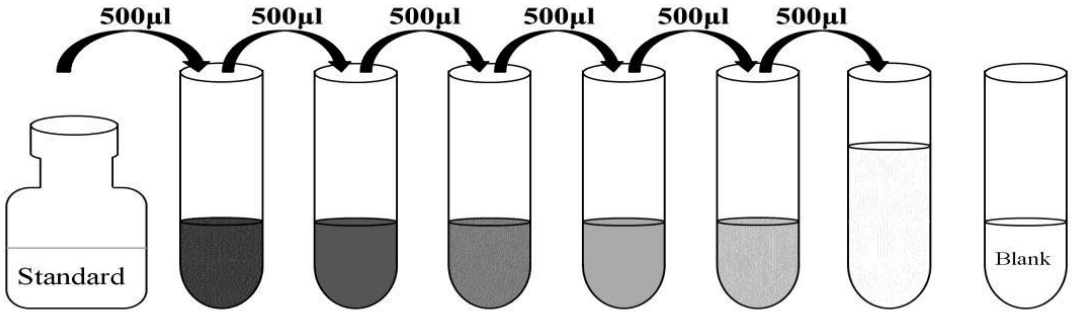
2.3.1.1.1 Principle

This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to MMP-9/Gelatinase B. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for MMP-9/Gelatinase B and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to

each well. Only those wells that contain MMP-9/Gelatinase B, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of MMP-9/Gelatinase B. The concentration of MMP-9/Gelatinase B in the samples was calculated by comparing the O.D. of the samples to the standard curve (figure 2-2).

2.3.1.1.2 Standard preparation

The standard vial was prepared within 15 minutes before use and then reconstitute the Standard with 1.0 mL of reference standard and Sample Diluent were let to stand for 10minutes until they dissolved fully. That reconstitution was produced a stock solution of 20ng/mL. Serial dilutions were prepared upon need. The recommended concentrations were as follows: 20, 10, 5, 2.5, 1.25, 0.625, 0.313 and 0 ng/mL. The undiluted standard serves as the highest standard (20 ng/ml) and the sample diluent serves as the zero (0 ng/mL) (figure(2-1).



Figure(2-1) Standard curve dilutions for MMP-9 (ng/ml)

2.3.1.1.3 Standard curve

Standard curve for MMP-9 in present study is shown below.

Table (2-2):Dilutions for standard curve of MMP-9 concentration in ng/ml

Absorbance	0.07	0.131	0.189	0.281	0.642	1.085	1.76	2.89
Concentration of MMP-9 (ng/ml)	0	0.31	0.63	1.25	2.5	5	10	20

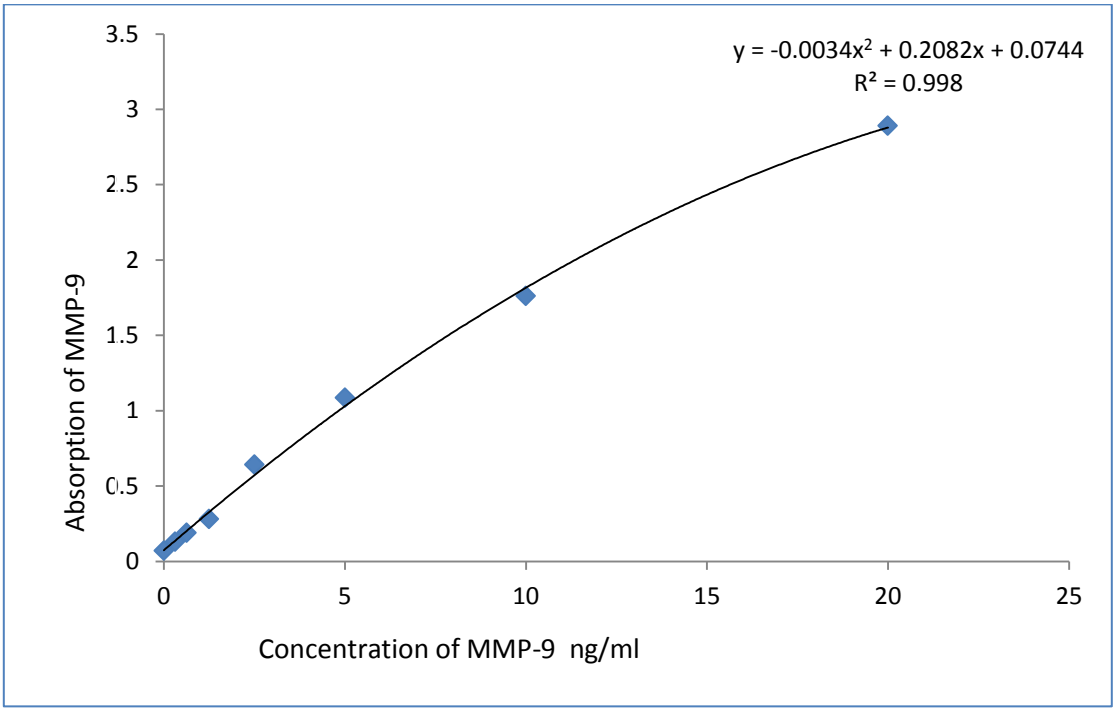


Figure (2-2) The standard curve of Matrix Metalloproteinase-9 concentration

2.3.1.2 Determination of Serum Human IL-18/IL-1F4 (Interleukin 18) by ELISA Technique:

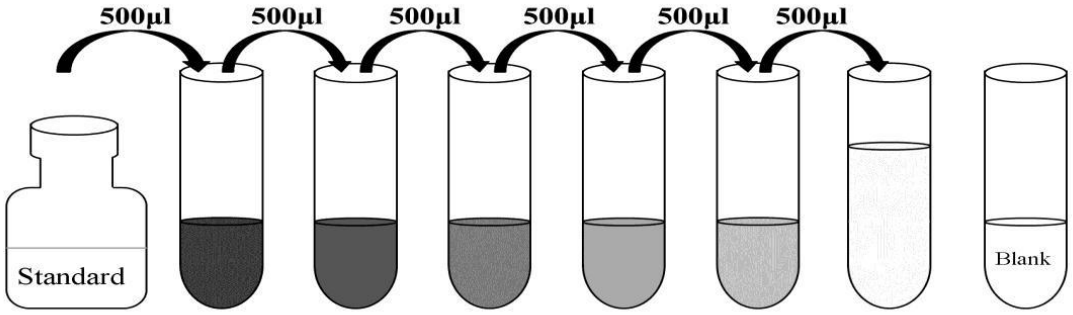
2.3.1.2.1 Principle

This ELISA kit uses Sandwich-ELISA as the method. The micro ELISA plate provided in this kit was been pre-coated with an antibody specific to IL-18/IL Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for IL-18/IL- and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain IL-18/IL, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The OD value is proportional to the concentration of IL-18/IL-1F4. You can calculate the concentration of IL-18/IL-1F4 in the samples by comparing the O.D. of the samples to the standard curve.

2.3.1.2.2 Standard preparation

The standard vial was prepared within 15 minutes before use, and then reconstitute the Standard with 1.0 mL of reference standard and Sample Diluent. Let it to stand for 10minutes. The reconstitution was produces a stock solution of 1000pg/mL . A serial dilutions were made. The recommended concentrations were as follows:1000, 500, 250, 125, 62.5, 31.25, 15.625

and 0 pg/mL . The undiluted standard serves as the highest standard (1000pg/mL). The Sample Diluent serves as the zero (0 pg/mL)(figure(2-3)).



Figure(2-3) Standard curve dilutions for IL-18 (pg/ml)

2.3.1.2.3 Standard curve

Standard curve for S. Interleukin-18 in present study is shown in figure (2-4).

Table (2-3) Dilutions for standard curve of IL-18 in pg/ml

Absorbance	0.052	0.111	0.17	0.26	0.5	0.927	1.681	3.03
Concentration of IL-18(pg/ml)	0	15.63	31.25	62.5	125	250	500	1000

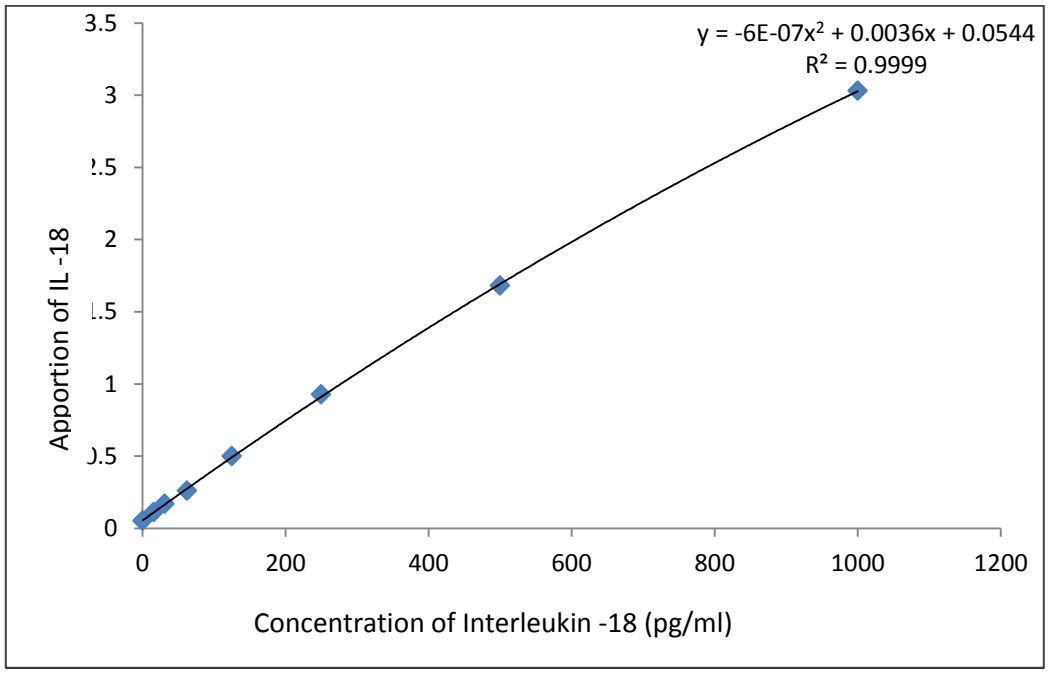


Figure (2-4)The standard curve of S. Interleukin-18 concentration

2.3.1.3 Determination of serum Superoxide Dismutase (SOD) level

Superoxide Dismutase (SOD) level was measured by enzyme linked immunosorbent assay technique:

2.3.1.3.1 Principle

This ELISA kit uses competitive-ELISA method. The microtiter plate provided in this kit has been pre-coated with SOD. During the reaction, human SOD in the sample or standard competes with the a fixed amount of SOD on the solid phase supporter for sites on the Biotinylated Detection AB specific to human SOD.Excess conjugate and unbound sample or standard were washed from the plate, and Avidin conjugated to Horseradish Peroxidase (HRP) is added

to each microplate well and incubated. Then a TMB substrate solution was added to each well. The enzyme- substrate reaction was terminated by the addition of sulfuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm ± 2nm. The concentration of human SODin the samples was then determined by comparing the OD of the samples to the standard curve.

2.3.1.3.2 Standard curve preparation of SOD

The standard vial was centrifuged at 10,000×g for 1 minute, and then reconstituted with **1.0** ml of Reference Standard &Sample Diluent. The lid was tightened and the standard was let to stand for 10 minutes and turned it upside down for several times. This reconstitution is to produced a stock solution of 4000pg/ml. Then serial dilutions were made as needed. The recommended concentrations were as follows: **4000, 2000, 1000, 500, 250, 125, 62.5, 0 pg/ml.** The standard was prepared within 15 minute before use (Figure(2-5)).

2.3.1.3.3 Standard curve

Standard curve for SOD in present study is shown in figure(2-5).

Table (2-4): Dilutions of SOD standard curve in pg/ml

Absorbance	2.467	2.01	1.72	1.31	0.98	0.77	0.51	0.401
Concentration of SOD(pg/ml)	0	62.5	125	250	500	1000	2000	4000

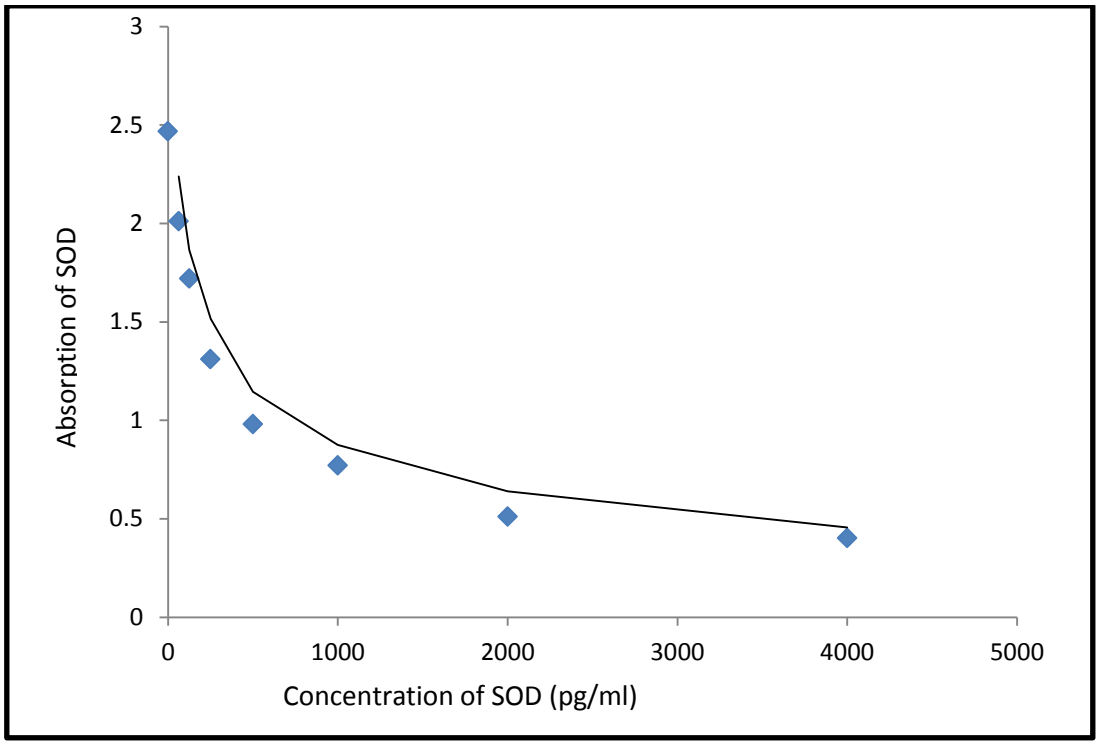


Figure (2-5):The standard curve of SOD concentration (pg/ml)

2.3.1.4 Assessment of biochemical Prameters

2.3.1.4.1 Determination of Random Serum Glucose

Random serum glucose was determined by an enzymatic method by using Spectrum, Egyptian Kit. The samples were measured at 510 nm (492-550). Normal range reference (70-130 mg/dl)

2.3.1.4.2 Determination of Serum Urea

Serum urea was determined by an enzymatic method using Biomaghreb Tunisia kit at 590 nm. Normal reference range (15-40 mg/dl).

2.3.1.4.3 Determination of Serum Creatinine

Serum creatinine was determined by an enzymatic method using Spectrum Egyptian kit. The samples were measured at 492 nm. Normal reference range (0.7-1.3 mg/dl).

2.3.1.4.4 Determination of serum trace elements

2.3.1.4.4.1 Determination of serum Zinc

Serum zinc was measured by the colorimetric method using zinc Spectrum Egyptian kit. The samples were measured at 560 nm (500-550). The normal reference range (140-270 mg/dl).

2.3.1.4.4.2 Determination of serum copper

Serum cupper was measured by the colorimetric method using copper Spectrum Egyptian kit. The samples were measured at 580 nm (490-550). The normal reference range (60-165 mg/dl).

2.3.1.4.4.3 Determination of serum magnesium

Serum magnesium was measured by the colorimetric method using magnesium Biomaghreb Tunisia kit. The samples were measured at 520 nm (490-550). The normal reference range (60-165 mg/dl).

Part two: Immunohistochemistry study

2.4.1 Umbilical cord tissue collection and processing

One Centimeter piece of umbilical cord was taken from mid-way between periphery and the cord insertion and transferred immediately to be fixed with 10% formalin and processed in paraffin blocks.

2.4.1.1 Preparation of the paraffin section

According to Bancroft and Stevens (1987) and Baker *et al.,(*1998), the umbilical cord tissues were histologically prepared for paraffin section as follows:

Fixation, dehydration, clearing, impregnation, embedding, sectioning, de-waxing, hydration, staining and mounting.

2.4.1.1.2 Fixation

umbilical cord samples fixed in formalin solution (10%) prepared previously as the following:

- Formaldehyde (BDH) 40%---------- (100 ml)
- Tap water ---------- (900 ml)

These specimens were fixed for 48 hours at room temperature.

2.4.1.1.3 Dehydration

In order to ensure adequate dehydration of the samples, the umbilical cord tissues are immersed sequentially in the following solutions at room temperature for the indicated times:

- 70% alcohol for hours (after 2 washes)

- 90% alcohol for overnight(6 hours)
- 100 % alcohol for 1-2 hours

2.4.1.1.4 Clearing

The clearing of the specimens had been performed by transferring these specimens into xylene (BDH, Analar); two exchanges were implemented for 30-45 minute to ensure a good tissue transparency.

2.4.1.1.5 Impregnation and embedding

An electric wax dispenser (Model No. 222 Lipshaw, figure 2.3) was utilized for embedding the sections in a labeled baths of a molten paraffin wax (E. Merck) melting point 56 Cº; two changes were performed 2 hours each using an embedding oven (Fisher scientific Model 615G). Then the specimens were transferred to be blocked in paraffin wax using a labeled stainless steel embedding moulds (L shape).

2.4.1.1.6 Sectioning

Serial sections of 5 µm thickness were cut using the electrical microtome (Richert –Jung, 2030 MOT Biocut, figure 2.4) ; these thin sections (about 3-5 sections per slide) were transferred to a water bath (figure 2.8) with a temperature of 50 c° before attaching the sections to the slides . Five sets of slides were made; one for staining with haematoxylin and eosin stain and the others for immunohistochemical studies, which were collected on a positively charged slides.

Figure 2-6 Electric paraffin dispenser used in this study

Figure 2-7 Reichert-Jung Microtome system used in sectioning placental tissue

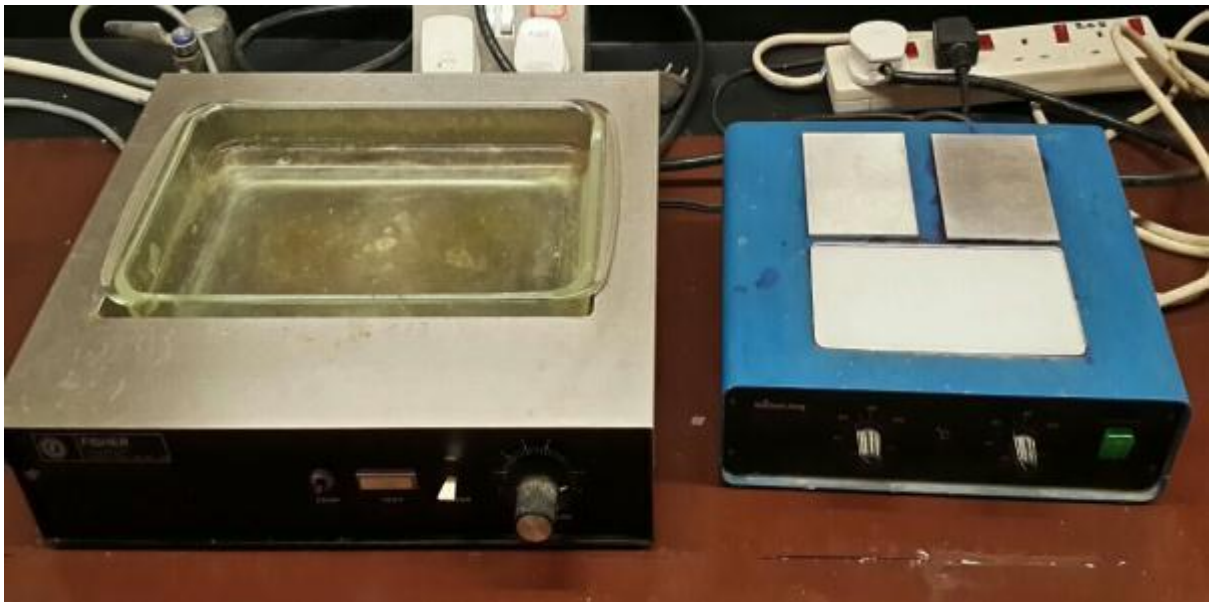


Figure 2-8 Fisher Scientific Model 615G water bath used in this study

2.4.1.1.7 De-waxing and hydration

This procedure was performed by transferring the slide to an oven set on 57C° and kept for 15 minutes after which the slide were treated with xylene for 30 minutes which was followed by passing the slide through graded concentration of ethyl alcohol baths (100%, 90% and 70%) which was done for 1 minute each and then washed with distilled water.

2.4.1.1.8 Staining: method of the histological staining

The haematoxylin and eosin staining method had been used for the general histological studies for its comparative simplicity. The Harris modified haematoxylin (Fisher), which contained alum as a mordant and chemically ripened with mercuric oxide, was used regressively for nuclear staining (blue-black).

The staining solution was prepared as follows:

- Haematoxylin (Fisher)---------- (5g)
- Absolute ethyl alcohol (Fluka)---------- (50 ml)
- Potassium alum (BDH) ----------(100g)
- Distilled water ----------(950 ml)
- Mercuric oxide (Fluka)---------- (2.5g)
- Glacial acetic acid (May and Baker) ----------(40 ml)

The haematoxylin was dissolved in absolute alcohol, and then added to the alum which had been previously dissolved in warm distilled water in a flask. The mixture was rapidly brought to the boiler and the mercuric oxide was then added. The stain was rapidly cooled by plunging the flask into cold water. When the solution is cold, the glacial acetic acid was added. The stain was then strong, and was filtered before use (Bancroft &Stevens, 1982).

Eosin-yellowish 1% weight / volume (Fluka) was used with the alum haematoxylin as a counter stain, to demonstrate the general histological architecture, and to distinguish between the cytoplasm of different cell types and between different connective tissue fibers and matrices (red-pink). The staining solution was prepared as follows:

- Eosin Yellowish (Fluka)----------(10 g)
- Tap water ----------(990 ml)

The staining procedure was done as the following:

- After the slide being de-waxed and hydrated; they were stained in the alum haematoxylin for 3-5 minute and then washed well in running tap water for about 5 minutes.
- Stained in 1% eosin yellowish for 1-3 minute, and washed in running tap water for 3-5 minutes.
- Dehydrated in graded alcohol (70%, 80%, 90%, and 100%) 1 minute for each, cleared in xylene (2-3 minutes) and mounted by permount (DPX).

2.5 Immunohistochemistry Guide for Slide-mounted Paraffin Sections

2.5.1 De-waxing and hydration

This procedure was performed by transferring the slide to an oven set on 57C° and kept for 15 minutes after which the slide were treated with xylene for 30 minutes which was followed by passing the slide through graded concentration of ethyl alcohol baths (100%, 90% and 70%) which was done for 1 minute each and then washed with distilled water.

2.5.2 Deparaffinizing and rehydration

1. Immerse the slides in Xylene Ⅰ and Xylene Ⅱ successively for 10 minutes respectively.

2. Immerse the slides in anhydrous ethanol Ⅰ, anhydrous ethanol Ⅱ, 95% ethanol, 80% ethanol, and 70% ethanol successively for 5 minutes respectively, and then use deionized water to wash the slides for 2 times, each time for 2 minutes.

2.5.3 Antigen retrieval (optional)

3. Put the slides into the repair box, and then add 0.01M citric acid buffer (PH6.0) to make the tissue immersed.

4. Repair the antigen with medium power microwave for 10 minutes (Start timing when the liquid boils), and don't make the tissue dried during the process.

5. Take out the repair box from microwave oven, and naturally cool it down. Take out the slides when the repair liquid is cooled down to room temperature, and wash the slides for 3 times with PBS (PH7.4), each time for 3 minutes (Don't flush the tissue directly during the washing process in order to avoid breaking up the tissue).

2.5.4 Inactivation

6. Add the 3% H2O2 that is prepared well in advance to the slides drop by drop to block endogenous peroxidase, and then incubate them at room temperature for 15 minutes (Use ionized water to dilute 30% H2O2), finally use PBS to wash the slides for 3 times, each time for 3 minutes.

2.5.5 Antibody incubation

7. Blot up PBS with absorbent paper, and add 5% normal serum (Sharing the same or similar species with secondary antibodies) drop by drop on the sections, then block it at 37℃ for 30 minutes.

8. Wipe dry the liquid around the tissue on the slides with absorbent paper, and use an oil pen to draw a circle around the tissue, and then add the diluted primary antibodies drop by drop. Add PBS to the section of controls if the negative controls are required. After adding primary antibodies, put the slides into wet

box to be incubated at 4°C overnight. (The optimum dilution ratio of the antibodies should be pre-determined through experiments in advance)

9. Wash the slides with PBS for 3 times, each time for 2 minutes, and then add HRP-conjugated secondary antibodies after wiping dry the slides with absorbent paper, finally incubate the slides at 37℃ for 30 minutes.

2.5.6 Signal detection

10. Wash the sections with PBS for 4 times, each time for 3 minutes, and wipe dry the sections with absorbent paper, then add DAB substrate reagent that is prepared freshly drop by drop to each section, and observe them under a microscope. The positive signal appears brown-yellow or brown in color. The time should be well controlled to avoid the color appears too deep. Wash the section with tap water to terminate the reaction.

11. Hematoxylin counterstaining: Immerse slides in Harris hematoxylin solution for about 30 seconds to 1 minute, and then transfer slides into ethanol solution with 1% HCl after washing with water, finally wash them with water. *(Optional)*

2.5.7 Dehydration and mounting

12. Firstly immerse the slides in water and wash them, and then put the slides into the following reagents in order to dehydrate and permeate: 70% ethanol, ethanol, 90% ethanol, ethanol, anhydrous ethanol Ⅰ, anhydrous ethanol, Xylene Ⅰ and Xylene Ⅱ. Put the slides in each reagent for 2 minutes, and finally air dry the sections in the fume cupboard.

13. Drop resinene beside the section, and then cover them with the cover glass. In order to avoid air bubble, firstly lay one side of the cover glass flat and then gently lay another side flat. Finally dry the sealed sections by laying them in the fume cupboard

14. Observe the dried sections and collect images under a microscope.

2.6 Immunohistochemistry (IHC) technique for detection of Matrix Metalloproteinase (MMP-9) andInterleukin-18 (IL)

Immunohistochemistry technique is used for the detection of a specific antibody bound to an antigen in tissue sections.

2.6.1 Principle of the test

Antigens in tissues are detected by two stage processes:

a) The binding of the primary antibody to specific epitope and the subsequent detection of this binding by colorimetric reaction then these sections incubated with primary antibody.
b) The bound primary antibody is then detected by a serial addition of complement, horse radish peroxidase- (HRP) conjugate and diaminobenzidine (DAB) substrate. The DAB substrate offers the greatest sensitivity of all the HRP colorimetric chromogens. The positive reaction will result in a permanent, insoluble and brown colored precipitate at antigen site in tested tissue which has high contrast in photographs.

2.6.2 Materials

In the present study, (Expose Mouse and Rabbit Specific HRP/DAB detection IHC Kit (ab80436) from (abcam, UK) was used (figure 2-12).

It is a micro-polymer detection system. It is advantageous over a polymer detection system as a smaller detection complex is formed rather than a polymer backbone aiding better tissue penetration. The contents of this kit can be explained as shown in table 2.2.

Table 2-5 Contents Expose Mouse and Rabbit Specific HRP/DAB detection IHC Kit

Components	**Quantity**
DAB chromogen 50x	**1 x 0.5ml**
DAB substrate	**1 x 15ml**
Goat anti-Rabbit HRP Conjugate	**1 x 15ml**
Hydrogen Peroxide Block	**1 x 15ml**
Mouse Specifying Reagent (Complement)	**1 x 15ml**
Protein block	**1 x 15ml**

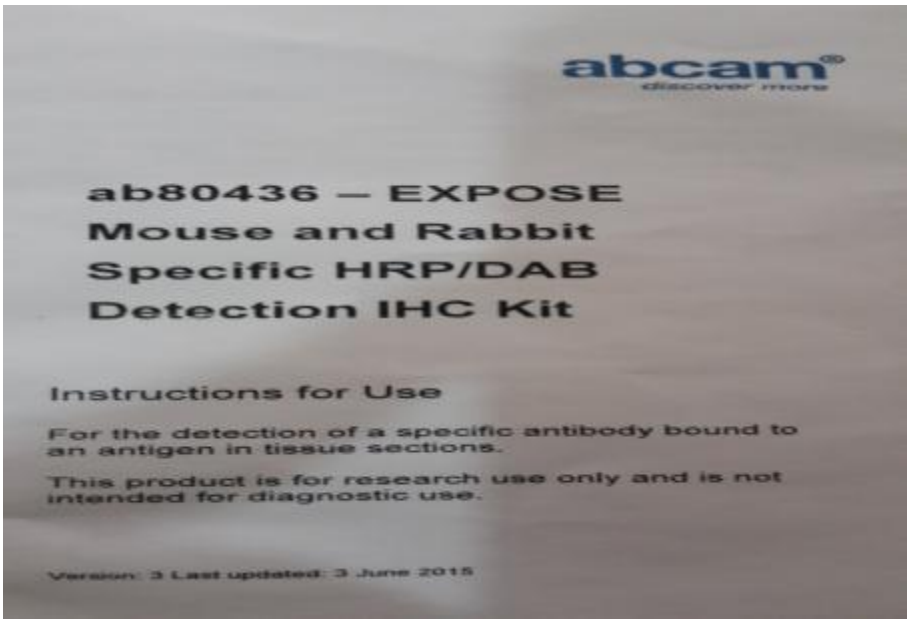


Figure 2-9 Shows the IHC Kit (ab80436)) from (abcam, UK) used in this study.

2.6.3 Other material required

Other reagents and equipment that was used in IHC were shown in Table 2-6 and 2-7.

Table (2-6) Reagents that was used in IHC

Reagents	Source
Absolute Ethanol	Scharlau, Spain
Antigen retrieval solution: Citrate buffer pH 6.0	Dako, Denmark
Deionized distilled water	Bioneer Korea
Mayer`s Hematoxylin histological staining reagent	Dako, Denmark No:S3309
Mounting medium No:S3025	Dako, Denmark
PBS	Oxoid-England
Oil immersion microscope	Olympus , Japan
Xylene	Scharlau, Spain

Table (2-7) Equipment that was used in IHC

Equipment	Source
Automatic Micropipette 5-50 µl,10-100 µl,100-1000 µl	Slamed, Germany
Cover slips	Dako, Denmark
Incubator	Memert, Germany
Glass staining Jars	China
Graduated Cylinder	Uk
Positively charged microscopic slids (Silanized slides) S3003	Dako, Denmark
Slide holders (ten slide each)	China
Sterile Eppendorf tube	Eppendorf, Germany
Sterile Tips	Eppendorf, Germany
Timer with alarm	Germany

2.6.4 Polyclonal Antibody used in this study

In this study polyclonal antibodies were used as primary antibody as shown in table (2-7).

Table (2-8) The following polyclonal antibody were used to examine umbilical cord tissue:

biomarker	Laboratory Kit	Manufacturer	Country
MMP-9	Matrix Metalloproteinase (MMP-9) polyclonal Antibody (Clone 1F83)	Japan Institute for the Control of Aging (JaICA)	Japan
IL-18	Interleukin-18 (IL) polyclonal Antibody	Japan Institute for the Control of Aging (JaICA)	Japan

2.6.5 Preparation of tissue sections and reagents

The following steps were undertaken in order to prepare tissue sections and reagents:

1. Absolute ethanol was diluted in distilled water to prepare 90%, 70% and 50% concentrations of ethanol.
2. Substrate chromagen solution was prepared by adding 30 µl DAB chromagen to 1.5 ml of DAB substrate, mixed by swirling. This is according to the manufacturer instructions.
3. Primary antibody: The primary antibody was diluted with PBS (add (50) µl from PBS to (1)µl from primary antibody) to each mono or polyclonal antibody.

2.6.6 Immunohistochemistry procedure

Paraffin embedded tissues were cut into 5µm thickness for each antibody in this study and then placed on positively Silanized slides (Dako, Denmark), slides were left overnight to dry at room temperature.

2.6.7 Immunohistochemistry tissue staining protocols

1. Detection of Matrix Metalloproteinase -9 Polyclonal antibody **(MMP-9)**

The following steps were undertaken:

1. Slides baking: the slides were placed in vertical position in drying oven (hot air oven) at 60 °C Over night for melting of paraffin and evaporation of water droplets.

2. Deparaffinizing tissue sections: the slides were immersed sequentially into the specified jars containing approximately 200 ml of each of the following solutions at room temperature.

- Xylene two times for 5 minutes.
- Absolute ethanol for 5 minutes.
- 100% ethanol for 5 minutes.
- 90% ethanol for 5 minutes.
- 70% ethanol for 5 minutes.
- Distilled water for 5 minutes.

3. Antigen retrieval: The slides were washed with distilled water and placed in a heat – resistant staining jar containing antigen retrieval solution with citrate buffer (Ph 6.0). The slides were fully covered with the solution and put in an oven operated for 10 minutes to boil tissue section, it was affirmed that retrieval solutions still covered the slides. After that, the slides bath was removed from oven and slides were allowed to cool at room temperature for 20-30 minutes, and then rinsed with several changes of distilled water. The slides were placed in distilled water jar for 5 minutes.

4- Peroxidase block: Excess liquid was tapped off. Using absorbent wipe, around the specimen was carefully wiped to remove any remaining liquid and to keep reagents within the prescribed area. Two to three drops of Hydrogen Peroxide were applied to cover sections, and then slides were placed in humid chamber and incubated at room temperature for 10 minutes. After that, it was rinsed gently with distilled water from wash bottle and placed in fresh buffer bath for two times.

5- Excess liquid was tapped off and slides wiped as before. Protein blocking reagent was added to cover specimen. Slides were incubated at room temperature for 5-10 minutes, and then washed for one time in buffer.

6- Diluted primary Antibody: 20µl of antibody solution was applied according to manufacturer`s protocol to cover the sections in concentration: 1µl/50µl for MMP-9. Later on, the slides were placed in humid chamber then incubated at 25 C° for two night. The slides were washed for three times in buffer.

7- Complement solution was applied and incubated for 10 minutes at room temperature and then the slides were washed 2times in buffer.

8- HRP conjugate was applied and incubated for 15 minutes at room temperature and then the slides washed for four times in buffer.

9- Substrate chromagen solution was applied on tissue sections incubated for {1-10} minutes at room temperature and then the slides were rinsed for four times in buffer.

10- The slides were immersed in a bath containing counter stain (Mayer`s Hematoxylin) for 1 minute at room temperature and then the slides were rinsed gently with distilled water.

11- The slides were sequentially dehydrated by applying:

- 70% ethanol for 3 minutes.
- 90% ethanol for 3 minutes.
- 100% ethanol for 3 minutes.
- Absolute ethanol for 3 minutes.
- Fresh Xylene for 5 minutes.

12- Two drops of mounting medium (Dako) were applied to the sections and the slides were covered with cover slips and left to dry and then examined by light microscope at 10X and 40 X.

Figure (2-10) Matrix Metalloproteinase -9 (MMP-9) Polyclonal antibody Vial

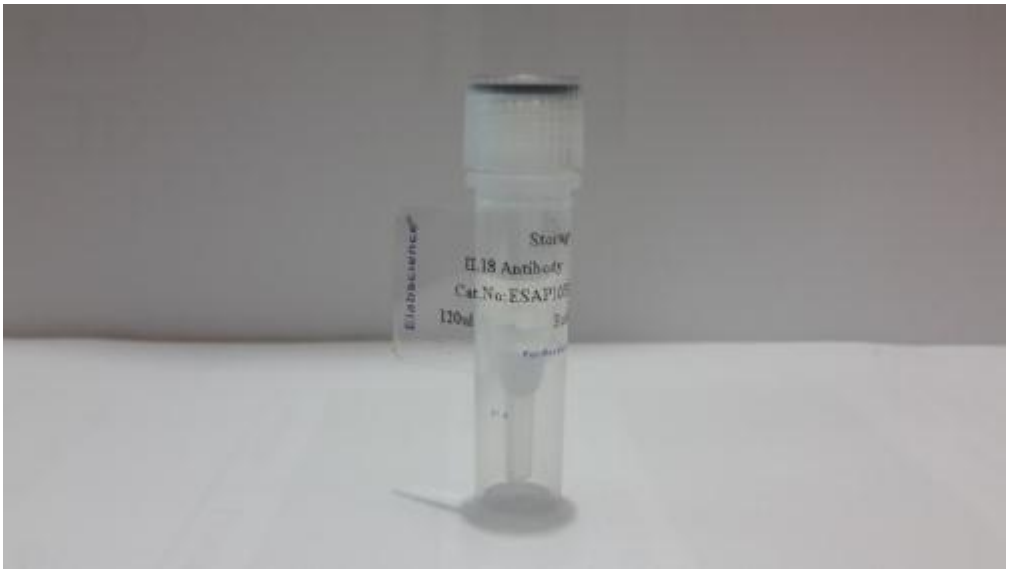


Figure (2-11) Interleukin-18(IL-18) Polyclonal antibody Vial

2. Detection of Interluekin-18(IL-18) Polyclonal antibody in tissue section

Same protocol that was previously used for the detection of Matrix Metalloproteinase -9 (MMP-9) was also used; except the concentration was changed to 1µg/20 µl for IL-18.

Figure (2-12) The contains of detection kit for both MMP-9 and IL-18

2.6.8 Digital Imaging

The sections were then examined and different fields of the tissue section were selected for imaging. Pictures were taken by using microscopic digital optic camera DSC-TX5 with new image software as shown in the Figure 2.11.

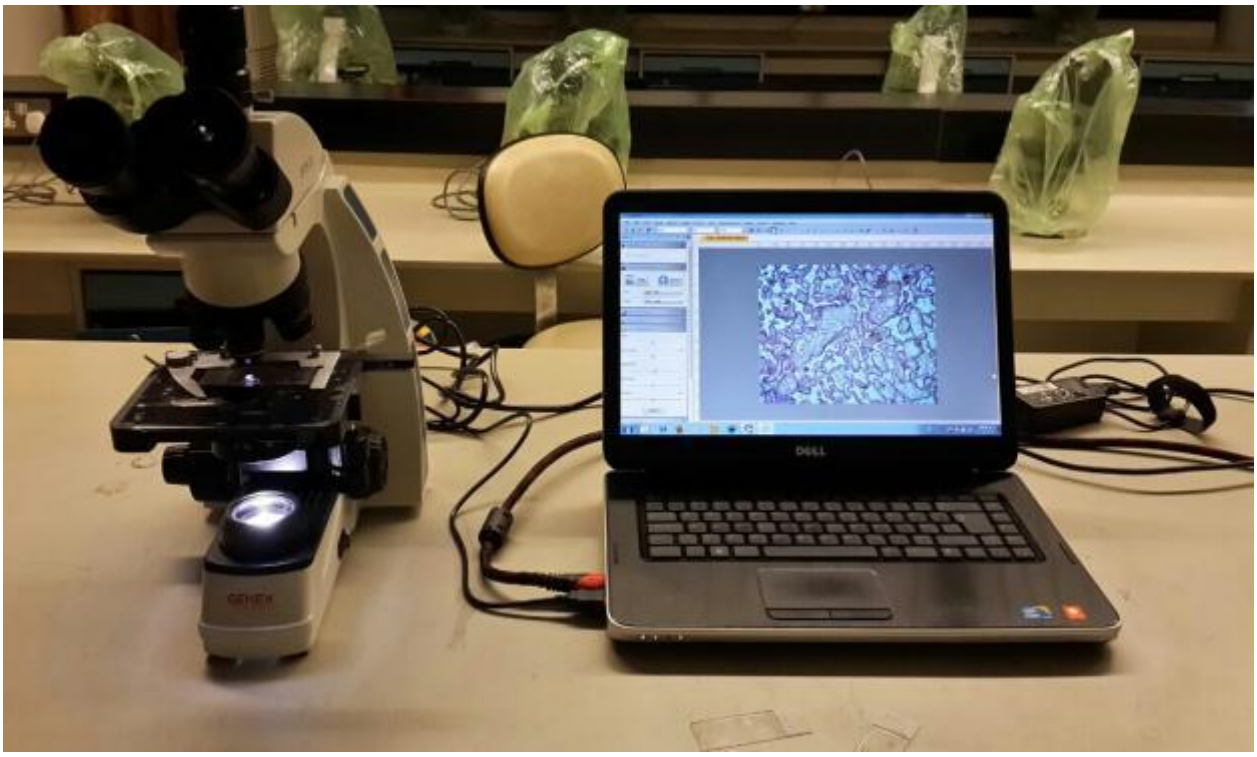


Figure (2-13) Microscopic digital optic camera DSC-TX5 that was employed to photograph histological images in this study.

2.7 Statistical Methods

The data were encoded and entered into SPSS statistical package (v. 22). Data cleaning and management was then conducted to ensure that no mistakes were made during the data entry phase.

Data analysis was then conducted. Descriptive data analysis was performed to describe the characteristics of study sample. The mean and Standard Error (S.E.) was used to describe the continuous variables while frequencies and percentages were used to describe categorical variables. Bivariate analysis was performed. P value less than 0.05 was considered

significant. Chi-square tests were done to examine the statistical difference in the demographic variables between the study groups. Pearson correlation test was performed to examine the association between oxidative stress biomarkers and pregnancy duration.

Moreover, the Receiver Operating Characteristics (ROC) curve was calculated to assess the sensitivity and specificity of the used kits in classifying the true positive versus false positive cases in this study. The ROC curve plots were presented as well.

Finally, a series of histological images were produced and presented to show the umbilical cord of MMP-9 and IL-18 levels among normal vaginal delivery and cesarean section delivery groups respectively.

Immunhistochemical assessment for MMP-9 and IL-18 using Aperio positive pixel count program.

For Immunhistochemical reaction software was used. The Aperio image scope software V12.1.0.5029 was used to assess the quantity of the immunhistochemical reaction for both IL-218 and MMP-9 .

The quantitation of the amount of special color that occur after the reaction was done on the pictures of the slides for each type of marker . This software have different types of parameters . The parameter used for quantitation of the color was selected and the reaction color which was brown then 3 grads (ranges) of this color intensity was selected (weak positive , positive and strong positive and the procedure was as followed.

Pixel count algorithm program is put to a picture by considering the following stages:

1- Open the algorithm program by clicking twice on the icon of the image scope program

2- The image then converted to the algorithm program by clicking the command file then the open image

3- After loading picture to algorithm program, view option is chosen then analysis.

4- From the option of analysis the algorithm option is chosen.

5- To from margins surrounding the islets of Langerhans the free-hand pen in the toolbar of image scope is chosen.

6- After that the selected annotation layer command was chosen as an area of analysis and terminally the image analyzed by touching the run analysis command in the widow of analysis

Chapter Three

Results

Results

Sixty four women were involved in this study, 31 women were delivered by cesarean section and 33 were delivered by normal vaginal delivery . Age of women involved in this study was nearly comparable and with no significant differences ($p>0.05$). About 75.8% were housewives and the rest had jobs as shown in table (3-1).

No significant difference was also found between groups in their weeks of gestation ($p>0.05$) (table 3-1).

Table (3-1):Age , Job and Weeks of gestation in studied groups.

groups		Job		
		Housewife	Worker	Total
NVD	No.	25	8	33
	% within groups	75.8%	24.2%	100.0%
C/S	No.	23	8	31
	% within groups	74.2%	25.8%	100.0%
P value		0.885		

groups	Age		
	Mean	±SE	P value
C/S	29.1	1.21	0.103
NVD	26.43	1.1	

groups	Weeks of gestation		
	No	Mean	±SE
NVD	33	37.42	0.087
C/S	31	37.26	0.08
P value	0.167		

NVD normal vaginal delivery *C/S cesarean section*
SE standard error *No. number*

3.1 Obstetric history of study group

In table (3-2), regarding gravida, para and abortion ; a highly significance different was found between para and abortion among women delivered by C/S and NVD while no significant difference was found between their gravida .

Table (3-2): Gravida, Para and abortion in studied groups

Groups		Gravida						Total
No. of pregnancy		0.00	2.00	3.00	4.00	5.00	6.00	
NVD	No.	0	13	6	10	2	2	33
	% within groups	0%	39.4%	18.2%	30.3%	6.1%	6.1%	100.0%
C/S	No.	0	4	10	9	6	2	31
	% within groups	0%	12.9%	32.3%	29.0%	19.4%	6.5%	100.0%
P value		0.101						

Groups		Para							Total
No. of pregnancy		0.00	1.00	2.00	3.00	4.00	5.00	6.00	
NVD	No.	0	0	13	6	10	2	2	33
	% within groups	0%	.0%	39.4%	18.2%	30.3%	6.1%	6.1%	100.0%
C/S	No.	0	4	6	11	10	0	0	31
	% within groups	0%	12.9%	19.4%	35.5%	32.3%	.0%	.0%	100.0%
P value		**0. 035***							

Groups		Abortion				Total
No. of pregnancy		.00	1.00	2.00	3.00	
NVD	No.	33	0	0	0	33
	% within groups	100.0%	0.0%	0.0%	0.0%	100.0%
C/S	No.	14	9	7	1	31
	% within groups	45.2%	29.0%	22.6%	3.2%	100.0%
P value		**0.001****				

*P value < 0.05 is significant

**p value < 0.01 is highly significant

3.2 Biochemical investigations

3.2.1 Determination of serum superoxide dismutase (SOD) concentration in study groups

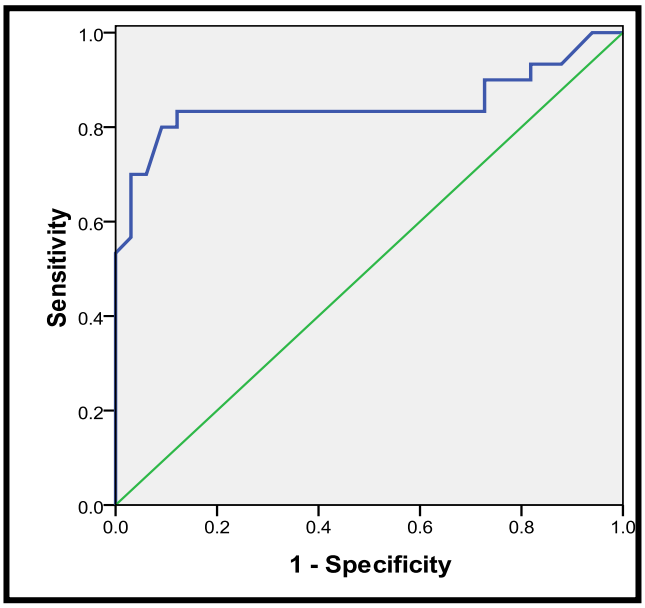
The mean (± SE) value standard error of serum SOD concentration in women delivered by C/S & women delivered by NVD were showed in table (3-3). Serum SOD concentration was significantly lower in women delivered by NVD compared with women delivered by C/S (P<0.001**).

Table (3-3):Serum superoxide dismutase concentration in studied groups.

Groups		Mean	±SE	P value
SOD pg/dl	C/S	1851.03	153.68	**P<0.001****
	NVD	947.94	45.50	

**p value < 0.01 is highly significant

The area under the corresponding ROC curve for serum SOD concentration was compared between women with C/S and NVD for the prediction of type of delivery (figure (3-1)). ROC analysis revealed that serum SOD concentration had a significant area under the curve (AUC) 0.847, indicating that a threshold of 1200 pg/dl gave sensitivity of 83% and specificity 88% . Higher levels of serum SOD concentration was associated with increased rate of C/S .



SOD pg/dl	Area	Cutoff point	Sensitivity	Specificity	P value	95% CI	
						Lower Bound	Upper Bound
	0.847	1200	83%	88%	**0.001****	0.736	0.959

**p value < 0.01 is highly significant

Figure (3-1): Estimated area under curve (AUC) and predictive cut-off point values for the serum SOD concentration with the receiver operating characteristics (ROC) curve analysis between women with C/S and NVD .

3.2.2 Serum interleukin-18 concentration in study groups

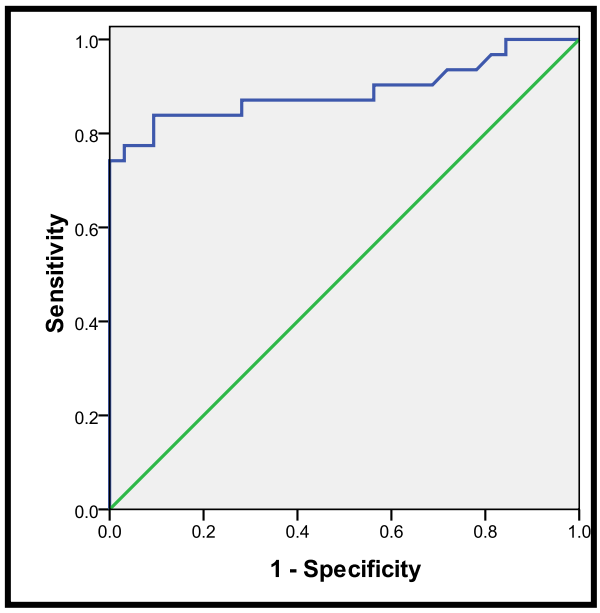
Serum IL-18 concentration in women delivered by C/S & women delivered by NVD were shown in table (3-4). A significant reduction in serum IL-18 concentration was found in women delivered by NVD compared with women delivered by C/S (P<0.001**).

Table (3-4): Serum IL-18 concentration level in studied groups.

Groups		Mean	±SE	P value
IL-18 pg/ml	C/S	485.78	32.01	**P<0.001****
	NVD	216.75	15.33	

**p value < 0.01 is highly significant

The area under the corresponding ROC curve for Serum IL-18 concentration was compared between women with C/S and NVD for the prediction of type of delivery (figure (3-2)). ROC analysis revealed that Serum IL-18 concentration had a significant area under the curve (AUC) 0.890, indicating that a threshold of 300 pg/ml gave sensitivity of 82% and specificity 91% . Higher levels of serum IL-18 concentration were associated with increased rate of C/S .



IL-18 pg/ml	Area	Cutoff point	Sensitivity	Specificity	P value	95% CI	
						Lower Bound	Upper Bound
	0.890	300	82%	91%	**<0.001****	0.800	0.981

**p value < 0.01 is highly significant

Figure (3-2): Estimated area under curve (AUC) and predictive cut-off point values for the serum IL-18 concentration with the receiver operating characteristics (ROC) curve analysis between women with C/S and NVD .

3.2.3 Serum Matrix Metalloproteinase-9 concentration in study groups

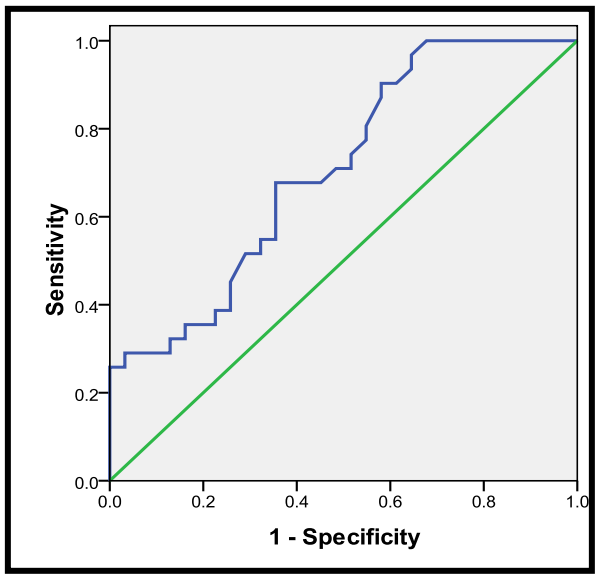
A highly significant decrease in serum MMP-9 concentration was found in women delivered by NVD when compared with women delivered by C/S (P<0.001**)(Table (3-5)).

Table (3-5): Serum MMP-9 concentration level in studied groups.

Groups		Mean	±SE	P value
MMP-9 ng/ml	C/S	15.24	0.54	**P<0.001****
	NVD	12.51	0.60	

**p value < 0.01 is highly significant

The area under the corresponding ROC curve for serum MMP-9 concentration was compared between women with C/S and NVD for the prediction of type of delivery (figure (3-3)). ROC analysis revealed that serum MMP-9 concentration had a significant area under the curve (AUC) 0.708, indicating that a threshold of 13.5 ng/ml gave sensitivity of 68% and specificity 65%. Higher levels of serum MMP-9 concentration was associated with increased rate of C/S .



MMP-9 ng/ml	Area	Cutoff point	Sensitivity	Specificity	P value	95% CI	
						Lower Bound	Upper Bound
	0.708	13.5	68%	65%	**0.005****	0.580	0.835

**p value < 0.01 is highly significant

Figure (3-3): Estimated area under curve (AUC) and predictive cut-off point values for the serum MMP-9 concentration with the receiver operating characteristics (ROC) curve analysis between women with C/S and NVD .

3.2.4 Serum magnesium concentration in study groups

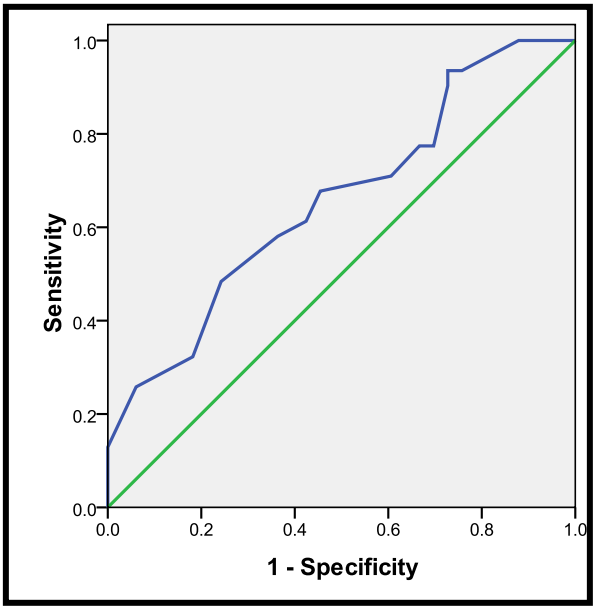
As shown in table (3-6), a significant increase in Mg concentration was found in sera of women delivered by NVD when compared with those delivered by C/S ($P<0.05$).

Table (3-6): Serum Mg mg/dl concentration in studied groups.

Groups		Mean	±SE	P value
Mg mg/dl	C/S	1.47	0.08	**0.021***
	NVD	1.77	0.09	

*p value < 0.05 is significant

The area under the corresponding ROC curve for serum Mg concentration was compared between women with C/S and NVD for the prediction of type of delivery (figure (3-4)). ROC analysis revealed that serum Mg concentration had a significant area under the curve (AUC) 0.657, indicating that a threshold of 2 mg/dl gave sensitivity of 90% and specificity 73%. Less of serum Mg concentration was associated with increased rate of C/S .



Mg mg/dl	Area	Cutoff point	Sensitivity	Specificity	P value	95% CI	
						Lower Bound	Upper Bound
	0.657	2.0	90%	73%	0.031*	0.524	0.791

*p value < 0.01 is significant

Figure (3-4): Estimated area under curve (AUC) and predictive cut-off point values for the serum Mg mg/dl concentration with the receiver operating characteristics (ROC) curve analysis between women with C/S and NVD .

3.2.5 Serum Copper concentration in studied groups

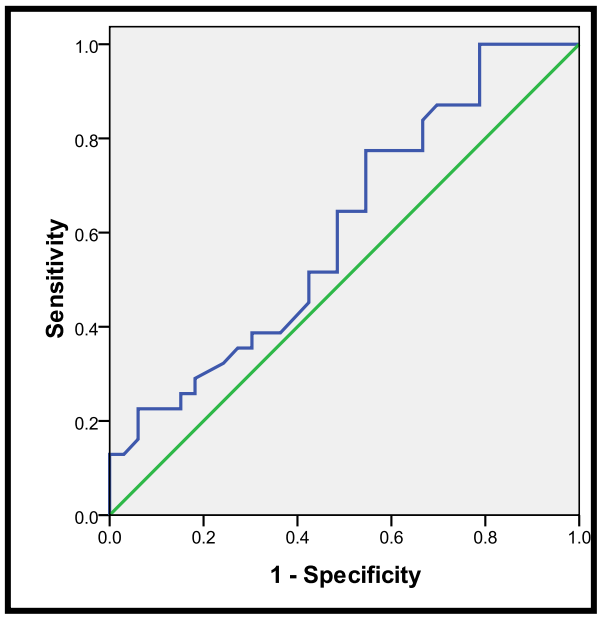
Regarding determination of cupper in this study; a significant increase in serum Cu concentration was found in women delivered by NVD compared with women delivered by C/S (P>0.05) table (3-7).

Table (3-7): Serum Cu (µg/dl) concentration in studied groups.

Groups		Mean	±SE	P value
Cu µg/dl	C/S	89.97	6.78	**0.05***
	NVD	113.21	9.34	

*p value < 0.05 is significant

The area under the corresponding ROC curve for Cu was compared between women with C/S and NVD for the prediction of type of delivery (figure (3-5)).ROC analysis revealed that serum Cu concentration had a significant area under the curve (AUC) 0.607, indicating that a threshold of 100 µg/dl gave sensitivity of 58% and specificity 52%. Less of serum Cu concentration was associated with increased rate of C/S .



Cu µg/dl	Area	Cutoff point	Sensitivity	Specificity	P value	95% CI	
						Lower Bound	Upper Bound
	0.607	100	58%	52%	0.143	0.468	0.745

Figure (3-5): Estimated area under curve (AUC) and predictive cut-off point values for the serum Cu **µg/dl** concentration with the receiver operating characteristics (ROC) curve analysis between women with C/S and NVD .

3.2.6 Serum Zinc concentration in studied groups

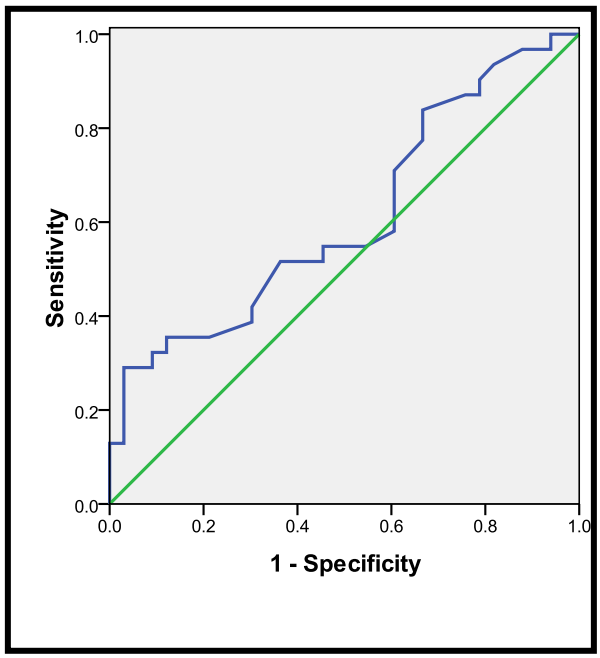
Serum Zn concentration in women delivered by NVD was significantly increased compared with Zn concentration in C/S women table (3-8) ($P<0.05$).

Table (3-8): Serum Zn µg/dl concentration level in studied groups.

Groups		Mean	±SE	P value
Zn **µg/dl**	C/S	197.39	3.29	**0.035***
	NVD	205.27	1.71	

*p value < 0.05 is significant

The area under the corresponding ROC curve for Serum Zn concentration was compared between women with C/S and NVD for the prediction of type of delivery (figure (3-6)).ROC analysis revealed that serum Zn concentration had a significant area under the curve (AUC) 0.610, indicating that a threshold of 205 µg/dl gave sensitivity of 55% and specificity 54%. Less of Serum Zn concentration was associated with increased rate of C/S .



Zn µg/dl	Area	Cutoff point	Sensitivity	Specificity	P value	95% CI	
						Lower Bound	Upper Bound
	0.610	205	55%	54%	0.129	0.471	0.750

Figure (3-6): Estimated area under curve (AUC) and predictive cut-off point values for the serum Zn µg/dl concentration with the receiver operating characteristics (ROC) curve analysis between women with C/S and NVD .

3.2.7 Serum FBG, Urea and Creatinine concentration in studied groups

As shown in table (3-9); no significant differences were found between concentration of FBG, urea and creatinine among women delivered by or C/S or NVD.

Table (3-9)Serum (FBG, urea, Cr)mg/ml concentrations in studied groups.

Groups		Mean	±SE	P value
FBS mg/dl	C/S	113.58	6.95	0.119
	NVD	96.86	7.95	
Groups		Mean	±SE	P value
S.Urea. mg/ml	C/S	41.21	1.64	0.431
	NVD	39.14	1.90	
Groups		Mean	±SE	P value
S.Cr mg/ml	C/S	0.68	0.04	0. 385
	NVD	0.64	0.03	

p value < 0.05 is significant *NVD normal vaginal delivery*
C/S cesarean section *SE standard error*

3.3 Correlation between Study Variables in women with C/S and NVD

Table (3-10) and (3-11): shows the Correlation between study variables in women with C/S and NVD. Serum IL-18 shows a significant negative correlation with S. Creatinin in women with C/S (P<0.05)**.** Also, S.Cu have a significant negative correlation with S. Mg in women with C/S (P<0.05). the significant positive correlation was found between S.urea and S. Creatinin in both groups (P<0.05). [figures (3-7)(3-8) and (3-9)]respectively .

In women with C/S and NVD, a significant negative correlation was found between S.Zn and Mg (P<0.05) as shown in figure (3-10).

Figures (3-11)(3-12):show the correlation between study variables in women with C/S and NVD. Serum IL-18 shows no correlation with serum trace elements as well as there are no correlation between serum MMP-9 and serum trace elements .

Also figure (3-13) shows no correlation between serum SOD and serum trace elements in both studies groups.

Table (3-10):Correlation between Study Variables in women with C/S

S/C	r	P
S.IL-18 + S. Creatinine	-0.581	0.003**
S.Mg + S.Cu	-0.388	0.031*
S.Urea+ S. Creatinine	0.454	0.026*
S.Mg + S.Zn	-0.353	0.05*

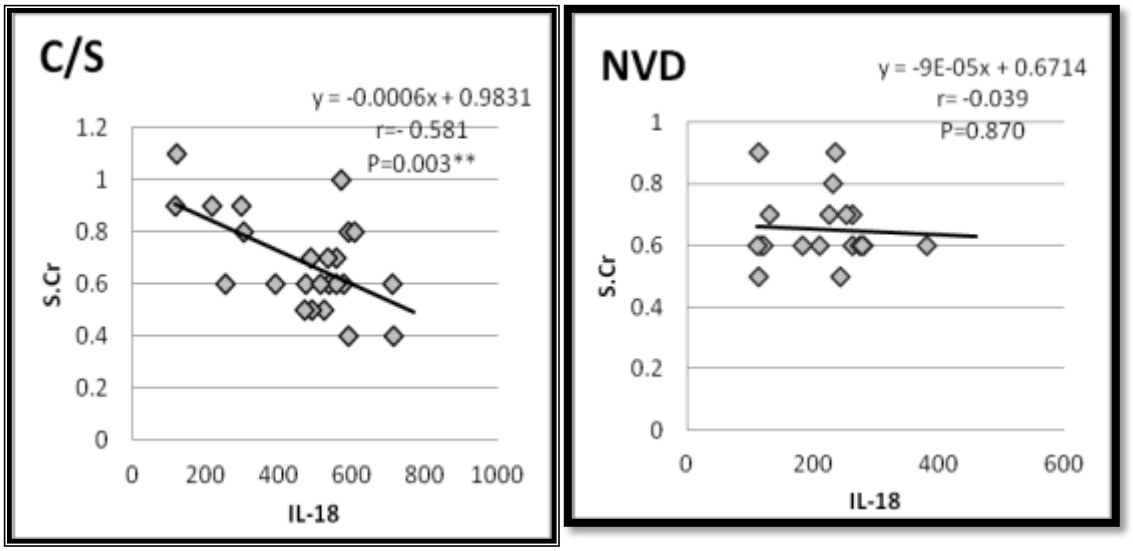
****. Correlation is significant at the 0.01 level (2-tailed) , *. Correlation is significant at the 0.05 level (2-tailed) And r.Correlation Coefficient**.

In the NVD group a significant negative correlation was found between S.Mg and S.creatinin while the significant positive correlation was found between S.urea and S. Creatinin (table (3-11)).

Table (3-11):Correlation between Study Variables in women with NVD

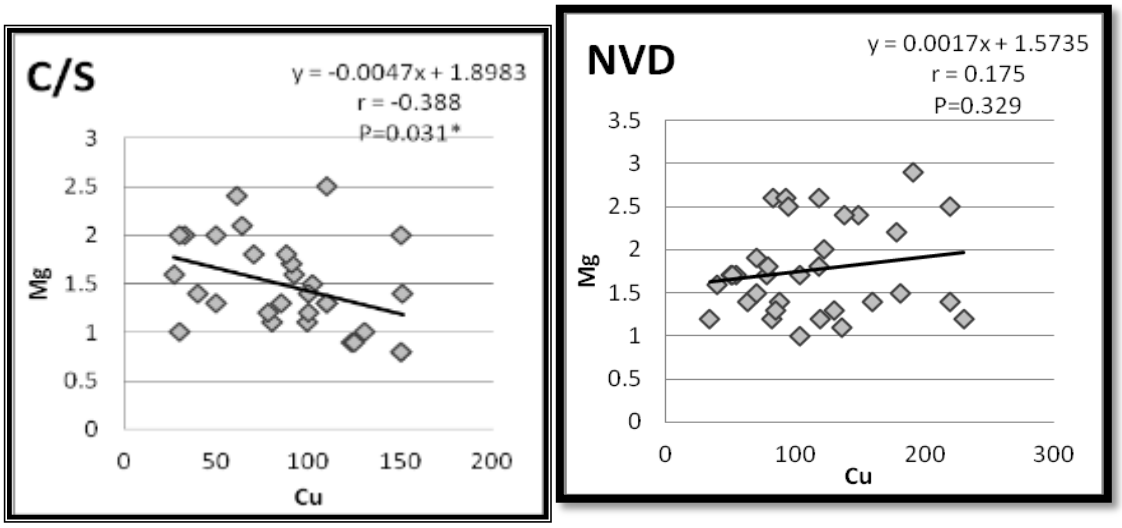
NVD	r	P
S.Mg + S.Zn	- 0.353	0.044*
S.Urea+ S. Creatinine	0.438	0.047*

***. Correlation is significant at the 0.05 level (2-tailed) and r.Correlation Coefficient.**



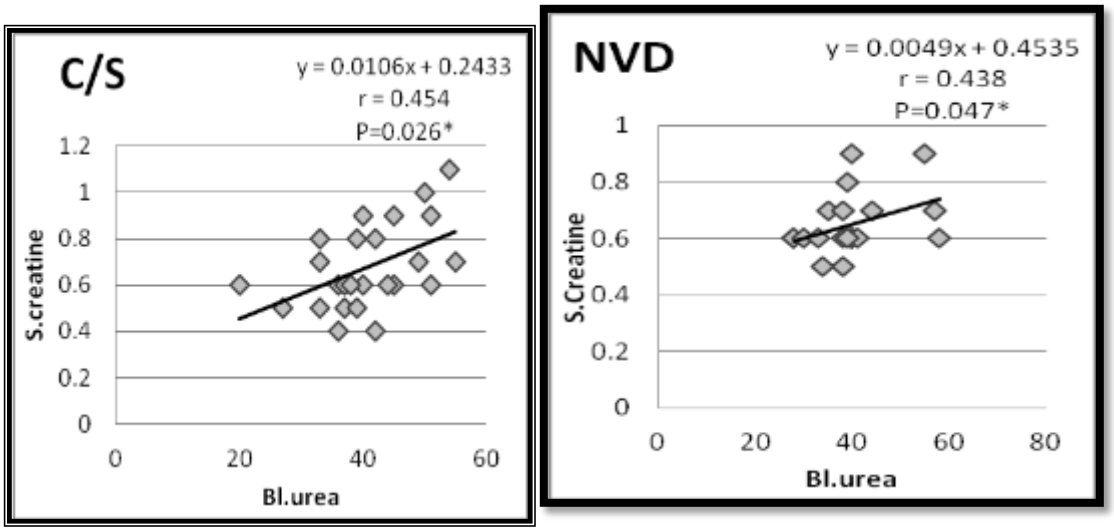
***p value < 0.05 is significant r: Correlation coefficient**

Figure (3-7) Correlation between S.cr(mg/dl) and S. IL-18(pg/ml) levels in both women with C/S and NVD



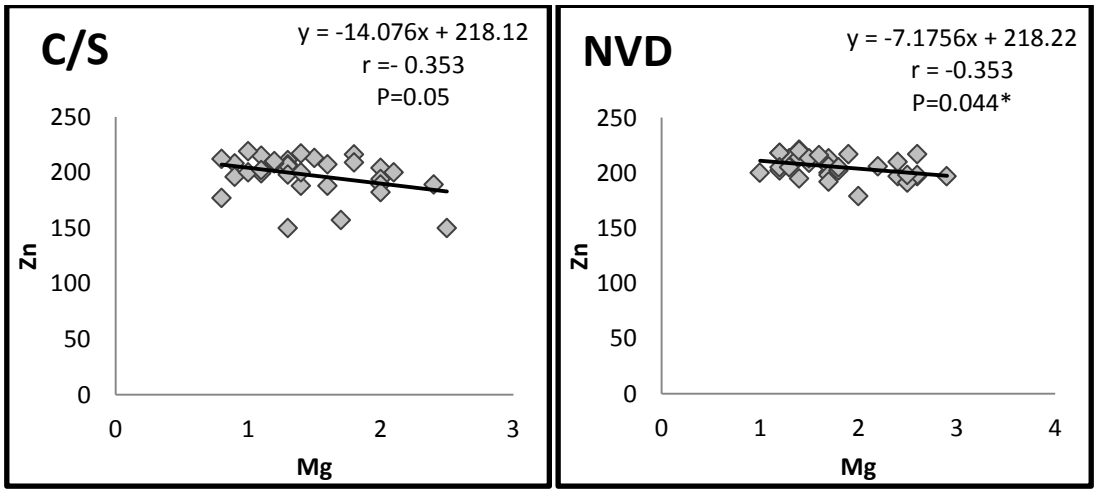
***p value < 0.05 is significant r: Correlation coefficient**

Figure (3-8) Correlation between S. Mg(mg/dl) and S.Cu(µg/dl) levels in both women with C/S and NVD



***p value < 0.05 is significant** **r Correlation coefficient**

Figure (3-9) Correlation between S. urea(mg/dl) and S. creatinine(mg/dl) levels in both women with C/S and NVD



***p value < 0.05 is significant** **r Correlation coefficient**

Figure (3-10) Correlation between S. Mg(mg/dl) and S. Zn(µg/dl) levels in both women with C/S and NVD

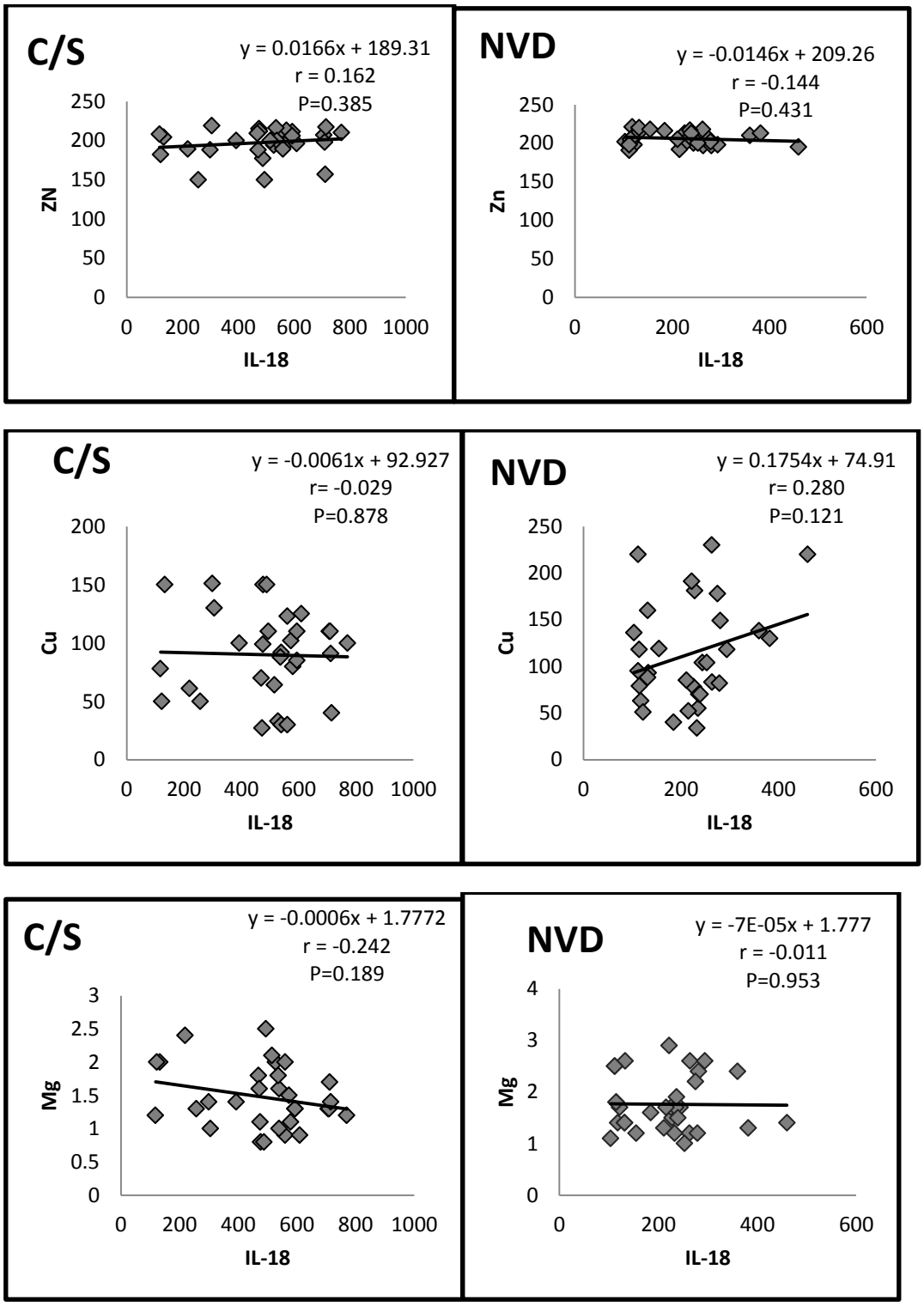


Figure (3-11) Correlation between S. IL-18 (pg/ml) and S. trace elements levels in both women with C/S and NVD

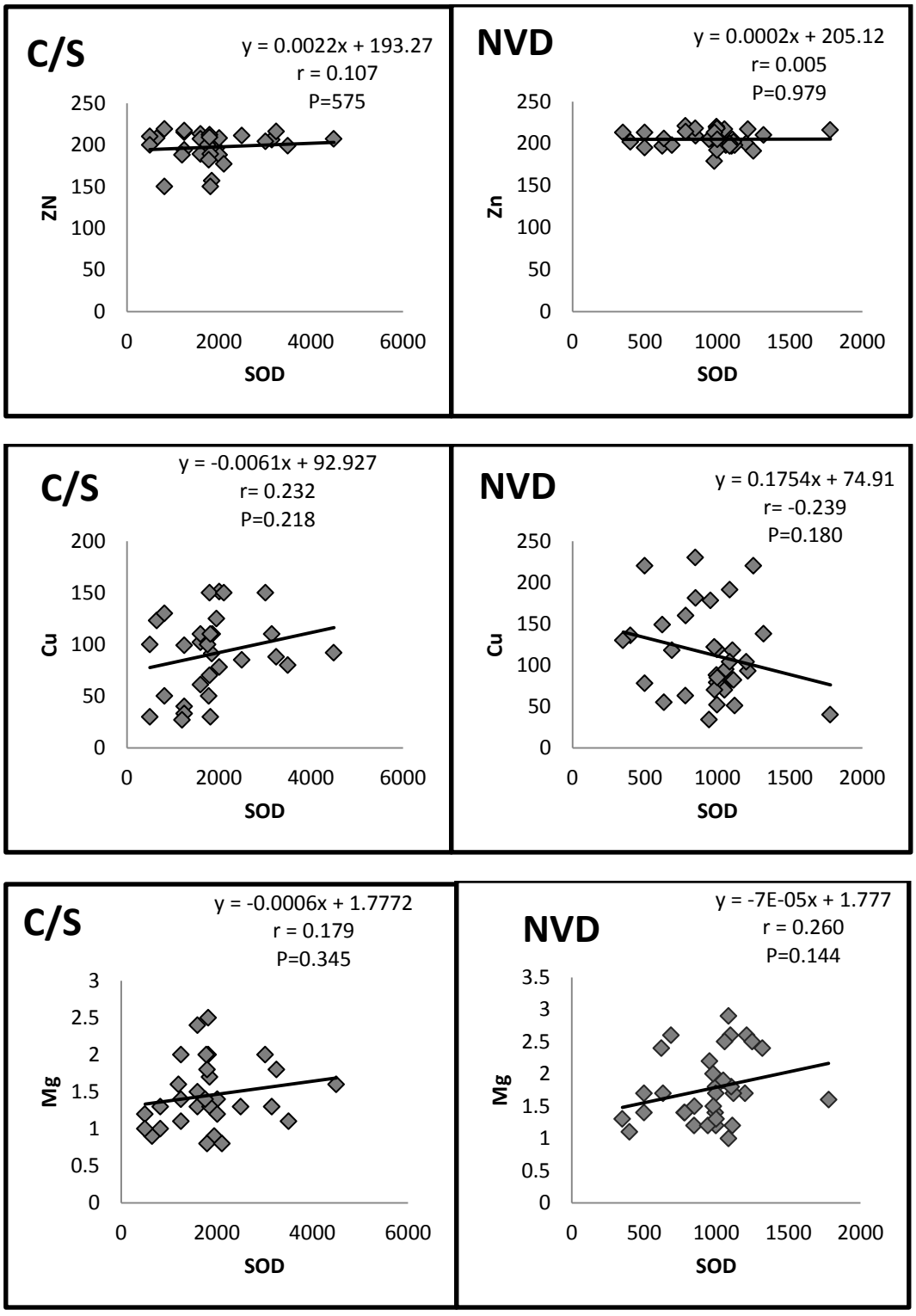


Figure (3-12) Correlation between S. SOD (pg/ml) and S. trace elements levels in both women with C/S and NVD

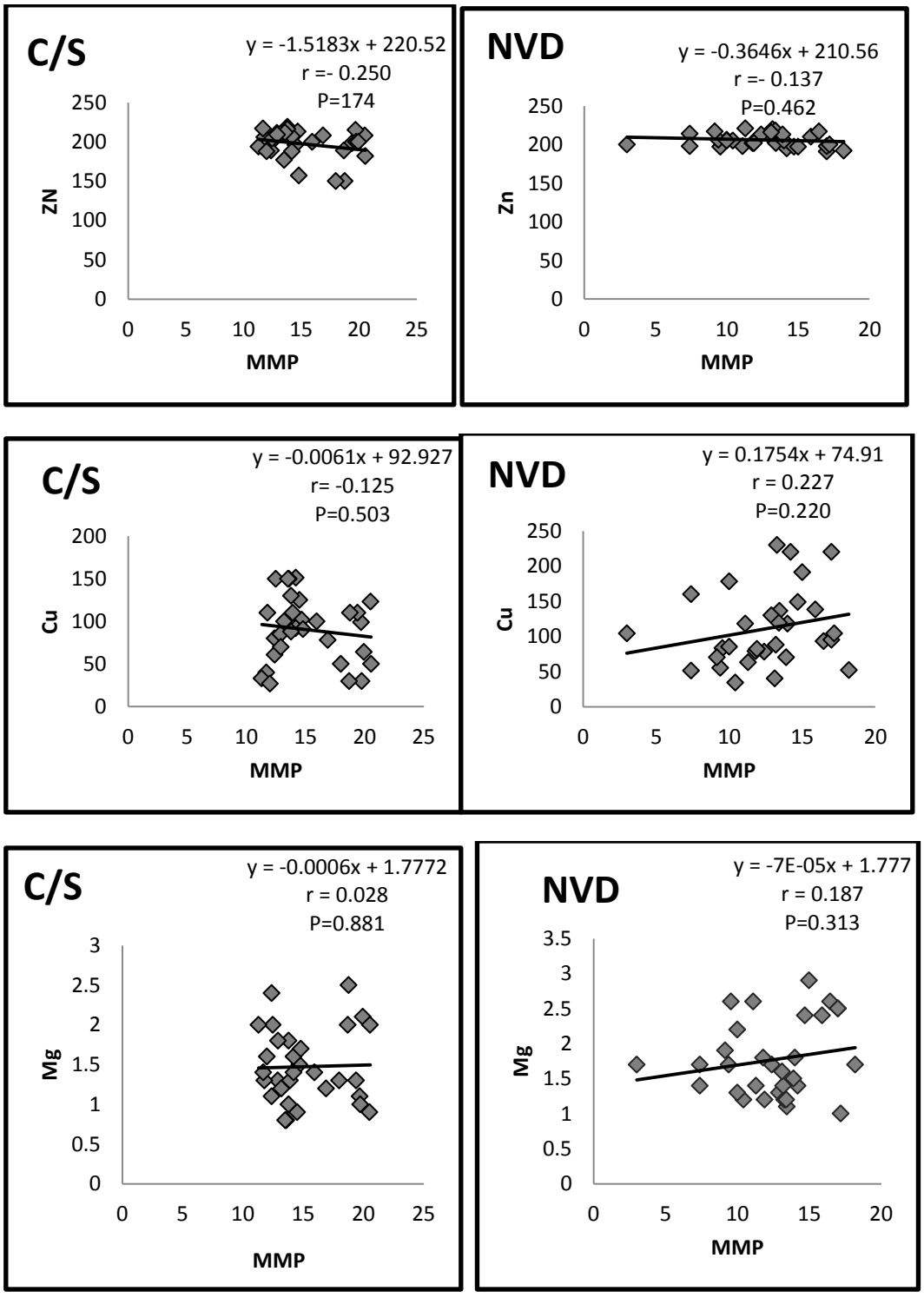


Figure (3-13) Correlation between S. MMP (ng/ml) and S. trace elements levels in both women with C/S and NVD

3.4. Association of C/S with Study Variables

To examine the relationship of the measured parameters with the C/S, a binary logistic regression analysis was performed**.** In this analysis, SOD , IL18, MMP-9, Mg and Zn were significantly associated with the C/S (P<0.05). Increased SOD, MMP-9 and IL18 were associated with increased odds ratio for the C/S . While Mg and Zn were associated with decreased odds ratio for the C/S (P<0.05) table (3-12) .

Table (3-12): Binary logistic regression analysis for C/S as the dependent variable in studied groups.

Parameters		P value	Odds Ratio	95% CI for Odds Ratio	
				Lower Bound	Upper Bound
C/S	SOD	0.0001	1.003	1.002	1.005
	IL-18	0.0001	1.012	1.006	1.017
	MMP	0.004	1.332	1.095	1.621
	Mg	0.026	.293	.100	.863
	Cu	0.057	.989	.978	1.000
	Zn	0.049	.960	.921	1.000
	Age	0.106	1.069	.986	1.159
	FBS	0.130	1.015	.996	1.035
	S.urea	0.405	1.031	.959	1.110
	S. cr	0.378	6.013	.111	325.326

The reference category is: women with NVD and *p value < 0.05 is significant

3.5 Histochemical Study

3.5.1 Histological Study

Cross sections of umbilical cord stained with H & E showed a general arrangement of three blood vessels embedded in a matrix which in Wharton's jelly. The vessels where two arteries and one vein (Figure 3- 14).

The Extracellular matrix (ECM) located between these blood vessels was found to be arranged in a lamellated way ,in a concentric arrangement surrounding these three blood vessels (Figure 3-15).

A closer look to the blood vessels showed a well identified three tunica's of blood vessels (Tunica Intima, Tunica Media, Tunica Advantatia).

The ECM lamelated arrangement was examined at a higher magnification and it showed a well identified cells with a darlely stained nuclear and cytoplasmic process all around the cells (Figure 3-16).

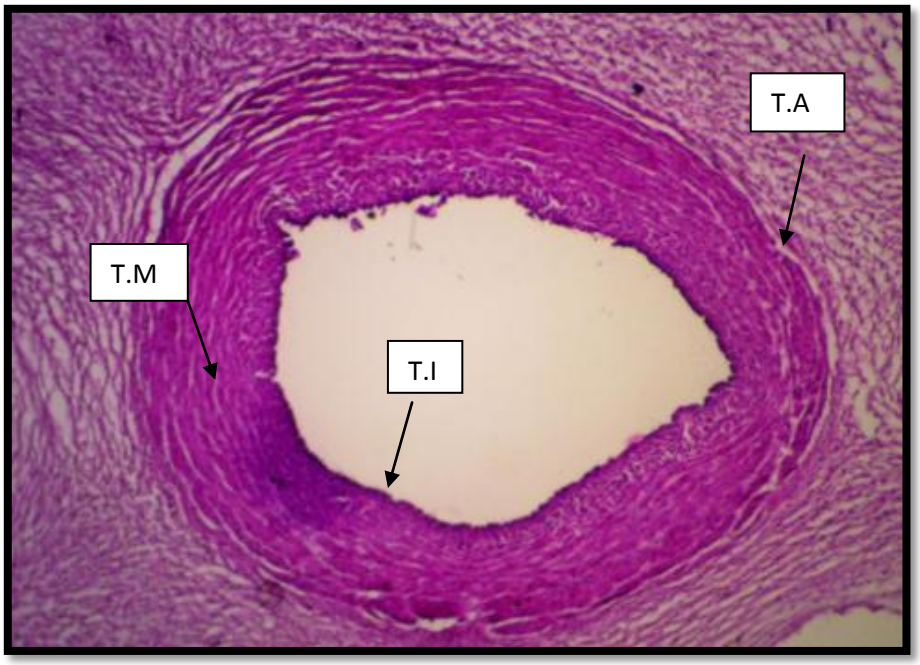


Figure (3-14) A cross section of the umbilical cord showing blood vessel and the matrix in between them. T.I (Tunica Intima),T.M(Tunica Media), T.A(Tunica Advantatia)H&E,NVD,X 40

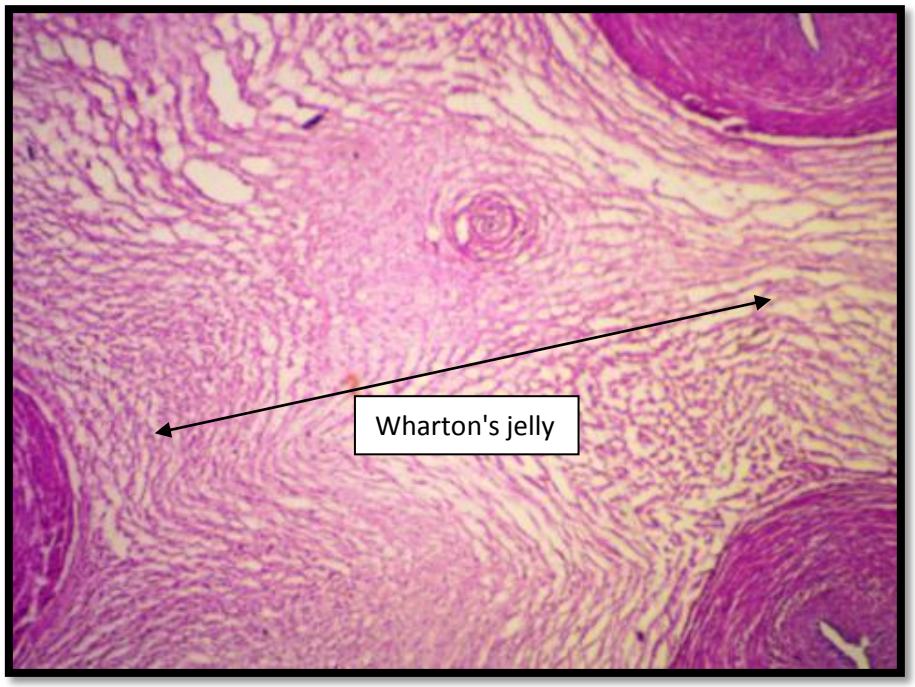


Figure (3-15) A cross section of the umbilical cord showing the well identified matrix arranged in packed lamella surrounding blood vessel. H&E,NVD,X 100

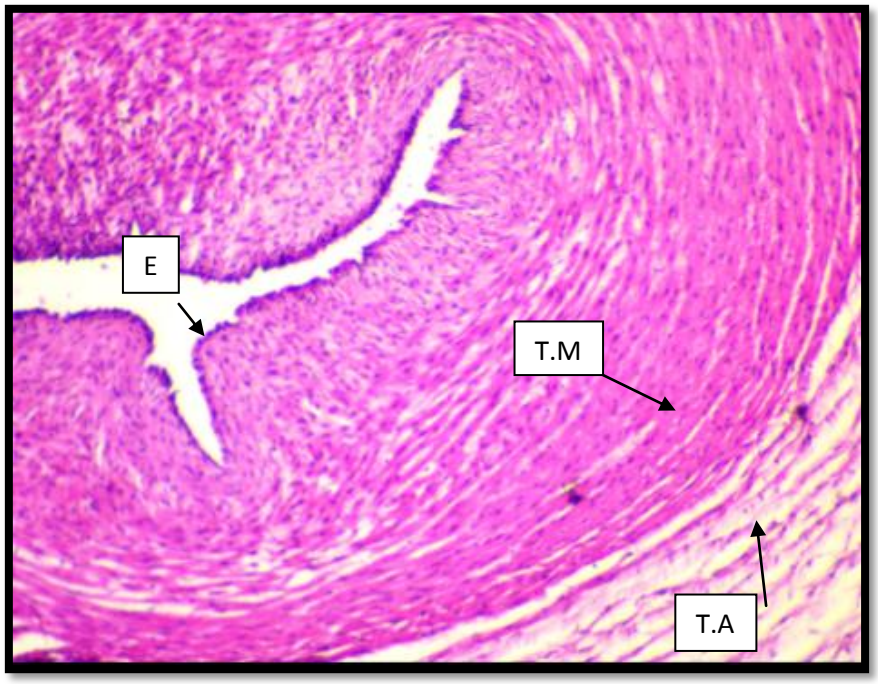
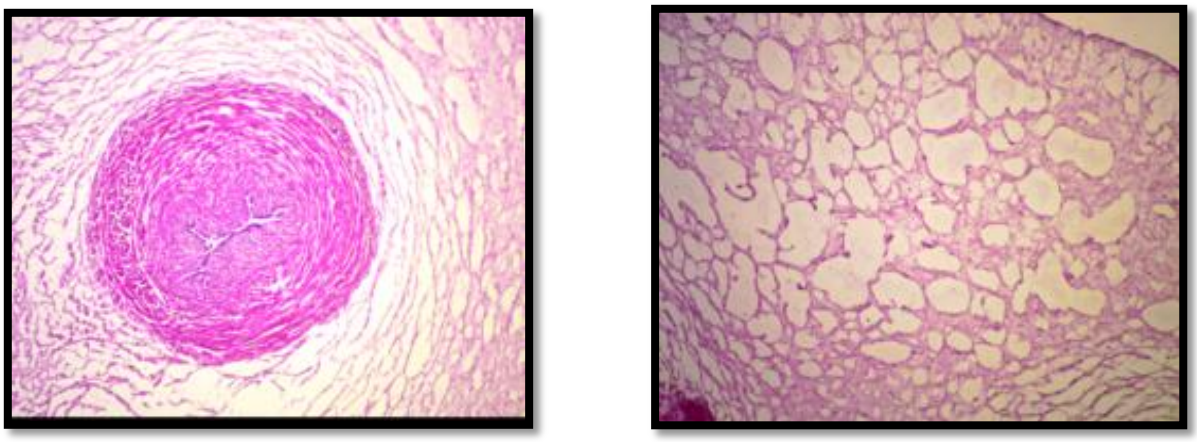


Figure (3-16) A cross section in the umbilical cord showing the distinction three layer of blood vessel. T.I (Tunica Intima),T.M(Tunica Media), T.A(Tunica Advantatia).H&E,NVD,X 100

The blood vessels arrangement , position and tunica did not show any differences between NVD and C/S cords.

On the other hand the matrix showed a stricking feature of being separated in wide spaces trapping .The cell in an empty surrounding in cord tissue of C/S as seen in (Figure 3-17 A&B). While the matrix in cord tissue of NVD showed a well-organized , highly pocked cord used lamelleted arrangement (Figure 3-18).



A B

Figure (3-17): Cross section in umbilical cord in C\S showing the separation of matrix in certain places with wide spaces in H&E , C\S, A:X10;B:X 40

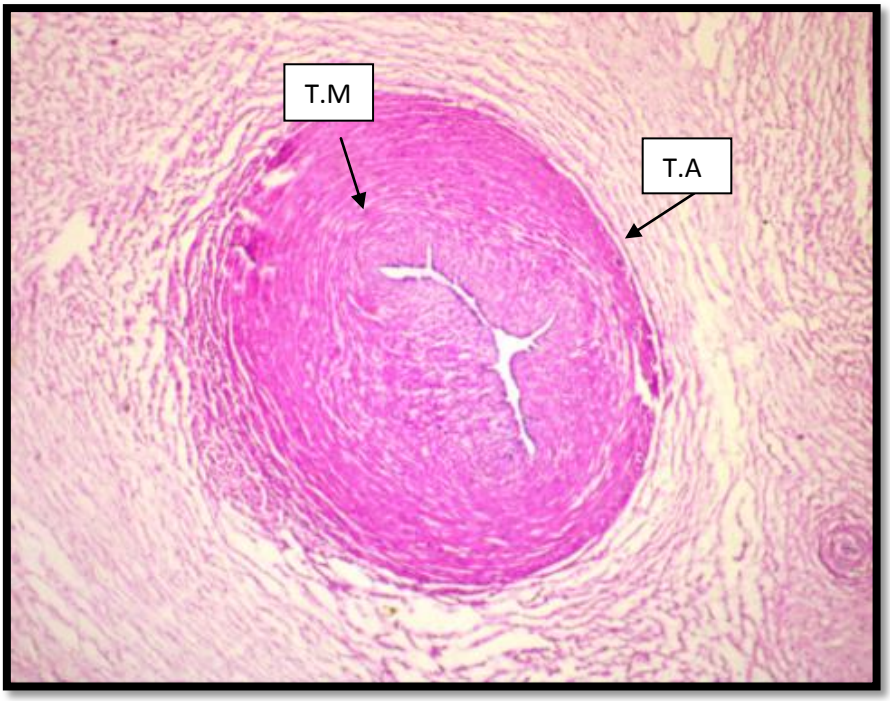


Figure (3-18) : A cross section in the umbilical cord in NVD showing a well arranged. Lamellated matrix with no spacing and separation. T.M(Tunica Media), T.A(Tunica Advantatia)

H&E,NVD, X 40

3.6 Immunohistochemistry study:

3.6.1 Matrix Metalloproteinas-9

Using MMP-9 and IL-18 Antibodies the reaction detected as fine small brownish at the site of reaction.

The staining reactivity of the immune-reaction was detected to be very low in the matrix of cord tissue for women underwent NVD. The fine brownish granules was not seen and the matrix was clear and nuclei was darkly stained with the counter (Figure 3-19).

While MMP-9 and IL-18 reactivity showed a well identified reaction in cord tissue of women underwent C/S as small fine brownish granules in the matrix , and the reactivity was the same in disorganized matrix are seen in cord tissue of (Figure 3-20).

Morphmetrical analysis of these immunohistochemistry reactivities using A perioposative pixel count. Algorithm was used in the study to evaluate the changes and the intensity of the reaction between these two types of tissue.

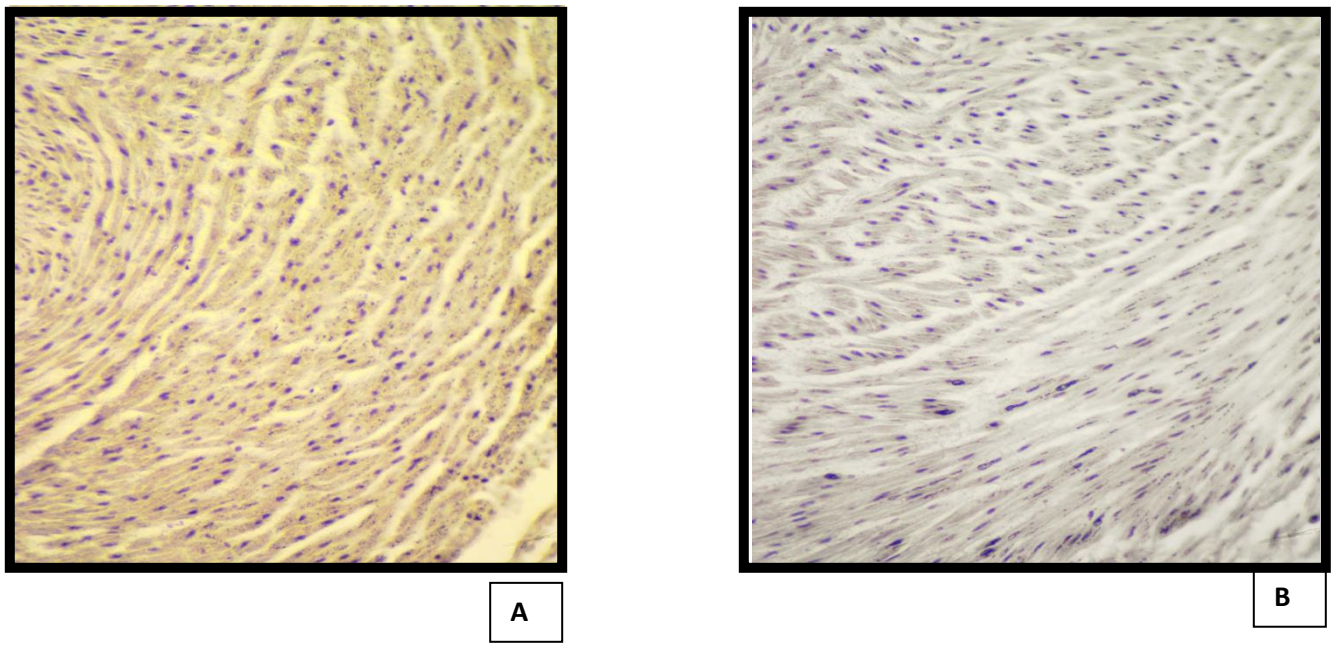


Figure (3-19) A: Showing reaction of Matrix Metalloproteinase -9 (MMP-9) in cesarean section
B: Showing low reaction of the tissue in normal vaginal delivery
MMP-9,X 40

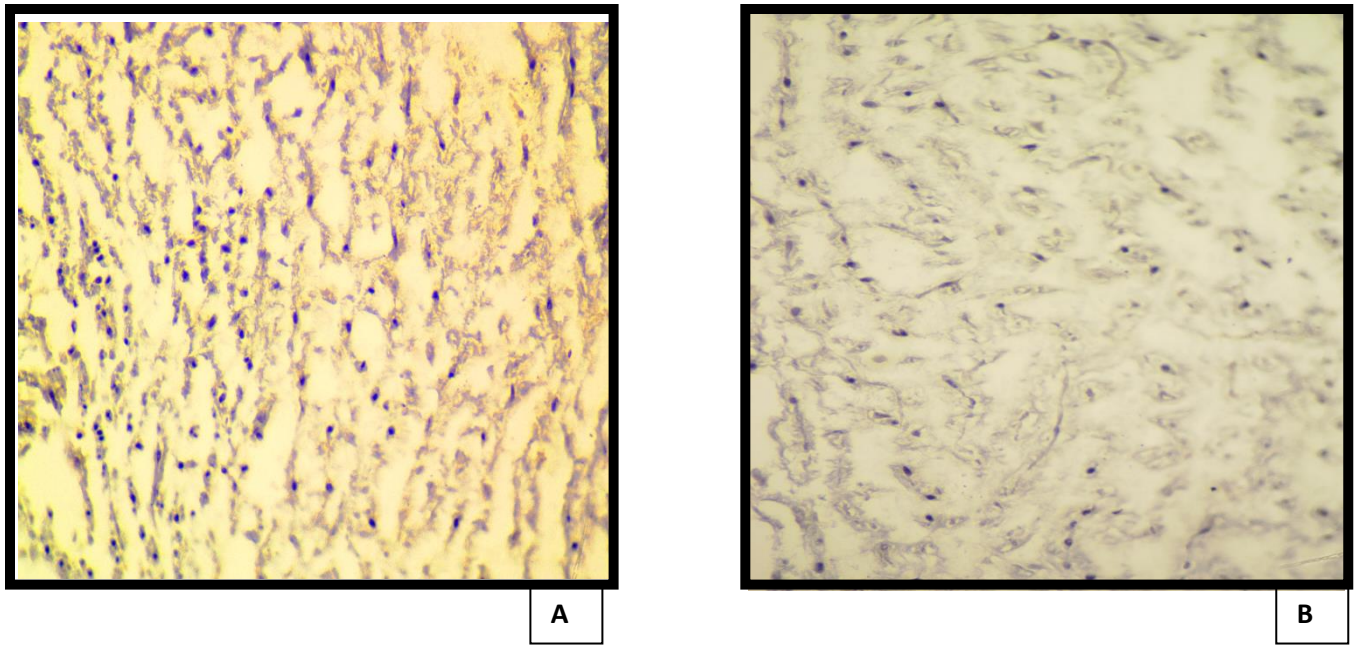


Figure (3-20) A: Showing positive reaction of Interleukin-18(IL-18) in cesarean section
B:Shawing Interleukin-18(IL-18) low reaction in normal vaginal delivery
IL-18, X 40

Quantification of MMP-9 and IL-18 reactivity using position pixel Count software (Aprio)

The Aprio software was used to calculated the mean positivity of the number of pixel of MMP-9 and IL-18 in umbilical cord section.

The reactivity of MMP-9 was recorded, it showed a high number of pixel and a high positivity in section of C/S cord with a mean of (176.54 ± 11.9). While NVD cord tissue section showed a low reactivity and positivity with a mean of (48.1 ± 6.8). And the P value wads < 0.001 as seen in table (3-13).

Table (3-13): Intensity of the reaction for MMP-9 recorded by number of pixels

	NVD	C/S	P VALUE
Intensity of the reaction recorded by number of pixels	**Mean ± SE (10^3)**	**Mean ± SE (10^3)**	P < 0.001
	48.1 ± 6.8	116.54 ± 11.9	

IL-18 staining reactivity in cord tissue was assessed using Aperio soft wear the quantification of the reaction was calculated by using the mean positivity percentage in the matrix between the vessel of the cord.

The immune reactivity of IL-18 showed a high mean in women underwent C/S with a mean of 48.08 ± 2.5 and those with NVD showed a mean of 16 ± 2.05 as seen in table (3-14).

Table (3-14): Intensity of the reaction for IL-18 recorded by number of pixels

	NVD	C/S	P VALUE
Intensity of the reaction recorded by number of pixels	**Mean ± SE (10^2)**	**Mean ± SE (10^2)**	P < 0.001
	16 ± 2.05	48.08 ± 2.5	

Chapter Four

Discussion

4.1 Demographic and biochemical criteria of studied groups

The present study enrolled 64 pregnant women divided into two groups. Information about the demographic and the modes of delivery of study samples were elicited in order to adjust for any potentially confounding factors (Austin PC., 2011). All women delivered by normal vaginal delivery and women delivered by caesarean section, were watched in their age, pregnancy age, social and economic situation, most women were housewives and lived in areas with a convergent social levels.

The results study groups were not significantly different in all demographic and biochemical criteria which indicate that the results were not confounded by these factors, like age ,job and their home areas as well as in number of previous pregnancy while in number of gravida and number of previous abortion the result were significantly different between two groups. In general, all women enrolled in this study were within the reproductive age range((Austin PC., 2011).

Moreover, the present study also evaluated the biochemical criteria of study sample. Renal function test, and serum glucose were all measured to confirm the absence of renal dysfunction and diabetic mellitus in both groups. Women with abnormal values were excluded from this study .

The proteolytic activity of MMP-9 has been implicated in various physiological and pathological conditions. In the present study, the results showed that there was a significantly increased in the level of MMP-9 in cord blood of women undergoing cesarean section more than in cord blood of women underwent normal vaginal delivery as showed in table (3-5). This may be

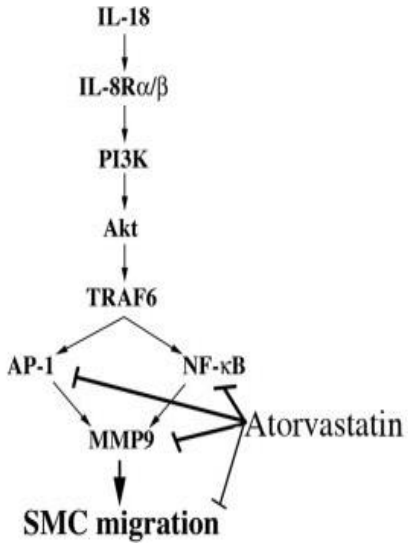
attributed to many reasons; one of them is the formation of fetal membranes represent a complex multilaminate tissue, composed of the amnion and chorion .These two closely adherent layers consist of several cell types, including epithelial cells, mesenchymal cells, and cytotrophoblast cells. The tensile strength of the fetal membranes depends on the integrity of these cells and their associated ECM (Uchide N., 2012).

Intensive disruption of the constituents in ECM may be associated with the rupture of fetal membranes both spontaneously and prematurely (Strauss JF., 2013). Most of the ECM and basement membrane components can be degraded by matrix metalloproteinases (MMPs), a group of structurally related, zinc-dependent enzymes (Lu P., 2011). These include MMP-2 and MMP-9 (also known as gelatinase A and B), which are capable of digesting collagen IV, a major component of basement membrane. These two MMPs have been identified in human fetal membranes and amniotic fluid. An increase in MMP-9 levels in the fetal membranes and amniotic fluid has been associated with term labor (Maymon., 2000), indicating a role for MMP-9 in human parturition. However, there is little information concerning changes in MMPs level with spontaneous preterm labor, and the cell sites of MMP-2and MMP-9 expression are not clearly known yet.

Another reason may attributed to increase in the level of interleukin -18 in group of cord blood of cesarean section women; IL-18 induced the transcription and mRNA expression of MMP-9 through a PI3K and Akt dependent AP-1- and NF-κB-mediated mechanism and stimulated the production of active MMP-9 (Ysani Chandrasekar., 2006). Also, they found the Atorvastatin significantly

inhibited IL-18- mediated NF-κB- and AP-1-dependent MMP-9 expression and smooth muscle cell (SMC) migration as in figure (Fig. 4-1).

These results suggest a causal role for IL-18 in atherogenesis and the possible therapeutic potential of atorvastatin for the inhibition of IL-18-mediated and MMP9-dependent SMC migration.(Chandrasekar., 2006)



Figure(4-1) schematic showing possible signal transduction pathways involved in IL-18-mediated SMC migration and that targeted by atorvastatin[ysani Chandrasekar., 2006].

Sundrani et al, (2012) have reported that MMP-9 levels was increased in preterm placenta as compared to term in women undergoing spontaneous vaginal delivery, However, MMP-9 levels were lower in preterm placenta as compared to term in women undergoing caesarean section. MMP-9 has been suggested to be important in separation of the placenta from the uterine wall during labor(Yarbrough, V. L.,2014). An increase in MMP-9 expression is suggested to contribute to degradation of the extracellular matrix ECM in the fetal membrane and placenta, thereby facilitating fetal membrane rupture and placental

detachment from the maternal uterus at term labor, both preterm and term (Vincent ZL.,2015).

In addition to Sundrani et al, (2012) another studies were disagreed with a study done by Fortunato et al. whom have also shown that MMP-9 is increased in the amniotic fluid of women with premature rupture of membrane (PROM) (Nhan-Chang CL.,2010). Other studies have reported increased activity of MMP-9 in fetal membrane (Enquobahrie DA.,2011) myometrium (Eckmann-Scholz C.,2012) and placenta (Vincent ZL.,2015) at the time of labor at term.

Findings of present study suggest that the mode of delivery may be one of the factors affecting the umbilical cord levels of MMP-9.

Also the increases the MMP-9 level in cord blood of women underwent cesarean section more than in cord blood of women with normal vaginal delivery may be due to significant decrease in the level of magnesium in cord blood of woman underwent cesarean section compared with cord blood of woman underwent normal vaginal delivery (table 3-1).

Study findings showed an inverse relationship between magnesium and MMP-9. Therefore, it's possible that magnesium affected MMP-9 production in the VSMCs through calcium mediated signal transduction . Mean serum level of magnesium concentration was significantly higher in woman underwent normal vaginal delivery compared with woman underwent cesarean section delivery as shown in table (3-6). In agreement with this result, Dobrowski et al found that magnesium is the most effectively reduced plasma MMP-9 concentration and they suggested that magnesium may effect on TIMP activity. Although

(Dobrowski et al.,2012) findings were agreed with study result ;but still this relation has not been documented.

Regarding correlation between Mg and MMP-9 ,Lee ;2004 found a negative correlation between magnesium and MMP-9 , It may resulted from N-methyl-D-aspirate (NMDA) receptor activity because magnesium is a non-competitive NMDA receptor antagonist(Lee., 2004). Glutamate activation of the NMDA receptor leads to increased cellular calcium, which activates a complex biochemical cascade that includes protein kinase activation, calpain-induced cytoskeletal breakdown, DNA fragmentation and reactive oxygen species accumulation (Dabrowski W., 2010). After Prolonged activation, neurons become damaged and subsequently die. The activation of NMDA receptors is reversibly modulated by MMP-9. However, the mechanism underlying MMP-9's regulation of the NMDA receptor is not clear. Some investigators have postulated that MMP-9 modulation of NMDA receptors requires proteolytic activity of MMP-9 and is not dependent on protein–protein interactions (Gorkiewicz ., 2010). Therefore, it assume that changes in serum umbilical cord magnesium concentration content can affect MMP-9 level; nevertheless, the interaction between Mg and remains to be elucidated (Dabrowski W., 2010) .

Also in this study, a negative correlation was found between zinc and magnesium in both woman underwent cesarean section and normal vaginal delivery (figure 3-13) while a positive correlation was found between copper and magnesium in woman underwent cesarean section; no correlation was found in normal vaginal delivery group (figure 3-11).

In the present study, a significant increase in zinc concentration was found in umbilical cord blood of women underwent normal vaginal delivery and cord blood of women underwent cesarean section as showed in tables (3-8).

Both Zn and Mg levels were increased in NVD group compared with C/S group. This result did not agreed with a study done by Kleopatra *et al* .They found that Zn and Mg concentration in serum of umbilical cord were remarkably decreased in NVD group compared with CSD group and they suggested that the uterus and skeletal muscles participated in this natural process. Their findings were similar to those found in athletes post-exercise (exercise provides an excellent model of tissue aseptic inflammation) .They attributed the decreases of their levels may be due to excretion in urine and sweet (Kleopatra et al., 2010).

The elevation in serum Cu concentration in NVD group against C/S group agrees with previous studies done by Elhadi 2015, where he found that the concentration of both elements (zinc and copper) were elevated in normal vaginal delivery group compared to cesarean section group.

In another study done by Lazer ,his findings agreed with this study when he observed that umbilical cord levels of zinc and copper levels were significantly higher in normal vaginal delivery compared with cesarean section delivery (Lazer *et al*., 2012).

Zinc and copper plays a vital role in the performances of the immune, antioxidant systems and affecting the complex network of the genes (nutrigenomic) with cytokines which are involved in the susceptibility to the diseases or disorders (Mocchegiani *et al*., 2014). It has been recently shown that

zinc and copper have a potent ability to induce TNF-α production (Cheknev SB., 2013). It is worth to be mentioned that some previous studies observed higher concentrations of IFN gamma, IL-4 and IL-10 in the peripheral and placental sera in the vaginal delivery compared with cesarean delivery (Palei AC.,2012).

The exact processes in human pregnancy which are involve in the initiation of labor are not clearly understood. during labor, the metabolic requirements increase sharply for both the mother and the fetus. Perhaps the uterotonin, prostaglandin, oxytocin, endothelin and platelet-activating factor that lead to contractility of the myometrium and the ripening of the cervix (Cunningham G, et al. 2010). On the other hand, the uterine activation/expression (in the presence of estrogen and progesterone) can be regulated by estradiol levels and matrix metalloproteinases (MMPs). Trace elements are important cofactors in the action of MMPs which are found in the decidua, fetal membranes and amniotic fluid and have several important biological processes including the initiation of labor and the parturition itself (Haneklaus M.,2013). Al-Saleh et al, 2004 reported an active and passive placental transport for zinc and copper, respectively between mother and fetus.

Superoxide dismutase was measured as antioxidant status in this study as shown in table (3-3). There was significantly increase in serum SOD concentration of cord blood of caesarean section women group more than found in normal vaginal delivery group .

Superoxide dismutase SOD occurs in the mitochondria (Mn-SOD) and the cytosol (Cu-SOD) of the cell and reduces superoxide radicals to hydrogen peroxides. In present study, serum of cord blood SOD from women of NVD was significantly lower than these of caesarean section. During fetal development

antioxidant defense mechanisms have generally been considered less active than those of adults (Kaur G., 2016). This may be due to a lower requirement for cellular antioxidant activity as the fetus is in an environment with a low partial pressure of oxygen (Kaur G., 2016) .

The increase in SOD of cord blood C/S women compared with SOD of NVD cord blood revealed increase in the antioxidant status (enzymatic type) ; this increase was combined with increase in the zinc and copper concentrations in the cord blood of NVD group suggested that there is increased antioxidant status (super oxide dismutase) in cord blood of caesarean section born babies and increased oxidative stress(which is indicated by raised zinc and copper) in cord blood of normal delivery born newborns.

These findings agreed with (Sajjad Y., 2000) that found that cord blood SOD concentration were significantly higher (P =0.0337) in cord infants who had been delivered by caesarean section when compared with vaginal delivery and they suggested that it may be a reflection of a relatively low level of SOD concentration in infants to the increase oxidative stress of labour.

The SOD is responsible for removing oxygen free radicals which occurs over a short period of time. The process of labour itself may be associated with production of oxygen free radicals (Mert, I., 2012), which may result in increased utilisation of SOD and explain the low levels of SOD in the NVD group, compared with the caesarean section group (Sajjad Y., 2000).

So, according to the present as well as majority of studies done in other parts of the world, caesarean section proves to be better than normal delivery, as in caesarean section the oxidative stress is less and antioxidant level is better, and there are studies which showed no significant difference between normal

vaginal delivery and caesarean section in respect to oxidative stress and antioxidant status. And there are studies which show a totally opposite findings. This part of the total oxidative stress which directly involves fetal outcome needs to be explored from many other angles including the social structure, the economic background, ethnicity, genetic preponderance, diet, nutritional status & susceptibility to oxidative stress. The studies which are in agreement with the result of the present study (Watanabe ., 2013).

Umbilical cord blood cytokine and chemokine profiles were compared between well-characterized term newborns delivered by CS or VD. The results of the present study support the view that fetal cytokine profiles were not elevated by spontaneous labor at term . Term labor and parturition have been associated with inflammatory activation and with altered local and systemic cytokine levels, although findings across studies according to cytokine profiles in cord blood were variable(Erdem, M., 2012). In contrast to the majority of previous reports, we could not show altering fetal cytokine concentrations in cord blood after VD compared with CS. Other groups measured various cytokines in cord blood of term newborns from uneventful pregnancies by means of ELISA and were able to demonstrate that vaginal delivery at term promotes mainly the production of pro-inflammatory cytokines such as IL-18 (Romero *et al* 2006). Methodological differences in detectable capacity of cytokine assays could potentially contribute to controversial study results: ELISA technology was used in this study and premixed kits with ROC analysis revealed that serum IL-18 concentration level had a significant area under the curve (AUC) 0.890, indicating that a threshold of 300 pg/ml gave sensitivity of 82% and specificity 91% . However, the biological relevance of circulating cytokines in low (physiological) concentrations remains unclear. It is also possible that results of

this study differ from others because it only included patients without evidence of infection or inflammation.

The precise mechanisms regulating parturition and onset of spontaneous term labor are complex. Labor involves highly interdependent physiologically processes mediated by interacting effects of hormones and cytokines, especially prostaglandins and pro-inflammatory cytokines (Clarissa, L.T.,2013). A growing body of evidence suggests that inflammatory immune response plays a central role in the mechanisms of parturition and in the cascades of events during spontaneous labor at term. Therefore, inflammatory cascades and immunological changes have been detected in cervix, myometrium, chorioamniotic membranes, and amniotic fluid during parturition and labor. Inflammatory activation at term labor was shown to be associated with a massive influx of immune cells into the gestational tissues, which is characterized by an increased local synthesis of cytokines and chemokines (Filipovich Y.,2015) . It is well established that placental and fetal membrane macrophages, lymphocytes, and decidual cells are the main sources of major inflammatory cytokines during labour (Gomez-Lopez N., 2013). Furthermore, the central role of the localized inflammatory response in myometrium and chorioamniotic membranes during labor is supported by gene expression analysis. Earlier studies demonstrated that the mode of delivery and labor alter separately cytokine concentrations in maternal and fetal compartments. the other study were disagreed with our finding like Pacora et al 2000. They determined IL-18 simultaneously in both compartments in dependence of labor at term. Although IL-18 concentration was increased in the amniotic fluid of patients at term in labor compared with those not in labor, the cytokine level measured in maternal serum and cord blood remained unaffected by labor. Findings of Gyarmati *et al* (2010) put an emphasis on the hypothesis of

the compartmentalization of cytokines during labor who investigated perinatal concentrations of 17 cytokines and growth factors in maternal serum by multiplexed Luminex methodology. In good accordance with present results, Cicarelli *et al* 2005 reported that mode of delivery and labor showed no systematic inflammatory response, as reflected by similar cytokine levels in the VD and CS groups. Also Tutdibi ;2012 findings suggest that, in healthy term neonates, the exposition to normal spontaneous delivery and labor is not associated with systemic activation of different inflammatory mediators compared with CS except for TGF- β 1.

The umbilical cord blood IL-18 concentration in cord blood women delivered by cesarean section and women delivered by normal vaginal delivery were showed in table (3-4), its concentration was significantly lower in women delivered by NVD compared with women delivered by CS ($P<0.001$).

The result in present study may attributed to fact that most of the patients have simple types of inflammations such as acne or tooth inflammations and other infections were discreet and clear, which may cause the increase of anti-inflammatories and interleukins(Kaye AD., 2014).

In addition to that, side effect of the anesthetics used during the cesarean section operation; and this may require other tests that may help us to understand the changes which are being produced in high level interleukins.

Another reason can cause high cytokines that women undergoing normal vaginal delivery will not need for injection, episiotomy or oxytocin and conducting injury. It is known that the injury and infections are usually associated with hypercatabolic response to injury.

There are two main groups of mediators are involved in this condition:

1- Hormones such as glucocorticoids, adrenaline and glucagon all these can mobilize carbohydrates and fat stores as sources of energy.
2- Cytokines such as interleukins (ILs) and tissue necrosis factor, which induce fever and gluconeogenesis (TNF) (Kumar ., 2013)

Umbilical cord blood cytokine were compared between term newborns delivered by CSD or NVD. Term labor and parturition have been associated with inflammatory activation and with altered local and systemic cytokine levels, although findings across studies according to cytokine profiles in cord blood are variable (Erdem, M., 2012).

The present study reveals several new and interesting key findings in pregnant women delivering at term in cesarean section and normal vaginal delivery. Which we found the umbilical cord serum IL-18 concentration level significantly lower in women delivered by NVD when compared with women delivered by cesarean section. And this result is disagree with other studies demonstrated that the mode of delivery and labor alter separately cytokine concentrations in maternal and fetal compartments(Jones RO.,2014.). (Pacora et al., 2000) determined IL-18 in both compartments in dependence of labor at term.

And the Gyarmati et al 2010 findings were the IL-18 concentration was increased in the amniotic fluid of patients at term in labor compared with those not in labor, the cytokine level measured in maternal serum and cord blood remained unaffected by labor.(Gyarmati et al., 2010)

4.2 Histological criteria in Umbilical cord tissue in groups

The histological examination of umbilical cord tissue for women underwent NVD and C/S revealed that the lowest reactivity with anti- IL-18 and anti MMP-9 monoclonal antibodies was found among women underwent NVD in comparison to those underwent C/S. This is believed to be due to the increase in umbilical cord mitochondrial activity with increasing gestational age, which results in increased reactive oxygen species (ROS) (Oliver C., 2011).

Our data demonstrate that the proinflammatory and pro-atherogenic cytokine IL-18 can induce human coronary artery smooth muscle cell SMC migration independently of other proinflammatory cytokines. IL-18 induced the transcription and mRNA expression of MMP-9 through a PI3K- and Akt dependent AP-1- and NF-_B-mediated mechanism and stimulated the production of active MMP-9(Fahlenkamp AV., 2011).

The postangioplastic restenosis, intimal thickening and hyperplasia are characterized by the proliferation of smooth muscle cells SMCs in the tunica media and their migration toward the luminal surface. In normal vessels, SMCs are largely confined to the tunica media, where they interact directly with components of the ECM. In addition to this physical interaction with the SMCs, ECM proteins regulate the expression, secretion, activation, and breakdown of various cytokines, chemokines, growth factors, and adhesion molecules by the adjacent cells (Isozaki T., 2015). ECM expression, deposition, and degradation are all tightly regulated by a balance between SMC motility and migration (Karagiannis GS., 2013)

MMPs are zinc-dependent proteases that are classified as collagenases, stromelysins, elastases, and gelatinases based on substrate specificity. Their

expression is regulated at both the transcriptional and post-transcriptional levels. They are synthesized as proenzymes and are activated following proteolytic cleavage. SMCs express the MMP-9 (gelatinase B). The excess activation of MMP-9 result in destruction of the ECM and leads to pathological remodeling and vascular restenosis (Fahlenkamp AV., 2011).

The IL-18 stimulated MMP-9 release without modulating TIMP1 levels (Zhou C.,2015), suggesting that IL-18 alters the MMP/TIMP balance in favor of MMP expression and induces ECM degradation. Bysani results also show that IL-18 stimulates MMP9 expression at both the transcriptional and post-transcriptional levels. IL-18 stimulated MMP-9 promoter-reporter activity, mRNA expression, enzyme activity, and secretion. Investigation into the possible signal transduction pathways involved in IL-18-mediated MMP-9 expression indicated that IL-18 induced Activator protein 1(AP-1) and NF-B activation was responsible for these responses (Tsai C.,2014).

Chapter six

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Appendix

Assay procedure for determination of MMP-9 concentration

The reagents and samples were Bring all to room temperature before used and the reagents were mixed thoroughly by gently swirling before pipetting.

1. A volume of 100µLof Standard, Blank, or Sample were dispensed into appropriate wells. Mixed gently and covered, then incubated for 90 minutes at 37℃.

2. Immediately add 100µL of Biotinylated Detection Ab working solution was added to each well was added.

3-The plate was covered with the Plate sealer and incubated for 1 hour at 37°C.

4. The microplate wells were washed and aspirated three times by filling each well with Washing Buffer (approximately 350µL) using an automated washer.

5. A volume of 100µL HRP Conjugate working solution was added to each well and covered with the new Plate sealer and Incubated for 30 minutes at 37°C.

6. The aspiration / wash process was repeated for five times as conducted in step 4.

7. A volume of 90µL Substrate Solution were dispensed into each well. Covered with a new Plate sealer. Incubate for about 15 minutes at 37°C.

8. The reaction was stopped by adding 50µLof Stop Solution to each well.

9. Optical density was determined for each well at once, by using a micro-plate reader set at 450 nm.

Assay procedure for determination of IL-18 concentration

All reagents and samples were brought to room temperature before use, and centrifuged again after thawing before the assay.

1. A volume 100µLof Standard, Blank, or Sample were dispensed into appropriate wells. Mixed gently and cover the plate with sealer, then incubated for 90 minutes at 37℃.

2. Immediately , 100µL of Biotinylated Detection Ab working solution was added to each well, and the plate was cover with the plate sealer then incubate for 1 hour at 37°C.

3. The microplate wells were washed and aspirated three times by filling each well with Washing Buffer (approximately 350µL) using an automated washer.

4. A volume 100µL of HRP Conjugate working solution was added to each well and covered with the plate sealer and incubated for 30 minutes at 37°C.

5. The aspiration / wash process was repeated for five times as conducted in step 3.

6. A volume of 90µL of substrate solution was dispensed into each well. Covered with a new Plate sealer then Incubate for about 15 minutes at 37°C.

7. The reaction was stopped by adding 50µLof stop solution to each well. Then, the color turns to yellow immediately.

8. The optical density (OD value) of each well was determined at once, by using a micro-plate reader set at 450 nm.

Assay procedure for determination of SOD concentration

1-A volume of 50µl of standard, blank, and samples were dispensed into appropriate wells.

2- Immediately 50µl of Biotinylated Detection Ab working solution was dispensed into each wells.

3-The plate was covered with the plate sealer and incubated for 45 minutes at 37°C.

4-The microplate wells were washed and aspirated three times by filling each well with wash buffer (approximately 350µl) using an automated washer.

5-The microplate wells were struck sharply onto absorbent paper to remove all residual water droplets.

6-A volume of 100µl of HRP Conjugate working solution was dispensed into each wells. Covered with a new plate sealer and incubated for 30 minutes at 37°C.

7-The aspiration/wash process was repeated for five times as conducted in step 4 and 5.

8-A volume of 90µl of substrate solution was dispensed into each wells. Covered with a new plate sealer and incubated for about 15 minutes at 37°C.

9-The reaction was stopped by adding 50µl of stop solution to each well. The blue color must be completely changed to yellow color.

10- The absorbance was read at 450nm with a micro-titer plate reader within 15 minutes.

Chapter Five

Conclusions and Recommendations

5.1 Conclusions

According to the findings reported in the present study, The following can be concluded:

1- Women delivered by cesarean section was associated with low oxidative stress status and high anti-oxidant enzymatic activity. While term pregnancy with NVD was associated with increase oxidative stress and decrease anti-oxidant activity.

2- There was a significant elevation in MMP-9 ,IL-18 in women who underwent cesarean section in comparison with women who underwent normal vaginal delivery.

3- This study concluded that normal vaginal delivery effect oxidative stress indicated by increase (Cu) ion concentration.

4- Serum concentration of SOD increased in cesarean section while decreased in normal vaginal delivery due to the physiological condition .

5- In women who underwent cesarean section decreased Mg^{+2} serum level may have an effect on MMP-9 concentration.

6- IL-18 serum level showed a very modest sensitivity and specificity in predicting delivery type.

7- MMP-9 serum level is not highly sensitive and specific in predicting delivery type.

8- SOD is highly sensitive and specific to predict women delivered by cesarean section based on serum enzymatic anti-oxidant activity.

9- Histological examination showed high MMP-9 and IL-18 concentrations in the umbilical cord tissue of women delivered by cesarean section in comparison to pregnancy with normal vaginal delivery.

5.2 Recommendations

Based on the conclusions provided from this study, it is recommended to:

1. Further investigate the role of remolding degradation enzyme (MMP-9) in different types of term labor should be undertaken to clarify the causal relationship; as the present study only described the correlation between high level of this enzyme and women undergoing cesarean section.
2. To determine the effect of antioxidant supplements, such as iron materials and vitamins during pregnancy and its impact on type of delivery.
3. Determined oxytocin hormone concentration during labour and its impact on type of delivery.
4. The relationship between circulatory and degradation enzyme (MMPs) levels in cord blood needs to be investigated genetically.
5. Genetic study need to be applied on MMP-9 enzyme.
6. Studied markers need to be measured in fetal circulation to correlate with maternal blood.
7. Study the effect of anesthetics used in Iraqi hospitals on MMP-9 level in women undergoing C/S.
8. Take blood samples before delivery to measure the level of these enzymes and if there is relation between them and mode of delivery

الخلاصة

ماتركس ميتالوبروتياز Matrix metalloproteinases اختصارا (MMPs) وهي انزيمات تعتمد على الزنك وتحتوي على اندوبيبتيداز endopeptidases . هذه الأنزيمات لها القدرة على إحداث التحلل في جميع أنواع البروتينات للمصفوفة خارج الخلية، ولكن أيضا يمكن معالجة عدد من الجزيئات النشطة بيولوجيا. ومن المعروفة انها تقوم بالمشاركة في انشقاق والانقسام لمستقبلات سطح الخلية، واطلاق المعقدات المسؤولة عن موت الخلية (مثل معقد FAS)، وعدم تفعيل السيتوكينات cytokines. وايضا خلال فترة الحمل تحدث تغيرات فسيولوجية خاصة مثل نضج عنق الرحم، وتمزق الأغشية الجنينية، وانفصال المشيمة والذي تتطلب إعادة تشكيل المصفوفة خارج الخلية (ECM) . السيتوكين مثل الانترلوكين -IL) (18يلعب دورا حاسما في دفاع المضيف، وانها قد تكون جزء في توفير حاجز لعنق الرحم/ ساقطي ضد الغزو الميكروبي من السائل الذي يحيط بالجنين وقد يكون بمثابة علامة لعنق الرحم للعدوى داخل الأمنيوتك Amniotic.

الإجهاد التأكسدي، وهي حاله تتميز بعدم التوازن بين الجزيئات المؤكسدة، بما في ذلك الأنواع الاكسجين التفاعلية (ROS)، وبين دفاع المضادات للأكسدة Antioxidant. هذه ROS قد يسبب إصابات الأنسجة مما يؤدي إلى تلف البروتينات الخلوية داخل الخلايا ، والحمض النووي DNA التي تورطت في مضاعفات الحمل في بوقت مبكر أثناء المخاض، ويمكن أن يؤثر على طريقة الولادة . استجابة الجسم المناعية تتضمن المضادات للأكسدة الأنزيمية وغير الأنزيمية التي تقلل من تأثير هذه الجذور الحرة. تهدف هذه الدراسة إلى تقييم العلاقة بين مستويات انزيم ماتركس ميتالوبروتياز-٩ (MMP-9)ووضعية الحمل.

الطرق: في هذه الدراسة تم تعيين ستة وأربعين النساء الحوامل (٣١ حاملا خضعن للولادة الطبيعية، ٣٣ حاملا خضعن للولادة القيصرية. . تم جمع نموذج الدراسة من صالات النسائية والتوليد في مدينة الامامين الكاظمين الطبية خلال فترة من حزيران من ٢٠١٥ الى تموز من ٢٠١٦.واخذت موافقة النساء الحوامل قبل الدخول لصالة العمليات، وتم اخذ عينات من الدم وقطعة من النسيج جمعت خلال خمس دقائق من الحبل السري.

تم جمع عينات الأنسجة الحبل السري أيضا أثناء الولادة. واستخدمت عينات دم وأنسجة لقياس الفلزي المصفوفة (MMP-9)، وانترلوكين ١٨ (IL-18)، وايضا تم استخدام نماذج الدم لقياس معدل جهد التأكسد في مصل الدم و المضادات للأكسدة (SOD) والعناصر النزرة.

النتائج: أظهرت النتائج أن تدهور في المستويات الحيوية ل(MMP-9 و IL-18) في الدم النساء اللواتي خضعن الولادة الطبيعية. و كانت عالية في النساء اللواتي خضعن للعملية القيصرية وبالإضافة إلى ذلك، فإن مستويات الأنزيمات المضادة للأكسدة الأقل بين النساء اللواتي خضعن للولادة الطبيعية وأعلى بين النساء اللواتي خضعن للعمليات القيصرية. وذكرت في الدراسة أيضا انخفاض كبير في مستوى العناصر النزرة المؤشرات الحيوية (الزنك والنحاس والمغنيسيوم) في الدم النساء اللواتي خضعن للعملية القيصرية مقارنة مع مجموعة النساء اللواتي خضعن الولادة الطبيعية.

الفحص النسيجي للنسيج الحبل السري من النساء الحوامل كشفه عن نتائج مطابقة لنتائج فحوصات الدم حيث بينت عن ارتفاع معنوي في تركيز انزيم MMP-9 والانترلوكين IL-18 في النساء اللواتي خضعن للعملية القيصرية مقارنة مع مجموعة النساء اللواتي خضعن الولادة الطبيعية.

الاستنتاجات: ارتبط الولادة الطبيعية مع زيادة الاكسدة وانخفاض في النشاط المضادة للأكسدة و في المقابل انخفاض الاجهاد التأكسدي و زيادة في النشاط الانزيمي المضادة للأكسدة (SOD) في الولادات القيصرية ،. وأضاف لذلك ارتبط الولادة الطبيعية بانخفاض معنوي في مستويات انزيم MMP-9 والسايتوكينات IL-18 مقارنة مع مجموعة النساء اللواتي خضعن الولادة الطبيعية. ومن ناحه اخرى أظهر الفحص النسيجي هنالك تركيزات عالية MMP-9 و IL-18 في الأنسجة الحبل السري للمرأة التي تخضع للعملية القيصرية بالمقارنة مع النساء اللواتي الولادة الطبيعية.

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تقييم الانزيمات المعدنية المحللة للبروتين -9 (MMP-9) والانترلوكين-18 (IL-18) في دم الحبل السري والنسج البيني في الولادات القيصرية و المهبلية الطبيعية

أطروحة مقدمة الى

مجلس كلية الطب/جامعة النهرين

كجزء من متطلبات الحصول درجة الدكتوراه في فلسفة

الكيمياء الحياتية السريرية

من قبل

خوله عبد الحمزه شمران مشجل

بأشراف

الأستاذ الدكتورة
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الأستاذ الدكتورة
مي فاضل ماجد الحبيب

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